ISSN 2222-0682 (online)

World Journal of *Methodology*

World J Methodol 2022 January 20; 12(1): 1-63

Published by Baishideng Publishing Group Inc

World Journal of Methodology

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Bimonthly Volume 12 Number 1 January 20, 2022

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INDEXING/ABSTRACTING

The *WJM* is now abstracted and indexed in PubMed, PubMed Central, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Rui-Rui Wu*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Ji-Hong Liu*.

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Bimonthly

EDITORS-IN-CHIEF
Bruno Megarbane

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2222-0682/editorialboard.htm

PUBLICATION DATE
January 20, 2022

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ONLINE SUBMISSION
https://www.f6publishing.com

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World J Methodol 2022 January 20; 12(1): 1-19

DOI: 10.5662/wjm.v12.i1.1

ISSN 2222-0682 (online)

MINIREVIEWS

Ophthalmological instruments of Al-Halabi fill in a gap in the biomedical engineering history

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Author contributions: Saad MN designed the study, drafted the article, prepared the figures and tables, granted permission for figures reproduction, designed the outline, and read and approved the final version of the article to be published.

Conflict-of-interest statement: There is no conflict of interest associated with the single author contributed his efforts in this manuscript.

Country/Territory of origin: Egypt

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Open-Access: This article is an open-access article that was selected by an in-house editor and

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Abstract

Al-Halabi is an intriguing ophthalmologist who invented numerous surgical instruments for treating various eye diseases. The illustrations of such instruments in his invaluable book "*Kitab Al-Kafi fi Al-Kuhl*" reflect his willingness to teach. Moreover, he included in his book a magnificent illustration of the anatomical structure of the eye. The book reflects Al-Halabi's medical practice and teaching and shows several advanced medical techniques and tools. His invaluable comments reflect his deep experimental observations in the field of ophthalmology. The current article provides proof that Al-Halabi is one of our early biomedical engineers from more than 800 years ago. Al-Halabi represents a ring in the chain of biomedical engineering history. His surgical instruments represent the biomechanics field. Al-Halabi should be acknowledged among the biomedical engineering students for his various contributions in the field of surgical instruments.

Key Words: Al-Halabi; Biomedical engineering education; Biomedical engineering history; Ophthalmological instruments

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Core Tip: Medieval Islamic ophthalmological instruments are a rich, complex, and understudied subject. This topic is interesting and deserves more attention than it has had. The book of Al-Halabi is indeed one of the interesting books on ophthalmology written in Arabic. The ophthalmological instruments included in Al-Halabi's table represent an untold story about the contributions of Muslim and Arab scholars in the field of ophthalmology. The aim of the present article is to fill in one of the gaps to some extent in biomedical engineering history. The ophthalmological instruments represent the biomechanics field.

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Received: January 13, 2021
Peer-review started: January 15, 2021
First decision: October 17, 2021
Revised: October 25, 2021
Accepted: December 11, 2021
Article in press: December 11, 2021
Published online: January 20, 2022

P-Reviewer: Au SCL
S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

Citation: Saad MN. Ophthalmological instruments of Al-Halabi fill in a gap in the biomedical engineering history. *World J Methodol* 2022; 12(1): 1-19
URL: https://www.wjgnet.com/2222-0682/full/v12/i1/1.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i1.1

INTRODUCTION

Studying the biomedical engineering history has greatly influenced the creative and inventive sense of biomedical engineering students[1]. Biomedical technologies have a very long history, among which are the ancient surgical instruments[2]. Bronze age epilation forceps were found in Ur, Iraq. The forceps, used in the treatment of trachoma, date back to ca. 2600 BC[3]. Other epilation forceps were discovered in tombs of the New Kingdom of Egypt (1570-1070 BC). Moreover, pots containing copper, zinc oxide, lead, and antimony sulphates were uncovered at the same tombs. These pigments were utilized for treating conjunctivitis[4]. It is argued that the first "book" on medicine in the world which is a medical papyrus (1553-1550 BC) was discovered in Luxor (Thebes), Egypt, in 1872 by the German archeologist Georg Ebers (1837-1898). In the eye treatment section, the only surgical procedure mentioned in the papyrus that has survived was applying to the eyelid onions, myrrh, gazelle excrement, and blood from lizards or bats after epilation through the use of feathers of a vulture[3].

Furthermore, in Egypt, several surgical needles could be seen carved in stone from the relief on the internal facade of the second wall in Kom Ombo Temple, constructed during the Ptolemaic dynasty (180-47 BC). Moreover, W.M. Flinders Petrie (1853-1942) (English Egyptologist) has discovered a series of ancient cooper needles with no hooks or apertures in King Khasekhemwy's tomb (ca. 2690 BC) in the Royal Necropolis at Abydos, Upper Egypt, in 1900. These needles represent the earliest known ancient surgical instruments for dislodging the cataract away from the pupil[5]. These needles could be found in the National Museums in Liverpool, England[6].

Hippocrates (Greek physician d. 370 BC) recommended using wool rounded around a wooden rod soaked in a caustic solution for scraping the eyelids when treating trachoma. Celsus (Roman encyclopaedist d. 50 AD) included in the medical work of his encyclopedia the recommendation of using a hook, a threaded needle, and a scalpel in the treatment of pterygium. Galen (Greek physician d. 210 AD) utilized a cuttlefish bone for roughness in treating trachoma and as an agent to dissolve pterygium. Aëtius of Amida (Byzantine Greek physician, mid-5th century to mid-6th century) applied a blunt hook, a tiny hook, a horse-hair and linen thread through a needle, and a knife in the treatment of pterygium[7]. Numerous gaps exist in the biomedical engineering history, one of which the present article aims to fill in through providing an overview of Al-Halabi's contributions.

AL-HALABI'S BIOGRAPHY

Al-Halabi (middle of the 13th century)[8,9] - whose full name was Khalifah ibn Abi Al-Mahasin Al-Halabi[10] and who was known as Khalifa Ben Abi Al-Mahassin[11], Khalifa ibn Abi Al-Mahasen Al-Halabi[12], Halifa B Abi L-Mahasin[13], and Halifa[14] for short - was named after Aleppo (Arabic: Halab), a city in the northern part of Syria, known for its thriving trades and wealth in the middle ages before the Mongol onslaught (in 1260 AD)[11]. It was mostly where he was born and died[15,16]. He mentioned two incidents that happened to him in Aleppo in 1252 and 1254 AD (650 and 652 AH)[11,15]. Aleppo was, as it has been for centuries, an important city and the capital of a principality[17]. It appears that Al-Halabi must have had a wide spectrum of knowledge about medical sciences as can be derived from the list of references that he cited. He was considered the first ophthalmologist to use a magnet in order to remove metallic foreign body from the eye by Wafai and Kalaji as well as Hirschberg, as he extracted a couching needle that was broken in a patient's eye during the surgery [14-16,18]. He realized that an instrument may be substituted by another because of unavailability[15].

AL-HALABI'S BOOK

The Sufficient Knowledge in Ophthalmology (Arabic: Kitab Al-Kafi fi Al-Kuhl), expected to be written within the period from 1256 to 1275 AD, is the only known book by Al-Halabi[11,13,14]. It is one of the masterpieces of Islamic ophthalmological medicine and surgery. The book's scientific value is incomparable to European manuscripts till the beginning of the nineteenth century[14]. The book describes in a separate chapter the measurements, weights, and sizes used at that time by physicians. It quotes 73 authors and 41 books before its time[9,18]. He referenced famous Arabian or Muslim authors such as Al-Razi, Ibn Sina (Avicenna), Al-Tabari, Hunayn ibn Ishaq, Al-Ghafiqi, Ibn Zuhr (Avenzoar), and Al-Zahrawi; Greeks such as Galen, Hippocrates, and Oribasius; and Indians[15,16].

The two copies of the book are to be found in the Bibliotheque Nationale in Paris, France (under the number 1043d. Arabe), and the Süleymaniye Kütüphanesi in Istanbul, Turkey (under the number Yeni Jami 924). The Paris manuscript was written by the Christian copyist Abd Al-Aziz ibn Abi Saeed Al-Masihi Al-Mawsili Al-Mutatabbeb in 1277 AD, meaning that it is very close to the time of the author. The Istanbul manuscript was written by the Muslim copyist Ahmad Al-Wali in 1560 AD, meaning that the manuscript was written around 300 years after the death of the author[15].

The book was disappointingly ignored until the French medical historian Lucien Leclerc (1816-1893) had described it briefly for the first time in 1876 in his book "*Histoire de la Médecine Arabe*"[19]. In 1905, the German ophthalmologist and historian Julius Hirschberg (1843-1925) with the orientalists J. Lippert (1839-1909) and E. Mittwoch (1876-1942) wrote a book entitled "*Arabian Ophthalmologists*" in which they studied the book with great detail[15,16,20].

The present article depends basically on the reproduction of Al-Halabi's book edited by M. Z. Wafai and M. R. Kalaji. They produced Al-Halabi's book within the combined series "*The Islamic Heritage in Ophthalmology*" in which they unearthed the Islamic glorious heritage and its valuable knowledge that served humanity for over thirteen centuries. They compared the two copies of the book (in Paris and Istanbul) and put in the text the most accurate words. They explained in the footnotes some words that could contain more than one meaning. They gave the modern names of the diseases that Al-Halabi described. The book was published by the Islamic Educational, Scientific and Cultural Organization, Rabat, Morocco in 1990[15].

THE INSTRUMENTS TABLE

Al-Halabi's book is the first book to place thirty-six surgical instruments in a very elegant table[9,21]. This table is considered to be well organized as each surgical instrument was placed in a special frame with the name of the instrument on the top and the way to use it underneath it, as shown in Figure 1[16,22]. This was the first time that an author put a table for the surgical instruments, unlike his predecessors who used to put the drawing of the instrument within the text[15].

Some of these instruments were known and used by earlier Arabic ophthalmologists, and a few authors also included illustrations of the instruments, but none was as comprehensively or as systematically as Al-Halabi[18]. For example, Al-Razi (d. 925 AD) recommended the rose leaf needle (No. 7 in Figure 1) for scraping off the chronic trachoma. Moreover, Al-Razi used hooks (No. 6 in Figure 1) to lift thick pannus, which was then excised with scissors[23]. Al-Zahrawi (d. 1013 AD) used spear (No. 9 in Figure 1) to treat symblepharon[24]. Al-Ghafiqi (d. 1165 AD) used drill (No. 12 in Figure 1) and small drill (No. 34 in Figure 1) in dacryocystorhinostomy[25]. Ammar ibn Ali Al-Mawsili (d. 1010 AD) invented a hollowed aspirating needle (No. 22 in Figure 1) for cataract surgery[26]. Ibn Al-Nafis (d. 1288 AD)[27] used the scalpel (No. 4 in Figure 1) or the rose leaf needle for scraping the eyelid in trachoma surgery. Besides, Ibn Al-Nafis used speculums (No. 3 in Figure 1), scissors (No. 2 in Figure 1), and hooks in the treatment of pannus. Furthermore, Ibn Al-Nafis applied the rounded couching needle (No. 16 in Figure 1) to penetrate pterygium and strip it away from the conjunctiva and cornea[28].

It is worth mentioning that Al-Halabi used the instruments with other diseases other than what he mentioned in the instruments table. So, for all the instruments discussed in the current article, the statements of use on the table itself were given first and then supplemented with other uses given elsewhere in his treatise (with page numbers given for those), as could be seen in Table 1. These instruments were as

Table 1 The descriptions of the uses of the instruments[15]

Instrument name	No. in Figure 1	Uses in his table	Other uses in his book with page numbers in reference[15] given
Scissors	1	Distichiasis	
Scissors	2	Pannus	Concretions (p.121, p.276); Ectropion (p.278); Stye (p.279); Distichiasis (p.282); Hordeolum (p.287); Hemangioma at the eyelid (p.287); Wart (p.289); Lacrimal caruncle swelling (p.291); Pterygium (p.293); Hemangioma at the conjunctiva (p.297); Iris prolapse (p.299); Superficial temporal artery (p.300)
Speculums	3	Only description	Pannus (p.295)
Scissors	5	Pannus, pterygium (a note with the myrtle leaf needle)	Granuloma at the conjunctiva (p.297)
Hooks	6	Pannus, pterygium, distichiasis	Symblepharon (p.277); Ectropion (p.278); Distichiasis (p.281); Hemangioma at the eyelid (p.287); Sebaceous cyst (p.290); Lacrimal caruncle swelling (p.291); Hemangioma at the conjunctiva (p.297); Granuloma at the conjunctiva (p.297); Superficial temporal artery (p.300)
Rose leaf needle	7	Hemangioma at the eyelid, sebaceous cyst, hemangioma at the conjunctiva (a note with the half rose leaf needle)	Trachoma (p.275); Symblepharon (p.277); Lagophthalmos (p.123, p.278); Ectropion (p.278); Wart (p.289)
Half rose leaf needle	8	Hemangioma at the conjunctiva, (Trachoma, concretions) (a note with the scraper)	Adhesions between the two eyelids (p.277)
Spear	9	Sebaceous cyst	Symblepharon (p.277); Ectropion (p.278)
Myrtle leaf needle	10	Pterygium, symblepharon, sebaceous cyst (a note with the spear)	
Axe	11	Bloodletting the supraorbital vein	
Drill	12	Dacryocystitis	
Raven's beak	13	Removing whatever sticks to the eye or the inner side of the eyelid	Conjunctival wound (p.213)
Lancet	14	Cataract	
Rounded couching needle	16	Could be substituted by the triangular needle	Pannus (p.295); Hemangioma at the conjunctiva (p.297); Cataract (p.305)
Scalpel	18	Hypopyon, chemosis	Adhesions between the two eyelids (p.277); Allergic dermatitis (p.288)
Scraper	19	Trachoma, concretions	
Lancet	20	Lipoma, chalazion	Concretions (p.276); Chemosis (p.285); Blepharitis (p.289); Hypopyon (p.298); Superficial temporal artery (p.300); Cataract (p.307)
Sickle	21	Adhesions between the two eyelids, ectropion	
Hollowed aspirating needle	22	Cataract	
Gooseneck speculum and tailor's needle	24	Distichiasis	
Gatherer	27	Wart	'Foreign body' fallen into the eye (p.215), Stye (p.279), Hordeolum (p.287),
Gatherer	28	Distichiasis, 'foreign body' fallen into the eye	
Cautery	29	Supraorbital vein, superficial temporal veins	Superficial temporal artery (pp.300-301)
Cautery	30	Dacryocystitis	
Cautery	31	Distichiasis	
Small drill	34	Dacryocystitis	
Awn-tongs	35	'Foreign body' fallen into the eye	

functional as the seventy-six instruments invented by the Austrian ophthalmic surgeon Wilhelm Czermak (1856-1906) as considered by Hirschberg[14].

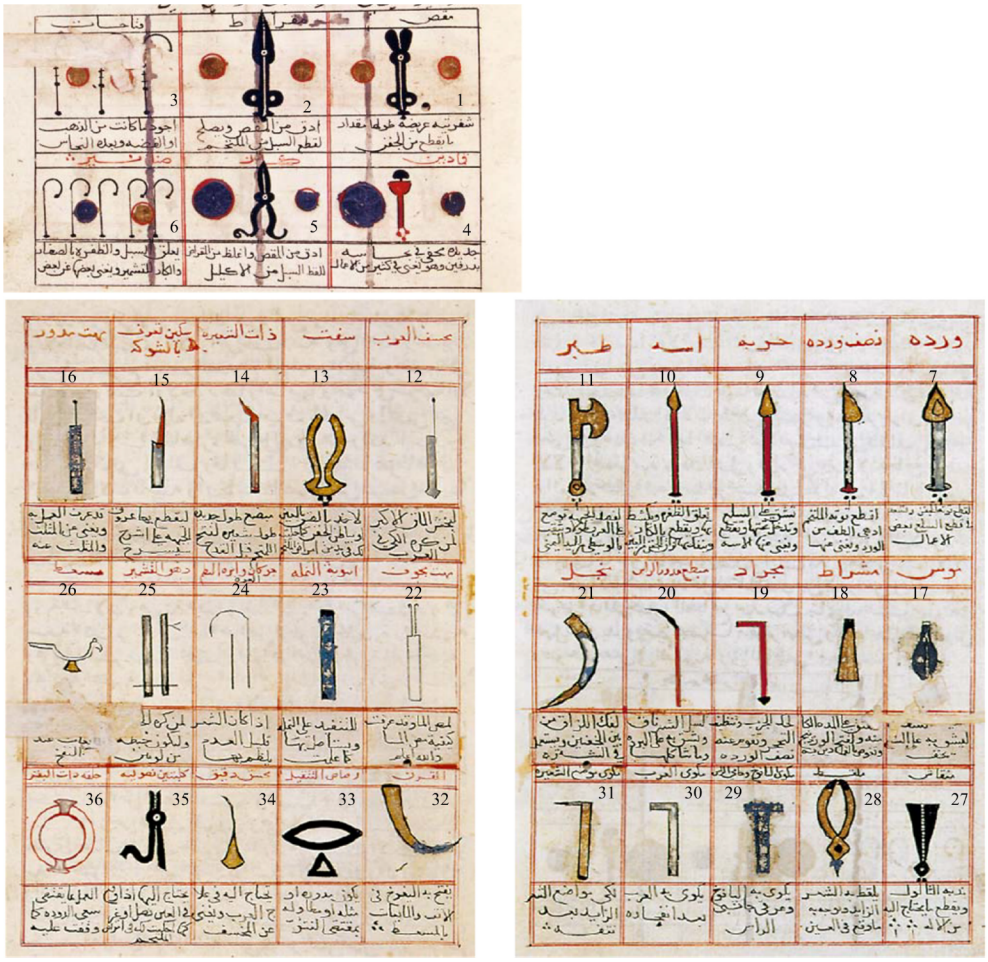

Figure 1 Ophthalmological instruments from Al-Halabi's book, the Istanbul manuscript of the Süleymaniye Kütüphanesi, Yeni Cami 924.
Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 6. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

CONTRIBUTIONS IN EYELID DISEASES

Al-Halabi utilized a needle in the treatment of trachoma (called granular conjunctivitis or Egyptian ophthalmia, which is a contagious, chronic inflammation of the mucous membranes of the eyes, caused by *Chlamydia trachomatis*; it is characterized by swelling of the eyelids, sensitivity to light, and eventual scarring of the conjunctivae and corneas of the eyes). The patient was lied down and Al-Halabi stayed beside the diseased eye. A nurse stayed beside Al-Halabi for handing him the required surgical instrument. The shape of the tip of the needle resembled the rose leaf with a small, short, pointed end, as illustrated in Figure 2[22]. He started stripping the scabies by the needle from the medial canthus to the lateral canthus. He noted that the ophthalmologist should preserve mildness and tranquility to prevent eye damage. As an alternative to the needle, a scraper can be used. It looks like a right-angled bolt extrator, as seen in Figure 3[22]. It is utilized for scratching scabies[15].

Moreover, Al-Halabi used the rose leaf needle when treating lagophthalmos (a condition in which a complete closure of the eyelids over the eyeball is difficult or impossible). If lagophthalmos was a result of a healed ulcer or a strained suture of a wound leading to eyelid attraction, the rose leaf needle was used to incise the place of the healed ulcer or the strained suture. Then, a piece of cotton was inserted at the place of the incision[15].

He used a lancet in order to remove chalazion (a cyst that appears on the eyelid because of a blocked meibomian gland)[8]. If medications did not heal chalazion, an incision should be applied to it. If the chalazion was at the upper eyelid, it should be extended downwards. If the chalazion was at the lower eyelid, it should be extended upwards. At this point, a horizontal incision was applied to the chalazion through the

Figure 2 Rose leaf needle (instrument number 7 in Figure 1) used in treatment of trachoma, lagophthalmos, symblepharon, hemangioma at the eyelid, and sebaceous cyst. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 45. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 3 Scraper (instrument number 19 in Figure 1) for scratching scabies and for digging out concretions. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 49. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

use of a lancet with a rounded tip (Figure 4) in order to cleave the chalazion[22]. If the chalazion was inclined to the outer side of the eyelid, the incision should be applied on the outer side of the eyelid, and vice versa. Finally, the chalazion should be removed using a spoon[15,29].

The lancet as well could be used in the treatment of chemosis (abnormal edematous swelling of the mucous membrane covering the eyeball and lining the eyelids) and lipoma (benign fatty tumor occurring at the inner side of the upper eyelid between its layers). In case of lipoma, Al-Halabi warned the ophthalmologists that they should be cautious of the penetration of the eyelid leading to the puncture of the tarsal cartilage of the eyelid, and a perforated cornea and iris. Furthermore, the lancet could be used in the incision of blepharitis (inflammation of the eye glands and eyelash follicles along the margin of the eyelids).

The same lancet was applied for removing the concretions (small, separated tumors at the inner side of the eyelid). A horizontal deep incision was conducted through the use of the lancet for digging out the stone formations. Besides, the scraper could be used as a substitution for the lancet. Al-Halabi mentioned that the ophthalmologist should be careful while using either the lancet or the scraper to prevent the penetration of the eyelid. Scissors may be used after the eradication of the concretions to cut off the incision slits. This procedure slows the healing of the incision in order to prevent the reappearance of the concretions. The scissors (Arabic: Miqrad) have a flattened, sharp, and straight shape, with the pivot tempered, as shown in Figure 5[15,22].

Al-Halabi used a tool that resembles to some extent an extremely small sickle (see Figure 6) in the treatment of the adhesions between the two eyelids[22]. If the two eyelids were closely adhered, a small incision should be performed using a fine scalpel (instrument number 18 in Figure 1) or a needle at the origins of the eyelashes to let the tip of the sickle be introduced between the two eyelids. The shape of the tip of the needle resembles half the rose leaf (instrument number 8 in Figure 1). He started opening the incision from the direction of the medial canthus towards the lateral canthus[15].

Figure 4 Lancet with rounded tip (instrument number 20 in Figure 1) used in treatment of chalazion, chemosis, lipoma, blepharitis, concretions, hypopyon, headache, and cataract. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 49. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 5 Scissors (Miqrad) (instrument number 2 in Figure 1) used in treatment of concretions, ectropion, stye, hordeolum, lacrimal caruncle swelling, pannus, pterygium, hemangioma, iris prolapse, and headache. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 48. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

If the adhesions were between the eyelid and the conjunctiva or the cornea (symblepharon), two hooks were utilized for holding up the eyelid. If the adhesions were at the upper eyelid, it should be extended upwards. If the adhesions were at the lower eyelid, it should be extended downwards. Al-Halabi used the rose leaf needle to separate the adhesions. He stated that the ophthalmologist should use delicacy when the adhesions were between the eyelid and the conjunctiva. A tremendous delicacy should be applied when the adhesions were between the eyelid and the cornea in order to prevent making punctures in the corneal layers. These punctures could cause iris prolapse (protrusion of the iris or part of the iris through an injury in the cornea).

When the adhesions are closer to the bones of the eyebrow, it is harder to separate them, and a longer tool should be used like the needle or the spear. The shape of the tip of the needle resembles the myrtle leaf with a small, long pointed end appropriate for cleaving the adhesions, as presented in Figure 7[22]. The spear has a big long pointed end, as shown in Figure 8, and could replace the myrtle leaf needle[15,22].

Al-Halabi used hooks (instrument number 6 in Figure 1), the spear, and the scissors (Miqrad) in the treatment of ectropion (eversion or turning outward of the margin of the eyelid). If the ectropion was due to the growth of a superfluous fleshy tissue on the inner side of the eyelid, two or three hooks were inserted in the fleshy tissue for holding up the eyelid. Then, the tip of the spear was inserted under the fleshy tissue to

Figure 6 Sickle (instrument number 21 in Figure 1) for splitting adhesions between the two eyelids. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 52. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 7 Myrtle leaf needle (instrument number 10 in Figure 1) used in treatment of symblepharon, and pterygium. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 47. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 8 Spear (instrument number 9 in Figure 1) used in treatment of symblepharon, ectropion, and sebaceous cyst. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 45. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

warn it off the eyelid skin. Finally, the scissors were utilized for dissecting the fleshy tissue. Al-Halabi pointed out that the ophthalmologist should use tremendous delicacy in order to prevent the laceration of the eyelid skin and its dissection with the fleshy tissue[15].

He used the scissors (Miqrad) and a gatherer for treating stye (inflammation of the sebaceous gland of an eyelash) or wart (small, benign growth caused by a viral infection of the skin). The gatherer which has two broad heads (instrument number 27 in Figure 1) was utilized for holding stye or wart from its middle and extending it away from the eyelid. Then, the scissors were used for cutting it out of its roots. In the case of the wart, the scissors could be replaced by a horse-tail hair. In order to saw off the wart, the horse-tail hair was placed under the gatherer[16]. Actually, a recent study indicated that the horse-tail hair is about 0.2 mm thick, and it recommended using it in ophthalmic surgeries[30].

In addition, the scissors and gatherer were utilized for eradicating hordeolum (suppurative inflammation of a gland of the eyelid). Furthermore, the scissors were used for uprooting the hemangioma at the eyelid (benign tumor found on the eyelid, composed of dilated blood vessels, and often encapsulated within a fibrous shell) with the aid of a hook. The rose leaf needle could substitute the scissors.

The rose leaf needle was used in sebaceous cyst removal. The function of the needle was to make a horizontal incision at the liquefied tissue. Then, a hook and the rose leaf needle were utilized for removing the pus. The spear was applied for a deeper, T-shaped incision when the inflamed area was bigger and deeper. The myrtle leaf needle could substitute the spear. Al-Halabi elucidated that the liquefied tissue should be completely removed in order to avoid the reappearance of the sebaceous cyst[15].

CONTRIBUTIONS IN DISTICHIASIS TREATMENT

Al-Halabi used annexation technique in the treatment of distichiasis (congenital, abnormal, accessory row of eyelashes, often causing severe discomfort from contact with the eye). If the eyelashes number was from one to five, a very thin tailor's needle could be used. A thread was twisted through the needle hole and around itself to make a buttonhole. Another thread was entered in the buttonhole to make it ready for annexation. The needle was applied at the margin of the eyelid from its inner side to the center of the distichiasis. The abnormal eyelashes were entered in the buttonhole with the aid of a tip of a gooseneck speculum (instrument number 24 in Figure 1). Then, the needle was pulled slowly and carefully to tighten the buttonhole on the eyelashes. Finally, the needle was extended quickly away of the eyelid, and the eyelashes were epilated at the middle of the buttonhole[15].

Another technique for treating distichiasis was the use of glue. If the abnormal eyelashes were long enough and their number was from one to five, they were pasted to the nearest normal eyelashes. The components of the glue are listed in Table 2[31, 32]. First, the glue was placed on a plate made of bronze. Second, the glue was melted on a soft flame. He noted that the flame should be near the ophthalmologist and ready to be used in order to prevent the glue from cooling down while being used. Third, an eye stick or a tip of a hook was utilized for applying the glue on the misdirected eyelashes. Finally, these eyelashes were extended to allow them to paste to the normal eyelashes for preventing the irritation of the eyeball. If the glue reached undesired normal eyelashes, it was removed by rubbing[15].

Moreover, distichiasis could be treated by cauterization. If the aberrant eyelashes number was from one to five despite being long or short, they should be plucked out first through the use of the gatherer. It could also be used in pulling out any "foreign body" that has fallen into the eye, as shown in Figure 9[22]. Then, a cautery was applied to the position of the pulled-out eyelashes. The cautery was pointed to be appropriate for the narrow places, as seen in Figure 10[22]. Al-Halabi preferred gold as a material of the cautery as the disease was cleared quickly and blisters did not appear at the cauterized position. When the eyelash was plucked out, the cautery was heated till its color turned red. Then, it was directed to the position of the pulled-out eyelashes. It should be slightly deeper to destroy the lash follicle[15].

In case of abnormal eyelashes number being more than five, their place on the eyelid should be cut using scissors. First, the eyelid was rolled up and incised at the location of the abnormal eyelashes from the medial canthus to the lateral canthus. A tailor's needle and a thread were used to sew the incision. Then, Al-Halabi placed the amount of the eyelid's skin between the two blades of the scissors. The scissors (Arabic: Miqass) (see Figure 11) should have two broad blades with their length equal to or more than the amount of the eyelid's skin that should be removed[22]. He distinguished the Miqass scissors from the Miqrad scissors that the first type has thicker and longer blades than the second being appropriate for removing the amount of the eyelid's skin in one cut[15].

CONTRIBUTIONS IN MEDIAL CANTHUS DISEASES

Al-Halabi used a hook and scissors (Miqrad) in treating lacrimal caruncle swelling. The hook was utilized for hanging up the swelling. Then, the swelling was excised by the scissors. Finally, he warned the ophthalmologists that they should be cautious of the excision of a normal part of the lacrimal caruncle causing epiphora[15].

In case of dacryocystitis (congenital displacement of lacrimal tissue results in subconjunctival cysts), he placed a cautery that looks like a right-angled screwdriver, as shown in Figure 12[22]. Its shape is appropriate for the target with a smooth rounded tip contact. This cautery was used to cauterize the lacrimal gland fistula after its rupture until the vessels stop bleeding. He preferred gold as a material of the cautery. The cautery was heated till its color turns red. Then, it was directed to the

Table 2 Medicinal natural products used in distichiasis treatment[15,31,32]

Common name	Scientific name	Arabic name	Effects
Mastic	Pistacia lentiscus	Mustaqy	Antiseptic, anti-inflammatory, analgesic, sedative
Sarcocolla	Astragalus Sarcocolla L	Aanzarout	Anthelmintic, emollient
Aloe	Aloe vera	Sabr	Wound healing, antimicrobial

Figure 9 Gatherer (instrument number 28 in Figure 1) used in treatment of distichiasis. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 53. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 10 Pointed cautery (instrument number 31 in Figure 1) for cauterizing the places of superfluous eyelashes after they have been pulled out. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 51. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

position of the fistula till boiling. The cauterized position was wiped with a rag. Next, cauterization was repeated several times till the crust on the lacrimal bone was peeled. Finally, the cauterized position was cleaned from rot[15].

A drill was utilized in case the patient refused the cauterization solution. It consists of a handle and a long shaft with a small sharp pointed end, as seen in Figure 13[22]. It was used to clean the entire corner of the eye. In addition, a perforation was made at the tear-producing gland fistula in the nasal direction. A high strength should be applied until the blood flows from the nose and the mouth. Al-Halabi discussed that the perforation should not be directed upward as this would be the incorrect direction. Moreover, he noted that the ophthalmologist's working hand should be inclined to the nose (not to the eye) in order to avoid damage to the eye layers. Through this, a smaller drill (instrument number 34 in Figure 1) was wrapped in cotton which should be dry or soaked in ox fat or in verdigris ointment (corrosive, anti-inflammatory effects). He pointed that the verdigris became less effective if used after a year of its manufacture date. This would then be exchanged every day until the cotton was extracted clean in order to reach the bone pureness. With some details, the opening of

Figure 11 Scissors (Miqass) (instrument number 1 in Figure 1) used in treatment of distichiasis. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 46. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 12 Cautery (instrument number 30 in Figure 1) used in treatment of dacryocystitis. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 43. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 13 Drill (instrument number 12 in Figure 1) for cleaning the entire corner of the eye. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 43. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

the wound should be widened through the use of the smaller drill, and the cotton should be exchanged as it is expected that the cotton would be extracted from the wound with small dirty fractures of the bone[15].

CONTRIBUTIONS IN PANNUS TREATMENT

Al-Halabi used eye speculums, hooks, scissors (Miqrad), couching needle with a rounded tip, dove's feather, and eye stick in the treatment of pannus (membrane of fine blood vessels and fibrous tissue that spreads down over the peripheral cornea in trachoma and other inflammatory corneal disorders causing loss of vision)[34]. The patient was lied down, and he stayed behind the patient. A sessile pillow was put under the patient's head to make it slightly declined[15].

A tool, which looks somewhat like the eye speculum nowadays (instrument number 3 in Figure 1), was used to keep the patient's diseased eye open. Al-Halabi expressed his preference of using two thumbs of a skilled nurse instead of two eye speculums. The disadvantage of using the eye speculums from Al-Halabi's viewpoint was the obstruction of the eye speculums to the movement of the scissors (Miqrad) while cutting off the pannus. Three hooks were applied to hang up the pannus, one from the medial canthus, one from the center of the conjunctiva, and one from the lateral canthus. The hooks were applied near the upper eyelid[15].

A tip of the scissors (Miqrad) was utilized for cutting off a part of the membrane from the lateral canthus. Then, the couching needle was introduced to saw off the pannus. Al-Halabi recommended that the couching needle should be made of red bronze (instrument number 16 in Figure 1). A dove's feather might substitute the couching needle. The Miqrad scissors were used again to pick out the membrane until reaching the medial canthus. At this part, the three hooks were applied near the lower eyelid, and the same procedure was repeated till the pannus was completely removed. He noted that the ophthalmologist should watch out the cornea while performing this procedure[15].

A tiny eye stick was wrapped in cotton which should be soaked in egg yolk and rose oil (calms painful sores and constricts and cools wounds). He recommended that the rose oil should not be used after two years of its manufacture date as it got expired. The eye stick should be applied at the middle of the eye and extended in the directions of the two eyelids in order to prevent any adhesions that might occur[15].

Al-Halabi described another way to get rid of the pannus. One hook was applied to hang up the pannus. The scissors (Arabic: Kaz) were used to make a cut in the membrane. The Kaz scissors were thinner than the Miqass scissors and thicker than the Miqrad scissors, as shown in Figure 14[23]. Consequently, the hook was raised up while connected to the membrane, another hook was inserted, and the Kaz scissors were applied again. This procedure was repeated many times until the membrane was gathered by the Kaz scissors as one piece[15].

A third technique was elucidated by Al-Halabi for removing the pannus. Several hooks were used to hang up the pannus. The number of hooks ranged from six to twelve depending on the size of the eye. The insertion of the hooks started from the medial canthus near the upper eyelid and ended near the lower eyelid in a circular shape. A tip of the Miqrad scissors was used for cutting off a part of the membrane from the lateral canthus near the upper eyelid, and then near the lower eyelid. Then, the couching needle was introduced to saw off the pannus. At this point, the Kaz scissors were applied to make a circular incision in the membrane. The pannus would be extracted in a shape that looks like a signet ring[15].

CONTRIBUTIONS IN CONJUNCTIVA DISEASES

Al-Halabi used a hook, a smooth dove's feather, myrtle leaf needle, Miqrad scissors, and Kaz scissors in treating pterygium (a pink, fleshy tissue that grows on the conjunctiva)[33]. The hook was utilized for raising the pterygium from its center. If the pterygium loosely adhered to the conjunctiva, a smooth dove's feather was inserted under the hook to saw off the pterygium. If the pterygium hardly adhered to the conjunctiva, two or three hooks could be added to the sides of the pterygium. Then, the myrtle leaf needle was used for sawing off the tissue. Finally, the Miqrad scissors or the Kaz scissors were used for cutting off the pterygium[15].

Al-Zahrawi used a horse-tail hair to saw off the pterygium. The dove's feather and the horse-tail hair were utilized due to the inability of the technology of that era to manufacture a man-made instrument with the required thickness and sharpness[24]. Al-Halabi stated that the ophthalmologist should use extreme delicacy in order to avoid penetrating the cornea or the medial canthus. He asked the ophthalmologist to beware of the total removal of the pterygium to avoid its reappearance. Furthermore, he asked the ophthalmologist to take care of the adhesiveness of the pterygium with

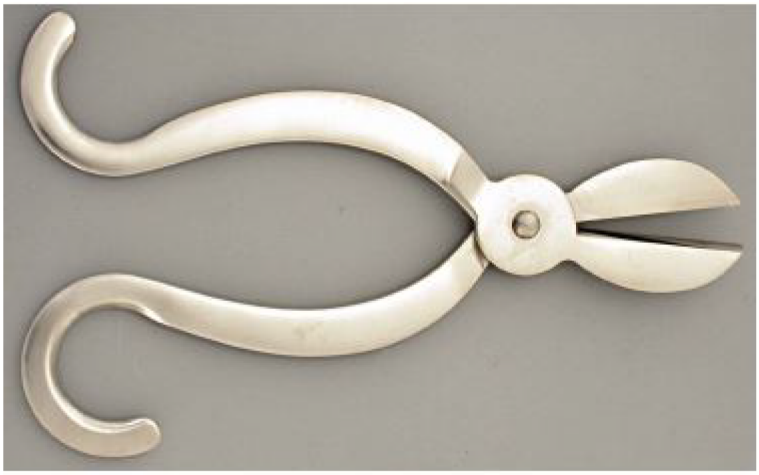

Figure 14 Scissors (Kaz) (instrument number 5 in Figure 1) used in treatment of pannus, pterygium, and granuloma at the conjunctiva.
Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 48. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

the conjunctiva. If the pterygium extremely adhered to the conjunctiva, the surgical instruments should not be applied at all in order to avoid eye damage[15].

Al-Halabi used a hook, a couching needle, the half of the rose leaf needle, and the Miqrad scissors in treating hemangioma at the conjunctiva. The hook was applied to raise up the hemangioma. He stated that the ophthalmologist should use tremendous delicacy while raising up the hemangioma because it has a spongy consistency, and the hook might turn up during the treatment. The couching needle was inserted under the blood vessels, and he started sawing off the hemangioma. The half of the rose leaf needle could replace the couching needle in sawing off the hemangioma. Finally, the scissors were utilized for uprooting the hemangioma[15].

Al-Halabi used a hook and the scissors (Kaz) in the treatment of granuloma at the conjunctiva (growth appearing like a nodule, consisting essentially of granulation tissue, and occurring as a result of localized inflammation). The hook was utilized for holding up the granuloma. If the size of the granuloma was large, two or three hooks should be applied. The Kaz scissors were applied to eradicate the granuloma[15].

He used a Raven's beak in the treatment of conjunctival wound (presence of wound or laceration of the conjunctiva with swelling and edema of the wound edges). The Raven's beak is a slim gatherer with two heads, as displayed in Figure 15[22]. The Raven's beak was used to extract an extremely small piece of wood that penetrated the eyeball and was fallen between the sclera and the eye bones. In addition, the Raven's beak could be used for removing whatever sticks to the eye or the inner side of the eyelid. The awn-tongs (see Figure 16) were utilized when an awn (either a hair- or bristle-like appendage) or a similar object fell down into the eye[15,22].

CONTRIBUTIONS IN CORNEA AND IRIS DISEASES

Al-Halabi used a scalpel and a lancet in treating hypopyon (inflammatory cells in the anterior chamber of the eye). The scalpel was applied between the cornea and the conjunctiva to make an incision. He noted that the ophthalmologist should be cautious of the penetration of the iris. The lancet was applied at the position of the incision for the expulsion of the inflammatory cells. He used a needle, a thread, and scissors (Miqrad) in the treatment of the iris prolapse. A needle with a thread was inserted beneath the protrusion from the medial canthus and extracted from the lateral canthus. The two ends of the thread were drawn out of the eye. Then, the scissors were used to cleave the protrusion[15].

Figure 15 Raven's beak (instrument number 13 in Figure 1) for removing whatever sticks to the eye or the inner side of the eyelid. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 52. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 16 Awn tongs (instrument number 35 in Figure 1) used when an awn or something of that kind falls into the eye. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 53. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

CONTRIBUTIONS IN HEADACHE AND MIGRAINE TREATMENT

In case of pain in the temporal muscles, headache, and migraine due to eye diseases, Al-Halabi used scissors (Miqrad), a hook, a lancet, and a cautery in the bloodletting and cutting the superficial temporal arteries and the posterior auricular veins[13]. A cord was tightened around the patient's throat with great mildness. Then, the patient was asked to throttle himself/herself with leniency. This procedure was conducted to make the arteries and the veins of the head visible and to target them. In case of the superficial temporal artery, he suggested shaving the temple, and spotting the artery with ink—this made the artery visible. After targeting the artery, the patient was asked to stop throttling himself/herself, and the cord was released to reduce the amount of the seepage blood while working[15].

Then, he attracted the skin over the artery using two fingers of his left hand. At this point, an incision was performed in the skin through the use of scissors (Miqrad). Consequently, the artery was extracted from its position and eradicated through the

use of a hook's tip. In case the artery was slim, a lancet was inserted under it to cut it off. Equally, the artery might be cut off using the scissors (Miqrad) instead of the lancet. In case the artery was thick, Al-Halabi used a needle and a thread made of silk or linen so that the artery would be tied in two places. Then, the lancet was utilized for opening an aperture between the two ligatures so that the desired amount of blood would flow out. After bloodletting, the artery was ligated and the blood flow was controlled[15].

Al-Halabi provided the cauterization by a flathead cautery instead of the eradication of the artery through the use of the lancet or the scissors. The same procedures were conducted for the posterior auricular vein except that the cautery was smaller than that for the superficial temporal artery. His usage of smaller cautery for the posterior auricular vein is in line with modern knowledge, as the thickness of the outer walls and the layers of muscle and elastic fibers of the veins is less than that of the arteries. As a disinfection procedure, he recommended the use of medical packs made of cotton after cauterization[15].

In case the previous procedures were unfruitful in treating migraine and headache, the supraorbital vein and the superficial temporal veins should be cauterized. First, the head was shaved from the middle of the scalp towards the forehead. Al-Halabi described how to know the position of the supraorbital vein in the forehead. The patient was asked to put the nail of his/her thumb of the right hand on the apex of the nose. Then, his/her forefinger was extended on the nasal septum towards the forehead. The maximum point the forefinger could reach was the position of the supraorbital vein. Next, this position was massaged using rough linen pack until it became red. At this point, a cautery was applied on the position longitudinally and horizontally in order to make a cross-shaped cauterization. The cautery looks like a fleshy olive and was made of iron, as shown in Figure 17[22]. The same procedures were performed on the superficial temporal veins. A knife that resembles to some extent an extremely small axe (see Figure 18) might replace the cautery for the supraorbital vein[22]. The knife was utilized for bloodletting the supraorbital vein. It was placed lengthwise on the vein, and the severing was conducted with the middle finger of the right hand[15].

THE ANATOMICAL STRUCTURE OF THE EYE

Al-Halabi's book was the first book to give a remarkable illustration of the anatomy of the brain, the eyes, and the visual pathway among them, as displayed in Figure 19[9, 22]. The illustration presented the eyes, the optic chiasm, the cerebral ventricles, the pericranium, the dura mater, the pia mater, the olfactory nerves, and the petrosal bone [34]. The illustration showed that the left eye is controlled by the right part of the brain, and vice versa. All the ocular coats (cornea, sclera, choroid, zonules, and the retina) and the three humidities (vitreous, crystalline lens, and aqueous) are clearly illustrated and labelled. In this drawing, the conjunctiva seemed to originate from the pericranium and the sclera from the dura mater[16].

He drew the optic nerves as hollow, parallel lines, stemming from the back of the sclera to meet the optic chiasm and continue their course posteriorly through the brain tissue until finally reaching the occipital lobe[35]. Two parallel lines extended from the back of the lens to the sclera and optic nerve, almost nearing the description of Cloquet's canal. Although Al-Halabi drew a small circle in the middle of the triangle behind the chiasm, he did not mention or give the pituitary gland a name.

The American neuroanatomist and neurologist Stephen Lucian Polyak (1889-1955) considered all the European diagrams of the eye until the end of the 16th century, including those by Leonardo da Vinci (1452-1519)[36], to be dependent on Arabic models[37]. Al-Halabi's drawing is more detailed and informative than Leonardo da Vinci's one (Codex Atlanticus: Biblioteca Ambrosiana, Milan, vol. 3, fol. 628). Al-Halabi's drawing remained a reference to all the books dealing with the anatomy of the eye until the German physician D. W. Soemmerring (1793-1871) drew a cross section of the eye in 1827. The American Academy of Ophthalmology used a modified version of Al-Halabi's drawing as the emblem for the 1987 annual meeting without giving credit to him[11,15,16].

J. Hirschberg stated that "*First of all one must appreciate that the Arab ophthalmologists since Hunain had made real efforts to exploit the anatomy, the physiology and the pathology of the brain for their patients. Therefore we do not wish to criticize them for having dragged the optic nerve crossing unnaturally to the front in this imaginary stylized representation of the brain in order to be able to illustrate it at all; we also do that in our diagrams*" and "*In any*

Figure 17 Olivary-shaped cautery (instrument number 29 in Figure 1) used for headache, and migraine. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 50. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

Figure 18 Axe (instrument number 11 in Figure 1) for bloodletting the supraorbital vein. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 50. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

case we see in this venerable picture, which probably goes back to models at least from the time around 1000 A.D., a cautious attempt to represent what D. W. Soemmerring insightfully arranged in his classic illustration in 1827" commenting on Al-Halabi's illustration[22].

CONTRIBUTIONS IN CATARACT SURGERY

Al-Halabi detailed cataract operation representing twelve sorts of cataract in a table [14]. He dedicated a chapter of his book to the surgeries for removing the cataract. He described cataract operations, the required instruments, and the steps to be taken after the operation. He included his own experience which was described in good detail. He is so confident in his own talents that he had the courage to operate the cataract surgery on a one-eyed man for forty days[11,13]. Moreover, he surprisingly reported that he performed a successful cataract surgery for a predatory bird that was owned by his servant. However, the bird's head movement after the surgery allowed the reappearance of the cataract[15].

He preferred the use of the hollowed cataract needle for aspiration[33], on the solid three-edged couching needle or the rounded couching needle for its safety in the operation. First, a lancet with a rounded tip (instrument number 14 in Figure 1) was utilized for puncturing the outer coats. Then, the hollowed needle (instrument number 22 in Figure 1) was applied to the iris without perforating the cornea. Extracting the lens by suction, using the hollowed needle that resembles to some extent the aspirating syringe nowadays as what could be understood from Al-Halabi's description has the benefit of excluding the possibility of the lens falling back into the eye[8,15].

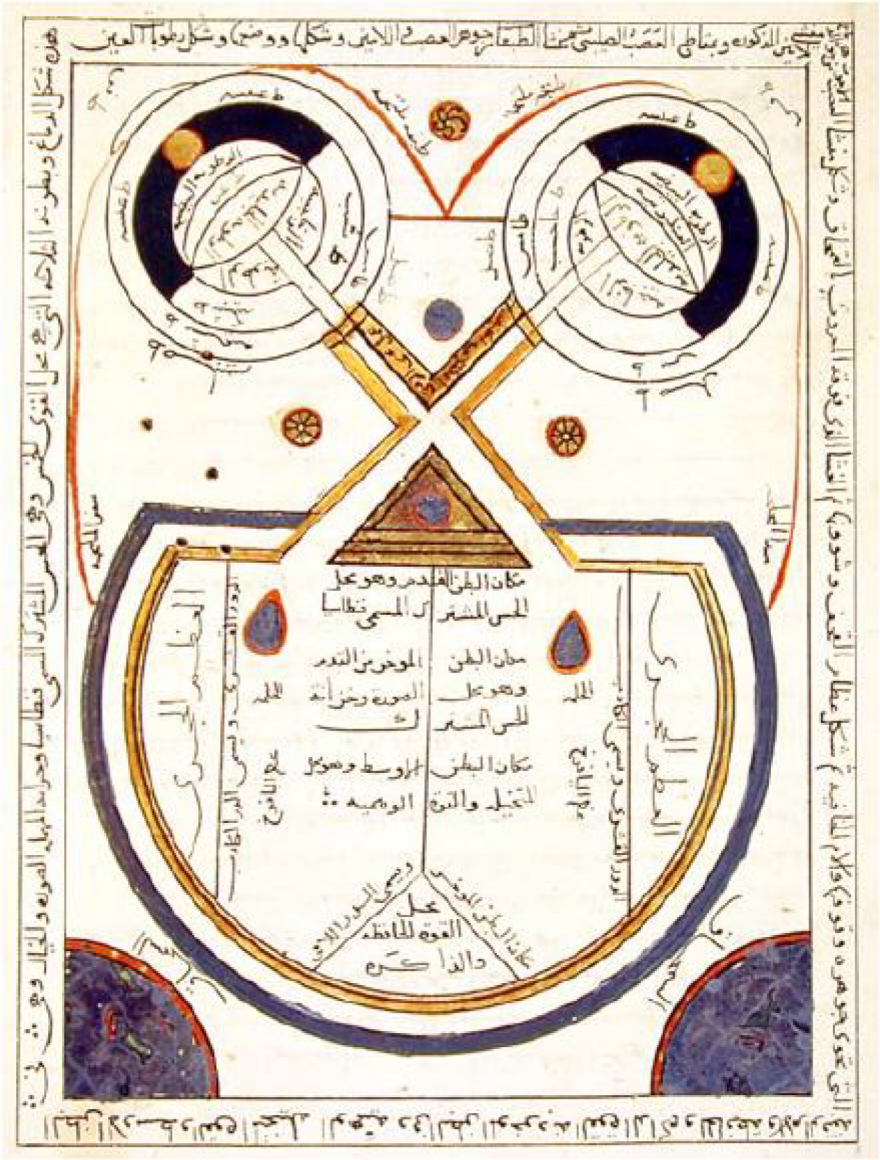

Figure 19 Optic nerve crossing together with that of the eye and the brain from Al-Halabi's book, the Istanbul manuscript of the Süleymaniye Kütüphanesi, Yeni Cami 924. Citation: Sezgin F, Neubauer E. Volume IV: Catalogue of the collection of instruments of the institute for the history of Arabic and Islamic sciences. In: Sezgin F. Science and technology in Islam. Frankfurt am Main, Germany: Institute for the History of Arabic-Islamic Science, 2010: 27. Copyright ©The Author(s) 2010. Published by Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften). The author has obtained the permission for figure using from the Institute for the History of Arabic-Islamic Science (Institut für Geschichte der Arabisch-Islamischen Wissenschaften) (Supplementary Material).

CONCLUSION

Al-Halabi is an early biomedical engineer who invented various ophthalmological instruments. He is the first ophthalmologist to use a magnet for removing metallic foreign body from the eye. He utilized his instruments in the treatment of different eye diseases such as trachoma, lagophthalmos, chalazion, chemosis, symblepharon, ectropion, hordeolum, distichiasis, pannus, pterygium, and cataract. Al-Halabi's book was the first to give a remarkable illustration of the anatomy of the brain, the eyes, and the visual pathway among them. Al-Halabi detailed cataract operation representing twelve sorts of cataract in a table.

ACKNOWLEDGEMENTS

The current research article could not come to light without the reproduction of Al-Halabi's book edited by Wafai MZ (MD, FACS, FICS, and FRCOphth) and Kalaji MR (PhD). Dr. Wafai is a chief of Vitreo-Retina Division, King Khalid Eye Specialist

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Finally, recognition must be given to the Frankfurt and Istanbul Museums for the History of Science and Technology in Islam. The Istanbul Museum has copies of the instruments that are in the Frankfurt Museum, and both museums are founded by the same person, Professor Dr. Fuat Sezgin. Institute for the History of Arab-Islamic Sciences, which runs the Frankfurt Museum, kindly and generously authorized the reproduction of the photos in this article.

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World Journal of Methodology

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World J Methodol 2022 January 20; 12(1): 20-31

DOI: 10.5662/wjm.v12.i1.20

ISSN 2222-0682 (online)

MINIREVIEWS

Severe acute respiratory syndrome coronavirus 2 pandemic related morbidity and mortality in patients with pediatric surgical diseases: A concerning challenge

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Author contributions: Vaos G conceived, wrote, and revised the manuscript; Zavras N performed the literature search and analyzed the collected data.

Conflict-of-interest statement: Dr. Vaos has nothing to disclose.

Country/Territory of origin: Greece

Specialty type: Pediatrics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

The global spread of the novel severe acute respiratory syndrome coronavirus 2 has had serious consequences in terms of patient morbidity and mortality and overburdened health care systems as well as the socioeconomic implications. In the absence of effective therapies and vaccinations during the viral outbreak, the major and most concise means to control viral spread is spread prevention. Although information concerning the impact of severe acute respiratory syndrome coronavirus 2 on pediatric surgical patients has greatly expanded, relevant comprehensive studies are scarce. However, pandemic related morbidity has increased, while under normal circumstances mortality could have been minimized.

Key Words: SARS-CoV-2; COVID-19; Pandemic, Pediatric surgery; Children; Morbidity; Mortality

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Core Tip: Severe acute respiratory syndrome coronavirus 2 pandemic related morbidity and mortality have been increased in children. Moreover, pandemic may manifest additional clinical problems. Pediatric surgeons must be aware of the different forms and symptoms in children affected by coronavirus disease 2019 infection.

Citation: Vaos G, Zavras N. Severe acute respiratory syndrome coronavirus 2 pandemic related morbidity and mortality in patients with pediatric surgical diseases: A concerning challenge.

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Received: February 23, 2021
Peer-review started: February 23, 2021
First decision: June 17, 2021
Revised: July 1, 2021
Accepted: November 15, 2021
Article in press: November 15, 2021
Published online: January 20, 2022

P-Reviewer: Fei C, Morenikeji OB
S-Editor: Wu YXJ
L-Editor: Filipodia
P-Editor: Wu YXJ

World J Methodol 2022; 12(1): 20-31
URL: https://www.wjgnet.com/2222-0682/full/v12/i1/20.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i1.20

INTRODUCTION

It has been approximately 1 year since the outbreak of novel pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in the city of Wuhan in China. The disease was subsequently named coronavirus disease 2019 (COVID-19)[1]. The global spread of this new virus forced the World Health Organization to declare it a pandemic on March 11, 2020[2]. It is estimated that up to June 23, 2021, about 180079074 people worldwide have already been infected, and the number of confirmed deaths had reached 3900967[3].

In an attempt to restrict the accelerated transmission of the disease, governments and health organizations across the world adopted various measures[4]. The pediatric surgical community responded accordingly, both globally and locally. The American College of Surgeons and the American Pediatric Surgical Association proposed certain criteria for the management and prioritization of pediatric surgical procedures, taking into account elective procedures that could be postponed and balancing the risk between disease progression and viral vulnerability[5,6]. Similarly, the European Association of Urology issued a statement of recommendations for pediatric urological cases during the SARS-CoV-2 era[7]. In the same spirit, the Spanish Association of Pediatric Surgery published and announced elective surgery restrictions, with the exception of emergencies and oncological cases[8]. Several organizations in the United Kingdom also provided evidence-based guidelines for elective pediatric surgical services[9]. In addition to these measures, many hospitals at the local level established extensive protocols for the security, protection, and proper management of sick children and their parents[10].

Apart from the resultant drop in elective surgeries amid SARS-CoV-2 cases[11,12], many pediatric surgical centers also noted a decline in emergency cases[11]. Moreover, the pandemic has seen the emergence of a new clinical entity, defined as multisystem inflammatory syndrome in children (MIS-C), which is considered by many to be a complication of SARS-CoV-2[13].

This study aims to identify the influence of the SARS-CoV-2 pandemic on morbidity and mortality among children with surgical diseases.

METHODS

We selected all related articles regarding the morbidity and mortality of surgical pediatric patients during the SARS-CoV-2 pandemic by searching PubMed, Google Scholar, and Mendeley search network (www.mendeley.com) from January 1, 2020 to June 23, 2021. The search terms "COVID-19 and pediatric surgery" or "SARS-CoV-2 and pediatric surgery" or "COVID-19 and pediatric surgery and morbidity" or "SARS-CoV-2 and pediatric surgery and morbidity" or "COVID-19 and pediatric surgery and mortality" or "SARS-CoV-2 and pediatric surgery and mortality" were used. Articles in full texts including reviews, original articles, case reports, case series, and letters to the Editor were screened without language restrictions. Abstracts, recommendations, strategies, and opinions were excluded.

RESULTS

A data-based search retrieved 939 articles from the period January 1, 2020 to June 23, 2021. After subtracting duplicates, out of the 809 articles that emerged, we assessed 118 manuscripts in full text and finally reviewed 46 studies (Figure 1). The details of these studies are described below and summarized in Table 1.

The literature search revealed 24 articles[14-35] concerning the influence of SARS-CoV-2 in children with acute appendicitis (AA). More specifically, we found 14 retrospective studies[14-27], one letter to the editor[28], two case series[29,30], one brief communication[31], and four case reports[32-35]. In many series[17,20,24], the diagnosis of AA was delayed for various reasons, such as fear of contact with SARS-

Table 1 Morbidity and mortality in pediatric surgical patients in the pandemic era

Ref.	Journal	Patient number	SARS-CoV-2 test	Disease	Treatment	Outcome	Study details
Place R *et al*[14]	JAMA Network Open 2020; 3: e2027948	90	N/A	CAA: 35CAA + abscess: 8	OT: 35; Abscess drainage with IA: 8	Successful	*Retrospective study*. The authors noted increased number of CAA compared with the same period in 2019
Kvasnovsky CL *et al*[15]	J Pediatr Surg 2020(Epub head of print)	55	Positive: 3 (without symptoms)	NOT: 25 (2 with CAA); OT: 30 (CAA: 13, Simple AA:17)	NOT: 25 pts (3 CAA); OT: 30 (13 CAA); 1 patient SARS-CoV-2+: OT; 2 patients SARS-CoV-2+: NOT	Successful	*Retrospective study*. 45.5% of all patients: NOT protocol to minimize operative resources; The majority of children (78.2%) did not meet previous criteria for non-admissions comparable to pre- SARS-CoV-2 era
Gerall CD *et al* [16]	J Pediatr Surg 2020 (Epub head of print)	89 (41: pre SARS-CoV-2 era, 48: SARS-CoV-2)	Positive: 4 (excluded from the study)	UAA and CAA	NOT: Antibiotics 3 in the pre- SARS-CoV-2 era *vs* 7 during pandemic; OT: 33 in the pre- SARS-CoV-2 era *vs* 23 during pandemic	Successful	*Retrospective study*. It compares children's' symptoms and complications in pro- *vs* SARS-CoV-2 era. Patients in SARS-CoV-2 era: -Duration of symptoms: longer; -Increased number of imaging findings for perforation, increased LOS, increased time until resolution of symptoms.
Snapiri O *et al* [17]	Acta Pediatr 2020; 109: 1672-1676	7	N/A	CAA (perforated, abscess)	OT: 4; NOT: 4 (abscess drainage)	Successful	*Retrospective study*. Delayed diagnosis: Insufficient initial evaluation, telemedicine: 3, parental concerns)
Fisher JC *et al*[18]	Ann Surg 2020 (Epub head of print)	57 patients SARS-CoV-2 era *vs* control: 1292	Positive: 11/28	CAA in the SARS-CoV-2 era: 45% *vs* 27% in the control group	OT: UAA: 30; CAA: 20; NOT: 7	Successful	*Retrospective study*. Comparison of clinical characteristics of children *vs* pre- SARS-CoV-2 era. Main findings: Higher duration of symptoms and perforation rates in the SARS-CoV-2 era. No differences between perforation rates and LOS among positive or negative SARS-CoV-2 children.
La Pergola F *et al* [19]	Front Pediatr 2020; 8: 600320	86 *vs* 309 in the pre-SARS-CoV-2 era	Positive: 3	UAA: 59; CAA: 27	N/A	Successful	*Retrospective study*. COVID-19 era *vs* previously (2017-2019); -No differences: in the prevalence of the AA, duration of symptoms and CAA
Raffaele A *et al* [20]	Br J Surg 2020; 107: e529-e530	14	Positive: None	UCC: 7; CAA: 71	OT: 13/14; NOT: 1 (abscess drainage)	Successful	*Retrospective study*. -Delayed presentation in the ED *vs* previous years, delayed admission to OT due to COVID-19 test preoperatively
Montalva L *et al* [21]	Pediatr Surg Int 2020; 36: 1397-1406	108 (69 during lockdown)	Positive: 3	UAA: 24; CAA: 84	OT: UAA and CAA with peritonitis:94; CAA with abscess: 14 (drainage or medical treated)	Successful	*Retrospective cohort study*. The authors found increased cases of AA during the period of lockdown compared to pre-lockdown era. LOS, complication rates, re-admissions and peritoneal abscesses similar
Bellini T *et al*[22]	Acta Pediatr 2021 (Epub head of print)	27 in the SARS-CoV-2-era *vs* 75 control group	Positive: None	UAA: 14; CAA: 13 *vs* UAA: 50; CAA: 25	N/A	Successful	*Retrospective study*. CAA cases significantly more when compared with previous 3 yr due to delayed admissions (P = 0.004)
Zampieri N *et al* [23]	Minerva Pediatr 2020;	N/A	N/A	N/A	N/A	N/A	*Retrospective study*. The authors found decreased number cases of AA during lockdown *vs* post-lockdown period ($P < 0.05$) possibly due to the less exposure to co-factors
Velayos M *et al* [24]	Ann Pediatr (Barc) 2020; 93:	Pre- SARS-CoV-2-era: 41; Post- SARS-CoV-2:	Positive: 1	CAA: -pre- SARS-CoV-2: 3; -post- SARS-CoV-2: 8	OT: All patients	Successful	*Retrospective study*. Increased number of CAA in SARS-CoV-2 era compared to pre- SARS-CoV-2 era due to delayed

	118-122	25					diagnosis (P = 0.019), LOS increased in the CAA SARS-CoV-2 group
Malhotra A *et al* [25]	Pediatr Inf Dis J 2021; 40: e49-e55	10	Positive: 10	CAA+MIS-C-: 5; UAA: 5	OT: 8; NOT: 2	Successful	*Retrospective study*. CAA associated with MIS-C
Cai *et al*[26]	Front Pediatr 2020;8: 1-9	5	Positive: 5	1 patient: CAA + MIS-C	OT	Successful	*Retrospective study*. CAA associated with MIS-C
Schäfer FM *et al* [27]	Front Pediatr 2021; 9: 683607	514	N/A	CAA			
Zvizdic Z *et al* [28]	J Pediatr Surg 2021; 56: 196-200	6	Positive: None	AA	OT	Successful	*Letter to the Editor*. Decreased admissions of AA compared to pre- SARS-CoV-2 era. Hypothesis: Correlation with decreased exposure to microbes due to lockdown
Lishman J *et al* [29]	J Pediatr Infect Dis 2020; 39: e472-e473	4	Positive: 4	UAA: 4; CAA: 2; MIS-C: 3	OT: 3	Successful	*Case series*. AA with MIS-C
Meyer JS *et al*[30]	J Pediatr Surg Case Rep 2021; 64: 101734	4	Positive: All	UAA: 2; CAA: 2	OT: 4	Successful	*Case series*. Possible association of SARS-CoV-2 with AA
Lee-Archer P *et al*[31]	J Pediatr Child Health 2020; 56: 1313-1314	48	N/A	UAA: 25; CAA: 23	OT	Successful	*Brief communication*. Increased number of CAA compared to previous years (2014-2019), parental concerns
Wang H *et al*[32]	Chin J Pediatr Surg 2020; 41: 299-302	1	Positive	UAA +; pneumonia	OT		*Case report*. UUA associated with pneumonia of the right lung
Harwood R[33]	J Surg Case Rep 2020; 9: 1-3	2	Positive: 1	CAA + MIS-C	OT	Successful	*Case report*. CAA associated with MIS-C
Shahbaznejad L [34]	BMC Pediatrics 2020; 513	10	Positive: 10	1 patient: UAA with MIS-C	OT	Successful	*Case report*. UAA associated with MIS-C
Alsuwallem AB *et al*[35]	Cureus 2020; 12: e8677	1	Positive	CAA	OT	Successful	*Case report*. CAA associated with COVID-19 Infection
Mehl SC *et al*[36]	Pediatr Infect Dis J 2021	1	Positive	NEC	NOT	Successful	*Case report*. Full term neonate with NEC secondary to SARS-CoV-2 infection
Rohani P *et al*[37]	J Pediatr Surg Case Rep 2021; 61: 101667	1	Positive	NEC	NOT	Successful	*Case report*. Gastrointestinal SARS-CoV-2 manifestation
Moazzam Z *et al* [38]	J Pediatr Surg Case Reports 2020; 59:101533	1	Positive	Intussusception	Pneumatic reduction	Successful	*Case report*. Gastrointestinal manifestation of SARS-CoV-2
Rajalakshmi L *et*	Indian J Pract	1	Positive	Intussusception	Pneumatic reduction	Successful	*Case report*. Gastrointestinal manifestation of SARS-CoV-2

al[39]	Pediatr 2020; 22:236						
Martinez-Castañoi[40]	Pediatr Emerg Care 2020;36: e368	1	Positive	Intussusception	Hydrostatic reduction	Successful	*Case report*. Gastrointestinal manifestation of COVID-19
Makrinioti H *et al*[41]	J Pediatric Infect Dis Soc 2020; 9: 504-506	2	Positive: 2	Intussusception; Intussusception + malrotation	Pneumatic reduction; Surgical reduction + ladd procedure	Death; Successful	*Case reports*. Fatal gastrointestinal manifestation of SARS-CoV-2; Gastrointestinal manifestation of SARS-CoV-2
Bazuaye-Ekhuyasi EA *et al*[42]	Emerg Radiol 2020; 27: 761-764	1	Positive	Intussusception	Hydrostatic reduction	Successful	*Case report*. Gastrointestinal manifestation of SARS-CoV-2
Guerrón N *et al*[43]	Global Pediatr Health 2021; 8: 1-3	1	Positive	Intussusception	Hydrostatic reduction	Successful	*Case report*. Gastrrointestinal manifestation of COVID-19
Osorno JF *et al*[44]	Global Pediatr Health 2021; 8: 1-3	1	Positive	Intussusception (delayed presentation)	Laparotomy	Successful	*Case report*. Gastrrointestinal manifestation of COVID-19
Kawalec AM[45]	Burns 2020; 46: 1713-1714	Increased admissions in ED compared to previous year	N/A	Increased TBSA burns, house fire burns and PICU admissions *vs* previous year	Outpatient care, hospitalization, PICU	N/A	*Retrospective study*. Need for a family plan during pandemic
Demicran M[46]	Burns 2020 (Epub ahead of print)	Increased admissions and hospitalizations compared to previous year	N/A	Increased TBSA burns, increased all kinds of burns	Outpatient care, hospitalization	N/A	*Retrospective study*. Burn care material must be ready
Sethuraman U[47]	Burns 2020 (Epub head of print)	Increased admissions in ED *vs* all visits	N/A	Increased TBSA	Outpatient care, hospitalization, PICU	1 death	*Retrospective study*. Parents should keep children away from hot liquids and surfaces
Pelizzo G *et al*[48]	Healthcare 2021; 9: 551	84 (pandemic era: 52previous pre-pandemic period: 32)	Positive: 1	TBSA < 10%: 32; 10%-15%: 11; > 15% >: 9	34/52: Discharge; 18/52: Burn Service Area; (10/18: Ward; 8/18: PICU)	Successful	*Retrospective study*. A higher number of admissions during pandemic was noticed compared to the same period in the previous year. An appropriate planned service and care ensure a safe and feasible hospitalization without risks of infections and major complications
Marino-Mateo L *et al*[49]	Actas UrolEsp 2020; 44: 659-654	45	Positive: 0	Pelviureteric junction obstruction, spina bifida, lithiasis, hypospadias	49 interventions	Successful	*Retrospective study*. A stratification of the urological based on the different phases of pandemic and EAU was conducted
Cesaro S *et al*[50]	Pediatr Blood Cancer 2020; 67: e8466	247	Positives: 10	Solid tumors, leukemia	Ceased chemotherapy and radiation for 12-26 d	Successful	*Retrospective study*. Mild or asymptomatic patients with positive tests may continue therapy
Hrusak O *et al*[51]	Eur J Cancer 2020;132: 11-16	200	Positives: 9	Hepatoblastoma: 2; Wilms tumor: 1; Ewing's sarcoma: 1; osteosarcoma: 1; cervical rhabdoid: 1; ALL: 1	Antibiotics and/or hydroxychoroquine, lopinavir, ritonavir	Successful	*Retrospective study*. Children on anticancer therapy may have mild or asymptomatic course of infection with SARS-CoV-2. In this case anticancer treatment should not be delayed or postponed

Madhusiidhan PP *et al*[52]	Pediatr Blood Cancer 2020; e28843	578	Positive: 98; No symptoms: 73	Neuroblastoma: 5,Solid tumor: 16; Others: 77	Mechanical; ventilation: 7; Supplemental oxygen: 25; SARS-CoV-2 direct treatment: 98	Successful: 94; Death: 4	*Multi-institutional cohort study*. Low morbidity and mortality among oncologic patients but higher than in general pediatrics. Significant impact of pandemic: Delay in therapy in 67% of positive patients; Overall delays: Chemotherapy 54%, surgery 46%, transplant 30%

CAA: Complicated acute appendicitis; OT: Operative treatment; IA: Interval appendectomy; NOT: Non-operative treatment; AA: Acute appendicitis; UAA: Uncomplicated acute appendicitis; LOS: Length of hospital stay; MIS-C: Multisystem inflammatory syndrome in children; ED: Emergency department; NEC: Necrotizing enterocolitis; TBSA: Total body surface area; ALL: Acute lymphoblastic leukemia; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; PICU: Pediatric intensive care unit.

CoV-2 patients, telemedicine, fear of traveling, and disruption of the local health system; this led to an increase in the number of cases with complicated AA (CAA). While an association between AA and SARS-CoV-2 has been speculated[26], other studies have also reported 13 children with CAA diagnosed in the context of MIS-C [29,33,34]. It is worth noting that one case reported the coexistence of AA and pneumonia[32].

Two children aged 7 wk and 6 years respectively, both positive for SARS-CoV-2 infection, presented with clinical signs of acute abdomen. The clinical and diagnostic investigation showed necrotizing enterocolitis (NEC), which was successfully treated with antibiotics. The authors considered infection to be responsible for this emergency clinical manifestation[36,37].

Seven articles[38-44] referred to cases of ileocolic intussusception. Eight infants, aged 4-10 mo, presented with clinical symptoms related to intussusception (abdominal cramps, bilious vomiting, currant jelly stools) and were confirmed by ultrasound. On admission, seven infants were found to be positive for SARS-CoV-2, and one was found to be positive on the third postoperative day; four[38,40-42] had relatives with suspected or confirmed SARS-CoV-2 infection. Reduction (pneumatic or hydrostatic) was attempted in seven patients, six of whom had a successful outcome. Only two cases were treated surgically. Notably, in one case, at laparotomy, malrotation was found in addition to intussusception, and a Ladd's procedure was performed[41]. In the other case, intestinal resection with consequent ileostomy and mucous fistula was performed due to intestinal ischemia and peritonitis[44]. Moreover, following successful pneumatic reduction of intussusception, one female infant with documented SARS-CoV-2 infection went on to develop acute respiratory infection and multiorgan complications during hospitalization, including abdominal ascites and intestinal failure, which subsequently led to her death[41].

A total of four articles was extracted for SARS-CoV-2 in children with burns[45-48]. All of them disclosed useful information. Specifically, they recorded an increase in admissions of children with moderate and severe fire-related burns in comparison to earlier years, while the greater difference in burned total body surface area was statistically significant[46-48].

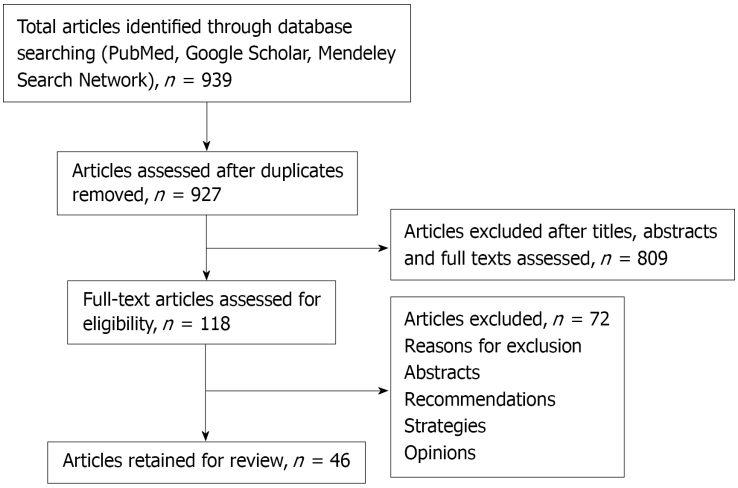

Figure 1 Flow chart of articles selection.

One article from Spain was included[49] that reported clinical and surgical data, complications, and readmissions of all children who underwent procedures for various urological conditions. The authors developed an escalation program based on the different phases of the pandemic and the European Association of Urology recommendations. They concluded that this strategy allowed them to manage successfully urological diseases.

Three studies[50-52] that screened pediatric oncology/hematology patients for SARS-CoV-2 infection were evaluated. In the first study, 334 nasopharyngeal swabs were taken from 247 patients, only 10 (4%) of whom tested positive for SARS-CoV-2 infection (eight patients were asymptomatic and two had a mild fever). In nine patients, chemotherapy was postponed until they had tested negative (time period ranges from 12-26 d), while only one patient on chemotherapy and radiation was positive after 30 d[50]. In a multicenter survey performed in 25 countries involving 200 suspected patients, only nine tested positive for SARS-CoV-2 infection[51]. Notably, none required admission to pediatric intensive care or mechanical ventilation. Finally, a retrospective, multicenter study among 13 institutions including 578 patients examined test-positive SARS-CoV-2 patients aged ≤ 21 years receiving active anticancer treatment[52]. Among those 578 patients, 98 (16.95%) were positive, 78 (79.6%) of whom were symptomatic, and four died (4%). Delay of anticancer therapy occurred in 67% of these patients. Overall, these studies raised the question concerning the benefits of discontinuing or delaying chemotherapy in mild or asymptomatic SARS-CoV-2 positive patients.

DISCUSSION

This study provides descriptive data on pediatric surgical patients infected with the novel coronavirus SARS-CoV-2. The data show that the impact of SARS-CoV-2 seems to be multifactorial as it interferes directly with human health due to the vulnerability of the virus and indirectly with the resources to access care, thereby increasing morbidity and mortality[12].

SARS-CoV-2 infection can affect all ages of children with median age of infection of 6.7 years (1 d to 15 years)[53], with no gender predominance[54]. The angiotensin-converting enzyme 2 is the main host receptor of SARS-CoV-2 and is frequently expressed in ciliated epithelial cells in human lungs. The second most common site of angiotensin-converting enzyme 2 receptors is the gastrointestinal cells[53]. Notwithstanding, gastrointestinal symptoms attributed to SARS-CoV-2 infection are more prevalent in children, while respiratory involvement is more common in adults[55,56]. In line with this finding, Meyer *et al*[30] speculated a conceivable association of SARS-CoV-2 and AA based on the assumption of the predominant association of gastrointestinal infection and SARS-CoV-2. However, in the era of SARS-CoV-2, other factors such as the fear of contact with persons positive for SARS-CoV-2, difficulty in visiting health centers, and insufficient evaluation *via* telemedicine may constitute strong reasons for delayed diagnosis of CAA[17,20,24].

The predominant association of gastrointestinal infection and SARS-CoV-2 might also explain the cases of intussusception[38-44] and the two cases of NEC[36,37]. Intussusception is the most common cause of intestinal obstruction in infancy[57]. Although in most cases it is thought to be idiopathic, a preceding viral infection due to adenovirus and rotavirus has been reported in approximately 30% of cases[58]. Notably, all cases described here were positive for SARS-CoV-2 infection, while a previous history of upper respiratory tract infection was diagnosed in three cases[38-40]. In the two cases of NEC, the association of SARS-CoV-2 test positivity with coexistence of pneumatosis intestinalis and bloody stools suggests ischemic necrosis in both cases and hence NEC[36,37].

The increase in the number of children presenting with burns during the outbreak could be ascribed to the fact that the lockdown obliged children to stay home where they may have had less surveillance, since parents were constrained to work from home and were thus unable to keep a close watch on children. Educational programs are needed to increase parents' knowledge concerning safety behaviors during a prolonged stay at home[45-48].

The impact of SARS-CoV-2 outbreak on children with urological problems is not known. An escalation program based on different phases of the pandemic has been proposed by Merino-Mateo *et al*[49] for the management of urological problems. However, the lengthy postponement of certain crucial surgical procedures, such as cryptorchidism, or obstructive uropathies including ureteropelvic junction obstruction, ureterovesical junction obstruction, or neurogenic bladder may lead to loss of a testicle function or loss of renal function. Three articles referred to testicular torsion[59-61]. The conclusions were contradictory, as increased rate of orchidectomies was noticed in the two articles due to delayed presentation[59,60], while in the remaining one article the authors found early presentation in testicular torsion and no differences in rates of orchidectomies between SARS-CoV-2 period and preceding era[61].

Children with cancer face significant health problems in view of the rapid changes in the health system and restrictions in accessing medical support. Compromised immunity due to malignancy and the unknown behavior of SARS-CoV-2 lead to further insecurity. Although the studies mentioned above[54-56] did not show significant consequences from the virus itself, it was clear that postponement of therapies owing to the heavily burdened health system could result in insufficient medical support.

In May 2020, the Centers for Disease Control in the United States expressed concern for a new entity termed MIS-C associated with SARS-CoV-2[34,62]. MIS-C is defined by clinically severe illness requiring hospitalization that presents with fever, elevated inflammatory markers, and multisystem organ dysfunction in the setting of recent proven or probable SARS-CoV-2 infection and the absence of a plausible alternative explanation[34]. However, there are no data available as to whether the mechanism that can lead to severe respiratory failure resembles that of MIS-C. Current data raise the suspicion for a distinct entity related to severe SARS-CoV-2 infection[62]. MIS-C-related AA was suspected in three studies[25,33,34] in which the coexistence of CAA and positive was tested.

This study is not without its limitations inasmuch as it is based on all types of articles, most of which included retrospective studies, letters to the Editor, case reports, and case series, all of which were written during the pandemic within a short period of time in an effort to share experiences and divulge information that could help the scientific community. Furthermore, in order to achieve a better understanding, a substantial number of studies compared their results with those to pre- SARS-CoV-2 era, which can lead to a significant bias. Another limitation is that most data were collected within 204 mo. Consequently, there is a lack of acceptable follow-up that would have helped us gain precise knowledge as concerns post-operative outcomes and readmissions of patients treated during the pandemic.

CONCLUSION

Pediatric surgical practice during the SARS-CoV-2 pandemic is challenging. Summarizing the information and results of studies, we conclude that SARS-CoV-2 infection could have a negative influence on virtually the entire pediatric surgical spectrum. Morbidity has increased for various reasons in children with burns, urological problems, and cancer including fear of contracting the virus in health centers, lockdown, telemedicine, postponement of medical consultation and elective surgeries, or unknown manifestations of SARS-CoV-2. Under normal circumstances,

mortality could have been minimized. The delay in presentation and consequent management of AA has resulted in an increased number of CAA. The impact of SARS-CoV-2 on the gastrointestinal system has further exacerbated the manifestation of common pediatric surgical conditions such as AA, intussusception, and NEC. Further studies and research are needed to overcome the demands of this period.

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 January 20; 12(1): 32-42

DOI: 10.5662/wjm.v12.i1.32

ISSN 2222-0682 (online)

MINIREVIEWS

Liver transplant allocation policies and outcomes in United States: A comprehensive review

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Author contributions: Latt NL and Niazi M researched, planned, outlined and wrote the entire manuscript; Pyrsopoulos NT advised the outline, reviewed and edited the manuscript.

Conflict-of-interest statement: Dr. Pyrsopoulos reports grants from MALLINCKRODT, grants from VALEANT, grants from GILEAD, grants and other from BAYER, grants from BEIGENE, grants from GENFIT, grants from PROMETHEUS, grants from GRIFOLS, grants from INTERCEPT, grants from ZYDUS, grants and other from EISAI, other from ROCHE, grants from ABBVIE, grants from NOVARTIS, outside the submitted work.

Country/Territory of origin: United States

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

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Abstract

Liver transplant allocation policies in the United States has evolved over 3 decades. The donor liver organs are matched, allocated and procured by the Organ Procurement and Transplantation Network which is administered by the United Network of Organ Sharing (UNOS), a not-for-profit organization governed by the United States human health services. We reviewed the evolution of liver transplant allocation policies. Prior to 2002, UNOS used Child-Turcotte-Pugh score to list and stratify patients for liver transplantation (LT). After 2002, UNOS changed its allocation policy based on model for end-stage liver disease (MELD) score. The serum sodium is the independent indicator of mortality risk in patients with chronic liver disease. The priority assignment of MELD-sodium score resulted in LT and prevented mortality on waitlist. MELD-Sodium score was implemented for liver allocation policy in 2016. Prior to the current and most recent policy, livers from adult donors were matched first to the status 1A/1B patients located within the boundaries of the UNOS regions and donor-service areas (DSA). We reviewed the disadvantages of the DSA-based allocation policies and the advantages of the newest acuity circle allocation model. We then reviewed the standard and non-standard indications for MELD exceptions and the decision-making process of the National Review Liver Review Board. Finally, we reviewed the liver transplant waitlist, donation and survival outcomes in the United States.

Key Words: Liver transplant; Allocation; Distribution; Waiting list; Policies; Acuity circles; Transplant exceptions; National Review Liver Review Board

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Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

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Received: February 24, 2021
Peer-review started: February 24, 2021
First decision: May 6, 2021
Revised: June 21, 2021
Accepted: November 12, 2021
Article in press: November 12, 2021
Published online: January 20, 2022

P-Reviewer: Zhu YY
S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

Core Tip: The liver transplant donor allocation and distribution polices have evolved over three decades. The liver donor distribution policy has recently changed from donor-service area-based policy to the acuity circle model. The new policy is believed to work more efficiently and equitably for waitlist candidates across the United States.

Citation: Latt NL, Niazi M, Pyrsopoulos NT. Liver transplant allocation policies and outcomes in United States: A comprehensive review. *World J Methodol* 2022; 12(1): 32-42
URL: https://www.wjgnet.com/2222-0682/full/v12/i1/32.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i1.32

INTRODUCTION

In the United States, organ transplantation is regulated by the United Network OF Organ Sharing (UNOS). UNOS is a not-for-profit and scientific organization which manages the Organ Procurement and Transplantation Network (OPTN), the sole network which is responsible for procuring, matching and allocating donated human organs in the United States by maintaining the national organ transplant database (UNet)[1,2]. UNOS was first established by the United States Congress *via* the National Organ Transplant Act in 1984. OPTN began its operations in 1986. In 2000, "the Final Rule" was published by the United States Department of Health and Human Services establishing federal regulations on OPTN policies including listing requirements, organ procurement, identification of organ recipient, allocation of donated organs, designated transplant program requirements, reviews, evaluation and enforcement of transplant programs[3]. Similar to UNOS, National Health Service Blood and Transplant is responsible for the matching, procurement and allocation of organ transplantation in the United Kingdom[4]. In Europe, the Eurotransplant network is responsible for organ procurement and allocation[5].

The two primary goals of OPTN/UNOS are (1) The equitable distribution of donated orangs; and (2) The appropriate care of minority candidates. It is crucial not to discriminate transplantation on age, gender, ethnicity and socioeconomic backgrounds. The National Organ Transplant Act published in 1984 indicates UNOS to establish medical criteria when organs are being allocated. The medical criteria were put in place to ensure justice and reinforce equity. The Final Rule which was later published in 1999 elaborated the national framework for matching, allocation and distribution of the donated organs. The OPTN must institute equitable allocation policies which are based on medical judgement in order to achieve the best outcomes of donated organs and to promote the access to transplantation. Policy 3.6 requires the OPTN to standardize medical criteria for determining suitable transplant candidates and set priority rankings based on objective and measurable medical criteria. The rankings mush be sequenced from the most medically urgent to the least and the geographic area must be feasible for organ distribution in order of decreasing medical urgency[6].

CHILD-PUGH SCORE

Prior to 2002, UNOS used Child-Turcotte-Pugh (CTP) score to list and stratify patients for liver transplantation (LT). CTP score was first developed in 1973 to risk stratify patients undergoing portosystemic shunt surgery. CTP score includes blood tests such as serum total bilirubin, serum albumin, international normalized ratio (INR) as well as the severity of symptoms such as ascites and hepatic encephalopathy[7]. One of the disadvantages of using symptoms in CTP scoring was the lack of standardization of perceived symptom severity. Different transplant physicians might interpret the severity of ascites and hepatic encephalopathy in different manners. Moreover, CTP score was not able to predict which patients were in greater need of an orthotopic LT (OLT).

MODEL FOR END-STAGE LIVER DISEASE SCORE

In 2001, Kamath *et al*[8] from Mayo Clinic developed the model for end-stage liver disease (MELD), a mathematical model using all objective tests such as serum bilirubin, INR, serum creatinine, to predict poor survival among cirrhotic patients undergoing transjugular intrahepatic portosystemic shunts[8]. MELD score was also shown to accurately predict disease severity and survival outcome in patients with chronic liver disease[9]. Brown *et al*[10] demonstrated that the MELD score is superior to CTP score in estimating pre-OLT disease severity and optimize the timing of OLT [10]. The OPTN/UNOS committees developed the LT allocation policy based on MELD score. This allocation policy was approved by the OPTN/UNOS Board of Directors in November 2001 and went into effect in February 2002[11].

MELD-SODIUM SCORE

Hyponatremia is the independent risk factor which negatively impacts the survival in patients with cirrhosis. The degree of hyponatremia correlates with the severity of chronic liver disease. In 2005, Biggins *et al*[12] from University of California San Francisco demonstrated that serum sodium level < 126 mEq/L at liver transplant listing or while listed for OLT is a strong independent predictor of mortality. The researchers concluded that addition of serum sodium to MELD score can increase the accuracy to predict 3- and 6-mo mortality in patients with cirrhosis[12]. In their 2008 study, Kim *et al*[13] studied OPTN data of 1781 participants who underwent OLT and 422 who died within 90 d after registration on the waiting list. The MELD and serum sodium combined score was significantly higher than the MELD score alone in patients who died on the waiting list. This data indicated that the priority assignment of MELD-sodium score might have resulted in OLT and prevented mortality[13].

The MELD-sodium score Policy 9.1 was approved by the OPTN/UNOS Board of Directors in June 2014 and implemented in January 2016[14].

PEDIATRIC END-STAGE LIVER DISEASE SCORE

The pediatric end-stage liver disease (PELD) score calculates the pediatric version of the MELD score for liver cirrhosis severity. In addition to serum bilirubin and INR, patient's age, growth failure and serum albumin are included in the mathematical formula of PELD score, contrast to MELD score[15].

Evolution of liver transplant organ allocation systems

Liver transplant organ allocation systems have evolved tremendously over two decades to reduce disparity, increase equity and access to liver transplant based on new evidence-based data with the primary goal of increased "best use" of donated livers.

Allocation priorities based on UNOS status

Patients who are listed as status 1A on UNOS waiting list have acute onset liver failure and are deemed not likely to survive more than a few days without an OLT. Status 1B is reserved for extremely sick, chronically ill pediatric patients with cirrhosis who are younger than 18 years of age-pediatric population. Status 1A and 1B patients are usually less than 1% of overall waitlisted patients at any given time.

Historical background of liver distribution–donor-service areas-based policy

Prior to the current and most recent policy, livers from adult donors were matched first to the status 1A adult patients, then to status 1B pediatric patients located within the boundaries of the same region as the donor hospital but could be outside of donor-service area (DSA). There are 11 UNOS regions and 58 DSAs catered by various organ-procurement organizations (OPO) in the United States. "Share-35 rule", implemented in June 2013, mandated that the waitlisted patients with MELD-sodium score of 35 or above would be offered donated livers outside of the OPO and within the same region. While "Share-35" policy increased the number of OLT by 6% and the number of regional sharing by 11%, there was no impact on the overall waitlist mortality, the post-transplant survival and the overall liver discard rate. The UNOS/OPTN leadership considered a concept of restructuring 11 UNOS regions to 8 districts to

lower geographic disparity, waitlist mortality and the high variability of MELD-sodium at the time of transplant among various liver transplant centers across the regions. The statistical model for "Region Redistricting" was limited by the minimum number of transplant centers *per* district which was set to be 6 centers and maximum of 3 h allotted travel time between the DSAs in the same district.

New liver distribution policy-acuity circles

Due to the disadvantages of DSA-based liver distribution policy and geographic inequities in access to OLT, the OPTN Board of Directors mandated a thorough review process for system improvement since 2014. The new liver policy was proposed by transplant experts, reviewed, and debated by organ recipients, donor families with thousands of public comments on UNOS website. The priority of the new distribution policy is to ensure that the organ distribution is equal for waitlisted candidates where they liver or wish to seek a transplant. DSA-based donation boundaries had led waitlisted patients to get more than one wait list (*i.e.*; dual listing practice) or travel to different regions in the country to get access to transplant. The new process is simple with a measure of distance from donor hospital to the transplant hospital in nautical miles, eliminating DSAs. The sole benefit of the December 2018 policy is that it is projected to save more lives by lowering waitlist mortality by 100 lives annually[16].

According to UNOS data, organ donation from deceased donors set an all-time high record in 2020 despite the global COVID-19 pandemic. 36548 organs from deceased donors were transplanted resulting in 33309 people receiving life-saving transplants [17]. The implementation of the December 2018 acuity circles (AC) policy is projected to reduce the impact of where waitlist candidates live, or what hospital they choose for their care. The new policy is believed to work more efficiently and equitably for waitlist candidates across the United States.

AC–status 1A/1B

Under the new policy, livers from all deceased donors are offered for status 1A and 1B candidates listed at transplant hospitals within a radius of 500 nautical miles from the donor hospital.

AC-non-donating upon cardiorespiratory death donors younger than age 70

For the deceased liver donors which are not donating upon cardiorespiratory death (DCD) and under age 70, waitlist candidates with MELD or PELD score of 37 or higher are prioritized after status 1A/1B candidates. The initial offers will go out to the candidates at transplant hospitals within a radius of 150 nautical miles from the donor hospital. The next sequence offers will go out to the candidates within a radius of 250 nautical miles from the donor hospital. Then, the offers will go out to the candidates within a radius of 500 nautical miles from the donor hospital. The MELD/PELD score ranges will progressively continue from 33 to 36, from 29 to 32 and from 15 to 28.

AC-DCD donors and/or adult donors older than 70

For the deceased liver donors who are donating DCD and/or adult donors older than 70 years of age, the new liver distribution policy prioritizes the candidates more local to the donor hospital with earlier access to transplant. The candidates with MELD or PELD of 15 or higher are offered these donated livers after status 1A/1B candidates. The sequence of distribution is for candidates within a radius < 150 nautical miles, then 150-500 nautical miles and lastly > 500 nautical miles from the donor hospital[17].

Challenges of the new liver distribution policy

The new liver distribution policy with AC allocation was approved in December 2018 after a lawsuit was filed in New York by patients who stated that their wait time was longer than other patients with lower MELD score in other parts of the country. The new AC policy went into effect on May 14, 2019. However, a United States federal judge in Atlanta, Georgia temporarily blocked the new policy on May 17, 2019, citing that waitlist candidates and hospitals in less-populated areas would suffer if the AC distribution model rules remained in effect. The liver allocation policy was reverted to DSA-based distribution on May 23, 2019. On February 4, 2020, the OPTN/UNOS re-instated the new AC model distribution policy. This model was supported by the Scientific Registry of Transplant Recipients' (SRTR) 2018 analysis which projected that the AC model would decrease the variability of MELD score at the time of transplant (MMaT) across DSAs. The model predicted a substantial decrease from 9.97 to 4.33 based on historical statistics[17].

Chyou *et al*[18] compared the center- and DSA-level changes in the 6-mo period pre-AC model era (August 8, 2019 to February 3, 2020) and post-AC era (March 5, 2020 to August 31, 2020) using OPTN/UNOS data. The focus was on non-status 1A adult deceased donors on following metrics: Transplant volume, MMaT, procurements requiring flights and termed "flight-consistent distance" procurements. The volume of adult non-status 1A deceased liver donors decreased by 2.7% during this post-AC era. The DSA-level MMaT ranged from 18.5 to 32 in the pre-AC era while it ranged from 18 to 33 in the post-AC era. The median change in MMat was +1 MELD point. The DSA-level variance in MMaT was unchanged: 12.2 pre-AC era *vs* 12.1 post-AC era. The number of "flight-consistent distance" procurements increased: 42.5 % pre-AC era *vs* 60.5% post-AC era. The post-AC era has coincided with the coronavirus disease 2019 (COVID-19) global pandemic and the transplant volumes could be affected by the COVID-19 restrictions and hospital constraints. However, these early data have raised the concern that the AC model projection based on mathematical simulations may not match the real-world transplant metrics. Longer-term data are needed to evaluate the benefits of the AC distribution model[18].

NATIONAL LIVER REVIEW BOARD

The OPTN/UNOS Liver and Intestinal Organ Transplantation Committee has established regulation/guidance for both hepatocellular carcinoma (HCC) and non-HCC adult MELD exceptions and extensions requests. MELD exception policies allow opportunity to have a diseased donor liver transplant for the patient whose natural MELD score does not reflect the true liver related mortality risk. These MELD-exceptions could be standardized or non-standardized. All standardized MELD-exceptions requests do not need to be approved by the National Liver Review Board (NLRB) but all non-standardized MELD exceptions must be submitted to the NLRB.

In January 2017, the OPTN/UNOS liver and Intestinal Organ Transplantation Committee proposed NRRB and on May 14, 2019, NLRB replaced the Regional Review Boards (RRB) for each individual 11 OPTN regions for MELD exception scores approval. According to the briefing paper from OPTN/UNOS, the need for this change was warranted secondary to wide range (75.8% to 93.5%) of MELD exception requests being approved among different regions[19]. The NLRB is a nationwide peer review system that provides fair and increase consistency in providing MELD exception scores candidates of all liver transplant programs in United States and has eliminated the regional differences for granting MELD exception points. NLRB reviewers are assigned from a pool of nationwide liver transplant physicians and surgeons. NLRB has three boards, one for HCC exception requests, 2nd for non-HCC exception request and 3rd for pediatrics. NLRB is responsible for approval or denying exception points for patients who do not qualify for standardized MELD exception points. The liver transplant program may request MELD exception points to NLRB, if the calculated MELD score does not accurately reflect the severity of the candidate's disease. The candidate's respective transplant center must submit a request to NLRB with specific MELD score and justification why candidate's current status does not accurately reflect urgency for LT. The initially all cases are reviewed by five randomly assigned reviewers and four out five has to approve the request. According to the current OPTN policy[20]: (1) The NLRB is responsible to review MELD exceptions/extensions requests within 3 wk after the request has been submitted to the OPTN. If the NLRB is unable to complete the decision within 3 wk, candidate will be assigned the requested MELD score; (2) The candidate's transplant program as a right to appeal within 2 wk to the NLRB if the MELD exceptions/extension request has been denied. The appeal must be reviewed by the NLRB within 3 wk after submission to the OPTN, if the decision could not be reached within 3 wk, the candidate will be assigned the requested MELD score; (3) Upon denial of appeal by the NLRB, the candidate's transplant program has a right to further appeal to the appeals review team (ART) within 1 wk after denial notification. Each ART team has 9 members but 5 needs to be present at given time to review the case and must review the request within 2 wk after submission to OPTN. If ART unable to make the final decision within assigned 2 wk' period, candidate will be assigned requested MELD exceptions/extension points; and (4) Upon denial the MELD exception/extension request by ART, the candidate's respective liver transplant program has a right to appeal within 1 wk after denial notification to Liver and Intestinal Organ Transplantation Committee.

MELD-exception for HCC

HCC is the 5th most common cancer and 3rd most common cancer related death in both sexes and in all ages[21]. Incidence of HCC in United States is rapidly rising secondary to chronic hepatitis C related cirrhosis. LT is an effective and curative treatment for non-resectable HCC since removal of both tumor and cirrhotic liver will maximize recurrence-free patient survival. MELD score predicts 3 mo' mortality for majority of the patient with cirrhosis but unfortunately underestimates mortality in the patients with HCC and hence high probability of weight list mortality and weight list dropout secondary to tumor progression while waiting for OLT[22].

Since HCC patients historically have low MELD score, without MELD exception points, realistically will not be able to get diseased donor LT. The liver transplant allocation system designates MELD exception points to patients with HCC if they meet MILAN criteria, which is defined as one lesion to 5 cm or up to 3 lesions each ≤ 3 cm without radiologic evidence of macrovascular invasion or metastatic disease[23]. The MELD exception points for patients with HCC, decreases wait-list mortality and increases priority for LT. By for the commonest indication of MELD exception point is HCC in united states.

The 1st HCC exception points policy was implemented on February 27, 2002. Since then, significantly high number of patients with HCC have been transplanted. Secondary to donor organ shortage and high number of patients being transplanted for HCC, needing multiple revisions of UNOS MELD-exception allocation policy for HCC over the last 2 decades (Table 1). In comparison to policy change in October 2015 which focused on timing of exception and incremental increase in tumor MELD exception points with maximum points of 34, the most recent organ allocation policy change in May 2019 does not allow incremental increase in MELD exception points. The current organ allocation policy mandates to list the patient with actual Na-MELD of the patient and after 3 mo, request a MELD extension. Once 6 mo' observation period is finished and the patient is still in with in MILAN criteria, the patient will be granted HCC-MELD exception points. The maximum points are median MELD at transplant (MMaT) 2. The MMaT remains fixed score and does not increase every 3 mo. By using previous 12 mo' data, the median MELD is recalculated every 6 mo and subsequently MMaT is readjusted. The purpose of this change was to promote more balanced allocation of donor organs between HCC and non-HCC patients on liver transplant wait list. 6 mo wait list observation period for HCC patients also will provide better understanding to assess the tumor biology.

The OPTN/UNOS Liver and Intestinal Organ Transplantation Committee has established regulation for adult MELD exceptions for HCC. The following is the summary of summary of current UNOS Policy for HCC exception points[20].

Documentation of number and sizes by multiphasic CT or MRI of all OPTN class 5 lesions (5A or 5B), ruling out metastatic disease, AFP and candidate being not eligible for resection (Tables 2 and 3).

Wait listed patient within MILAN criteria (T2 lesion) and AFP ≤ 1000 ng/mL will be eligible for standardized MELD exception points. If AFP > 1000 ng/mL with T2 lesion, candidates may be treated with local-regional therapy (LRT): (1) After treatment if AFP < 500 ng/mL, eligible for standardized MELD exception points; and (2) After treatment if AFP > 500 ng/mL, candidate would need to be referred to and NLRB for MELD exception points.

Standardized MELD exception points if the lesions meet the down staging protocols (Tables 2 and 3) and after LRT the lesion meets the definition of T2 lesion, demonstrated on CT or MRI. If candidates do not meet initially the downstaging protocol and subsequently down staged to T2 lesions must go through NLRB for MELD exception points.

After initial automatic approval of MELD exception points, extensions of HCC exception points would need to be requested every 3 mo. Automatic MELD exception points will be granted as long as lesions do not progress beyond T2 criteria, AFP < 500 ng/mL.

The candidates who meet the standardized MELD score exception, will be granted calculated MELD score on initial and first extension request. After 6 mo (second extension request), the candidate will be granted 3 points below MMaT.

Non-HCC standard MELD-exceptions

In February 2002, non-HCC MELD exception points policy was implemented which allowed exception points for hepatopulmonary syndrome (HPS), familial amyloidosis and primary oxaluria. Subsequently familial amyloidosis and primary oxaluria were removed in 2009 form UNOS/OPTN policy as standard MELD exceptions. According

Table 1 Model for end-stage liver disease exception points granted

Year of policy implementation	MELD exception points granted	
	T2 lesion (A single nodule with diameter ≥ 2 cm and ≤ 5 cm or 2-3 lesions each between 1-3 cm)	T1 lesion (A single nodule ≥ 1 cm and < 2 cm)
February 2002	29 points	24 points
February 2003	24 points	20 points
April 2004	24 points	No exception points
March 2005	22 points	No exception points
October 2015	Natural MELD score at the time of listing	No exception points
	28 points after 6 mo with maximum 34 exception points	
May 2019	MMaT-3	No exception points

MELD: Model for end-stage liver disease; MMaT-3: Median MELD at transplant-3.

Table 2 Lesions eligible for downstaging protocols

Number of lesions	Size	Description
1	> 5 cm and ≤ 8 cm	
2-3	At least one lesion > 3 cm and all ≤ 5 cm	Total diameter of all lesions ≤ 8 cm
4-5	Each < 3 cm	Total diameter of all lesions ≤ 8 cm

Table 3 Organ procurement and transplantation network imaging classification for class 5 lesions in patients with cirrhosis

OPTN class	Description	Comments
0	Incomplete are technically in adequate study	No MELD exception points
5A	Lesion size ≥ 1 cm and ≤ 2 cm	Increased contrast enhancement in the late hepatic arterial phase along with either: (1) Wash out during late contrast phases and peripheral rim enhancement (capsule or pseudocapsule); and (2) Biopsy consistent with HCC
5A-g	Lesion size ≥ 1 cm and ≤ 2 cm	Increased contrast enhancement in the late hepatic arterial phase along with growth ≥ 50% documented on serial CT or MR obtained ≤ 6 mo apart
5B	Lesion size ≥ 2 cm and ≤ 5 cm	Increased contrast enhancement in the late hepatic arterial phase along with either: (1) Wash out during late contrast phases; (2) Peripheral rim enhancement (capsule or pseudocapsule); (3) Growth ≥ 50% documented on serial CT or MR obtained ≤ 6 mo apart in the absence of ablative therapy; and (4) Biopsy consistent with HCC
5T	Prior local regional therapy for HCC	Any residual lesion or perfusion defect at the site of prior class 5A, 5A-g, 5B lesion

OPTN: Organ procurement and transplantation network; MELD: Model for end-stage liver disease; HCC: Hepatocellular carcinoma.

to the OPTN policy change from November 2009 several non-HCC conditions were granted standardized MELD-exceptions, without the need to go through the evaluation process by RRB and now RRB has been replaced by NLRB. This OPTN/UNOS policy change was the results of recommendations made by the MELD exception study group and conference (MESSAGE) in 2006[24,25].

Following is the summary of all non-HCC conditions eligible for standardized MELD exception points according to the current OPTN/UNOS policy and MELD extension request are valid for 90 d after submission[26] (Table 4).

Cholangiocarcinoma

In order to be eligible for MELD exception points of MMaT-3, the candidate must meet all the criteria. The center needs to have written protocol including selection criteria,

Table 4 Conditions eligible for non-hepatocellular carcinoma standard model for end-stage liver disease-exceptions

Condition	Requirements for exception points	MELD score assigned
CCA	Un-resectable hilar CCA with biopsy/cytology consistent with malignancy or CA19-9 > 100 U/mL or aneuploidy	MMaT-3
	Center must have written protocol regarding selection of criteria, neoadjuvant therapy, operative staging for metastatic disease	
	Imaging to exclude metastatic disease	
HPS	Evidence of portal hypertension without any evidence of underlying significant pulmonary disease	MMaT-3
	PaO2 < 60 mmHg on room air	
	ECHO or lung scan confirming intra-pulmonary shunt	
POPH	Evidence of portal hypertension along with MPAP > 35 mmHg and PVR > 3 woods unit	MMaT-3
	MPAP < 35 mmHg and PVR < 5.1 woods unit post treatment of pulmonary hypertension	
FAP	Biopsy proven amyloid along with TTR gene mutation and able to walk independently	MMaT-3
	Must be on heart transplant wait list or EF > 40% on ECHO within 30 d	
Cystic fibrosis	Genetic analysis confirmation needed	MMaT-3
	FEV1 below 40% of predicted FEV1 with 30 d prior to initial request	
HAT	HAT within 2 wk of OLT	40
Primary hyperoxaluria	AGT deficiency proven on liver biopsy/genetic analysis	MMaT
	On kidney transplant list with eGFR ≤ 25 mL/min on two instances 42 d apart	

CA19-9: Carbohydrate antigen 19-9; FEV1: Forced expiratory volume at one second; TTR: Transthyretin; AGT: Alanine glyoxylate aminotransferase; MELD: Model for end-stage liver disease; CCA: Cholangiocarcinoma; HPS: Hepatopulmonary syndrome; MPAP: Mean pulmonary artery pressure; FAP: Familial amyloid polyneuropathy; HAT: Hepatic artery thrombosis; PVR: Pulmonary vascular resistance; POPH: Portopulmonary hypertension.

neoadjuvant therapy prior to transplant and operative staging to exclude metastatic disease. Needs to be unresectable hilar cholangiocarcinoma (CCA) and meeting the diagnostic criteria for CCA with malignant appearing stricture on cholangiography with either biopsy/cytology consistent with malignancy or aneuploidy or carbohydrate antigen 19-9 > 100 U/mL without cholangitis. Imaging studies showing one lesion < 3 cm without metastatic disease. After completion of neoadjuvant therapy, operative staging to assess involvement of regional nodes and peritoneal metastases prior to considering for transplant. The biopsy of the original lesion must be avoided secondary to high risk of tumor seeding.

Cystic fibrosis

The candidate will be eligible for MELD exception points of MMaT-3 if has genetic analysis confirmation for cystic fibrosis (CF) and the forced expiatory volume at one second (FEV1) is < 40% of predicted FEV1 within one month prior to initial exception request. After 90 d' extension request needed to be submitted.

HPS

The candidate will be eligible for MELD exception points of MMaT-3 if the candidate has evidence of portal hypertension (ascites, varices, splenomegaly, or thrombocytopenia) in the presence of intrapulmonary shunt confirmed with contrast echocardiogram (ECHO) or lung scan. Also, the partial pressure of oxygen (PaO_2) < 60 mmHg on room air within one month prior to submission of initial MELD exception request along with no underlying significant primary lung disease. To be eligible for MELD extension, the candidate must have PaO_2 < 60 mmHg within last 1 mo.

Portopulmonary hypertension

To be eligible for MELD exception points of MMaT-3, the candidate must have evidence of portal hypertension along with mean pulmonary artery pressure (MPAP)

> 35 mmHg and pulmonary vascular resistance (PVR) > 3 woods unit or ≥ 240 dynes/sec/cm^5. The candidates must have documentation of treatment for pulmonary hypertension with improvement in MPAP < 35 mmHg along with post treatment PVR < 5.1 woods unit or 400 dynes sec/cm^5. For MELD extension request cardiac catheterization needs to be repeated every 3 mo with confirmation of MPAP < 35 mmHg.

Familial amyloid polyneuropathy

The candidate will be eligible for MELD exception points of MMaT-3 if the candidate has biopsy-proven Amyloid along with confirmation of transthyretin (TTR) gene mutation with good functional status (able to ambulate without assistance). The candidate must be on liver transplant list or has ejection fraction (EF) > 40% on ECHO been performed within last 1 mo. To be eligible for extension, the candidate must be on active heart transplant list and ECHO showing EF > 40% within last 4 mo.

Primary hyperoxaluria

MELD exception points of MMaT will be granted if the candidates have alanine glyoxylate aminotransferase (AGT) deficiency on liver biopsy sample analysis or genetic mutation analysis and on active kidney transplant list with estimated the glomerular filtration rate (eGFR) ≤ 25 mL/min on two instances at least 42 d apart.

Hepatic artery thrombosis

The candidate will be eligible for MELD exception points of 40 if hepatic artery thrombosis (HAT) is within 2 wk after LT and does not meet the criteria of status 1A which includes HAT within 7 d of liver transplant along with aspartate aminotransferase ≥ 3000 and at least 1 of the following (INR ≥ 2.5 or arterial pH ≤ 7.3 or venous pH ≤ 7.2 or lactate ≤ for mmol/L).

Liver transplant outcomes in the United States

The OPTN/SRTR publishes annual liver transplant outcomes report in the United States every year. The most recent reported liver transplant outcomes in the United States were from the year 2018 and they were published in January 2020[27].

Waiting list outcomes

The deceased donor OLT rate had increased to 54.5 *per* 100 waitlist-years in 2018 regardless of the recipients' geographic location (metropolitan and rural), gender and age. This trend has been rising since 2012. Historically, minorities such as Asian and Latino liver transplant candidates were not favored to received OLT, compared to their Caucasian and African American counterparts. This gap has narrowed to 10%, 44 and 48 *per* 100 waitlist-years for Asians and Latinos, compared to 56 and 62.5 for Caucasians and African Americans. The OLT rate was 66% higher for HCC candidates than for non-HCC candidates. This gap has been steadily narrowing since 2006. The overall median time from UNOS listing to transplant was 10.8 mo. The overall pre-OLT mortality rate was 13.2 *per* 100 waitlist-years in 2018. Age 65 or older candidates, candidates listed with acute liver failure, candidate listed with status 1A and MELD ≥ 35 had higher waitlist mortality. However, pre-OLT waitlist mortality of candidates listed at status 1A or MELD ≥ 35 had decreased since the regional "Share 35" rule implemented in 2013. Pre-OLT waitlist mortality also varied significantly by candidates' DSA regions geographically, ranging from 6.5 to 37.4 *per* 100 waitlist-years. That was one of the chief reasons for the UNOS/OPTN to change the liver distribution policy from DSA-based allocation model to the AC model.

Liver donation outcomes

The number of deceased liver donations continued to increase in 2018. There were total of 7766 deceased liver donations. The use of hepatitis C virus (HCV) exposed donor livers has increased steadily since 2013. Approximately 8% of deceased donor OLTs in 2018 were from HCV donors. This trend has increased due to the effective HCV direct-acting antiviral (DAA) therapies and increased anoxic brain deaths from drug overdose secondary to national opioid epidemic. The liver donor organ discard rate has been trending down since 2012. The liver donor organ discard rate in 2018 was 8.4%. HCV-exposed donor livers were more likely to be utilized.

Liver transplant outcomes

In 2018, the annual volume of OLTs was the highest in the United States history, recording 8250 transplants in a single year. In comparison, this number was a huge 31% increase from 2008 when only 6319 OLTs were performed. The percentage of

DCD donations also increased to 6.9% in 2018, compared to 4.8% in 2008. Although the majority of OLT recipients were Caucasian males between 50 to 64 years of age, the number of Asian and Latino transplant recipients increased by 15% and 11% respectively. The two most common diagnoses were alcohol-associated liver disease and cryptogenic disease, which are, in many cases, undiagnosed burnt-out non-alcohol steatohepatitis in etiology. The third most common diagnosis for OLT was HCC. The number of OLT recipients with HCV continued to decline in 2018. Only 10% of OLT recipients had the primary diagnosis of HCV-related chronic liver disease.

Overall and graft survival of OLTs continued to rise in 2018. 1-year and 3-year liver graft failure rates of deceased donor liver transplant were 8.8% and 16% respectively. 1-year and 3-year graft failure rates of living donor liver transplant were 7.8% and 14.6% respectively. 5-year overall and graft survival outcomes for the recipients with HCV diagnosis were comparable to the recipients with other etiologies. This trend was due to the effective DAA therapies for recurrent HCV infection after OLT. While the OLT recipients with HCC had better 1-year graft survival rate than the recipients with non-HCC diagnosis (90% *vs* 88%), they both had a similar 5-year graft survival rate (77% *vs* 76%)[27].

CONCLUSION

In summary, we reviewed the evolution of liver transplant allocation policies in the United States over 3 decades; from CPT score system to MELD score to MELD-sodium score. We reviewed the liver transplant distribution policies; from older DSA-based distribution to the newer AC model and its potential advantages and drawbacks. We also reviewed the indications for both standard and non-standard MELD exceptions granted by the National Liver Review Board. Finally, we reviewed the liver transplant waitlist, donation and survival outcomes in the United States.

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 January 20; 12(1): 43-53

DOI: 10.5662/wjm.v12.i1.43

ISSN 2222-0682 (online)

MINIREVIEWS

Interrogating the interplay of angiogenesis and immunity in metastatic colorectal cancer

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Author contributions: Foukas PG and Koumarianou A designed the research; Kampoli K and Ntavatzikos A performed the bibliographic research and drafted the manuscript; Arkadopoulos N, Foukas P and Koumarianou A critically revised the manuscript for important intellectual content.

Conflict-of-interest statement: All authors declare no conflicts-of interest related to this article.

Country/Territory of origin: Greece

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

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Abstract

Colon cancer is the third most common malignancy and the fifth most frequent cause of death from neoplastic disease worldwide. At the time of diagnosis, more than 20% of patients already have metastatic disease. In the last 20 years, the natural course of the disease has changed due to major changes in the management of metastatic disease such as the advent of novel surgical and local therapy approaches as well as the introduction of novel chemotherapy drugs and targeted agents such as anti-epidermal growth factor receptor, anti-BRAF and antiangiogenics. Angiogenesis is a complex biological process of new vessel formation from existing ones and is an integral component of tumor progression supporting cancer cells to grow, proliferate and metastasize. Many molecules are involved in this proangiogenic process, such as vascular endothelial growth factor and its receptors on endothelial cells. A well-standardized methodology that is applied to assess angiogenesis in the tumor microenvironment is microvascular density by using immunohistochemistry with antibodies against endothelial CD31, CD34 and CD105 antigens. Even smaller molecules, such as the micro-RNAs, which are small non-coding RNAs, are being studied for their usefulness as surrogate biomarkers of angiogenesis and prognosis. In this review, we will discuss recent advances regarding the investigation of angiogenesis, the crosstalk between elements of the immune microenvironment and angiogenesis and how a disorganized tumor vessel network affects the trafficking of CD8+ T cells in the tumor bed. Furthermore, we will present recent data from clinical trials that combine antiangiogenic therapies with immune checkpoint inhibitors in colorectal

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Received: May 30, 2021
Peer-review started: May 30, 2021
First decision: July 31, 2021
Revised: August 17, 2021
Accepted: December 28, 2021
Article in press: December 28, 2021
Published online: January 20, 2022

P-Reviewer: Bogach J, Valiveti CK
S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Wang JJ

cancer.

Key Words: Vascular endothelial growth factor; Circulating tumor cells; Colorectal cancer; MicroRNAs; Microvascular density; Crosstalk; Angiogenesis; Immunity

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Core Tip: Colon cancer is one of the most common malignancies with a poor prognosis in patients with metastatic disease. Because of the need to find more effective treatments, researchers are focusing on deciphering the mechanisms used by the cancer cell for survival, food and metastasis. The main events in this process are neo-angiogenesis and immune escape through the interplay of growth factors involved in both pathways. This review presents the events involved in these pathways with a focus on their prognostic and predictive value.

Citation: Kampoli K, Foukas PG, Ntavatzikos A, Arkadopoulos N, Koumarianou A. Interrogating the interplay of angiogenesis and immunity in metastatic colorectal cancer. *World J Methodol* 2022; 12(1): 43-53
URL: https://www.wjgnet.com/2222-0682/full/v12/i1/43.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i1.43

INTRODUCTION

Colorectal cancer (CRC), the third most common cancer in both genders, accounts for 9% of new cancer diagnoses in men and 8% in women and is the third leading cause of cancer death in both sexes. Although it is more common in those over the age of 70, a significant proportion of patients are of middle age. The 5-year survival rate of patients with localized disease CRC is 90%. However, this rate is significantly lower in patients with metastatic disease, reaching 14% and 15% in those with colon and rectal cancer, respectively[1].

Due to the poor prognosis of metastatic CRC (mCRC), the need for novel therapeutic approaches in these patients is urgently needed. A step towards this direction was made possible by the introduction of antiangiogenetic agents, but there are several unmet needs to better define patient profiles benefiting from such an approach. Moreover, despite this and other new treatments, the prognosis of patients with mCRC is still poor, and research is focusing on biomarkers with predictive and prognostic value (Table 1). Despite extensive research, in everyday practice only mutations in *RAS*, *BRAF*, *NTRK* and *HER2* genes as well as the level of microsatellite instability have found application in the targeted therapy of mCRC[2,3]. As the complex process of carcinogenesis and metastasis is continuously defined, this knowledge is expected to lead to the discovery of new therapies.

In this review, we will discuss recent advances in CRC regarding the investigation of angiogenesis, the crosstalk between the immune microenvironment and angiogenesis and the ways through which cancer cells escape the host immune system. Furthermore, we will present recent data from clinical trials that combine antiangiogenic agents with immune checkpoint inhibitors.

ANGIOGENESIS

Vascular endothelial growth factor

Angiogenesis is a complex mechanism of new vessel production that the cancer cell uses to ensure the supply of oxygen and nutrients and thus to multiply and generate evolving solid tumors with distant metastases[4].

There are two main regulators of angiogenesis that are essential for the development of CRC, hypoxia factor-1α and vascular endothelial growth factor (VEGF). Hypoxia factor-1α is a proangiogenic factor and is found in the tumor microenvironment. It is secreted by the cancer cell under hypoxic conditions and affects a wide

Table 1 Factors related to angiogenesis and immunity and studied as biomarkers in colorectal cancer

Factor	Biologic material	Pathway	Significance	Ref.
VEGF	Tissue, blood	Angiogenesis	Prognostic & predictive	Bendardaf *et al*[63] 2017, Des Guetz *et al*[7] 2006, Ferroni *et al*[64] 2006, Pascual *et al*[65] 2018, Tsai *et al*[66] 2013, Tsai *et al*[67] 2015, Boussios *et al*[68] 2019, Zygoń *et al*[23] 2017, Mohamed *et al*[69] 2019
VEGF polymorphism	Tissue, blood	Angiogenesis	Prognostic & predictive	Mousa *et al*[9] 2015
HIF-1α	Tissue	Angiogenesis	Prognostic	Baba *et al*[5] 2010
CTCs	Blood	Angiogenesis	Prognostic & predictive	Arrazubi *et al*[34] 2019, Burz *et al*[25] 2018, Cabel *et al*[28] 2017, Tan *et al*[33] 2018, Wang *et al*[35] 2019, Zhang *et al*[70] 2017
CTCs	Blood	Angiogenesis	Predictive	Nakamura *et al*[3] 2018
MicroRNA	Tissue, blood, stools	Angiogenesis	Prognostic & predictive	Balacescu *et al*[40] 2018, Boussios *et al*[68] 2019, Peng *et al*[41] 2017, To *et al*[38] 2018
MVD	Tissue	Immunity	Prognostic	den Uil *et al*[71] 2019, Des Guetz *et al*[7] 2006, Mohammed *et al*[72] 2020, Zhu *et al*[19] 2017, Zygoń *et al*[23] 2017

VEGF: Vascular endothelial growth factor; HIF-1α: Hypoxia–inducible factor 1-alpha; CTCs: Circulating tumor cells; MVD: Microvascular density.

variety of signaling pathways, including the upregulation of the VEGF cascade[4-6]. VEGF has several important functions, the most important one being the increase of vascular permeability and the induction of new blood vessels through its binding to endothelial cells and by promoting their proliferation[7,8].

VEGF comprises a group of glycoproteins that, together with placental growth factor, interact with three VEGF receptors (VEGFR1, VEGFR2, VEGFR3) and two neuropilin co-receptors (NRP1, NRP2). VEGFRs are tyrosine kinase receptors found in endothelial vascular cells. The binding of the glycoprotein to its receptor results in the initiation of a sequence of events that ultimately result in the formation of new vessels [3].The ligation of VEGF-A with VEGFR-2 is the most important step in the activation of angiogenesis in CRC[9].

Bevacizumab is a monoclonal antibody targeting VEGF-A and the first antiangiogenic agent to be used against metastatic cancer. Bevacizumab was approved in 2004 in the United States and in 2005 in Europe for use in patients with mCRC. Its mechanism of action is mediated through the inhibition of the interaction of VEGF-A with VEGFR, and thus bevacizumab inhibits the signaling pathway that promotes neovascularization[10]. Finding biomarkers that could predict the response to antiangiogenic therapy so that it could be used only in patients who would benefit from its administration is a currently unmet need.

Due to the dominant role of VEGF in angiogenesis, researchers investigated whether the expression of this factor could be a predictive biomarker for patients receiving antiangiogenic therapy. One study indicated that high VEGF baseline levels associated with worse response to bevacizumab treatment and progression-free survival[11]. In 2013 Hegde *et al*[12] showed that there is no statistically significant relationship between plasma VEGF-A levels and the clinical response to bevacizumab. Therefore, it has no predictive value in metastatic colon cancer. Another exploratory analysis investigating epithelial and stromal VEGF expression, assessed by in situ hybridization and immunohistochemistry on tissue microarrays and whole tumor tissue sections, suggested that in patients with mCRC the addition of bevacizumab to chemotherapy improves survival regardless of the level of VEGF expression[13]. Mavericc was the first prospective mCRC study using gene expression data from blood (plasma VEGF-A protein levels) to evaluate the efficacy of mCRC chemotherapy regimens indicating that high plasma VEGF levels were associated with shorter treatment duration of response and progression-free survival[14]. More interesting, VEGF polymorphisms have also been studied, and it appears that they could possibly be used as predictive agents in mCRC in patients treated with irinotecan and bevacizumab[15]. In another study, VEGF-A (c.*237C>T) was associated with a significantly better time to treatment failure[16]. Another study investigating the predictive role of VEGF-A indicated a significant association of rs833061 single nucleotide polymorphism with the overall response rate in advanced CRC patients treated with cytotoxic chemotherapy plus bevacizumab[17].

Microvascular density

An important indicator used in translational studies to assess the degree of neovascularization of the tumor is the microvascular density (MVD). MVD appears to increase as it progresses from normal mucosa to adenoma and from adenoma to cancer, and this is explained by the intense angiogenesis that aims to meet the neoplastic cells need for oxygen[18]. MVD was found to be higher in primary tumors than in metastases[5, 18], while its levels within the tumor were associated with an increased risk of distant metastases[19]. The assessment of MVD includes pan-endothelial cell markers, also expressed in normal tissues, such as CD31 and CD34, as well as endothelial markers expressed on the surface of proliferating endothelial cells, such as CD105[18,20]. Endoglin is expressed mainly in vascular endothelial cells during active angiogenesis, while it is only weakly expressed or absent in pre-existing vascular endothelial cells, making this marker an important indicator of neoangiogenesis[18].

A systematic review and meta-analysis have indicated that increased VEGF and MVD expression markers are associated with an increased incidence of metastasis in CRC patients treated with surgery and chemotherapy[21]. An attempt was also made to correlate MVD with clinicopathologic features, such as sex, age, location, grade of differentiation, infiltrated lymph nodes and distant metastases, but with contradictory results. A negative correlation was found in two studies that investigated MVD in relation to the above variables[22,23], but in two other studies MVD staining was positively associated with tumor invasion, lymph node metastases[18] and distant metastases[19].

Since MVD is a biomarker for the quantification of angiogenesis, the question arises whether it can be used as a predictor of the treatment outcome with the antiangiogenic agent bevacizumab.

In 2006, Jubb *et al*[13], reported a clinical study of 813 patients with mCRC and found no association between elevated MVD or VEGF expression and the clinical outcome in relation to bevacizumab treatment. Although the predictive value of MVD in relation to bevacizumab response has been recognized in other cancers such as advanced ovarian cancer[24], in mCRC this has not yet been demonstrated.

CIRCULATING TUMOR CELLS

It has been postulated that cancer cells circulate in the peripheral blood of patients with metastatic disease[25,26]. It is reasonable to expect that the isolation and study of these cells can provide information about the metastatic potential of primary disease and an assessment of their value as prognostic and predictive biomarkers[25].

The mechanism by which cancer cells enter the circulation and acquire the ability to metastasize is not fully understood. However, this process appears to be activated by tumor hypoxia, which also activates angiogenesis[27].

It has been estimated that the frequency of circulating tumor cells (CTC) is about 1 per 1 mL of peripheral blood[28] or otherwise 1 g of tumor releases 10^6 cells into the bloodstream[25]. Despite the large number of cells released into the bloodstream daily, a small number can be detected and isolated. This is partly due to the fact that these cells are covered by platelets and coagulation factors[29]. However, with the advent of new methods, it is now more feasible to isolate circulating cancer cells and study them [30]. Liquid biopsy, the isolation of CTCs or tumor cell-free DNA from peripheral blood is only minimally invasive compared to tumor biopsy and can be repeated many times for the monitoring of genomic changes that contribute to cancer progression and/or resistance to chemotherapy[31].

Although CTCs have been isolated in the blood of patients with polyps of the colon, the number of CTCs measured in the blood of patients with colon cancer is statistically significantly higher[28]. Furthermore, a smaller number of CTCs is detected in well-differentiated tumors compared to the less differentiated counterparts. The number of CTCs does not seem to be related to the tumoral histologic subtype, whereas it seems to be related to the anatomical location, being higher in cancer of the rectum and sigmoid colon compared to other sites[32]. Circulating cancer cells is an independent prognostic factor for the survival of patients with CRC[33]. In patients with mCRC and liver secondaries treated with complete resection of the primary tumor site and liver metastases, the presence of two or more CTCs/7.5 mL of blood preoperatively was an indicator of poor disease outcome and low survival[34]. Furthermore, according to another recent study, the CTC-positivity rate was an independent predictive factor of progression-free survival and overall survival in patients with advanced disease treated with chemotherapy. In addition, the CTC concentration was related to the

pathological stage of the disease, the presence of metastatic disease, the depth of tumor invasion, the presence of lymphatic invasion and high serum carcinoembryonic antigen levels[35].

MicroRNAs

In recent years microRNAs, have been studied as biomarkers for diagnosis, prognosis and treatment resistance in patients with CRC. MicroRNAs are small non-coding molecules consisting of 18 to 25 nucleotides that control the expression of many target genes, either by inhibiting their expression or by stimulating it. Thus, by affecting the expression of oncogenes it is possible to either inhibit or promote oncogenesis[36]. These molecules can be detected not only in tissues but also in the serum and feces of cancer patients. They are found extracellularly either as a result of cancer cell death or due to extracellular secretion by cancer cells[37]. MicroRNAs target the 3' untranslated region of target genes, thereby degrading and controlling their expression[36]. MicroRNA interaction with target genes and their mRNA is affected by single nucleotide polymorphisms in the 3' untranslated region of these target genes, which also affect their expression. These polymorphisms have been studied to predict treatment outcomes, such as resistance to chemotherapy[38].

MicroRNAs are extremely stable molecules because they are stored in extracellular structures or bound to lipoproteins[38]. This feature and the fact that they do not require invasive methods for their detection make them potential ideal diagnostic and prognostic biomarkers.

The association of microRNAs with CRC was first described by Michael *et al*[39] in 2003. In this study, the authors showed that microRNA-143 and microRNA-145 levels were reduced in precancerous adenomatous lesions and CRC compared with normal mucosa. Since then, several research studies and meta-analyses have been published, emphasizing the importance of microRNAs in cancer[40].

In addition to oncogenesis, there are microRNAs that target regulatory molecules that lead to angiogenesis. These molecules, known as "angiomiRs," either promote or suppress angiogenesis, thereby indirectly affecting tumor formation and metastasis.

MicroRNA-21 is the most representative of neoangiogenesis as it has been studied in many types of cancer and by several researchers. In a meta-analysis published in 2017, Peng *et al*[41] analyzed data from 57 studies and concluded that microRNA-21 has a diagnostic sensitivity of 64% and a specificity of 85%, making it a potential prognostic indicator for patient survival. According to this study, peripheral blood microRNA-21 levels can be used as an indicator of CRC detection, and tissue levels can be an indicator to predict patient survival.

In addition to microRNA-21, there are many other microRNAs that target regulatory molecules leading to angiogenesis. Such molecules are microRNA-126, microRNA-30, microRNA-182, microRNA-194, microRNA-23b, microRNA-27a, microRNA-27b, microRNA-29b, microRNA-143, microRNA-145 and the complexes microRNA17-92, microRNA15a/16-1, microRNA-885-3p and microRNA885-3p[42].

MicroRNAs in the stool are the least studied but have been proven stable enough to correlate with the stage of the disease and have a high sensitivity and specificity in distinguishing patients from healthy individuals[38].

Long non-coding RNAs are made up of about 200 nucleotides and have also been studied as prognostic biomarkers. Although not translated into proteins, they act competitively by binding to common microRNA binding sequences and trapping them to alter the expression of their target genes. Available data suggest that long non-coding RNAs play a role not only in CRC development but also in metastasis[43].

THE CROSSTALK BETWEEN ANGIOGENESIS AND IMMUNITY

Tumor development and progression are highly dependent on the vascular network that penetrates the tumor bed and supplies proliferating malignant cells with oxygen and nutrients[44]. Although several mechanisms contribute to the constant development of the new vascular network, *i.e.*, neoangiogenesis, most new vessels are considered to be formed by the sprouting from parental ones[45]. The process of neoangiogenesis is triggered by hypoxia and deprivation of nutrients and is regulated by many proangiogenic and antiangiogenic factors such as VEGF-A, fibroblast growth factor, platelet-derived growth factor, transforming growth factor and others[45-47]. Compared to normal tissue vasculature, tumor neoangiogenesis is characterized by abnormalities in structure and function, driven by the imbalance between pro-angiogenic, mainly VEGF, and antiangiogenic factors in the tumor microenvironment

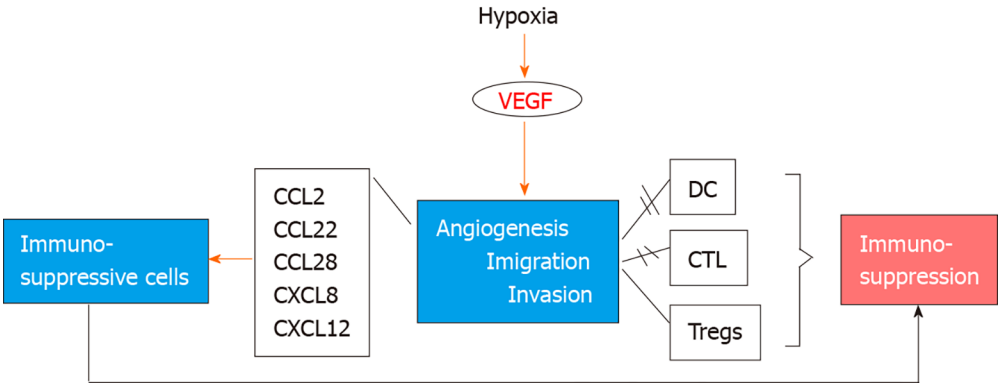

Figure 1 The sequence of events following hypoxia and vascular endothelial growth factor secretion leading to immune system escape and carcinogenesis. VEGF: Vascular endothelial growth factor; CCL: C-C motif chemokine ligand; CXCL12: C-X-C motif chemokine ligand 12; DC: Dendritic cells; CTL: Cytotoxic T lymphocytes; Tregs: Regulatory T cells.

[48]. The abnormal structure and function of the tumor vasculature significantly affect the anti-tumor immunity, facilitating immune evasion in many different aspects (Figure 1). Overexpression of VEGF, produced by tumor cells, platelets and inflammatory cells such as neutrophils and monocytes, promotes the formation of an immature vascular network with increased leakiness, which in combination with the increased physical compression in the tumor bed leads to impaired blood perfusion and reduction of delivering oxygen and cytotoxic T cells in the tumor area[8,49]. Moreover, hypoxia/acidosis induced growth factors and cytokines such as transforming growth factor-β and VEGF suppress the activity of cytotoxic T cells, suppress the antigen presenting capacity of dendritic cells, reprogram macrophages into a protumorigenic phenotype and upregulate the expression of programmed cell death-ligand 1 by tumor cells, myeloid-derived suppressor cells and dendritic cells and macrophages, further increasing immune evasion in the tumor microenvironment [8,50-52]. Of note, hypoxia-induced chemokines such as C-C motif chemokine ligand 2, C-C motif chemokine ligand 22, C-C motif chemokine ligand 28, C-X-C motif chemokine ligand 8 and C-X-C motif chemokine ligand 12 recruit immunosuppressive cells in the tumor microenvironment such as myeloid-derived suppressor cells, regulatory T cells and M2 macrophages[53] (Figure 1). In addition, tumor endothelial cells, in contrast to normal vasculature, express FasL and acquire the ability to kill effector CD8+ T cells but not regulatory T cells[54,55].

Immunotherapy is now a key therapeutic weapon in the treatment of many cancers, such as melanoma, lung and urothelial cancer and has significantly improved patients' prognosis. Immunotherapies target immune checkpoints that are abnormally expressed in many patients and aim to kill the tumor indirectly by boosting the anti-tumor immune responses. Cytotoxic T-lymphocyte-associated protein 4 and programmed cell death protein 1 with its ligand programmed cell death-ligand 1 are primarily involved in inhibitory immune signaling and are essential regulators of cancer immune evasion. Current clinical practice includes mainly two types of immune checkpoint inhibitors such as anti-cytotoxic T-lymphocyte-associated protein 4 (ipilimumab and tremelimumab) and anti-programmed cell death protein 1/programmed cell death-ligand 1 (nivolumab, atezolizumab, pembrolizumab) monoclonal antibodies[56]. However, in CRC these therapies have not proved to mediate similar effects, except in tumors with microsatellite instability[57].

As the immunosuppressive tumor microenvironment is additionally induced in part by the dysfunctional vascular network, a window for therapeutic application opens for the combination of immunotherapies and antiangiogenics. This strategy has been exploited in several clinical trials for different tumor types[51], such as non-small cell lung cancer (atezolizumab and bevacizumab)[58], renal cell carcinoma (axitinib and pembrolizumab or cabozantinib and nivolumab)[59,60], endometrial cancer (lenvatinib and pembrolizumab)[61] and hepatocellular carcinoma (atezolizumab and bevacizumab)[62].

Regarding CRC, ongoing clinical studies (Table 2) are investigating the effectiveness of combinations of antiangiogenic agents and immune checkpoint inhibitors. It is possible that such combinations could be applied in the future treatment of mCRC.

Table 2 Clinical trials related to antiangiogenic agent therapy and immunotherapy in colorectal cancer

Status	Study title	Drugs	Country
Recruiting	A study evaluating the efficacy and safety of multiple immunotherapy-based treatment combinations in patients with metastatic colorectal cancer (Morpheus-CRC)	Regorafenib, atezolizumab	United States
Recruiting	Study of chemotherapy combination with autologous cell	Bevacizumab, oxaliplatin, capecitabine; Biological component: PD1-T cells	China
Recruiting	Treatment of colorectal liver metastases with immunotherapy and bevacizumab	Atezolizumab, bevacizumab, oxaliplatin	Korea
Recruiting	Neoadjuvant treatment in rectal cancer with radiotherapy followed by atezolizumab and bevacizumab (TARZAN)	Atezolizumab, bevacizumab	Netherlands
Not yet recruiting	Chemotherapy and immunotherapy as treatment for MSS metastatic	Capecitabine, oxaliplatin, bevacizumab, pembrolizumab	France
Not yet recruiting	QL1101 in combination with JS001 in patients with pMMR/MSS refractory metastatic	Bevacizumab, tripleitriumab	China
Not yet recruiting	Comparison of sintilimab to XELOX	Sintilimab *vs* XELOX + bevacizumab	China

CRC: Colorectal cancer; PD-1: Programmed cell death protein 1.

CONCLUSION

Due to the poor prognosis of patients with mCRC, research has focused not only on finding prognostic and predictive factors but also on new therapeutic combinations. Immunohistochemistry methods have been instrumental in finding molecules that could be used as predictors, but molecular biology and immunology have been most informative in dissecting the mechanisms by which the cancer cell survives and spreads. Understanding how the immune and vascular microenvironments interact has opened new horizons in cancer treatment. Although such combination therapies for CRC have not yet been approved, the results of clinical trials are eagerly awaited.

Finding new molecular targets for different approaches including immunotherapy may enrich treatment options for CRC in the future.

ACKNOWLEDGEMENTS

Part of the expenses and materials for this narrative review were provided by the Hellenic Society of Medical Oncology and the Hellenic Study Group of Psycho-neuroimmunology in Cancer. The authors express their gratitude to Professor Dimitrios T Boumpas for expert revision of the manuscript.

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 January 20; 12(1): 54-63

DOI: 10.5662/wjm.v12.i1.54

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Retrospective Study

Phenomenology of obsessive-compulsive disorder in children and adolescents: Sample from a tertiary care center in Istanbul, Turkey

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Author contributions: Cifter A and Erdogdu AB designed the project and created data collection tools; Cifter A examined the patient files; Cifter A and Erdogdu AB did the analysis, interpreted the data and wrote the paper for publication; Erdogdu AB critically revised the paper.

Institutional review board statement: The study protocol was approved by the Marmara University School of Medicine Clinical Research Ethics Committee (Protocol No: 09.2019.360, date: April 5, 2019).

Informed consent statement: Written informed consent was obtained from parents or legal guardians of the patients.

Conflict-of-interest statement: None declared.

Data sharing statement: No additional data are available.

Country/Territory of origin: Turkey

Specialty type: Psychiatry

Provenance and peer review: Invited article; Externally peer

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Abstract

BACKGROUND

Obsessive-compulsive disorder (OCD) is a heterogeneous disease in many respects and exhibits this diversity in terms of phenomenology. It also displays several different characteristics in children compared to adults.

AIM

To describe the socio-demographic and phenomenological features of children with OCD and to investigate the impact of these features on response to pharmacotherapy.

METHODS

This retrospective study was carried out with 150 children and adolescents who had been diagnosed with OCD between 2014 and 2018. Data was collected by examining the files of the patients with diagnosis of OCD and similar disorders from the hospital database. Yale-Brown Obsessive-Compulsive Scale for Children was used for the assessment of obsession-compulsion subtypes. The Clinical Global Impression (CGI) scale was used to evaluate the severity of the disease (CGI-S) and global improvement (CGI-I). The predictors of treatment response were evaluated using linear regression analysis. The level of significance for all statistic tests was set as $P < 0.05$.

RESULTS

The sample was divided into prepubertal (44%) and adolescent (56%) age groups. The most prevalent obsessions were contamination and aggression obsessions, and the most frequent compulsions were washing and checking. While contamination was observed more commonly in the prepubertal age group, the religious obsession was seen more frequently in adolescents. Patients with aggression obsession presented a higher frequency of comorbid anxiety ($P = 0.022$) and mood

reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

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Received: June 10, 2021
Peer-review started: June 10, 2021
First decision: July 31, 2021
Revised: August 5, 2021
Accepted: December 2, 2021
Article in press: December 2, 2021
Published online: January 20, 2022

P-Reviewer: Pasquini M
S-Editor: Wu YXJ
L-Editor: Filipodia
P-Editor: Wu YXJ

(P = 0.047) disorder. CGI-I scores did not differ according to phenomenological subgroups (P > 0.05). A lower CGI-I score was linked to a lower CGI-S score (95% confidence interval 0.21-0.39, P < 0.001) and the prepubertal age of admission (95% confidence interval 0.03-0.87, P = 0.020).

CONCLUSION
The phenomenology of OCD shows differences depending on the age group and the comorbid psychiatric disorders. Earlier identification and treatment of OCD may help to prevent the impairment of the mental health of children and adolescents.

Key Words: Obsessive-compulsive disorder; Phenomenology; Comorbidity; Treatment response; Serotonin reuptake inhibitors

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Core Tip: We aimed to analyze the socio-demographic and phenomenological features of children and adolescents with obsessive-compulsive disorder and to investigate the impact of these features on the pharmacotherapy response. Contamination was the commonest obsession, and washing-cleaning was the most common compulsion. The type of obsession varied with the age group: Contamination was seen more frequent in prepubertal age group, whereas the religious obsessions in adolescents. Aggression obsession was associated with the comorbid anxiety disorders and depression. The treatment response deteriorated with the increase in severity of disease and the age of admission. No difference was observed between the phenomenological subgroups in case of treatment response.

Citation: Cifter A, Erdogdu AB. Phenomenology of obsessive-compulsive disorder in children and adolescents: Sample from a tertiary care center in Istanbul, Turkey. *World J Methodol* 2022; 12(1): 54-63
URL: https://www.wjgnet.com/2222-0682/full/v12/i1/54.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i1.54

INTRODUCTION

Obsessive-compulsive disorder (OCD) is a neuropsychiatric disorder characterized by recurrent, unwanted thoughts (obsessions) and rituals to reduce anxiety (compulsions), which may lead to significant distress in a person's life[1]. The age of onset varies between the ages of 2 and 46, and symptoms usually begin around the age of 10 [2].

In contemporary psychiatric terminology, the term *phenomenology* is used to describe the symptoms and signs of diseases[3]. As a disorder with heterogeneous features in many respects, OCD also shows its variability and diversity in terms of phenomenological features, which are affected by many structural and environmental factors, and shows several different characteristics in children compared to adults. Rosario-Campos *et al*[4] stated that aggression, sexual and religious obsessions are more common in adults, whereas symmetry-ordering obsession/compulsions are more common in the pediatric age group. In addition, comorbid psychiatric disorders have been associated with some phenomenological subgroups. While mood and anxiety disorders accompany aggression obsession more frequently, it is reported that symmetry-ordering obsession and compulsions are more common in patients with tic disorders[5].

Whilst phenomenology is affected by multifactorial components, it also plays an essential role in the treatment response of OCD. However, there are contradictory results in the literature about which subgroup responds better to treatment[6]. In clinical practice, some subgroups benefit from selective serotonin reuptake inhibitors (SSRI) and clomipramine, whereas some groups need an antipsychotic augmentation in treatment[7].

In this study, we aimed to analyze the socio-demographic and phenomenological features of children and adolescents with OCD and to investigate the influence of these features on the pharmacotherapy response.

MATERIALS AND METHODS

Procedures and study group

This research was conducted in the Marmara University School of Medicine, which houses the largest child and adolescent psychiatry clinic on the Asian side of Istanbul, Turkey. The study was carried out retrospectively by examining the files of the emergency and outpatient clinics of the Child and Adolescent Psychiatry Department.

For our research, files of patients with International Classification of Diseases-10 diagnostic code F42 and refractions (OCD and similar disorders) were collected from the hospital database from a total of 88710 outpatient and 3896 emergency /consultation admissions between 2014 and 2018. There was totally 1516 applications belonging to 642 patients, and the patient files were overviewed systematically according to date of admission to the clinic. Out of the files overviewed, 153 could not be accessed due to problems in file archiving, and 101 patients were excluded since the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders, 5th Edition for OCD were not fully met[1]. Also, the information in another 238 patient files was not taken into consideration due to lack of data. Finally, a total of 150 children and adolescents fully meeting the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition criteria were included in the study.

At least one drug treatment (SSRI, clomipramine, second generation antipsychotics) had been given to 118 (78.7%) children who had applied for at least two follow-up interviews, and seven of the children who started drug treatment discontinued the follow-up. One hundred and eleven children under medication continued their follow-up for an average of 5.5 mo. Children who had not used any psychotropic drugs received supportive psychotherapy.

Data collection tool

The data were collected through Patient Follow-Up Form from patient records. Obsession and compulsion subgroups were established based on the Yale-Brown Obsessive-Compulsive Scale for Children[8]. Obsessions such as the need to know or remember, fear of saying certain things, fear of not saying just the right thing, intrusive images and sounds were evaluated under the heading of "miscellaneous obsessions" in Yale-Brown Obsessive-Compulsive Scale for Children. Also, compulsions such as mental rituals, the need to tell, ask or confess, the need to take precautions to prevent damage to himself/herself and others, and the need to touch, tap or rub were evaluated as "miscellaneous compulsions."

Clinical Global Impression (CGI), one of the most commonly used clinician rated tools in psychiatry, measures the severity of the disease with CGI-S and the global improvement with CGI-I. The CGI-S score ranges from 1 (normal, not at all ill) to 7 (among the most extremely ill patients), and the CGI-I assesses the improvement from 1 (much improved) to 7 (much worse), which was recorded at the last interview following the treatment[9].

Statistical analysis

SPSS 20.0 for Windows (IBM, 2011, Armonk, NY, United States) was used during the statistical analyses. The Shapiro-Wilk test was used to evaluate the normality of data distribution. Parametric variables were analyzed with Pearson's χ^2 or Fisher's exact test, independent samples t test, one-way analysis of variance, and for the non-parametric variables Kruskal-Wallis and Mann-Whitney U tests were used. The predictors of treatment response were evaluated using linear regression analysis. The level of all significance for all statistical tests was set as $P < 0.05$.

Ethics

The study protocol was conducted in keeping with the code of ethics of the Declaration of Helsinki and was approved by the Marmara University School of Medicine Clinical Research Ethics Committee (Protocol No: 09.2019.360, date: April 5, 2019). Written informed consent was obtained from parents or legal guardians of the patients.

RESULTS

A total of 150 children, with a mean age of admission to the clinic of 11.90 ± 3.02 (min: 4, max: 17) and a mean age of onset of symptoms of 11.01 ± 3.36 (min: 3, max: 17), participated in our study. As the age of onset of the symptoms was in congruence with the age of admission and was prone to recall bias, we divided the study group into prepubertal/childhood (44.0%) and postpubertal/adolescent (56.0%) age groups based on the age at admission and accepted the onset of puberty as 12 years of age. The socio-demographic and clinical characteristics of the children are shown in Table 1. The socio-demographic features such as parental educational and employment status were similar in terms of sex ($P > 0.05$).

In the year prior to the admission, 35.3% of the children had experienced a stressful life event, which included the death of one of the nuclear or extended family members (10.7%), material loss (8.0%), serious illness of themselves or family members (8.0%), moving to another place (7.3%), an accident (3.3%) and parental divorce (0.7%). Before admission to our clinic, 22.0% of the children had been admitted to a mental health professional due to OCD, and 20.0% had shown psychiatric symptoms other than OCD. Out of all participants, 35.3% of the first-degree relatives had a history of psychiatric disorder, the most prominent of these being major depressive disorder (19.3%), which was followed by OCD and panic disorder (both 7.5%).

While 96.6% of the children had an obsession and 88.0% had a compulsion, 55.3% had more than one obsession, and 47.3% had more than one compulsion. The patterns of obsessions are shown in Figure 1 and compulsions are shown in Figure 2. There was no difference in the phenomenological subgroups between females and males ($P > 0.05$). Contamination obsessions were observed more commonly in the prepubertal age group ($\chi^2 = 4.658$, $P = 0.031$), whereas religious obsessions were more common in adolescents ($\chi^2 = 7.013$, $P = 0.008$). There was no variance in compulsions and other obsessions according to age group ($P > 0.05$). In terms of stressful life events, aggression obsession was observed more frequently in children who had recently lost one of their family members ($\chi^2 = 3.684$, $P = 0.05$), and superstitious obsession was more common in children who had recently been in or witnessed an accident ($\chi^2 = 12.312$, $P = 0.023$). Ordering-arranging compulsion was found to be more common in children who had moved in the last year ($\chi^2 = 4.718$, $P = 0.03$).

In respect of comorbid psychiatric disorders, patients with aggression obsession presented a higher frequency of comorbid anxiety disorders ($\chi^2 = 5.239$, $P = 0.022$) and major depressive disorder ($P = 0.047$, Fisher's exact test). Ordering-arranging compulsion was seen less frequently in children with comorbid disruptive behavior disorders ($\chi^2 = 6.042$, $P = 0.014$).

The baseline CGI-S and post-treatment CGI-I scores are shown in Table 2. A positive, moderately strong and statistically significant correlation was found between CGI-S and CGI-I scores ($r = 0.443$, $P < 0.001$). The CGI-I scores of the adolescents were higher than children ($t = -2.231$, $P = 0.027$), but there was no significant difference between CGI-S scores of age groups ($t = -0.894$ $P = 0.373$). While CGI-S scores were higher ($t = 2.342$, $P = 0.021$) in children with superstitious behaviors (mean: 4.42 ± 0.53) compared to those without (mean: 3.20 ± 1.37), CGI-I scores did not differ according to phenomenological subgroups ($P > 0.05$).

In the linear regression analysis, the CGI-S score and the age of admission were found to be the parameters that predicted the CGI-I score (Table 3).

DISCUSSION

To the best of our knowledge, this study is the largest from Turkey concerning the phenomenology of pediatric OCD. Also, the study data was derived from a child and adolescent psychiatry clinic in a tertiary university hospital rather than a specialized clinic for OCD and therefore should be more representative of all pediatric OCD patients. So, ranging from mild to moderate, all severity levels of OCD, including treatment-resistant patients, were represented in the sample, suggesting that the findings could be broadly applied to OCD.

In the present study, the most common obsession was contamination, followed by miscellaneous, aggression and religious obsessions, and the most common compulsions were washing/cleaning, control, the miscellaneous category and ordering-arranging. Many studies worldwide also indicated contamination as the commonest obsession and cleaning as the commonest compulsion[10-12]. However, some researchers revealed symmetry-ordering as the most prevalent phenomeno-

Table 1 Socio-demographic and clinical characteristics of children according to age group

Socio-demographic characteristics	Prepubertal	Postpubertal	Overall	Statistical analysis
	n (%)	*n* (%)	*n* (%)	
Sex (male)	40 (60.6)	43 (51.2)	83 (55.3)	$\chi^2 = 1.326$, $P = 0.250$
Educational level of mother				
Secondary school and lower	24 (32.9)	40 (54.1)	73 (49.7)	$\chi^2 = 6.704$, $P = 0.010$[1]
High school and above	49 (67.1)	34 (45.9)	74 (50.3)	
Employment status of mother	16 (24,6)	13 (15.5)	29 (19.5)	$\chi^2 = 1.953$, $P = 0.162$
Educational level of father				
Secondary school and lower	20 (35.1)	44 (51.2)	57 (39.9)	$\chi^2 = 3.583$, $P = 0.058$
High school and above	37 (64.9)	42 (48.8)	86 (60.1)	
Employment status of father	63 (96.9)	67 (82.7)	130 (89.0)	$\chi^2 = 7.459$, $P = 0.006$[1]
Parents live together	62 (93.9)	73 (86.9)	135 (90.0)	$\chi^2 = 2.032$, $P = 0.154$
Consanguinity	13 (19.7)	21 (25.0)	34 (22.7)	$\chi^2 = 0.593$, $P = 0.441$
Mental disorder in 1st degree relatives	24 (36.4)	29 (34.5)	53 (35.3)	$\chi^2 = 0.055$, $P = 0.815$
Comorbid psychopathology	48 (72.7)	53 (63.1)	101 (63.3)	$\chi^2 = 1.559$, $P = 0.212$
Anxiety disorders[a]	22 (33.3)	29 (34.5)	51 (34.0)	$\chi^2 = 0.023$, $P = 0.879$
Disruptive behavioral disorders[b]	23 (34.8)	18 (21.4)	41 (27.3)	$\chi^2 = 3.351$, $P = 0.067$
Neurodevelopmental disorders[c]	20 (30.3)	16 (19.0)	36 (24.0)	$\chi^2 = 2.567$, $P = 0.109$
Mood disorders (major depressive disorder)	1 (1.5)	6 (7.1)	7 (4.7)	$\chi^2 = 2.631$, $P = 0.105$
	mean ± SD			
Age gap between mother and father	3.34 ± 2.75	4.54 ± 4.18	4.00 ± 3.26	$t = -1.736$, $P = 0.085$
Birth order	1.69 ± 0.86	2.17 ± 1.37	1.95 ± 1.19	$t = -2.444$, $P = 0.016$[1]

[1]Indicates emphasis of significance.
[a]Generalized anxiety disorder, separation anxiety disorder, social anxiety disorder, specific phobias and panic disorder.
[b]Attention deficit/hyperactivity disorder, oppositional defiant disorder and conduct disorder.
[c]Articulation/phonation disorders, mental retardation, autism spectrum disorders, dyslexia and tic disorder.
SD: Standard deviation.

Table 2 Clinical Global Impression-Severity and Clinical Global Impression-Improvement scores of the children

CGI-S[1]			CGI-I[2]		
Score	**Remark**	**%**	**Score**	**Remark**	**%**
2	Borderline mentally ill	5.0	1	Very much improved	39.1
3	Mildly ill	19.3	2	Much improved	24.3
4	Moderately ill	54.3	3	Minimally improved	25.2
5	Markedly ill	19.3	4	No change	6.1
6	Severely ill	2.1	5	Minimally worsened	5.2

[1]Evaluated in the first session (baseline).
[2]Evaluated in the last session (post-treatment).
CGI-S: Clinical Global Impression-severity score; CGI-I: Clinical Global Impression-global improvement score.

logical subgroup[4,13], whereas Bryńska and Wolańczyk[14] described aggression as the most frequent obsession in adolescents. There was no sex difference between phenomenological subgroups in our study. Despite that, Tanidir *et al*[15] reported more contamination and somatic obsessions in males by using another diagnostic tool

Table 3 Regression model indicating variables affecting response to treatment in children with obsessive-compulsive disorder

Independent variables	Non-standardized coefficients		Standardized coefficients	*P*	Confidence interval 95%	*t*
	β	Standard error	β			
CGI-S	0.259	0.070	0.289	< 0.001	(0.121)-(0.397)	3.719
Age of admission[1]	0.507	0.238	0.165	0.035	(0.036)-(0.878)	2.127
R = 0.336; F = 9.347; P < 0.001; Durbin-Watson = 1.925						

[1]Age of admission was divided into two groups as prepubertal and postpubertal and categorically included in the analysis. CGI-S: Clinical Global Impression-severity score.

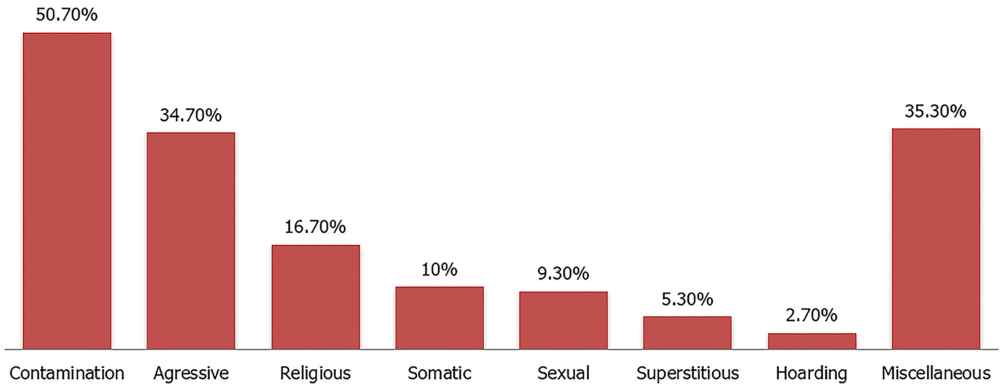

Figure 1 Pattern of obsessive symptoms among participants.

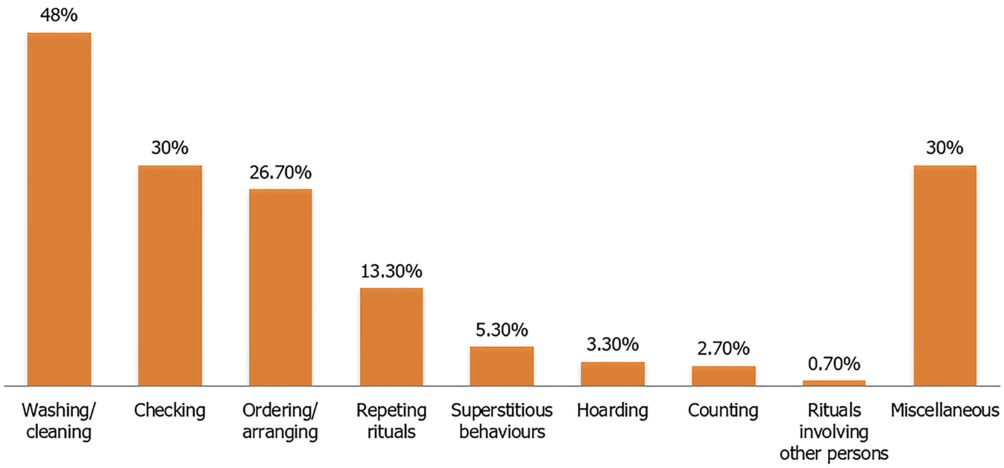

Figure 2 Pattern of compulsive symptoms among children.

(K-SADS-PL) on 110 children and adolescents in Istanbul. Mataix-Cols *et al*[16] found in their study in the United States that hoarding was common in girls, while sexual obsessions were more common in boys. The variance in results of such studies may be due to the wide range of age and methodologies and the cultural differences.

Regarding age group, contamination obsessions were related to prepubertal age group, whereas religious obsessions to adolescent age group. Consistent with our findings, studies conducted with children and adults asserted that contamination obsessions were seen in earlier ages[17], and religious obsessions were more apparent in older ages[15,18-19]. Exceptionally, Albert *et al*[20] related religious obsessions in adult OCD patients with the disease onset before the age of ten. Considering that the concepts of abstract thought and religion in children become more elaborated during adolescence, it is expected that religious obsessions would be seen more frequently in this period.

During our follow-up, approximately two-thirds of the children were diagnosed with a psychiatric disorder other than OCD, and the most common comorbid disorder was anxiety disorders (34.0%). Similar pediatric OCD studies also reported high rates of comorbidity, and the commonest comorbid mental disorders were defined as anxiety disorders, attention deficit/hyperactivity disorder, and tic disorder[14,16,21]. Among the phenomenological subgroups, variations in terms of comorbidities were observed, and the aggressive obsession was more frequently seen in children with anxiety and mood disorders. Studies conducted in Western and Asian countries have related anxiety disorder with aggressive symptomatology of OCD[5,22-23]. Also, Storch *et al*[24] observed daily functional impairment in the presence of either comorbid anxiety disorder or aggressive obsessions in children with OCD. Additionally, we observed nearly one-third of the children had a history of psychiatric disorder in their first-degree relatives. In other studies, the rate of psychiatric family history in childhood-onset OCD cases varied from 35%-45%[13,25], and the earlier age of onset of symptoms has been strongly associated with the familiality of the disorder [26].

In the light of our results, the treatment response deteriorated with the increase in severity of disease and the age of admission. Congruent with our results, Masi *et al*[27] highlighted that a better response to SSRIs is related to less severe illness in pediatric OCD. In a systematic review, early pharmacotherapeutic intervention was found to be the most remarkable indicator of treatment response in pediatric OCD[28]. Notwithstanding, some researchers did not find a significant relationship between age of onset/admission and response to pharmacotherapy in children with OCD[29,30]. Furthermore, in our study group maternal educational level and paternal employment level were found significantly higher in the prepubertal age group, which are supposed to be critical protective factors for a child's mental health and might be associated with better therapy outcomes.

Between the phenomenological subgroups, no difference was observed in case of treatment response. Results of the studies concerning which single subgroup responds better to SSRIs are controversial[29,31], and this may be due to the heterogeneous nature of the disorder. Therefore, in future studies, response to pharmacotherapy of OCD symptoms in children and adolescents should be examined in homogeneous subgroups with a dimensional approach rather than in single phenomenological groups.

There are also some limitations to the study. First, some of the children were excluded from the study due to lack of data, failure to meet the diagnostic criteria and problems in file archiving, all of which limited the number of participants. Second, retrospective data collection from the files might cause reporting bias. Third, due to its nature, the sample might have some ascertainment biases such as medication discontinuation due to fear of stigmatization, type of responders to SSRIs in terms of OCD phenomenology, family characteristics, and underlying neuropathology, *etc*. Lastly, data concerning the children who dropped-out would provide a comparison of the results with the follow-up group and could strengthen our results.

CONCLUSION

In the present study, it has been shown that obsessive-compulsive disorder in children and adolescents show their heterogeneity in terms of phenomenology. There are variations in the phenomenology of obsessions depending on the age group and comorbid mental disorders. During the follow-up of pediatric OCD patients, comorbid psychiatric disorders associated with certain phenomenological subgroups should be considered. The response to pharmacotherapy was associated with a younger age of admission and lower severity of disorder, which emphasizes the value of therapeutic interventions in the early stages of the disease in order to limit the impairment of social functioning and prevent the development of secondary mental disorders in adulthood.

ARTICLE HIGHLIGHTS

Research background

As a disease with heterogeneous features in many respects, obsessive-compulsive disorder (OCD) shows variability in terms of phenomenology.

Research motivation

Phenomenology of obsessions and compulsions are affected by many structural and environmental factors and shows several different characteristics in children compared to adults.

Research objectives

To identify the most common phenomenological subgroups of pediatric OCD and to determine the relationship of these subgroups with familial and clinical characteristics of children and the treatment response.

Research methods

Data of 150 children and adolescents, who had been diagnosed with OCD between 2014 and 2018, were examined retrospectively.

Research results

Contamination obsession was observed more frequently in the prepubertal age group, whereas religious obsessions were more frequent in adolescents. The treatment response deteriorated with the increase in severity of disease and the age of admission.

Research conclusions

Variations in phenomenology of obsessions are found in terms of age groups. The response to pharmacotherapy was found to be better in patients in the prepubertal age group and with lower severity of disease.

Research perspectives

Earlier diagnosis and therapeutic interventions in OCD may limit the impairment of mental health of children and adolescents.

ACKNOWLEDGEMENTS

The authors would like to thank all the children and their families whose participation made this study possible.

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ISSN 2222-0682 (online)

World Journal of *Methodology*

World J Methodol 2022 March 20; 12(2): 64-82

Published by Baishideng Publishing Group Inc

World Journal of Methodology

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World Journal of Methodology

Bimonthly Volume 12 Number 2 March 20, 2022

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INDEXING/ABSTRACTING

The *WJM* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Rui-Rui Wu*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Ji-Hong Liu*.

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Bimonthly

EDITORS-IN-CHIEF
Bruno Megarbane

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2222-0682/editorialboard.htm

PUBLICATION DATE
March 20, 2022

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World J Methodol 2022 March 20; 12(2): 64-82

DOI: 10.5662/wjm.v12.i2.64

ISSN 2222-0682 (online)

REVIEW

Radiological evaluation of patellofemoral instability and possible causes of assessment errors

Tugrul Ormeci, Ismail Turkten, Bayram Ufuk Sakul

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Garcia-Mansilla I, Mesregah MK, To K

Received: January 14, 2021
Peer-review started: January 14, 2021
First decision: October 17, 2021
Revised: October 27, 2021
Accepted: February 9, 2022
Article in press: February 9, 2022
Published online: March 20, 2022

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Abstract

Patellofemoral instability (PI) is the disruption of the patella's relationship with the trochlear groove as a result of abnormal movement of the patella. To identify the presence of PI, conventional radiographs (anteroposterior, lateral, and axial or skyline views), magnetic resonance imaging, and computed tomography are used. In this study, we examined four main instability factors: Trochlear dysplasia, patella alta, tibial tuberosity–trochlear groove distance, and patellar tilt. We also briefly review some of the other assessment methods used in the quantitative and qualitative assessment of the patellofemoral joint, such as patellar size and shape, lateral trochlear inclination, trochlear depth, trochlear angle, and sulcus angle, in cases of PI. In addition, we reviewed the evaluation of coronal alignment, femoral anteversion, and tibial torsion. Possible causes of error that can be made when evaluating these factors are examined. PI is a multi-factorial problem. Many problems affecting bone structure and muscles morphologically and functionally can cause this condition. It is necessary to understand normal anatomy and biomechanics to make more accurate radiological measurements and to identify causes. Knowing the possible causes of measurement errors that may occur during radiological measurements and avoiding these pitfalls can provide a more reliable road map for treatment. This determines whether the disease will be treated medically and with rehabilitation or surgery without causing further complications.

Key Words: Patellofemoral instability; Radiological evaluation errors; Trochlear dysplasia; Patella alta; Tibial tuberosity-trochlear groove distance; Patellar tilt

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Core tip: Patellofemoral instability (PI) is the disruption of the patella's relationship with the trochlear groove. For radiological evaluation, conventional radiographs, magnetic resonance imaging, and computed tomography are used. We examine the tibial tuberosity-trochlear groove distance, patellar height, and patellar tilt measure-ments that require surgical correction and are defined as principal factors, along with other assessment methods that allow the patellofemoral joint to be evaluated qualitatively and quantitatively. We discuss the radiological assessment of patients with PI and possible misconceptions. Knowing the possible causes of measurement errors and avoiding these pitfalls can provide a more reliable road map for treatment.

Citation: Ormeci T, Turkten I, Sakul BU. Radiological evaluation of patellofemoral instability and possible causes of assessment errors. *World J Methodol* 2022; 12(2): 64-82
URL: https://www.wjgnet.com/2222-0682/full/v12/i2/64.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i2.64

INTRODUCTION

Patellofemoral instability (PI) is the disruption of the patella's relationship with the trochlear groove (TG) as a result of abnormal movement of the patella, which is part of the extensor mechanism. Many factors can cause patellofemoral joint instability. Problems with the bony structure of the patella and trochlea [*e.g.*, the patella alta, increased length of the tibial tubercle–TG (TT-TG), rotational limb malalignment, and trochlear dysplasia (TD)], deficiency in static soft-tissue constraints [*e.g.*, medial capsular restrictions, medial patellofemoral ligament (MPFL)], or insufficiency of dynamic constraints [*e.g.*, vastus medialis obliquus (VMO)] can lead to PI[1,2].

The solution to this problem lies in understanding correctly the anatomical problem and the defective biomechanics. Generally, clinical examination is used in diagnosis, but radiological evaluation is also used in diagnosis and differential diagnosis. Therefore, accurate and reliable radiological measurements are important. In radiological evaluation, conventional radiographs (anteroposterior, lateral, and axial or skyline views), magnetic resonance imaging (MRI), and computed tomography (CT) are used[3-5].

Anatomy

The complex function of the knee joint is enabled by the bone structure, which consists of the femur, patella, and tibia as well as ligaments, tendons, and other soft tissue formations.

The patella is the largest sesamoid bone in the body. It fits in the TG of the femur and is part of the extensor mechanism. According to the length of its facets and the localization of the median prominence, Wibeeg[6] defined three types of normal patellar morphology.

In the distal femur, there are TGs in the center, medial and lateral facets on the sides.

The rectus femoris, vastus medialis, vastus lateralis and vastus intermedius muscles form the quadriceps muscle. The quadriceps tendon is included in the extensor mechanism, and this mechanism contributes to patellofemoral joint stability.

The VMO is the primary muscle structure that creates resistance to the patella's lateral tracking[1]. The lateral retinaculum acts as a secondary stabilizer against the medial translation of the patella.

Biomechanics

Normal patellofemoral movement is enabled by the harmony between the related bones and soft tissues. In the resting position, the patella is positioned slightly laterally. Between 0° and 30° of flexion, resistance to lateral patellofemoral translation is mainly provided by the MPFL[7-10]. At the beginning of the flexion, due to the harmony of the TG and patella, a slight shift of the patella to the medial side is observed. Stabilization increases with the placement of the patella in the trochlea at 20°-30° of flexion. When the flexion increases toward 60°, the proximal part of the patella also begins to associate with the trochlea[11].

When flexion exceeds 90°, there is some decrease in the joint reactive force as a result of the contact of the quadriceps tendon with the trochlea[12,13]. Between 90° and 135°, the patella rotates and the median ridge approaches the femoral condyle[14].

Radiological evaluation of the patellofemoral joint

In the evaluation of patellofemoral articulation, the patellar position, which is measured by the lateral patellofemoral angle (LPFA), patellar tilt, and patellar lateralization, is checked. TT-TG distance measurement is used for patellar translation[15]. Along with these, it must be determined whether there is TD, which is one of the main morphological disorders in patients with PI. Sulcus depth, sulcus angle, and lateral trochlear inclination (LTI) are the measurements used in the evaluation of TD[15]. The TT-TG distance, patellar height, and patellar tilt measurements are defined as principal factors in patients

with PI and require surgical correction[16].

Imaging algorithm of PI

Fithian *et al*[16] summarized the evaluation algorithm of patients presenting with PI, referring to Dejour *et al*[19]. According to this account, the examination starts with true lateral radiography and evaluation of anatomical factors. If they are found normal, axial patellofemoral views at 30° of knee flexion are taken. Here, other causes, such as anterior cruciate ligament (ACL) disorders that may cause complaints, are investigated. If no morphological correction is considered, CT is not required, and a nonoperative treatment method is chosen. Knowing the true lateral radiography is essential in the evaluation of patients presenting with PI. Anteriorly, parallel dense lines belonging to both condyles and the linear density of the base of the trochlear sulcus are posteriorly observed. These lines do not intersect with each other (Figure 1). This normal appearance is disturbed by the intersection of the dense lines of the condyles anteriorly or anterosuperiorly (crossing sign). These may be accompanied by a bump or prominence in the form of overflow in the anterosuperior contour. These findings vary depending on the severity of the dysplasia. On the lateral graph, the depth and continuity of the TG and the patellar height are evaluated. After that, the path to be followed can be determined according to the data collected.

High congruence angles are seen in the dysplastic trochlea on axial radiographs. Dysplasia can be identified visually even if the angle cannot be measured. However, the point to be considered here is that if the knee flexion angle is more than 45°, it may cause interpretation errors, since the relatively normal lower parts of the trochlea are displayed. Laurin *et al*[17] recommend taking axial radiographs at 20° (at this angle, the patella fits in the center of the trochlea) and evaluating the patellar tilt with the LPFA.

After Dejour *et al*[19] defined a significant statistical relationship between dysplasia and instability, CT entered routine use in patients with PI. If there is evidence of dysplasia on lateral radiography, anatomical correction may be required, and CT becomes necessary. In CT, along with other findings, TT-TG distance is checked, and patellar height is measured. The TT-TG measurement is the distance between the deepest points of the TG and the most prominent part of the TT in the horizontal plane. MRI has become one of the methods that can be used for diagnosis in the evaluation of patients with PI, as it can recognize potential flake fractures and measure TT-TG[18]. In addition to these, MRI can be used to evaluate the ligaments and muscle structures related to PI as well as the patellar and trochlear cartilage due to increased soft tissue resolution.

In this study, we mainly examine the four major instability factors (trochlear dysplasia, patella alta, TT-TG, and patellar tilt) identified by Dejour *et al*[19] for the classification of patellofemoral diseases and potential errors that can be made when evaluating them.

ASSESSMENTS AND MEASUREMENTS

Patellar height evaluation

For patellar stability, when the knee is flexed, the patella should fit quickly and fully into the TG. Patellar height is one of the main factors affecting this relationship. The patellar height ratio is important not only in the assessment of PI, but also in patella infera syndrome and after total knee replacement [20].

In common usage, the lower patella is called patella baja, and the higher one is called patella alta. When the quadriceps muscle contracts, the patella rises proximally over the trochlea; at this level, the support it receives from the groove decreases. In the presence of patella alta, even in advanced stages of knee flexion, the patella cannot fully settle into the groove. This increases the risk of lateral dislocation [21,22]. Another effect of patella alta is that it causes an increase in intra-articular pressure, which is more pronounced in the advanced stages of flexion[23]. There is an increased tendency to chondromalacia and dislocation due to this defect in the patella-trochlear location[5,24]. However, in patella baja, joint reactive forces increase on the patella, which causes limitation of movement and an increase in the risk of patellofemoral arthritis[25].

Various indexes have been defined for measuring patellar height (Table 1). These indexes refer to the tibia [*e.g.*, Insall-Salvati (IS), Caton-Deschamps (CD), and Blackburne-Peel (BP)] or the femur (*e.g.*, Blumensaat)[20]. Chareancholvanich *et al*[26] have defined the criteria that should be included in an optimal patellar height measurement index. According to this definition, a good index should have a femoral reference point that can accurately reflect the patellofemoral joint, should not be affected by the flexion angles of the knee, should be easy to measure, should have good intra- and interobserver variation, should be easily applied in lateral radio-graphy, and should be compatible with clinical data.

At the IS ratio[27], the patellar tendon (PT) length from the distal pole of the patella to the tuberositas tibia is measured (a in Figure 2A) and divided by the greatest diagonal length of the patella (b in Figure 2A). This ratio is 1 in normal knees[27]. It is important to make measurements on a true lateral radiograph. The length of the PT is measured from the posterior aspect of the tendon facing Hoffa's fat pad. This may be particularly important in cases where the PT is attached across a wide area to the

Table 1 Anatomical factors used in the evaluation of patellofemoral instability, their normal of value and range values

Anatomic factor	Normal value	Range
Patellar height		
Insall-Salvati[33]	1	(0.8-1.2)
Mod. Insall-Salvati[30]	Patella alta > 2	
Blackburne-Peel[22]	0.8	(0.5-1)
Caton-Deschamps[141]	< 1.2	(0.6-1.2)
PI[142]	0.49	0.18-0.80
Trochlear morphologic finding		
Trochlear depth[19]	≥ 5 mm	
Sulcus depth[15]	≥ 3 mm	
Sulcus angle[2,92,93]	< 144°	
LTI[54]	> 11°	
Patellar position		
LPFA[15]	≥ 8°	
Patellar tilt[17]	< 12°	
Patellar lateralization[15]	< 2 mm	
Patellar translation		
TT-TG distance[75,76]	≤ 10 mm	

PI: Patellotrochlear index; LTI: Lateral trochlear index; LPFA: Lateral patellofemoral index; TT-TG: Tibial tuberosity–trochlear groove.

inferior pole of the patella. The measurement is independent of knee flexion and is one of the most commonly used ratios. If the tendon cannot be seen sufficiently for any reason, the tibial tubercule can be taken as a reference point, but attention should be paid to bone problems that may occur at this level. In addition, this ratio is defined in direct radiography. While taking measurements in MRI and CT, evaluation should be made from the sagittal plane sections showing the tendon completely, and the longest diagonal measurement of the PT and patella should be taken. In order to obtain maximum patella and PT size, it is important to measure at the midtrochlear level[28]. Tibial tuberosity can be difficult to assess if it is not obvious (*e.g.*, in Osgood–Schlatter disease[22]) and after distalization procedures[29]. Measurement errors may be secondary to patella deformities (patella with nonarticular long distal parts or Cyrano type patella[12], Larsen–Johansson disease[22], or osteoarthritis). IS ratio measurement changes with TT osteotomy. Therefore, it can be used to adjust patellar height after surgery.

Grelsamer and Meadows[30] defined a modified IS ratio to address the problems caused by patellar deformities. This index is obtained by measuring the distance between the lower end of the articular face of the patella and TT (a in Figure 2B) and dividing this distance by the length of the articular face of the patella (b in Figure 2B). However, this method also has some disadvantages, as can be seen in IS measurement[20].

The BP ratio[22] has been reported to be independent of the tibial insertion of the PT and the patella shape. In this index, the ratio of the length of the articular surface of the patella (b in Figure 2C) to the distance between the tibial plateau line and the lower pole of the patella joint surface (a in Figure 2C) is measured. However, in this method, it may be difficult to detect the route through which the tibial plateau line passes[29,31].

The CD ratio[32] identifies the distance between the inferior point of the patellar articular face and the anterior superior border of the tibia (a in Figure 2D). This distance is then divided by the length of the articular surface of the patella (b in Figure 2D). With this measurement, it may be difficult to assess the end point of the distal patellar joint surface and the anterior superior tibial corner as well[29]. This difficulty becomes more pronounced in patients with osteoarthritis due to hyper-trophic changes[31]. The CD ratio is widely used and is not affected by knee flexion.

The Labelle-Laurin method[33] was described in reference to a lateral radiograph taken with the knee flexed at 90°. If the proximal end of the patella is above the line drawn tangent to the anterior cortical line of the femur, it is defined as patella alta. Although it is easy to evaluate, it has many disadvantages. Radiography is difficult to obtain without fluoroscopy. Due to femoral bending, drawing the tangential line to the femoral diaphysis is not easy[20]. In addition, only the patella alta can be evaluated with this

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Figure 1 Lines and contours seen in normal people on true lateral radiography. Anteriorly, parallel dense lines belonging to both condyles and linear density of the base of trochlear sulcus (arrows) just posteriorly are observed. These lines do not intersect with each other. There is no bump or prominence on the anterior aspect.

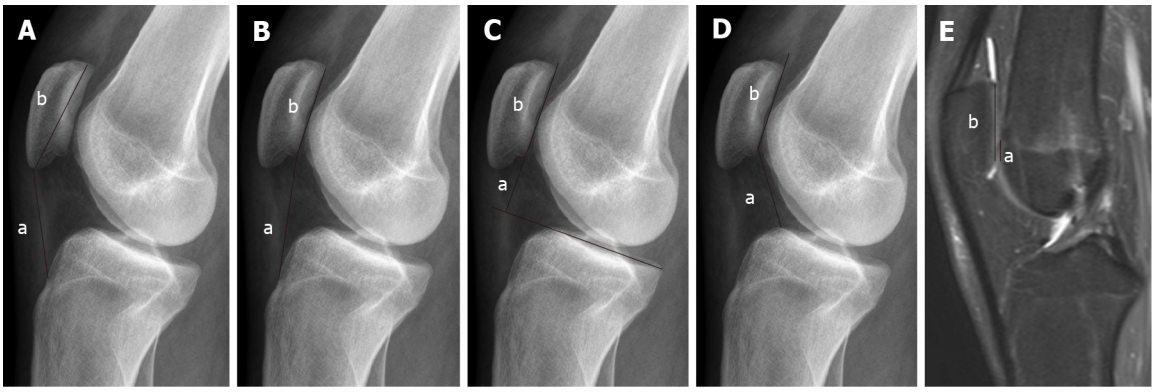

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Figure 2 Radiography. A: Insall-Salvati (IS) ratio (a: Patellar tendon length; b: Length of the patella); B: Modified IS ratio (a: The distance between the lower end of the articular face of the patella and TT; b: Length of the articular face of the patella); C: Blackburne-Peel ratio (a: The distance between the tibial plateau line and the lower pole of the patella joint surface; b: Length of the articular surface of the patella); D: Caton-Deschamps ratio (a: The distance between the inferior point of the patellar articular face and the anterior superior border of the tibia; b: Length of the articular surface of the patella); E: Magnetic resonance imaging, Patellotrochlear index (a: Length of the trochlear cartilage; b: Length of the patellar cartilage). These images are showing different measurement methods for evaluating the patellar height.

method. However, there are publications suggesting that it can be used in the evaluation of patellar height[34].

All these indexes are generally related to bone structure. However, the bone structure and the cartilage covering it may not always be compatible[35,36]. Therefore, it may be more logical to refer to cartilage, which is the main element directly exposed to stress forces in the joint.

Biedert and Albrecht[37] defined a new method that can be used in MRI for measuring patellar height. This is the patellotrochlear index (PTI), in which the distance that the patellar (b in Figure 2E) and trochlear cartilage (a in Figure 2E) overlap in parallel is measured in the sagittal plane. This method is said to be a reliable and reproducible method to show the patellofemoral relationship[37]. In this

measurement, the patellotrochlear articular congruence seems to be evaluated without being affected by differences in the shape and length of the trochlea, variations in bone structure, diseases, or operations. However, Ahmad *et al*[38] showed that PTI increases with the flexion angle of the knee and proposed a correction formula to solve this problem.

It is not easy to clearly say which measurement method should be used in patellar height assessment. The factors determining the patellofemoral joint relationship are the height of the patella and the length and depth of the TG groove. Therefore, when considered roughly, it may be more accurate to evaluate the articular surface of the patella according to the trochlea and tibiofemoral joint lines. The BP and CD ratios can fulfill this requirement. However, indexes referring to the femur (*e.g.*, Blumensaat and Bernageau) depend on the knee flexion angle and are poor in terms of measurement reliability[16].

The patellar shape or the position of the TT can affect the ratio of IS and minimally invasive surgery (MIS). IS and MIS may also be affected due to Osgood-Schlatter's disease, bone-PT-bone grafts used in ACL replacement, and transfer of the TT.

Another possible misconception when evaluating patellar height is that interobserver variability is often due to evaluation errors in the bone structure reference points used. In addition, misinterpretation may be caused by factors related to the deficiency of exposure technique factors affecting the quality of the radiography or failures of either the positioning or the system (film, cassette, and bath factors in conventional systems that are mainly due to the detector system features in digital systems).

The same index may give different results with different modalities. For example, the radiographically measured patellar length may be different in MRI. This difference may be due to the patella not being in the same slice with its entire size due to its shape and position in the knee, which may result in a decrease in reliability. It is recommended to use midsagittal sections to solve this problem[39,40]. Shabshin *et al*[41] proposed a method for measuring the patellar and PT length. They looked at the number of sections where the patella entered the image and then made the measurement from the middle slice.

However, this is a major problem in practice, especially in patients with PI, due to the lateralization of the patella. The patella, trochlea, tibia, and associated tendons cannot be shown in a single slice. Another problem may be experienced when measuring PT length. There may be osteophytes in the superior pole and enthesophytes in the inferior pole of the patella; these should not be taken into account during the measurement[42]. Measurements should be made at the posterior aspect of the PT [42]. X-ray measures the distance between the patella and TT, whereas MRI measures the tendon itself.

In their study on MRI, Miller *et al*[42] found that, although it was said to be unaffected by flexion, the upper limit for the SI index was 1.1 when the knee was flexed, whereas the upper limit was 1.3 when the knee was in extension.

These indexes were defined for radiography, and whether the normal limits used for them will also apply accurately to CT and MRI is another question. It can be seen that these indexes are studied in limited numbers for different modalities[39-43]. The IS, BP, CD and MIS indexes defined for radiography can also be applied to CT and MRI, but normal ranges may change in this case. In one study, patellar height indexes (IS, MIS, CD and BP) were found to be 0.1 factor higher in MRI than in radiography in patients with primary patellar instability[44]. However, another study suggests that for PT length measurement, it does not make any difference whether MRI or radio-graphy is used[45].

Although CT and MRI contribute significantly to the evaluation of patients with PI, they still recommend that radiographic evaluation should not be ignored[46].

In the Lyon school, patellar height is evaluated with the CD method through true lateral radiography [16]. But generally speaking, IS is the most commonly used index, as others are clinically inaccurate or time consuming[41].

Evaluation of trochlear dysplasia

The relationship between PI and TD has been known for a long time. When evaluating TD, some morphological features, such as trochlear depth, sulcus depth, sulcus angle, LTI, medial trochlea inclination, and trochlear angle can also be examined[15,47] (Table 1).

Dejour *et al*[19] defined specific radiographic features frequently seen in patients with PI. While the lateral trochlear ridge and the base of the groove did not coincide in normal individuals on lateral radiography, it was observed that these lines cross (at the crossing sign) in 95% of patients with PI[48]. The crossing point indicates the point where the lateral femoral condyle overlaps the trochlear floor; at this level, the trochlea is flattened (Figure 3). In fact, what is looked at in a lateral radiograph is the level of this flattening held by the groove and whether symmetry or asymmetry in the femoral condyles accompanies it. This shows the severity of dysplasia (Figure 3). In the examination of TD starting with lateral radiography, the crossing sign is evaluated as visually, the trochlear bump, and the trochlear depth are also evaluated as quantitative[19]. After the visual evaluation, the first quantitative evaluation is of the cortical beak (bump/prominence) measurement in the anterior (threshold value: 3 mm) (Figure 3). This is an important indicator of TD, and it increases as the degree of dysplasia increases[19].

Another quantitative criterion for TD in lateral radiography is the trochlear depth measurement. In the study of Dejour *et al*[19], a significant difference was found between the group consisting of PI and normal persons when measuring trochlear depth. In fact, with this, the more distal section of the groove is evaluated according to the level indicated by the crossing sign. For the trochlear depth measurement,

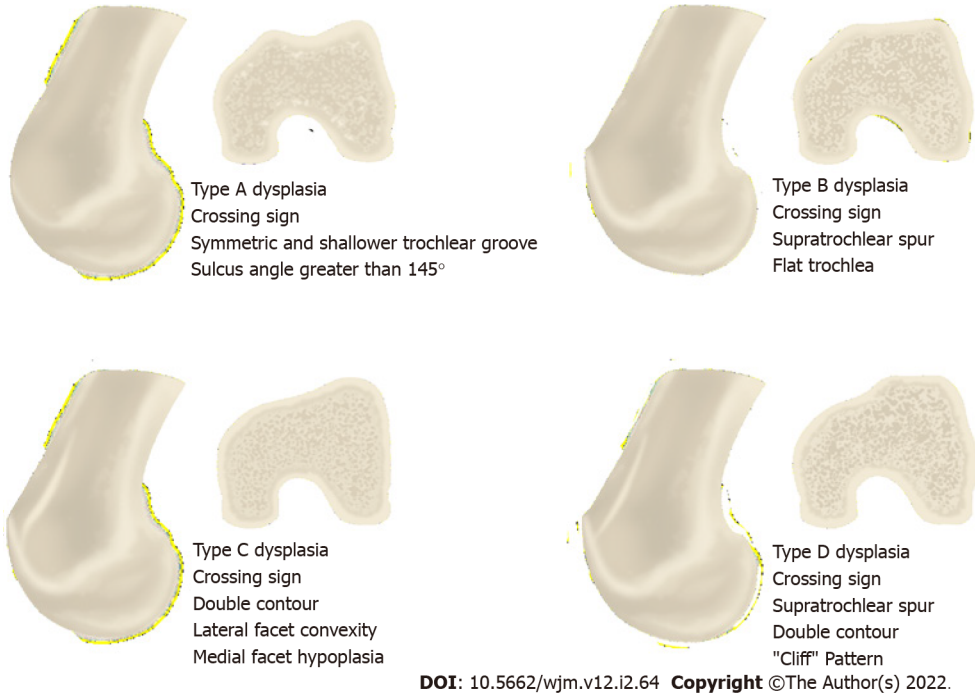

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Figure 3 Illustration showing the Dejour classification used in trochlear dysplasia in true lateral radiography and axial slice images.

a line perpendicular to the line taken tangentially to the posterior femoral cortex is drawn on the true lateral radiograph taken at 30° of flexion. A second line that makes an angle of 15° with this line is then drawn. Where this second line intersects the cortex anteriorly, the groove depth is measured. In this radiographic examination, the trochlear depth was found to be approximately 2.3 ± 1.8 mm in patients with PI[19]. For trochlear depth, values of ≤ 4 mm are considered pathological[19]. These anatomical defects prevent the patella from staying in the groove while the knee is flexed.

In order to evaluate the "crossing sign" in lateral radiography, the radiograph should be taken fully laterally, while the condyles should overlap each other in the posterior and look like a single condyle. Obtaining the radiograph even in a 5° rotation may affect the accuracy of the lateral radiography[49]. The difference between the lines formed by both condyles is < 2 mm, which indicates that the radiography is sufficient[50]. However, this is often not possible, and the radiography may need to be performed under fluoroscopy. This means extra time and additional radiation risk.

Knee axial radiographs have limitations, such as inadequate reproducibility, image distortion, and problems caused by low flexion angles[51]. Groove anatomy may not be shown correctly in axial X-ray images taken with the knee flexed at 30°[48]. Many researchers have worked on different angle values to provide better reproducibility[17,52].

In some cases, there may be mild dysplasia, and this may only affect the trochlea entrance. Thus, it may not be possible to evaluate these cases with radiography[53]. Proximal anomalies may also not be seen on lateral radiography due to superposition[54]. Achieving an optimal position on lateral radiography can be difficult. However, on CT imaging, it is easier to evaluate both the angle given to the knee and to establish a reference by aligning the posterior of the condyles. For this reason, the use of cross-sectional imaging methods has come to the fore (Figure 4). In addition, lateral radiography may not show the severity of TD as accurately as axial MRI[55,56].

The classification commonly used in the evaluation of TD is based on data obtained from lateral radiography and CT images[57]. According to D. Dejour's criteria for axial CT/MRI scans, TD is classified into types A (shallow trochlear sulcus), B (flat or convex trochlea), C (asymmetry of trochlear facets with hypoplastic medial condyle), and D (asymmetry of trochlear facets with cliff pattern)[58] (Figure 3). Dejour classification is qualitative evaluation, and its interobserver–intraobserver reliability is less than ideal[55,59-61]. There are different opinions in the studies conducted to increase this reliability. Lippacher *et al*[55] confirm that the four-grade TD classification defined by Dejour has low interobserver and intraobserver agreement, and they say that the results are better if the classification is applied as two-grade (low/high-grade dysplasia). Some studies have classified Dejour's Type A as low grade and Dejour's Types B-D as high-grade dysplasia[55,62].

Although the Dejour classification is widely known in the diagnosis of TD, the search for more reliable and reproducible methods continues. For this purpose, many measurement methods, such as the LTI, the trochlear depth index (TDI), the lateral condyle index, and PTI have been defined as having high sensitivity and specificity to be used in the evaluation of PI[50,54,63].

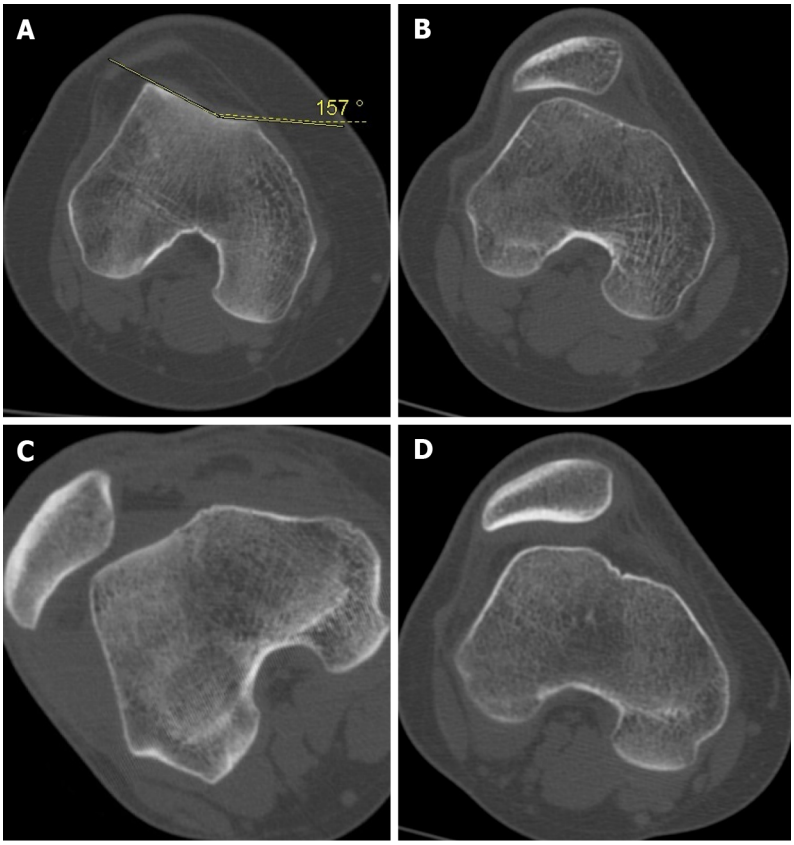

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Figure 4 Sample cases with trochlear dysplasia according to Dejour classification. A: Type A; B: Type B; C: Type C; D: Type D.

The measurements of LTI, trochlear facet asymmetry, and depth of TG are said to show good sensitivity and specificity[62]. Biedert and Bachmann[64] state that hypoplasia of the medial femoral condyle is a hallmark of TD.

For the evaluation of trochlear dysplasia, femoral width as described by Biedert and Bachmann[64], LTI as described by Carrillon *et al*[54], TDI as described by Pfirrmann *et al*[50], the medial condyle trochlear offset as described by Stepanovich *et al*[65], and the TT-TG distance as described by Schoettle *et al*[66] can be measured. Measuring can be difficult in severe dysplasia, but Schoettle *et al*[66] offer clues for how to do this.

As the degree of dysplasia increases, the clues and landmark points used in measurement may become faint, and therefore, some cases may be difficult to measure. Using the quantitative radiographic measurements and the Dejour classification system described above together provides convenience in diagnosis[56].

Evaluation of TT-TG distance

By superposing sections passing through the hip, knee, upper tibia, and ankle with CT, the alignment and rotational parameters of the lower limbs and components of the knee extensor mechanism can be evaluated. Dejour *et al*[19] evaluated patellar dislocation with lateral radiography and CT. However, examining the patellofemoral joint in CT while the knee is in extension is superior to radiography[67].

Today, instead of measuring the Q angle, the superimposed images of the TG and the TT are used in the axial images on CT, and the lateral offset of TT is evaluated (TT-TG measurement) (Figure 5) according to these. This measurement is more reliable than the Q angle and is frequently used in patients with patellar instability[19]. Brady *et al*[59] found a significant and positive relationship between TT-TG measurement and the degree of TD in the Dejour classification. However, TT-TG measurement is affected by many variables, such as torsion. For this reason, it should not be consi-dered as a definitive value, but as a part of a whole.

TT-TG measurement is widely used when making surgical decisions and determining the best procedure for patients with PI[60,66]. When measuring TT-TG distance, the deepest points of the TG and most prominent part of the TT are taken as bases. Different measurement methods have been described for the measurement of TT-TG distance[66,68]. There is uncertainty in defining the reference points to be used in measurement. To determine the level of TG measurement, in the axial images, the first image in which the notch is seen as a Roman arch should be selected[69], and the level should be confirmed by sagittal images if necessary.

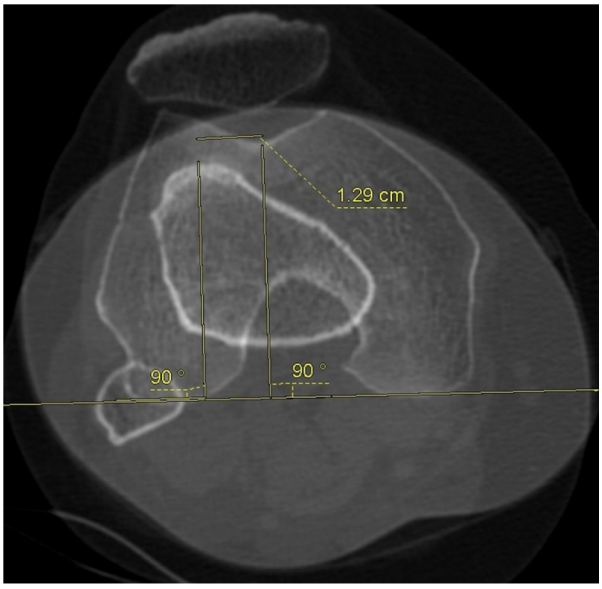

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Figure 5 Tibial tuberosity-trochlear groove distance measurement. Superimposed image of the trochlear groove and tibial tuberosity used in the axial images on computed tomography, and here the lateral offset of tibial tuberosity is evaluated.

The deepest point of the groove is taken as the reference point for TG in the horizontal plane. In severe TD, it can be difficult to detect the deepest point of TG. For these cases, Julliard and Ligeon[70] have defined the bidimensional reconstruction procedure. Brady *et al*[59] measured the TG at two levels on MRI. They measured the most proximal section (proximal TT-TG) where the trochlear cartilage was seen on axial views and the most distal section of the femur before the Roman arch was disrupted (distal TT-TG). No significant difference was found between the two measurements, and it was observed that both had high inter-rater reliability[59].

The selection of different reference points is mostly seen on the tibial side. There are discussions about the most prominent point of TT in studies[66,71]. Goutallie *et al*[68] identified the reference point for TT as the most anterior point of the tibial tuberosity. However, it is debatable where the reference point should be placed when the TT surface is wide rather than sharp. Deveci *et al*[72] took the midpoint of the dome of the TT as a reference when measuring TT rather than the most anterior part of the bone structure. Another controversy is whether the bone structure (TT) or the PT should be taken as the tibial reference. Schoettle *et al*[66] took the center of the PT as the reference point on the tibial side. Here, it can be discussed whether the front or center of the tendon should be evaluated as a reference point. Especially in cases where TT is lateralized and the tendon is more globularly adhered, it may be more appropriate to refer to the center of the tendon, as it is more suitable for the force vector.

Whichever is used as a tibial reference point (TT or PT), it is also important to determine which level should be used to take in the craniocaudal axis. For the PT-TG measurement, there are those who refer to the most proximal point as the place where the tendon inserts in TT[72]. The same level is accepted for TT-TG measurement[73]. The TT-TG value was defined as 13 mm in the normal population[74]. However, there are also publications that accept values of ≤ 10 mm as normal[75]. Park *et al*[76] reported the TT-TG distance as 13 mm for MRI in patients with PI. Values > 20 mm are considered determinants for the diagnosis of PI and the decision of surgery[19]. The PT-TG distance has been measured at a few millimeters larger than the TT-TG distance[72,77]. As can be seen, there are no definite limits for normal values, and there are differences according to modalities. Additionally, age and height also affect TT-TG distance[74]. Finally, in a study, TT-TG measurement was thought to be affected by knee flexion and rotation; as an alternative, TT-posterior cruciate ligament (PCL) measurement was made, but TT-TG measurement was found to be more reliable than TT-PCL measurement[59].

Evaluation of patellar tilt

The relationship between patellar tilt and TD is understood[78]: In PI evaluation, patella height and patellar tilt are examined in relation to the position of the patella[79-83]. Evaluations regarding patellar height were made above. In addition to this effect of the anatomical structure, patellar hypermobility may also occur due to problems in passive (ligamentous) and dynamic (musculous) soft tissue elements.

One of the hypotheses considered to explain patellar tilt refers to VMO dysplasia[19]. Laurin *et al*[17] also described in their study that a loss of cartilage in the lateral compartment of the patellofemoral joint causes lateral patellar tilt. Some authors associate the tight lateral structure with patellar tilt[19,78]. Generally, patellar tilt may be an indicator of tightness in lateral support structures, but it generally appears as weakness in medial soft tissue support structures[51]. Among all the causes of PI, patellar tilt

is the most difficult to correct. This is perhaps because it is associated with many factors affecting biomechanics[84]. Although patellar tilt (≥ 20) and patella alta (index: ≥ 1.2) detected in extension generally have values indicating quadriceps dysplasia[19], it is difficult to actually measure quadriceps dysplasia. A rough idea can be obtained by measuring the patellar tilt with CT and clinical evaluation [19].

Patellar tilt is the angle between the line joining the posterior femoral condyles (a in Figure 6A) and the transverse patellar axis (b in Figure 6A) in axial images[85]. This angle increases with quadriceps contraction. In one study, the sections passing through the mid-patella were taken when the knee was flexed at 15° in the relaxed state; when the quadriceps contracted, it was found that the patellar tilt increased by an average of 10° in the PI group[81]. Dejour *et al*[19] took 20 as an average value (mean patellar tilt), since it is not fully known whether the muscle is contracted when measuring in a patient with PI. However, minor forms of quadriceps dysplasia can be diagnosed by measuring the mean patellar tilt[81]. CT taken in extension shows the patellar dynamics in the first few degrees of knee flexion, where dislocation occurs.

Other parameters that can be used in the evaluation of PI patients

Evaluation of LTI, trochlear angle, and sulcus angle: The LTI angle is the angle between the line drawn tangent to the lateral facet and the line tangent to the posterior edge of the femoral condyles[86] (Figure 6B). Carrillon *et al*[54] set the threshold value for LTI as 11°. LTI measurement for TD allows both qualifying and quantifying assessments[54]. The LTI angle is important among trochlear morphology measure-ments. A decrease in this angle means flattening of the trochlea, that is, dysplasia.

While some publications refer to subchondral bone at the trochlear facet for LTI measurement[87], other publications refer to the slice where the cartilage was seen first and completely[88]. In the classical measurement method, the angle is calculated over the same section, while in some subsequent publications, it is recommended to be calculated by measuring at proximal and distal levels[88]. Proton density fat saturation or fat saturation T2 A sequences can be used for measurement.

The trochlear angle is the angle between the line passing posteriorly to both femoral condyles and the line joining the foremost parts of the medial and lateral facets (Figure 6C). The trochlear depth is found by measuring the maximal anterior–posterior diameter of the medial femoral condyle, the maximal anterior–posterior diameter of the lateral condyle, and the minimum distance of the deepest point of the trochlea with respect to the tangent line passing through the posterior of the condyles. It can be calculated according to the formula [(a + b)/2] - c[50] (Figure 6D). Reference points used in trochlear depth measurement differ in publications. Osseous surfaces were used as a reference in two studies[28,89]. However, it seems more appropriate to use the cartilage directly as a reference point because of possible cartilage–bone incompatibility[36,66].

Although it is not included in the Dejour classification, some authors also consider sulcus angle (SA) measurement as a characteristic part of the evaluation of TD[19,52]. However, although there is a strong relationship between the LTI and the trochlear angle with structural damage in the patellofemoral joint, a lesser relationship was found for the SA[47].

Axial knee radiographs can be used to evaluate the SA and patella position. The SA is defined as the angle between the lateral and medial facets of the joint[47,86,90,91] (Figure 7). An SA > 145°-150° is significant for TD[2,92,93]. The SA is measured from the first axial MRI section, where the medial and lateral trochlear facets can be fully observed[94]. These sections naturally show the more distal levels of the trochlea. However, dysplasia is most prominently seen at the trochlea entrance, that is, at the more proximal level. In the study by Dejour *et al*[19], 35% of the patients with PI had TD, although the trochlear angle was within normal limits. For this reason, it is useful to specifically control the proximal trochlear morphology, especially in cases where the SA is close to the normal ranges. Measurement of the SA may not be reproducible[95,96]. SA measurement can be used to evaluate an individual's risk status for PI in the future[97], but TD does not actually seem very reliable for assessing morphology.

There is also a relationship between the parameters described above. Smaller LTI values were found to be associated with larger TT-TG and SA values[46]. There is a positive relationship between patellar tilt and TD, and a negative relationship between patellar tilt and LTI. As LTI decreases, that is, as the lateral facet is tilted, especially when the knee is in extension, the patellar tilt should increase in order for the patellar lateral facet to adapt to it[46].

In the thesis of Levigne referenced by Fithian *et al*[16], it is stated that the most common cause of patients who underwent revision surgery due to PI was persistent patellar tilt, suggesting VMO dysplasia, and secondly, patella alta. However, as it was not within the scope of this study, a detailed analysis was not conducted on treatment and outcomes of PI.

Evaluation of patellar size and shape: It has long been debated whether there is a relationship between patellar size and shape and PI. Wibeeg[6] studied the effects of patellar shape in patients with PI and defined a grading system. However, Servien *et al*[98] found that there was no correlation between patellar shape and size and TD in their study comparing patients who were operated upon for PI with a control group. While the shape of the patella is examined during the evaluation of patients with PI, no significant difference has been defined that can be used to direct treatment. This is also the case for other secondary features associated with PI[16].

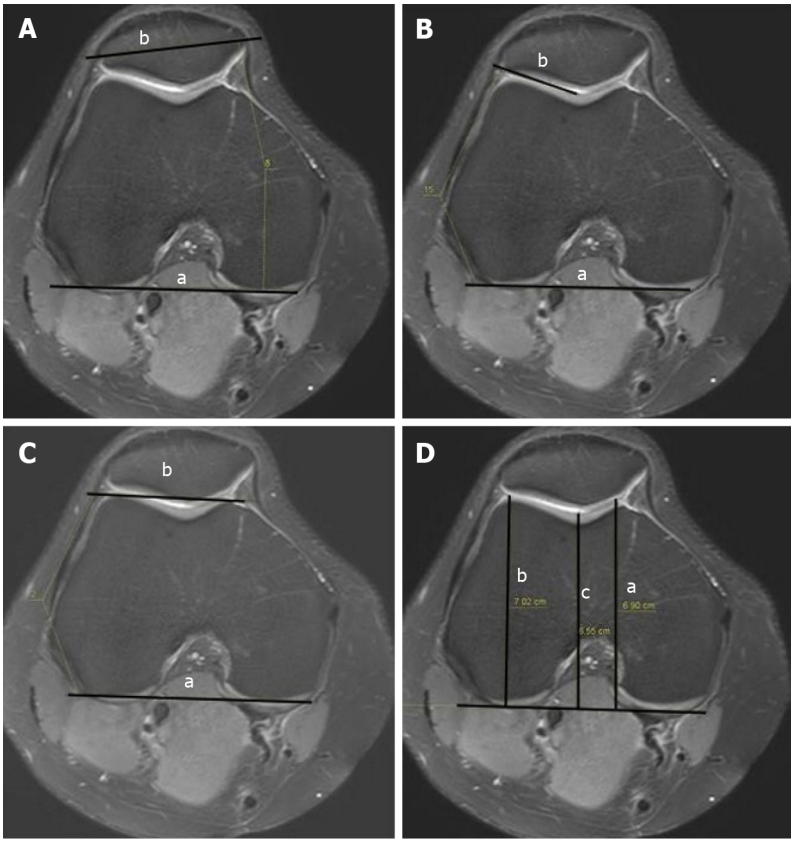

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Figure 6 Measurement. A: Patellar tilt (a: The line joining the posterior femoral condyles; b: The transverse patellar axis); B: Lateral trochlear inclination (a: The line tangent to the posterior edge of the femoral condyles; b: The line drawn tangent to the lateral facet); C: Trochlear angle (a: the angle between the line passing posteriorly to both femoral condyles; b: The line joining the foremost parts of the medial and lateral facets); D: Trochlear depth in axial magnetic resonance imaging (a: The maximal anterior-posterior diameter of the medial femoral condyle; b: The maximal anterior-posterior diameter of the lateral condyle; c: The minimum distance of the deepest point of the trochlea).

In addition to all these factors described above, although it is thought to be associated with recurrent patellar instability, there are other factors that are used in the treatment decision that cannot be directly treated and whose exact values are not defined. Genu recurvatum and ligament laxity, genu valgum, and femoral anteversion measurements are thus defined as secondary features[16].

Evaluation of coronal alignment, femoral anteversion, and tibial torsion: In this study, we examine in detail the four main factors of PI identified by Dejour *et al*[19] (trochlear dysplasia, patella alta, TT-TG, and patellar tilt). However, apart from the anatomical reasons described above for PI, deterioration of the coronal alignment of the lower extremity and torsional malalignment are also important causes.

Some reports in the literature state that there is a positive correlation between higher femoral antetorsion, TT-TG distance, and the degree of trochlear dysplasia in patients with recurrent PI[99,100]. It may be difficult to clinically detect torsion deformities and bone alignment disorders in patients with PI. Preoperative clinical and radiological evaluation of these factors is beneficial, as they may affect the outcome[101]. Franciozi *et al*[102] provide evidence that increased femoral antetorsion and valgus alignment increase lateral patellar instability.

The frontal mechanical axis can be evaluated using standardized weight-bearing full-leg radiographs. It is measured as the angle between the mechanical femoral and mechanical tibial axis as defined by Strecker *et al*[103]. Positive values indicate varus alignment, and negative values indicate valgus alignment. The quadriceps angle (Q angle), which is related to the alignment of the structures that make up the extensor mechanism (the quadriceps, patella, and tibial tubercle). The quadriceps or Q angle can be used to evaluate the forces involved in patellofemoral tracking. The Q angle is a valgus angulation, which is the angle between the lines drawn from the anterior superior iliac spine to the middle of the patella and from there to the tibial tubercle (Figure 8). This angle is measured slightly lower than standing radiographs when evaluated on radiographs taken in the supine position[104].

Although the exact value of the Q angle cannot be given in the literature, it is stated that a wide Q angle (≥ 15°-20°) predisposes patients to increased lateral patellar position[105,106]. The Q angle varies by gender[5]. On average, it measures 14° in men and 17° in women. This difference is due to the larger pelvis width of women, which causes an increase in the valgus angle in the knee[107]. Internal rotation and pronation also increase this angle[108]. There are some publications in the literature questioning the

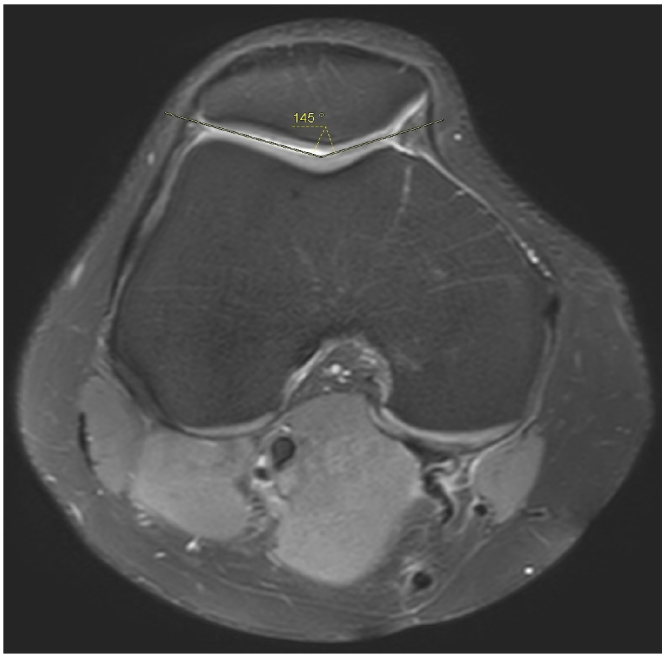

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Figure 7 Measurement of sulcus angle.

reliability of Q angle measurements[5,109]. For example, knee flexion angle can affect Q angle measurements. When comparing Q angles measured at 0° and 30° when the knee was flexed, it was found that the Q angle measured as little as 6° in flexion[110]. Although Hiemstra *et al*[111] defined the measurement method in axial views at 30° flexion, CT is currently used for Q angle measurement.

Torsional disorders, such as excessive internal femoral or external tibial torsion, exert force in the direction of lateralizing the patella, and these may show increased lateral patellar tilt and translation [112-114]. One study stated that increased femoral torsion is correlated with a dynamic Q-angle gait pattern, and this is seen more frequently in PI cases[99]. Femoral antetorsion and mechanical valgus axis showed a positive correlation[99].

Femoral torsion is a twist of the proximal femur relative to the distal femur and is performed using standardized axial slices through the hip, knee, and ankle. Femoral torsion was defined by Schneider *et al*[115], and it is the angle between the line connecting the femoral neck and femoral head center (femoral neck axis) and the line drawn parallel to it on the distal femur (tangential axis at the medial and lateral femoral condyles). Positive values indicated femoral antetorsion, whereas negative values indicated femoral retrotorsion. Kingsley and Olmsted[116] report the mean femoral anteversion angle as 8.0° (range, -20° to 38°). Several measurement methods have been defined depending on the different anatomical landmarks used while measuring femoral anteversion[117,118]. CT (a 3D reconstruction technique)[119,120] or MRI was used for the measurement of femoral anteversion[121,122]. Guenther *et al*[123], while measuring the femoral anteversion angle in MRI, used the advantage of MRI in planning by taking the line of the femoral neck axis parallel to the femoral neck. Studies have found different results in terms of the relationship between PI and femoral torsion. Imhoff *et al*[99] stated that there is an increased femoral torsion in high-grade TD. However, there are reports that neither femoral and tibial nor knee torsion are associated with trochlear dysplasia[124,125].

Likewise, there are different opinions about the relationship between PI and femoral rotation. While there are publications stating that there is an increased femoral antertorsion in patients with PI[112, 126], some articles report that there is no correlation between lateral patellar translation and femoral or tibial torsion[126,127].

Another rotational anomaly of the lower extremity is tibial torsion. This is the torsional angle between the proximal tibia and the distal tibia defined by Diederichs *et al*[126]. Pathology is mainly due to rotation in the proximal one quarter of the tibia[128]. Proximally, just before the head of the fibula, a line drawn parallel to the posterior tibial cortex is taken. Distally, the line connecting the middle of the medial and lateral malleolus is taken at the first slice passing through the talar dome[126]. Positive values indicate external tibial torsion, and negative values indicate internal tibial torsion. Methods using different landmarks have been described for the measurement of tibial torsion[129-131]. The normal values of external tibial torsion for individuals of European origin were found to be 24° to 30°[132].

Limb rotational deformities may be suspected on an anteroposterior X-ray, but measurements cannot be taken in this plane. In CT and MRI, rotational evaluations can be made using the hip, knee and ankle. Different measurement methods that can be used for tibial rotation measurement in cases with PI have

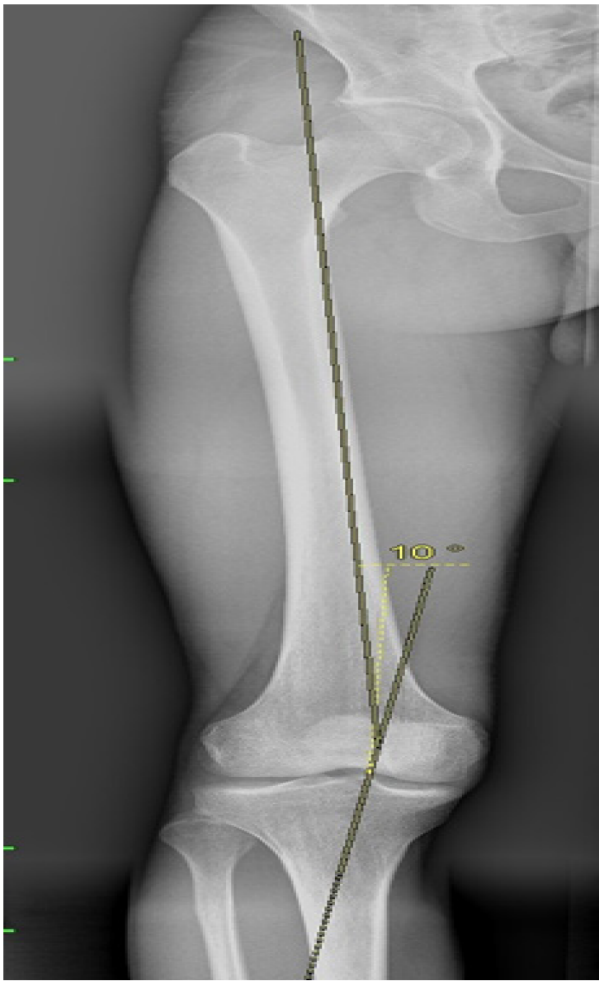

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Figure 8 Q angle measurement.

been described in the literature[133-136]. Proximally, the posterior axis of the tibia or the transcondylar axis can be used[137]. Distally, the transtibial[137] or bimalleolar axis can be used[138]. The difference in interobserver and intraobserver reliability mainly depends on the landmarks used in the distal measurement[139]. CT is generally accepted as the gold standard for lower extremity rotational deformities[117,140].

In conclusion, in the evaluation of PI, it will be beneficial to pay particular attention to TD, patella alta, and increases in both the TT-TG and lateral patellar tilt, although other factors are also considered. The effects of these factors can vary, so treatment may differ from person to person. Studies on various measurement methods and indices to be used in the evaluating both diagnosis and degree of TD are ongoing.

CONCLUSION

PI is a multifactorial problem. Many problems affecting the bone structure and muscles morphologically and functionally can cause it. It is necessary to understand normal anatomy and biomechanics to make more accurate radiological measurements and to identify causes. Knowing the possible causes of measurement errors that may occur during radiological measurements and avoiding these pitfalls can provide a more reliable road map for treatment. This determines whether the disease will be treated medically and with rehabilitation or surgery without causing further complications.

FOOTNOTES

Author contributions: Ormeci T contributed to the study concepts and design, manuscript preparation and writing; Turkten I contributed to the literature research and manuscript preparation; Sakul BU contributed to the manuscript editing and supervised the paper; and all authors read and approved the final manuscript.

Conflict-of-interest statement: Authors declare no conflict of interest for this article.

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S-Editor: Wang JJ
L-Editor: Kerr C
P-Editor: Wang JJ

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ISSN 2222-0682 (online)

World Journal of *Methodology*

World J Methodol 2022 May 20; 12(3): 83-192

Published by Baishideng Publishing Group Inc

World Journal of Methodology

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INDEXING/ABSTRACTING

The *WJM* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Wen-Wen Qi*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Ji-Hong Liu*.

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Bimonthly

EDITORS-IN-CHIEF
Bruno Megarbane

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2222-0682/editorialboard.htm

PUBLICATION DATE
May 20, 2022

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World J Methodol 2022 May 20; 12(3): 83-91

DOI: 10.5662/wjm.v12.i3.83

ISSN 2222-0682 (online)

MINIREVIEWS

Molecular and serology methods in the diagnosis of COVID-19: An overview

Marcel Silva Luz, Ronaldo Teixeira da Silva Júnior, Gabriella Almeida Santos de Santana, Gabriela Santos Rodrigues, Henrique de Lima Crivellaro, Mariana Santos Calmon, Clara Faria Souza Mendes dos Santos, Luis Guilherme de Oliveira Silva, Qesya Rodrigues Ferreira, Guilherme Rabelo Mota, Heloísa Heim, Filipe Antônio França da Silva, Breno Bittencourt de Brito, Fabrício Freire de Melo

Specialty type: Infectious diseases

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Pal A, India

Received: January 26, 2021
Peer-review started: January 26, 2021
First decision: July 18, 2021
Revised: July 31, 2021
Accepted: April 21, 2022
Article in press: April 21, 2022
Published online: May 20, 2022

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Abstract

Coronavirus disease-19 (COVID-19) has become a pandemic, being a global health concern since December 2019 when the first cases were reported. Severe acute respiratory syndrome coronavirus 2, the COVID-19 causal agent, is a β-coronavirus that has on its surface the spike protein, which helps in its virulence and pathogenicity towards the host. Thus, effective and applicable diagnostic methods to this disease come as an important tool for the management of the patients. The use of the molecular technique PCR, which allows the detection of the viral RNA through nasopharyngeal swabs, is considered the gold standard test for the diagnosis of COVID-19. Moreover, serological methods, such as enzyme-linked immunosorbent assays and rapid tests, are able to detect severe acute respiratory syndrome coronavirus 2-specific immunoglobulin A, immunoglobulin M, and immunoglobulin G in positive patients, being important alternative techniques for the diagnostic establishment and epidemiological surveillance. On the other hand, reverse transcription loop-mediated isothermal amplification also proved to be a useful diagnostic method for the infection, mainly because it does not require a sophisticated laboratory apparatus and has similar specificity and sensitivity to PCR. Complementarily, imaging exams provide findings of typical pneumonia, such as the ground-glass opacity

radiological pattern on chest computed tomography scanning, which along with laboratory tests assist in the diagnosis of COVID-19.

Key Words: COVID-19; Pandemic; Diagnosis; Polymerase chain reaction; Molecular biology; Serology

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Core Tip: Severe acute respiratory syndrome coronavirus 2 is primarily detected by PCR, which is the gold standard diagnostic method to detect viral RNA. On the other hand, techniques such as serology with detection of immunoglobulin M and immunoglobulin G antibodies, imaging, and laboratory tests also assist in the diagnosis of severe acute respiratory syndrome coronavirus 2 infection. Moreover, the reverse transcription loop-mediated isothermal amplification has similar specificity and sensitivity to PCR. In this review, we discuss the main diagnostic methods and their uses in the current pandemic.

Citation: Luz MS, da Silva Júnior RT, Santos de Santana GA, Rodrigues GS, Crivellaro HL, Calmon MS, dos Santos CFSM, Silva LGO, Ferreira QR, Mota GR, Heim H, Silva FAFD, de Brito BB, de Melo FF. Molecular and serology methods in the diagnosis of COVID-19: An overview. *World J Methodol* 2022; 12(3): 83-91
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/83.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.83

INTRODUCTION

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as responsible for severe cases of pneumonia in Wuhan, China, which culminated in the description of a new disease: coronavirus disease-19 (COVID-19)[1]. As a result of the large number of affected countries and high potential of viral infection, the World Health Organization declared COVID-19 as a pandemic in March 2020. Up to December 2020, 66243918 cases and 1528984 global deaths were officially confirmed[2]. SARS-CoV-2 is a single-stranded RNA, enveloped virus, which has the ability to attach to the angiotensin-converting enzyme 2 cell receptor due to the expression of the spike (S) protein on the viral envelope and then enter the host tissues[3]. The *Coronaviridae* family is made up of viruses historically known to cause diseases in animals and humans. The SARS and Middle East Respiratory Syndrome outbreak in 2002 and 2012, respectively, granted them wider visibility in the scientific community[4]. Furthermore, the dissemination potential of SARS-COV-2 is far higher than the others, due to structural differences in the S protein[5].

The most common signs and symptoms of COVID-19 include fever, dry cough, shortness of breath, myalgia, ageusia, anosmia, headache, rhinorrhea, nausea, vomiting, and diarrhea[6], and most patients showing severe symptoms are often affected by chronic disease. In addition, disturbed immune status, increased age, and obesity are strongly correlated to higher mortality rates[7]. Reverse transcription (RT)-PCR is considered the gold standard test for the diagnosis of COVID-19, due to its highly widespread and reliable technique performed in laboratories worldwide[8]. In addition, immunoenzymatic and immunochromatographic assays as well as reverse transcription loop-mediated isothermal amplification (RT-LAMP) are other diagnostic methods that have been applied in this field. Of note, clinical and epidemiological analysis, chest radiography and tomography, and laboratorial findings are crucial tools for an accurate diagnosis and appropriate evaluation of patients[9]. This article aimed to review the main aspects of COVID-19 diagnostic methods, providing updated information with an emphasis on molecular biology techniques and serology tests used in the detection of SARS-CoV-2 infection.

RT-PCR

RT-PCR is considered as the gold standard method in COVID-19 diagnosis, due to rapid detection with an average of 3-4 h and high sensibility and specificity[10]. The sample is usually taken through nasopharyngeal swab[11]. However, a systematic review and meta-analysis with 7 studies showed that bronchoalveolar lavage fluid had a higher positivity rate in the detection of SARS-CoV-2[12].

The test analysis usually starts from a sample collected from nasal and oropharyngeal swabs[13]. It is then divided in several steps that occur in different preset temperatures in order to provide RT and nucleic acid amplification[14]. The result is thus analyzed through probes marked with fluorescent dyes that enhance the sensitivity of the test[15]. The analysis of fecal samples, especially in children[16], may

be used as well, as the virus can remain viable for approximately 5 wk after patient respiratory samples are negative for SARS-CoV-2 RNA[17].

RT-PCR is considered the actual main method as a result of its fastness, reproducibility, and mitigation of false-positive results[18]. The test shows good sensitivity and specificity, such as 94% and 100%, respectively (Table 1)[19-28]. Studies have also pointed to a level of detection that can vary from 3.8 to 23.0 copies/mL of viral RNA and showed no cross-reactivity with circulating respiratory viruses [20].

However, in a study with 610 patients from Wuhan, China, 18 patients had a positive RT-PCR result after two consecutive negative results, which might be owing to insufficient viral material, test handling error, or incorrect collection processing[21]. This is an alert to the need for a pattern in sampling procedures and alignment between the test and the patient's clinical manifestation in order to achieve a higher diagnostic accuracy[22].

RT-LAMP

LAMP is a DNA amplification technique under isothermal conditions in a sample with 4 or 6 primers, which in contrast to RT-qPCR does not require a sophisticated laboratory apparatus, although it has similar specificity and sensitivity rates[29]. The visualization through pH-sensitive dyes, without the need of expensive instrumentation is also an advantage of this test[30]. As SARS-CoV-2 is an RNA virus, the test is therefore called RT-LAMP, due to the need for RTase to amplify RNA sequences[29].

The RT-LAMP is a fast test, providing results within 30 min[31]; moreover, unpurified samples can be directly used[32]. Studies also show that when the template has more than 200 copies of viral RNA, amplification curves appear within 15 min[33], which means an even quicker diagnosis. In addition, studies describe a level of detection of 2 copies of viral RNA in a 25 μL reaction[34], sensitivity rates that varies from 80%[26] to 97.5%[27], concomitantly with no cross-reactivity with other respiratory pathogens (high specificity)[33,35] and lower cost, all of which endorses that the diagnosis of COVID-19 through LAMP needs to be considered[36,37].

However, a meta-analysis including 138 articles showed that RT-LAMP sensitivity (86.3%) is lower than that of RT-PCR (96.2%)[38]. Furthermore, carry-over contamination, which can lead to false positive results, are common in LAMP reactions, probably as a consequence of aerosol formed from the products of the test[34]. This phenomenon highlights the need of laboratories with good practice of molecular biology and separate spaces to deal with the components of the test as well as more studies about the efficacy of all types of genes and primers used in this test.

SEROLOGICAL TESTS

Serological tests have become even more available during the COVID-19 pandemic. Consequently, research on their role as auxiliary diagnostic methods for SARS-CoV-2 infection has experienced exponential growth[39]. Thereby, these tests may support the COVID-19 diagnosis, especially when there is a longer period of symptoms with negative RT-PCR assays in a patient with a suspected infection by SARS-CoV-2[40]. Moreover, its use allied to RT-PCR greatly increases the diagnostic sensitivity[41].

Among the serological tests commonly used for diagnosis of COVID-19, the ELISA, the chemiluminescence immunoassay (CLIA), and the lateral flow immunochromatographic assay (LFA) stand out. The ease, agility, and point-of-care testing are great advantages associated with the use of these tests[42]. However, they may often show low sensitivity, require specialized equipment[39,42], or have cross-reactivity with other pathogens, such as SARS-CoV-1[43]. A study also showed a cross-reactivity of 26% in serological tests for COVID-19 during acute Zika virus infection[44].

The sensitivity of the test is strictly related to the elapsed time from the beginning of symptoms, being more useful 15 d after the onset of clinical manifestations, especially regarding the detection of isolated immunoglobulin (Ig) G[45]. In that context, a meta-analysis including 40 studies evaluated the presence of anti-SARS-CoV-2 IgG during the first symptomatic week, and the rates of false-negative diagnoses ranged from 44% to 87%[46]. Therefore, simultaneous analysis of IgM and IgG antibodies, as they have different emergence times, may increase the serological test sensitivity[39,47]. Although some studies compare IgG with other antibodies, such as IgA, to analyze any increase in the effectiveness of serological surveillance, the results are not promising[48].

The overall specificity for all types of antibodies was higher than 98%. The average sensitivity for IgG detection ranges from 80% to 85%, with CLIA being the most sensitive, followed by ELISA, and with much lower performance the LFA test[42,45]. IgM evaluation showed a sensitivity of 80.9% for CLIA, 84.5% for ELISA, and 51.4% with LFA. This study also demonstrated that in the use of combined IgM/IgG tests, the CLIA performance was higher than ELISA and LFA, with results of 97.3%, 90.5% and 85.8%, respectively[45].

Table 1 Coronavirus disease 2019 main diagnostic methods characteristics

Ref.	Diagnostic method	Sensitivity	Specificity	Time to result
Liu *et al*[23]	ELISA (IgM/IgG)	57.9%-90.7%	No cross-reactivity observed	About 100 min
Cai *et al*[24]	CLIA (IgM/IgG/IgM and IgG)	57.2%-81.5%	No cross-reactivity observed	ND
Montesinos *et al*[25]	LFA (IgM/IgG/IgM and IgG)	~70.0%	95.8%-100%	About 10 min
Scohy *et al*[52]	Antigen detection	30.2%	100%	About 15 min
Porte *et al*[54]	Antigen detection	93.9%	100%	About 15 min
Suo *et al*[19]	RT-PCR	94.0%	100%	ND
Österdahl *et al*[26]	RT-LAMP	80.0%	73%–100%	About 25 min
Dao Thi *et al*[27]	RT-LAMP	97.5%	99.7%	About 30 min
Ai *et al*[28]	Chest CT	97.0%	25%	ND

ELISA: Enzyme-linked immunosorbent assay; IgA: Immunoglobulin A; IgM: Immunoglobulin M; IgG: Immunoglobulin G; LFA: Lateral flow assay; CLIA: Chemiluminescence immunoassay; RT-PCR: Reverse transcription-polymerase chain reaction; RT-LAMP: Reverse transcription loop-mediated isothermal amplification; CT: Computed tomography; ND: Not described.

Sensitivity and specificity differences according to the viral protein analyzed are also documented: S protein is more specific, but the nucleocapsid and receptor-binding domain proteins are more sensitive in patients with mild infection[47,49]. Therefore, research on antibodies against different antigens may be useful in order to improve diagnostic methods, avoid false-negatives, and reach a higher diagnostic accuracy.

ANTIGEN DETECTION METHODS

Viral antigen is a molecule with immunogenic potential that can be targeted by diagnostic tests through a reaction with monoclonal antibodies. Several antigen detection tests have been developed as alternatives for the rapid diagnosis of the COVID-19[50,51]. The results of the test with nasopharyngeal secretions are ready within 15 min[52], and it can be performed either through immunochromatography, with rapid detection, or ELISA with better sensitivity[50].

The average sensitivity of antigen detection tests is around 50%–70%, and they are 100% specific[51,53]. Of note, the performance of those tests may be influenced by higher or lower viral loads as well as by the specific antigen used. In that context, studies have shown different results when this method was evaluated, and the sensitivity values varied from 30.2%[52] to 93.9%[54] Overall, higher rates of accurate diagnosis in antigen tests were greatly correlated with early infection, when the viral load of the upper respiratory tract is higher[55].

Therefore, although the COVID-19 diagnosis through antigen detection has a high specificity and is faster and cheaper than RT-PCR, the precise time of usage of this test is crucial for proper detection of the virus antigens[52]. That said, the current gold standard diagnostic test for COVID-19 is still more reliable because its use is associated with lower rates of false negative results[56]. Nevertheless, utilization of antigen detection tests, with additional research, could turn into a viable option in the current pandemic context.

COMPLEMENTARY DIAGNOSTIC METHODS

Chest computed tomography findings

Chest computed tomography (CT) has been used as an alternative and complementary method for COVID-19 diagnosis since CTs can detect pulmonary abnormalities even when RT-PCR results turn negative[57] for highly suspect cases with clinical symptoms[58] in the early days of infection[59].

The chest CT diagnosis works through analysis of the variation in imaging findings that occur according to the disease progression and severity[60]. The pulmonary imaging abnormalities start to appear around 4 d after the first symptoms, and their findings are more visible following the second week of clinical manifestations[58].

Accordingly, the most predominant COVID-19 pneumonia imaging changes are ground-glass opacity lesions with or without consolidations, peripheral and bilateral lung distribution of the disease, and multilobar lung involvement, predominantly in the lower lobes[61,62]. Some less common CT manifest-

ations are the crazy-paving pattern, ground-glass opacity with consolidation, interlobular septal thickening, and pleural effusion[63]. Chest CT scans are highly sensitive to COVID-19 lung abnormalities[64], mainly in high-risk symptomatic cases[65].

However, these imaging findings have low specificity[63]. A study performed with 1014 patients showed an average specificity of 25%[28], probably due to other viral pneumonias leading to similar imaging alterations, a fact that limits the use of this method in areas with high prevalence of other respiratory tract infections[64]. Moreover, chest CT exams can detect no abnormalities in some asymptomatic or mild symptomatic cases[63], making CT scans more of a complementary diagnostic test than a definitive one. Table 1 summarizes the sensitivity, specificity, time to result, and limitations of the diagnostic methods discussed in this review so far.

Laboratory findings

Patient reports from Wuhan showed recurrent cases of lymphocytopenia since the beginning of the infections in China[66]. Besides that, studies also show a relevant frequency of patients with leukocytosis during SARS-CoV-2 infection[67]. A meta-analysis showed that non-surviving patients had an expressive increase in leukocyte count, total bilirubin, serum ferritin, and interleukin 6 as well as a reduced lymphocyte count[68]. Thus, leukocyte series elevation can represent worse prognostic and high risk of unfavorable outcomes.

Increases in the levels of lactate dehydrogenase and C-reactive protein were also highlighted and associated with pulmonary and myocardial lesions, especially in severe patients[69]. Low serum albumin rates and high levels of alanine aminotransferase and aspartate aminotransferase points to possible liver complications, which is very common in acute phases of the disease in patients with a severe infection[70,71]. Furthermore, the association of these points with elevation in renal biomarkers (for example, creatinine), coagulation measures, and heart and muscle injury scores suggest potential progression to multiple organ failure in severe patients[68]. Thereby, elevated levels of D-dimer, fibrin degradation products, and fibrinogen can be observed during the course of the disease, with the D-dimer alterations being the most common[72]. A study related that levels of these coagulation parameters were observed in severe patients with worse prognosis, while mild disease or early stage patients had normal ranges[73,74]. In addition, thrombocytopenia is also a possible laboratory finding in COVID-19. A meta-analysis reported that platelet count was minor in severe disease and even smaller in non-surviving patients[74]. These findings might be indicative of disease progression and coagulation disorders, which means that the tracking of these signs is very important while managing patients[74,75].

Moreover, possible coinfections of SARS-CoV-2 and bacterial infections might cause neutrophilia and leukocytosis, associated with lymphocytopenia, without increasing inflammatory factors, such as D-dimer and C-reactive protein[76]. Therefore, laboratory findings have proven to be a helpful option as a complementary diagnosis. It is also suitable in the visualization of possible comorbidities in patients with COVID-19 and as an indicator of disease severity.

Several studies are being carried out to test the efficacy of drugs, foods, and mineral supplements against COVID-19. Lymecycline and famotidine, for example, are being studied as a potential treatment for COVID-19[77,78], due to a possible ability to bind some SARS-CoV-2 structures (M^{pro}, S protein, RdRp, and furin) and have an anti-inflammatory action[79], respectively. However, there is, up to this moment, not enough evidence and controlled clinical trials to affirm its efficacy against the disease. In addition, mineral supplements such as zinc apparently have some antiviral properties that could be used against SARS-CoV-2 infection, such as a capability of modulating the host's immune response and attenuating the cytokine storm caused by COVID-19[80]. However, a randomized clinical trial of 214 patients showed that zinc supplementation had no significant benefits[81].

CONCLUSION

Notably, the use of serology and antigen detection tests have important limitations since false negative results are common. Nonetheless, in a pandemic context, these methods are crucial for epidemiological surveillance. RT-PCR remains the gold standard test and should be preferred to diagnose COVID-19. However, the high potential of RT-LAMP, given that it is a fast and affordable test, should be considered in diagnostic propedeutics. In addition, the laboratory and imaging findings play important roles as complementary diagnostic tools aiding in patient management.

FOOTNOTES

Author contributions: Luz MS contributed to the conceptualization, methodology, validation, investigation, and writing – original draft; da Silva Júnior RT contributed to the validation, visualization, formal analysis, writing – reviewing and editing; Santos de Santana GA, Rodrigues GS, Crivellaro HL, Calmon MS, dos Santos CFSM, Silva LGO, Ferreira QR, Mota GR, and Heim H contributed to the methodology, writing, visualization, investigation, and

formal analysis; Silva FAF contributed to the methodology, visualization, investigation, and formal analysis; de Brito BB contributed to the methodology; de Melo FF contributed to the conceptualization, methodology, investigation, writing – original draft, and supervision.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other co-authors who contributed their efforts in this manuscript.

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S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Ma YJ

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 May 20; 12(3): 92-98

DOI: 10.5662/wjm.v12.i3.92

ISSN 2222-0682 (online)

MINIREVIEWS

Network meta-analyses: Methodological prerequisites and clinical usefulness

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Specialty type: Methodology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Elfayoumy KN, Egypt; Hasabo EA, Sudan; Yahaya TO, Nigeria

Received: October 19, 2021
Peer-review started: October 19, 2021
First decision: January 18, 2022
Revised: February 5, 2022
Accepted: March 26, 2022
Article in press: March 26, 2022
Published online: May 20, 2022

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Abstract

It is an undeniable fact that systematic reviews play a crucial role in informing clinical practice; however, conventional head-to-head meta-analyses do have limitations. In particular, studies can only be compared in a pair-wise fashion, and conclusions can only be drawn in the light of direct evidence. In contrast, network meta-analyses can not only compare multiple interventions but also utilize indirect evidence which increases their precision. On top of that, they can also rank competing interventions. In this mini-review, we have aimed to elaborate on the principles and techniques governing network meta-analyses to achieve a methodologically sound synthesis, thus enabling safe conclusions to be drawn in clinical practice. We have emphasized the prerequisites of a well-conducted Network Meta-Analysis (NMA), the value of selecting appropriate outcomes according to guidelines for transparent reporting, and the clarity achieved *via*

sophisticated graphical tools. What is more, we have addressed the importance of incorporating the level of evidence into the results and interpreting the findings according to validated appraisal systems (*i.e.*, the Grade of Recommendations, Assessment, Development, and Evaluation system - GRADE). Lastly, we have addressed the possibility of planning future research *via* NMAs. Thus, we can conclude that NMAs could be of great value to clinical practice.

Key Words: Network meta-analysis; Quality of evidence; Evidence-based medicine; Systematic reviews

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Core Tip: Systematic reviews with or without meta-analyses provide the highest quality of evidence, thus lying on the top of evidence-based medicine hierarchy. However, pair-wise meta-analyses present the inherent limitation of exclusively comparing direct evidence. By contrast, Network Meta-Analyses (NMAs) also consider indirect evidence, thereby offering additional useful information. Conducting an NMA, however, has certain requirements such as assuming that transitivity across the included studies exists. What is more, maintaining sufficient statistical power in the analyses is crucial. In addition, performing head-to-head statistical comparisons before setting up networks of interventions is a prerequisite for a methodologically sound NMA, and selecting not only positive but also negative outcomes is required. Lastly, implementing quality appraisal systems to grade the level of evidence is highly recommended. Should all the above criteria be fulfilled, then accurate clinical conclusions can be drawn from an NMA.

Citation: Christofilos SI, Tsikopoulos K, Tsikopoulos A, Kitridis D, Sidiropoulos K, Stoikos PN, Kavarthapu V. Network meta-analyses: Methodological prerequisites and clinical usefulness. *World J Methodol* 2022; 12(3): 92-98

URL: https://www.wjgnet.com/2222-0682/full/v12/i3/92.htm

DOI: https://dx.doi.org/10.5662/wjm.v12.i3.92

INTRODUCTION

Due to the plethora of different interventions for various clinical entities[1] identifying the most efficient and safe treatment is among the prime interests of a researcher[2-4]. In the case of conventional meta-analyses, only two interventions can be compared at a time, and only those evaluated in head-to-head trials[5-7]. What is more, intervention effect estimates can only be calculated from direct evidence[2]. In contrast to pair-wise meta-analyses, network meta-analyses (NMA) enable not only simultaneous direct comparisons of multiple interventions but also indirect comparisons provided a common comparator is shared between interventions[2]. This is even possible in the case of two interventions that have never been directly compared[2]. In addition, interventions may also be ranked utilizing the surface under the cumulative ranking (SUCRA) curves, thus allowing for judgments such as which treatment presents the highest probability of being the most effective[2]. It is underlined that identifying more than one highly efficacious treatment in an NMA is a common phenomenon given the subtle differences in treatment rankings of the modalities lying on the top of ranking probabilitiy tables. Overall, incorporating the results from network meta-analyses into clinical practice guidelines could help clinicians select the best available intervention to improve healthcare.

PREREQUISITES FOR A WELL-CONDUCTED NMA - THE ASSUMPTION OF TRAN SITIVITY AND HETEROGENEITY

For a systematic review of randomized evidence to qualify as a network meta-analysis, the assumption of transitivity must be fulfilled. To elaborate further, transitivity implies that it is possible to conclude hypothetical comparisons through a common comparator[6]. However, this is only possible in the absence of systematic differences between studies[8] with some degree of heterogeneity being permitted [6]. To illustrate further, heterogeneity is defined as a form of inter-study discrepancy due to differences that cause deviations in the observed effects other than sampling error[9]. However, when the discrepancy between studies exceeds that explained by clinical diversity, effects sizes cannot be safely estimated based on direct and indirect evidence and the distribution of effect modifiers needs to be examined[6].

PREREQUISITES FOR A WELL-CONDUCTED NMA- STATISTICAL POWER

It is worthy of note that the statistical power of a network of interventions should be sufficient to enable safe clinical conclusions to be drawn. To be more specific, the ratio between the number of included papers relative to the number of the competing interventions should be satisfactory. On top of that, the sample size per intervention arm as depicted by the size of the nodes in a network meta-analysis plot should also be robust enough (Figure 1). Lastly, prospective registration with systems such as the grade of recommendations, assessment, development, and evaluation system (GRADE) is valuable in assessing the heterogeneity and additional characteristics such as publication bias, indirectness, imprecision, the study limitations, and inconsistency[5].

CONDUCTING PAIR-WISE META-ANALYSIS PRIOR TO NMA

Of additional note, for a given dataset, researchers must conduct not only NMA but also traditional pair-wise meta-analyses. To be more precise, one can take advantage of early exploration of the results of conventional pair-wise meta-analyses before setting up networks of interventions. Authors should then proceed with the network meta-analysis to take advantage of indirect evidence synthesis for them to supplement their study results.

PREREQUISITES FOR A WELL-CONDUCTED NMA- SELECTING APPROPRIATE OUTCOMES

In determining primary and secondary outcomes, both positive and negative results should be considered. Outcomes of primary interest should be prioritized over outcomes of secondary clinical importance to ensure that the findings will be clinically relevant. For instance, laboratory tests are not routinely considered as primary endpoints as they tend to not directly inform decisions. However, they may play an explanatory and/or adjuvant role in explaining the intervention outcome[10].

FOLLOWING GUIDELINES FOR TRANSPARENT REPORTING

The PRISMA guidelines represent a checklist of 27 items that may be used when reporting a systematic review of health interventions with or without meta-anlysis[11]. Hutton *et al*[12], in 2015, has expanded the original list by including 5 additional items that apply to network meta-analyses. Firstly, the geometry and summary of the intervention networks have been incorporated in the methods, including a diagrammatic representation and a brief description. What is more, the findings of inconsistency assessment can be included in addition to the presentation of the networks' structure.

It should be noted that prospective registration (*e.g.* with PROSPERO database) of all NMAs is encouraged. By doing so, transparency is promoted and bias is prevented by avoiding unintended duplicate reviews[13]. It is also highlighted that adherence to a pre-existing protocol plays a crucial role in preventing selective outcome reporting[10,14,15]. In other words, registration of a systematic review in advance of study commencement precludes data manipulation and/or unethical reporting. Last but not least, prospective registration may enable researchers to assess whether the topic they intend to investigate has already been addressed by earlier authors, thus avoiding unnecessary research repetition.

SOPHISTICATED GRAPHICAL TOOLS IN NMA - DO WE NEED THEM?

Despite NMAs gaining popularity, a lot of criticism exists given their complex methodology discouraging clinicians from getting involved in this type of research[16]. This is due to the increased level of statistical and computational knowledge required. To tackle this issue, introducing graphical tools into the manuscript results in a significant increase in clarity and reproducibility[16].

What is more, competing interventions can be ranked from the most to the least effective *via* the use of SUCRA curves[2]. On the other hand, league tables enable a structured presentation of the result of each pair of comparisons with its corresponding 95% confidence intervals (Figure 2).

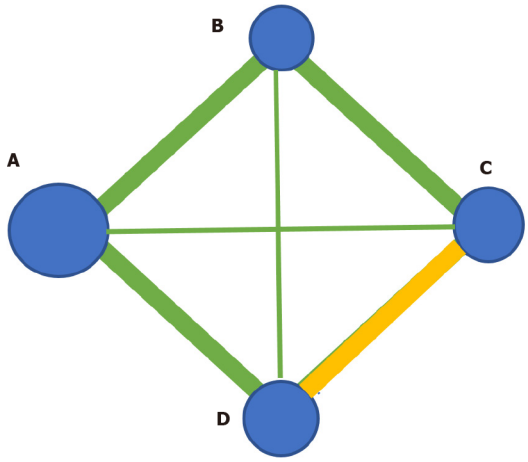

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Figure 1 A Network-Meta-Analysis plot example. Network meta-analysis plot including four competing interventions (*i.e.* A, B, C, and D). The nodes represent the included interventions with their size being proportional to sample size. The thickness of the edges connecting the nodes is reflected in the number of trials included in the given comparison. The edges depicted in green and yellow denote that the involved comparisons are at low and moderate risk of bias, respectively.

A				
-0.17 (-1.60, 1.25)	B			
-1.34 (-2.68, 0.01)	-1.16 (-2.99, 0.66)	C		
0.93 (-0.82, 2.68)	1.11 (-1.15, 3.36)	2.27 (0.16, 4.48)	D	
3.05 (1.41, 4.68)	3.22 (1.17, 5.27)	4.38 (2.39, 6.38)	2.11 (-0.28, 4.51)	E

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Figure 2 Hypothetical league table demonstrating standardized mean differences, from a network meta-analysis of five competing interventions, that is A-E. Statistically significant values are depicted in TextTitle.

PUBLICATION BIAS IN NMA AND ITS IMPACT ON CLINICAL ESTIMATES

It has been evidenced that detection of publication bias (that is typically reporting positive more often than negative results) in NMA is not uncommon. Inevitably, introducing this kind of bias in meta-analysis threatens the validity of the results of the study as an "overly rosy picture" may be painted. To elaborate further, the evaluation of small study effects acts as a proxy for the assessment of publication bias. For the above assessment, a sophisticated statistical tool namely a comparison-adjusted funnel plot can be implemented. Apart from funnel plots, researchers can also employ Egger's test to statistically evaluate the presence of small-study effects[16-18].

QUALITY APPRAISAL SYSTEMS AND TRANSITION TO CLINICAL PRACTICE

The GRADE system features 6 components[5], that are study limitations, heterogeneity, inconsistency, indirectness, imprecision, and publication bias[5,19]. The quality of evidence may be high, moderate, low, or very low. As a rule of thumb, randomized trials yield high-quality evidence, whereas observational studies more often than not offer a low quality of evidence with the risk of bias potentially affecting clinical judgment[20].

Potential limitations of randomized trials include failure to conceal allocation, failure to blind, loss to follow-up, and failure to appropriately consider the intention[20]. Guyatt *et al*[20], in 2011, also mentioned terminating a study early for apparent benefit, and selective reporting of outcomes according to the results. The indirectness may be due to patients deviating from those of interest, when the treatments have not been compared in head-to-head trials, and when there are different outcomes from those being expected from the study[21]. Furthermore, the contributions of biological and social factors to the magnitude of effect in the outcomes represents indirectness[21]. On the other hand, inconsistency is defined as a disagreement between direct and indirect evidence in NMA[19]. In addition, Salanti *et al* [19], have suggested the adoption of a quantitative approach to assess the risk of bias.

INVESTIGATING CLINICAL DIVERSITY IN NMA

It is an undeniable fact that a great many confounding factors can be encountered in a broad systematic review of randomized trials. Thus, conducting sensitivity analysis to delineate the impact of clinical heterogeneity factors is strongly recommended. For instance, the effect of low-quality trials, variation in intervention characteristics as well as differences due to variable outcome measurement tools needs to be considered in those secondary analyses. From a technical point of view, the researcher needs to improve the trial(s) with the above characteristics from the analysis, repeat the statistical tests and subsequently compare the new results with the findings of the primary analysis[22].

PLANNING FUTURE RESEARCH WITH NMA- IS IT POSSIBLE?

Directing the design of future studies based on NMA results appears to be of significant importance as mismanagement of resources can be overcome[23-25]. For a researcher to provide an estimate of whether the results of a subsequent trial are likely to change in the future, an interval plot should be considered. By visually inspecting an interval plot, an investigator can enable predictions on the efficacy of a particular intervention in a future trial[16,26,27].

IMPROVING INTERPRETATION OF NMA FINDINGS

To improve interpretability and clarity of the results of an NMA, researchers are encouraged to back-transform their data in a manner that interpretation of their results is improved. For instance, when it comes to Patient-Reported Outcome Measures, investigators can back-transform Standard Mean Differences to Mean Differences and subsequently assess their findings against the established minimal clinically important difference for a particular questionnaire[28].

CONCLUSION

Overall, NMAs play a crucial role in the decision-making process. As long as common methodological mistakes are avoided, researchers can produce reliable and accurate clinical conclusions.

FOOTNOTES

Author contributions: Karthavapu V and Tsikopoulos K were involved in the conceptualization of the study; Tsikopoulos A and Sidiropoulos K conducted the literature research and extracted relevant information; Tsikopoulos K and Kitridis D assessed the quality of the included studies; Christofilos SI and Stoikos PN were involved in the generation of tables and the writing of the paper; Karthavapu V supervised and revised the paper accordingly; Throughout the study, Christofilos SI was an intern of Professor Maniatis's group at University College London; all authors have read and approved the final manuscript.

Conflict-of-interest statement: All authors declare there is no conflict of interest.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

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World Journal of Methodology

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World J Methodol 2022 May 20; 12(3): 99-106

DOI: 10.5662/wjm.v12.i3.99

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Clinical and Translational Research

COVID-19 and thyroid disease: An infodemiological pilot study

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Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Hu H, China; Ozair A, India

Received: November 23, 2021
Peer-review started: November 23, 2021
First decision: February 8, 2022
Revised: February 11, 2022
Accepted: March 26, 2022
Article in press: March 26, 2022
Published online: May 20, 2022

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Abstract

BACKGROUND

Google Trends searches for symptoms and/or diseases may reflect actual disease epidemiology. Recently, Google Trends searches for coronavirus disease 2019 (COVID-19)-associated terms have been linked to the epidemiology of COVID-19. Some studies have linked COVID-19 with thyroid disease.

AIM

To assess COVID-19 cases *per se vs* COVID-19-associated Google Trends searches and thyroid-associated Google Trends searches.

METHODS

We collected data on worldwide weekly Google Trends searches regarding "COVID-19", "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)", "coronavirus", "smell", "taste", "cough", "thyroid", "thyroiditis", and "subacute thyroiditis" for 92 wk and worldwide weekly COVID-19 cases' statistics in the same time period. The study period was split in half (approximately corresponding to the preponderance of different SARS-COV-2 virus variants) and in each time period we performed cross-correlation analysis and mediation analysis.

RESULTS

Significant positive cross-correlation function values were noted in both time periods. More in detail, COVID-19 cases *per se* were found to be associated with no lag with Google Trends searches for COVID-19 symptoms in the first time period and in the second time period to lead searches for symptoms, COVID-19 terms, and thyroid terms. COVID-19 cases *per se* were associated with thyroid-related searches in both time periods. In the second time period, the effect of "COVID-19" searches on "thyroid' searches was significantly mediated by COVID-19 cases (P = 0.048).

CONCLUSION

Searches for a non-specific symptom or COVID-19 search terms mostly lead Google Trends thyroid-related searches, in the second time period. This time

frame/sequence particularly in the second time period (noted by the preponderance of the SARS-COV-2 delta variant) lends some credence to associations of COVID-19 cases *per se* with (apparent) thyroid disease (*via* searches for them).

Key Words: Data collection; Epidemiology; Thyroid; Medical informatics; Methods; Trends

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Core Tip: Google Trends searches for coronavirus disease 2019 (COVID-19)-associated terms have been linked to the epidemiology of COVID-19. In this study we aimed to assess worldwide COVID-19 cases *per se vs* COVID-19-associated Google Trends searches and thyroid-associated Google Trends searches for 92 wk. The study period was split in half and in each time period we performed cross-correlation analysis and mediation analysis. Significant cross correlation function factors for "COVID-19" and "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" were mostly found in the second time period, whereas COVID-19 cases *per se* were associated with "thyroid" searches in both time periods. In the second time period, which was characterized by the spread of SARS-CoV-2 delta variant, the effect of "COVID-19" searches on "thyroid" searches was significantly mediated by COVID-19 cases ($P = 0.048$). The observed time frame/sequence lends some credence to associations of COVID-19 cases *per se* with (apparent) thyroid disease.

Citation: Ilias I, Milionis C, Koukkou E. COVID-19 and thyroid disease: An infodemiological pilot study. *World J Methodol* 2022; 12(3): 99-106
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/99.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.99

INTRODUCTION

Digital epidemiology uses digital data which was not generated with the primary goal of serving epidemiological research[1]; such data are within the domain of "infodemiology"[2]. Google Trends (available at https://trends.google.com) searches may - according to some researchers - accurately reflect the epidemiology of infectious, acute, or chronic diseases, including, among others, coronary or thyroid disease[2-10]. Recently, Google Trends searches for COVID-19-associated terms have been tentatively linked to the epidemiology of COVID-19[11-17]. Some - but not all – clinical studies have linked COVID-19 with thyroid function abnormalities and more particularly with a form of subacute-like thyroiditis[18-22]. Since the use of Google Trends to study a wide range of medical topics is becoming more widespread and the available research on COVID-19-related thyroid disease is conflicting, with this work we aimed to look at the issue of COVID-19-related thyroid disease from a different angle, namely, that of digital epidemiology, since the latter may be a useful adjunct to classical epidemiology.

MATERIALS AND METHODS

Data and data collection

We collected data on worldwide weekly Google Trends searches, by means of their "relative search volumes" (RSVs). The latter is normalized internet search volume values over a given time period, with a minimum of 0 and a maximum of 100 (see also https://support.google.com/trends/). More in detail, we used the worldwide RSVs of the search terms in the English language for "COVID-19", "SARS-CoV-2", "coronavirus", "smell", "taste", "cough", "thyroid", "thyroiditis", and "subacute thyroiditis" for 92 wk, from January 26, 2020 to October 24, 2021. The search terms were chosen because of their ubiquity and uniformity in lay and medical terms. For the same time period, worldwide weekly COVID-19 cases' statistics, as provided by the Johns Hopkins University Coronavirus Resource Center (available at https://coronavirus.jhu.edu/map.html), were collected[23]. The study period was split in half: The first half corresponded to the time period with preponderance of the SARS-CoV-2 alpha variant and the second to the time period with preponderance of the delta variant (Figure 1).

Statistical analysis

In each of the aforementioned time periods, we performed cross-correlation analysis. The threshold for statistical significance of each cross-correlation factor value at the $P = 0.05$ level was set according to lag,

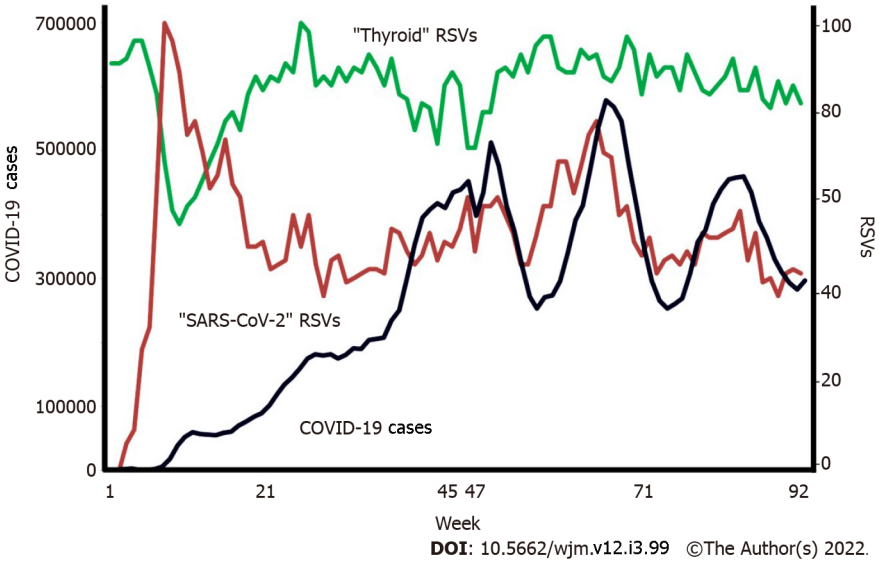

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Figure 1 Time series plot of selected study data: Worldwide coronavirus disease 2019 weekly cases and Google Trends relative search volumes for "severe acute respiratory syndrome coronavirus 2" and "thyroid" during the study period. Note the differences in magnitude, particularly during the second half of the study period. COVID-19: Coronavirus disease 2019; RSVs: Relative search volumes; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

thus the cross-correlation factor had to be higher than 0.290 at lag = 0 and 0.324 at lag = 8. A lag = 0 indicates contemporaneous correlation, a negative lag indicates that the first variable leads within a set time frame the second variable, and a positive lag indicates that the first variable follows (lags) within a set time frame the second variable. After the calculation of cross-correlation factor values, further evaluation among the variables was done with mediation analysis, implementing Sobel's test. Statistical analyses were done with Minitab v.17.1 (Minitab Inc, State College, PA, United States, 2010) and JASP v0.15 (JASP Team, University of Amsterdam, NL, 2021).

RESULTS

Worldwide, COVID-19 weekly cases *per se* gradually increased over time and showed wide fluctuations during the second half of the study period (Figure 1). The RSVs of the studied search terms also showed fluctuations (Figure 1 and Supplemental Figures 1 and 2). Significant positive cross-correlation factor values were noted in both time periods. More in detail, significant cross-correlation factors for "COVID-19" and "SARS-COV-s" were mostly found in the second time period (Table 1), whereas COVID-19 cases *per se* were associated with "thyroid" searches in both time periods. In the second time period, the effect of "COVID-19" searches on "thyroid" searches was significantly mediated by COVID-19 cases (Sobel test statistic $P = 0.048$).

DISCUSSION

COVID-19 cases *per se* were found to be associated with no lag with Google Trends searches for COVID-19 symptoms in the first time period and in the second time period to lead searches for symptoms, COVID-19 terms, and thyroid terms. Searches for a non-specific symptom or COVID-19 search terms mostly led Google Trends "thyroid" searches, in the second time period. This time frame/sequence particularly in the second time period, which was noted by the preponderance of the SARS-CoV-2 delta variant, lends some credence to associations of COVID-19 cases *per se* with (apparent) thyroid disease (*via* searches for them). Moreover, this finding, points to a possible higher probability of thyroid disease with SARS-CoV-2 delta variant compared to the alpha variant (and may also explain discrepancies regarding COVID-19 *vs* thyroid disease among previous relevant studies).

Digital health is in the spotlight as the COVID-19 crisis progresses[24,25]. At the same time, digital epidemiology is emerging at a very fast pace[25]. More and more of what we do and say - including epidemiologically relevant behaviors - is stored electronically, often in an accessible form. Internet data mining has a revolutionary impact on the way we monitor global health and health behaviors. Infectious and chronic disease data can be collected and disseminated in almost real time through a number of online sources. Google Trends provides a powerful measure of public interest in a topic,

Table 1 Positive cross correlation function values between variables; only significant values are presented (please see text for details)

		1st time period	2nd time period
COVID-19 cases *vs*	"Smell"	CCF: +0.644; Lag: 0	CCF: +0.540; Lag: 0
	"Taste"	CCF: +0.604; Lag: 0	CCF: +0.433 to +0.368; Lag: -2 to 0
	"COVID-19"	--	CCF: +0.412 to +0.315; Lag: -3 to 0
	"SARS-CoV-2"	--	CCF: +0.677 to +0.589; Lag: -2 to 0
	"Thyroid"	CCF: +0.323 to +0.315; Lag: -8 to -7	CCF: +0.412 to +0.343; Lag: -8 to -7
"COVID-19" *vs*	"Thyroid"	--	CCF: +0.374; Lag: -5
"SARS-CoV-2" *vs*		--	CCF: +0.323; Lag: -7

COVID-19: Coronavirus disease 2019; CCF: Cross correlation function; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

being a proxy of internet searches for it. The frequency of internet searches for disease terms may not reflect directly the epidemiological characteristics of a given disease, which is related and/or described by such search terms. Media coverage may skew subsequent internet searches. Nevertheless, the frequency of internet queries for various diseases' symptoms are correlated to a degree with physician visits for these diseases[26,27]. Google Trends has been used – despite its shortcomings – to monitor the yearly influenza epidemics[24,28]. Another source that has provided health data is Twitter. A smartphone application can be used to assess COVID-19 symptoms and may indicate future disease hotspots within 5-7 d[29]. The collection and classification of data ranging from the detection of suspected cases to the monitoring and assessment of pandemic risk is crucial. However, as this is a very evolving field, validation of digital health measures vis-à-vis input data, tentative associations, or predictive models is still needed. Regarding COVID-19, the influence of media on Google Trends RSVs has been studied and was found to be maximal after a week[30], whereas the effect of COVID-19 cases on "COVID-19" searches has been studied[31], and has been found to be most notable after 11.5 d[32]. Thus, with the lags in the observed cross-correlation factors, we believe that the Google Trends searches for COVID-19 and/or thyroid-related items may reflect personal interest fuelled by probable real disease (COVID-19 or thyroid disease).

Receptors for the SARS-CoV-2 virus are found in tissues beyond the respiratory system, such as the thyroid, thus an effect of COVID-19 on the thyroid is plausible[33]. Indeed, there is some evidence of thyroid dysfunction in patients with COVID-19, characterized by changes in hormone levels (low triiodothyronine or low thyrotropin levels) or laboratory results compatible with the presence of subacute thyroiditis[20,34]. Italian researchers observed that in the spring of 2020, 15% of COVID-19 patients (n = 93) admitted to the intensive care unit (ICU) at a hospital in Milan had changes in thyroid hormones. By comparison, only 1% of patients in the same period of 2019 (n = 101) had changes in thyroid hormones [19]. Considering the fact that viral infections can cause thyroiditis, the researchers began a monitoring program to look at thyroid function 3 mo after COVID-19 treatment. The researchers found that thyroiditis, in patients with moderate to severe COVID-19, was different from common subacute thyroiditis: Many patients had mild dysfunction and the rate of thyroid disease was higher in men. Thyroid dysfunction appeared to be associated with more severe COVID-19 disease. After 3 mo, thyroid function was normal in all followed patients (n = 53), with persistence of ultrasound findings of thyroiditis in one third of them[35]. Another study from Greece was based on the premise that the interpretation of thyroid tests in ill patients is hampered by changes that ensue in the context of non-thyroidal illness syndrome and studied thyroid function in cohorts of COVID-19 positive (n = 102, 46 in the ICU) and COVID-19 negative patients (n = 94, 41 in the ICU)[18]. The researchers noted a non-thyroidal illness syndrome pattern in 60% of ICU and 36% of ward patients (with no significant differences between COVID-19 positive and negative patients)[18]. The thyroid laboratory work-up was compatible with thyrotoxicosis in 14.6% of SARS-CoV-2 positive ICU patients *vs* 7.7% in SARS-CoV-2 negative ICU patients (P = NS) and, overall in 8.8% of SARS-CoV-2 positive *vs* 7.4% of negative patients. Thus, the authors concluded that a non-thyroidal illness syndrome pattern is common in COVID-19 but it relates to the severity of disease rather than SARS-CoV-2 infection, whereas a thyrotoxicosis pattern was less frequently observed and was not different between patients with and without COVID-19[18].

Our study has several limitations and its caveats have to be considered. We collected only Google Trends data for English-language searches; however, we have shown in an older study that searches in this language dwarf searches in all other languages[4]. Additionally, Northern hemisphere internet searches dwarf Southern hemisphere searches[4]. Analyses were done on a weekly worldwide basis since Google Trends searches for extended time periods are provided as such. From the literature, worldwide and weekly or monthly Google Trends data are considered to be more reliable than country-wide and daily data[36,37]. No periodicity in the data was assessed since the total time duration of data collection was rather short. As stated above, the datasets were split in half given the vast differences in

COVID-19 epidemiology in 2020-2021 due to the preponderance of different SARS-CoV-2 variants. Finally, we have to bear in mind the fact that Google Trends searches are limited to internet-literate persons, who are easily influenced by media items, although few (medical) research articles are reported by news outlets (targeting diverse audiences) and generate public interest[38].

CONCLUSION

Given the relatively recent onset of SARS-CoV-2 virus infection, the available monitoring data are limited in time and therefore long-term studies are needed to evaluate even longer-term effects on the endocrine glands. Research into the virus continues to grow, shedding more light on the real health risks posed by COVID-19. Ideally, it would be interesting to assess time and localization-delimited Google Trends searches with the corresponding thyroid disease incidence, as reported by physicians or as recorded in healthcare databases, to verify the associations observed. Understanding the nature of a pandemic of this magnitude means saving human lives and proper knowledge of ways to prevent further infection.

ARTICLE HIGHLIGHTS

Research background

Google Trends searches for symptoms and/or diseases may reflect actual disease epidemiology. Recently, Google Trends searches for coronavirus disease 2019 (COVID-19)-associated terms have been linked to the epidemiology of COVID-19. Some studies have linked COVID-19 with thyroid disease.

Research motivation

Since the use of Google Trends to study a wide range of medical topics is becoming more widespread and the available research on COVID-19-related thyroid disease is conflicting, with this work we aimed to look at the issue of COVID-19-related thyroid disease from a different angle, namely, that of digital epidemiology, since the latter may be a useful adjunct to classical epidemiology.

Research objectives

We assessed worldwide COVID-19 cases *per se vs* COVID-19-associated Google Trends searches and thyroid-associated Google Trends searches for 92 wk.

Research methods

We collected data on worldwide weekly GT searches regarding "COVID-19", "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)", "coronavirus", "smell", "taste", "cough", "thyroid", "thyroiditis", and "subacute thyroiditis" for 92 wk and worldwide weekly COVID-19 cases' statistics in the same time period. The study period was split in half (approximately corresponding to the preponderance of different SARS-COV-2 virus variants) and in each time period we performed cross-correlation analysis and mediation analysis.

Research results

Significant positive cross-correlation function values were noted in both time periods. More in detail, COVID-19 cases *per se* were found to be associated with no lag with Google Trends searches for COVID-19 symptoms in the first time period and in the second time period to lead searches for symptoms, COVID-19 terms, and thyroid terms.

Research conclusions

Searches for a non-specific symptom or COVID-19 search terms mostly led Google Trends thyroid-related searches, in the second time period. This time frame/sequence particularly in the second time period (noted by the preponderance of the SARS-COV-2 delta variant), lends some credence to associations of COVID-19 cases *per se* with (apparent) thyroid disease (*via* searches for them).

Research perspectives

Given the relatively recent onset of SARS-CoV-2 virus infection, the available monitoring data are limited in time and therefore long-term studies are needed to evaluate even longer-term effects on the endocrine glands. Research into the virus continues to grow, shedding more light on the real health risks posed by COVID-19. Ideally, it would be interesting to assess time and localization-delimited Google Trends searches with the corresponding thyroid disease incidence, as reported by "sentinel" physicians or as recorded in healthcare databases, to verify the associations observed. Understanding the nature of a pandemic of this magnitude means saving human lives and proper knowledge of ways

to prevent further infection.

FOOTNOTES

Author contributions: All authors conceived this work, searched the literature, analyzed the data, performed the analyses, and wrote this manuscript.

Institutional review board statement: The statement is not applicable since this is a web-based data study.

Clinical trial registration statement: The statement is not applicable since this is a web-based data study.

Informed consent statement: The statement is not applicable since this is a web-based data study.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

Data sharing statement: All the data for this study can be obtained from the publicly available sources https://coronavirus.jhu.edu/map.html&https://trends.google.com.

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S-Editor: Liu JH
L-Editor: Wang TQ
P-Editor: Liu JH

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World Journal of Methodology

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World J Methodol 2022 May 20; 12(3): 107-112

DOI: 10.5662/wjm.v12.i3.107

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Observational Study

Lutetium in prostate cancer: Reconstruction of patient-level data from published trials and generation of a multi-trial Kaplan-Meier curve

Andrea Messori

Specialty type: Microscopy

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Liang L, China; Ogino S, United States

Received: October 26, 2021
Peer-review started: October 26, 2021
First decision: December 17, 2021
Revised: December 18, 2021
Accepted: March 16, 2021
Article in press: March 16, 2021
Published online: May 20, 2022

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Abstract

BACKGROUND

Lutetium has been shown to be an important potential innovation in pre-treated metastatic castration-resistant prostate cancer. Two clinical trials have evaluated lutetium thus far (therap and vision with 99 and 385 patients, respectively), but their results are discordant.

AIM

To synthetize the available evidence on the effectiveness of lutetium in pre-treated metastatic castration-resistant prostate cancer; and to test the application of a new artificial intelligence technique that synthetizes effectiveness based on reconstructed patient-level data.

METHODS

We employed a new artificial intelligence method (shiny method) to pool the survival data of these two trials and evaluate to what extent the lutetium cohorts differed from one another. The shiny technique employs an original reconstruction of individual patient data from the Kaplan-Meier curves. The progression-free survival graphs of the two lutetium cohorts were analyzed and compared.

RESULTS

The hazard ratio estimated was in favor of the vision trial; the difference was statistically significant ($P < 0.001$). These results indicate that further studies on lutetium are needed because the survival data of the two trials published thus far are conflicting.

CONCLUSION

Our study confirms the feasibility of reconstructing patient-level data from

survival graphs in order to generate a survival statistics.

Key Words: Survival analysis; Individual patient data reconstruction; Kaplan-Meier curves; Meta-analysis; Prostate Cancer; Lutetium

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Core Tip: This paper describes the application of a new technique of individual-patient data reconstruction to the progression-free survival curves published in two trials evaluating lutetium in metastatic prostate cancer. Our analysis interpreted these survival data and showed discordant results between the two trials, that need to be addressed by further clinical research.

Citation: Messori A. Lutetium in prostate cancer: Reconstruction of patient-level data from published trials and generation of a multi-trial Kaplan-Meier curve. *World J Methodol* 2022; 12(3): 107-112
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/107.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.107

INTRODUCTION

Lutetium has been shown to be an important potential innovation in pre-treated metastatic prostate cancer, but the extent to which outcomes are improved by this treatment still needs to a fully investigated. Three studies have evaluated lutetium in this disease condition. One was phase II (therap trial[1]), the second was phase III (vision trial[2]); the third, which was an observational real-world study[3], differed from the first two because lutetium was given after radium-223.

In recent times, techniques that reconstruct individual patient data from the graphs of Kaplan-Meier curves have considerably improved in terms of performance and easy applicability[4]. One advantage is that the availability of these techniques permits to combine multiple survival curves published in different trials without using any meta-analytical statistics. An example of this approach is presented herein. Our objective was two-fold: 1) to quantify the gain in progression-free survival determined by lutetium: 2) to demonstrate the applicability of techniques of patient-level data reconstruction in addressing specific questions based on time-to-event endpoints without the need to employ any meta-analytic statistics.

MATERIALS AND METHODS

We applied the shiny technique of individual patient data reconstruction[4] to the Kaplan-Meier graphs of progression-free survival reported in the therap phase-II trial[1] and in the vision phase III trial[2]. Both trials were conducted in patients with metastatic castration-resistant prostate cancer previously treated for their metastatic disease. In the therap trial, the treatment group received Lu-PSMA-617 (6·0–8·5 GBq intravenously every 6 wk for up to six cycles) while the controls were given cabazitaxel (20 mg/m^2 intravenously every 3 wk for up to ten cycles). In the vision trial, the treatment group received ^{177}Lu-PSMA-617 (7.4 GBq every 6 wk for four to six cycles) plus protocol-permitted standard care while the controls received standard care alone. In the therap trial, progression-free survival was defined as the interval from randomisation to first evidence of pupil-size artefact progression defined by an increase of at least 25% and at least 2 ng/mL after 12 wk (as per PCWG316), radiographic progression using locally reported computed tomography and bone scanning [Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 and PCWG3 criteria for bone lesions], commencement of non-protocol anticancer treatment, or death from any cause. In the vision trial, the end-point was image based progression free survival.

The progression-free survival graphs of the two lutenium cohorts by Hofman *et al*[1] for the therap trial (99 patients; follow-up of 18 mo; 90 events) and Sartor *et al*[2] for the vision trial (385 patients; follow-up of 30 mo; 254 events). For each of these two Kaplan-Meier curves, the graph was digitalized and converted into x-y data pairs using Webplotdigitizer (version 4.5, https://apps.automeris.io/wpd/). Then, the shiny package (version: 1.2.2.0; subprogram "Reconstruct Individual Patient Data"; https://www.trialdesign.org/one-page-shell.html#IPDfromKM, see reference[4]) was used to reconstruct patient-level data on the basis of x-y data pairs, total number of enrolled patients, and total number of events. Finally, the pooled survival curves were generated from the reconstructed patient-level data and analyzed through standard Cox statistics. For this purpose we used three packages

("coxph", "survfit", and "ggsurvplot") under the R-platform. The hazard ratio (HR) was estimated.

RESULTS

The shiny procedure combined with standard Kaplan-Meier statistics allowed us to compare the 99 patients given lutetium in the therap trial with the 385 patients given lutetium in the vision trial.

Figure 1 shows the two Kaplan-Meier curves generated from reconstructed patient-level data. The HR estimated from these curves favored the patients of the vision trial and was 0.59 (95%CI, 0.46 to 0.75). The difference was statistically significant ($P < 0.001$).

DISCUSSION

When two or more randomised trials are available on a therapeutic issue and the clinical end-point is the form of time-to-event, synthetising the clinical evidence is a complex issue, and there is presently no consensus on which methodological approach should be preferred[5,6]. Pooling the values of HR is certainly the method most commonly used, but its important limitations have been widely recognised for many years (*e.g.* the inability to account for the length of follow-up, the inability to model variations of risk over time, the dimensionless nature of HR as opposed to the greater informative value of absolute parameters such as medians, *etc.*)[8]. The development of the restricted mean survival time has represented an advancement in this field[8,9], but the use of this parameter unfortunately remains low.

In this context, the marked improvement in performance of techniques that reconstruct individual-patient data[4] represents an important innovation, the role of which still needs to be fully evaluated. On the one hand, reconstructing individual-patient data is a mandatory pre-requisite to determine the RMST, and this explains the increased use of these reconstruction techniques when a single trial needs to be analysed[7]. On the other hand, another potential use of these techniques is being recognised when multiple trials are available: in such cases, these techniques offer a new methodological alternative to standard meta-analytic methods[5,6] and also to the more recent approaches where meta-analysis is based on the use of RMSTs[8,9].

The various parameters mentioned above (especially HR, RMST, and median) have been investigated for many years to identify their respective advantages and disadvantages, and the literature on this issue is wide[7]. In contrast, the literature on the use of reconstructed survival curves is still in its early stages[4,6], and this holds true particularly when multiple trials are analysed and pooled together.

The experience described herein offers a limited but useful contribution to the development of meta-analysis-like methods based on reconstructed survival curves.

The two control groups of the two trials differed in the treatment they received, and so were not included in our analysis, which was focused only on the two lutetium groups of the two trials. In comparing these two group with one another, our results raise the need to explain the statistically different outcomes shown by the HR and presented in Figure 1.

The inclusion criteria of the therap and vision trials were very similar, and so they likely had no substantial role in determining this difference. In fact, in the therap trial, patients had metastatic castration-resistant cancer and PET eligibility criteria for the trial were PSMA-positive disease, and no sites of metastatic disease with discordant FDG-positive and PSMA-negative findings; previous treatment with androgen receptor-directed therapy was allowed. In the vision trial, patients had metastatic castration-resistant prostate cancer previously treated with at least one androgen-receptor–pathway inhibitor and one or two taxane regimens and had PSMA-positive gallium-68 (68Ga)–labeled PSMA-11 and PET scans. While these differences in the inclusion criteria do not seem to suggest a better prognosis for patients included in either trial, a number of factors (*e.g.* environmental and lifestyle factors, tissue biomarkers, molecular pathological epidemiology, the microbiota, *etc.*) might have influenced tumor development and response to therapy. Hence, the discrepancies observed across the two trials included in our analysis might be explained by these factors. As regards innovative treatments such as lutetium, it should be stressed that molecular pathological epidemiology research has a growing role and is increasingly recognized to be a promising strategy to improve prediction of response to therapy.

In summary, the main strength of our analysis lies in the originality of the methodological approach that reflects the recent availability of very efficient patient data reconstruction techniques. The main limitation is represented by the indirect nature of the comparison between the two lutetium cohorts.

CONCLUSION

Our study indicates that further studies on Lu-PSMA-617 are needed because the survival data of the two trials published thus far demonstrate quite conflicting results. The example described in this paper

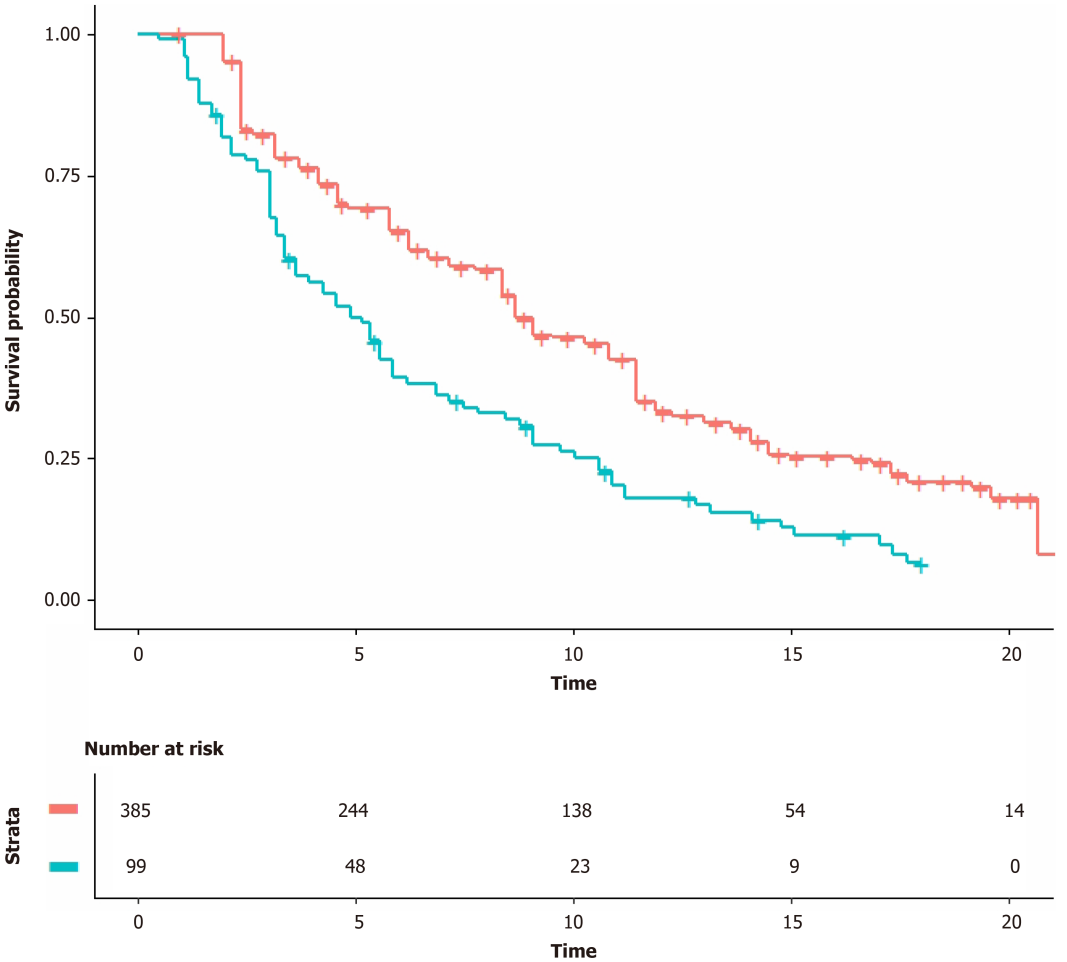

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Figure 1 Kaplan-Meier curves from reconstructed patient-level data. Pooled Kaplan-Meier survival curves obtained by reconstruction of individual patient data from two trials (therap[1] and vision[2]). Vision trial in red, therap trial in blue; time expressed in months. See text for details.

confirms the feasibility of reconstructing patient-level data from survival graphs in order to generate a survival statistics from these reconstructed data. To evaluate the advantages and disadvantages of this new methodological approach, further analyses will be needed.

ARTICLE HIGHLIGHTS

Research background

Two trials have been published to assess the effectiveness of lutetium in metastatic prostate cancer. The need to convert these effectiveness data into a pooled estimate represents a useful opportunity to test an innovative technique of individual patient reconstruction based on the analysis of Kaplan-Meier curves (shiny method).

Research motivation

The main motivation was to test the performance of the shiny method based on a real data-set.

Research objectives

Clarifying the effectiveness of lutetium in metastatic prostate cancer and confirm the reliability of the shiny method as a tool for reconstructing individual patient data.

Research methods

The clinical trials that have thus far evaluated lutetium in metastatic prostate cancer have been identified by standard literature search. A pooled survival curve has been generated from these trials by using the shiny technique of individual patient data reconstruction.

Research results

Two clinical trials were identified. A pooled Kaplan-Meier survival curve was generated that synthesizes the current evidence on the effectiveness of this treatment in this disease condition.

Research conclusions

A two-fold conclusion: First, lutetium is effective in metastatic prostate cancer; second, the Shiny technique can successfully be used to pool survival data from two trials without employing any meta-analytical method.

Research perspectives

The shiny technique has been confirmed to be a useful new tool for analyzing survival data from multiple trials and therefore deserves to be further applied in the analysis of clinical evidence.

FOOTNOTES

Author contributions: Messori A is the sole author, read and approved the final manuscript.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Data sharing statement: No additional data are available.

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L-Editor: A
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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 May 20; 12(3): 113-121

DOI: 10.5662/wjm.v12.i3.113

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Observational Study

Airway management training program for nurses *via* online course in COVID-19 preparedness

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Specialty type: Anesthesiology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Alberca RW, Brazil; Ng DTK, China

Received: December 17, 2021
Peer-review started: December 17, 2021
First decision: March 24, 2022
Revised: March 27, 2022
Accepted: May 7, 2022
Article in press: May 7, 2022
Published online: May 20, 2022

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Abstract

BACKGROUND

Nursing officers are an integral component of any medical team. They participate in taking care of basic airway management and assist in advanced airway management, specifically amidst the current coronavirus disease 2019 (COVID-19) pandemic.

AIM

To assess the efficacy of a standardized web-based training module for nurses in preparedness to fight against COVID-19.

METHODS

The training was held in three sessions of 1 h each, consisting of live audio-visual lectures, case scenarios, and skill demonstrations. The sequence of airway equipment, drug preparation, airway examination, and plans of airway management was demonstrated through mannequin-based video-clips.

RESULTS

Pre- and post-test scores as well as objective structured clinical examination scores were analyzed using Student's *t*-test and the Likert scale was used for feedback assessment. It was found that the mean score out of the total score of 20 was 8.47 ± 4.2 in the pre-test, while in the post-test it was 17.4 ± 1.8 (P value < 0.001). The participants also felt self-reliant in executing the roles of airway assistant (63.3%) and drug assistant (74.3%). Fear of self-infection with COVID-19 was also high, as 66% of participants feared working with the patient's airway.

CONCLUSION

Amidst this COVID-19 emergency, when the health care systems are being

persistently challenged, training of nursing staff in the safe conduct of airway management can ensure delivery of life-saving treatment.

Key Words: COVID-19; Nursing; Airway management; Online; Training; Preparedness

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Core Tip: The health care response systems are being persistently challenged by coronavirus disease 2019 (COVID-19). Nurses are actively involved in various tasks of airway management like preparation of airway equipment, drugs, and basic airway management. This study demonstrated a gross lack of knowledge regarding airway management despite receiving basic life support training. The participants felt more self-reliant and confident in executing the roles of airway assistant and drug assistant after the session. There is a need to train nursing staff from different subsets of practice in the safe conduct of airway management and simulation based online training program for health professionals can be employed for preparedness against COVID-19.

Citation: Gupta B, Jain G, Pathak S, Mishra P, Kumar H, Rao S. Airway management training program for nurses *via* online course in COVID-19 preparedness. *World J Methodol* 2022; 12(3): 113-121
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/113.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.113

INTRODUCTION

Coronavirus infection is a public health emergency of international concern[1]. The frontline health care workers are at a heightened risk of catching the disease[2]. Nearly 15% of coronavirus disease 2019 (COVID-19) patients require hospitalization and oxygen support and 5% require definitive airway management. As it primarily involves the respiratory system, the caring medical team should acquire airway management skills[3]. Nursing officers are an integral component of such team, taking care of basic airway management and assisting in advanced airway management[4]. The World Health Organization (WHO)'s prescribed norm is one doctor and three nurses for 1000 people. A wide disparity, however, prevails in the health care professional to population ratio, with developing nations having poorer statistics. The nurse/population ratio is 2.1 in India, as per the latest available WHO's global health workforce statistics[5]. This highlights our currently overloaded staff and also the necessity to keep our health care workers safe while dealing with COVID-19 patients as we cannot afford to lose the already worn-down workforce[6]. Acquiring adequate skills for airway management demands both technical proficiency and clinical knowledge. A lapse in judgment can contribute to increased morbidity and mortality in critically ill patients. Due to the threat of viral contamination during face-to-face training, online teaching is rising as the new norm of education. Thus, with the safety of health care workers as our chief priority, we designed an interactive online airway course to increase the ability of nursing officers in airway management of critically sick patients. The aim of the study was to assess the efficacy of a standardized web-based training module in preparedness to fight against COVID-19 and enhance Emergency Airway Response Team, knowledge, team dynamics, and personnel confidence.

MATERIALS AND METHODS

Study design

After institutional ethical approval (study registration No. AIIMS/IEC/20/283), we conducted a prospective, observational study over a period of 4 mo at the Advanced Center of Continuous Professional Development (CPD) Department through a dedicated online course conducted thrice weekly in our tertiary care institute. Our study was designed following the STROBE guidelines. A list of 30 participants (nursing officers) per session was prepared and we ensured a uniform representation from each department. Inability to attend the course due to prior commitment or network issues led to exclusion from that session and such participants were subsequently included in next scheduled course. Course content was diligently constructed to cover information regarding pandemic preparation, COVID-19 spread, risk alleviation, education about personnel protective equipment (PPE), protection required during airway procedures, signs of respiratory distress, indications of intubation, airway assessment, difficult airway predictors, airway management guidelines and sequence of plan, catalogue

of airway equipment and COVID-19 intubation kit, drugs, procedure of rapid sequence induction, mask ventilation using vice grip, steps of video-laryngoscopy, intubation, supraglottic airway placement, and front of neck access (FONA).

Through the online portal of "Google meet", the training was held in three sessions of 1 h each, consisting of live audio-visual relay of lectures, case scenarios, presentations, and skill station. The sequence of personal protection, airway equipment and drug preparation, designated COVID-19 isolation area for airway management, clinical airway examination with difficult airway assessment using MACHOCHA score, and plans of airway management (Plans A, B, C, and D) were demonstrated through simulator mannequin-based video-clips. The skill stations consisted of 1 h and included demonstration of preparation of appropriate equipment and drugs required for induction in a trolley, designation of negative pressure isolation room for intubation, team dynamics, plans of airway management, use of airway adjuncts, intubation using video-laryngoscope (Plan A), choosing appropriate size of supraglottic airway device and its insertion (Plan B), bag-mask ventilation using vice grip (Plan C), and equipment required (Plan D)-surgical scalpel cricothyroidotomy/FONA by the instructors *via* videos and skill stations. Participants could clarify their doubts by speaking through the microphone or writing it in the common chat window. To ensure an active participation, interaction of participants with instructors in the language that they were most comfortable with was encouraged. Each scenario was followed by a debriefing session, after which the participants were encouraged to enlist their achievements and shortcomings from the session.

Data analysis

The participants were provided with Google form links of "pre- and post-test questionnaire". Both the questionnaire forms were identical and consisted of 20 multiple-choice questions (1 mark each), which included specific theoretical questions related to airway management. The participants also had to answer 10 objective structured clinical examinations (OSCE), each consisting of one mark each. Each participant's performance during the skill stations was independently evaluated by two experienced instructors based on OSCE response. For a successful completion of training program, it was necessary for the participants to obtain 70% of marks in the post-test and more than 80% in OSCE assessment. A feedback form was filled at the end of the session, consisting of eight assertions on a 5-point rating Likert scale. The score of "5" indicated "strong agreement" with the statement while a score of "1" indicated that participants were in "strong disagreement" with it. Two faculty members, experts in airway management, validated the questionnaire and survey form at an independent level. An investigator who was blinded to the study protocols collected and then analyzed the outcome data. The basis for sample size estimates was convenience sampling.

Statistical analysis

The Statistical Package for the Social Sciences version 23.0 software (SPSS, IBM Corp. Armonk, NY, United States) was utilized to perform statistical analyses. A pre- and post-test questionnaire, specifically developed for this course, was analyzed as the primary outcome. The secondary outcome was evaluated as OSCE based assessment. The results are summarized as descriptive statistics and presented as the mean ± SD or mean ± SE. The Student's *t*-test was employed to analyze the data for intra- and inter-group comparisons. To assess the survey form, a mean Likert score was averaged to the total number of items. A P value < 0.05 was considered statistically significant.

RESULTS

A total of 1055 nursing officers were trained during the program. One hundred and nine participants who could not complete either the pre- or post-test were excluded from the analysis. Nine hundred and forty-six nursing officers were able to complete the pre- and post-test and thus included in the final analysis (Figure 1). The mean years of work experience of the participants was 4.01 ± 3.16 (mean ± SD). On analyzing the questionnaire, it was found that the mean score out of the total score of 20 was 8.47 ± 4.2 in the pre-test, while in the post-test it was 17.4 ± 1.8 (Figure 2); the difference was statistically significant (P < 0.001). Although 68% of our participants were trained in basic life support (BLS), questions in the pre-test, based on the specific knowledge of airway and plans for airway management, were frequently missed. The concept of team dynamics and role allocation was also alien to the majority of nursing officers. The overall knowledge and cognizance regarding airway management of COVID-19 patients improved significantly following the session (P < 0.001). Approximately 92% of the participants accurately responded to specific questions related to airway management in the post-test. There was improvement in OSCE based assessment and all participants could score above 80% in OSCE.

Participants were asked to provide feedback at the end of session. Amongst the various questions asked in feedback, one was pertaining to the part of training which they found most helpful. The video demonstration of airway procedures, preparation of airway trolley, and medications was the most cherished by the nursing officers. After attending the program, 79% of participants felt that they were familiar with airway management techniques and protocols for COVID-19 patients. The participants

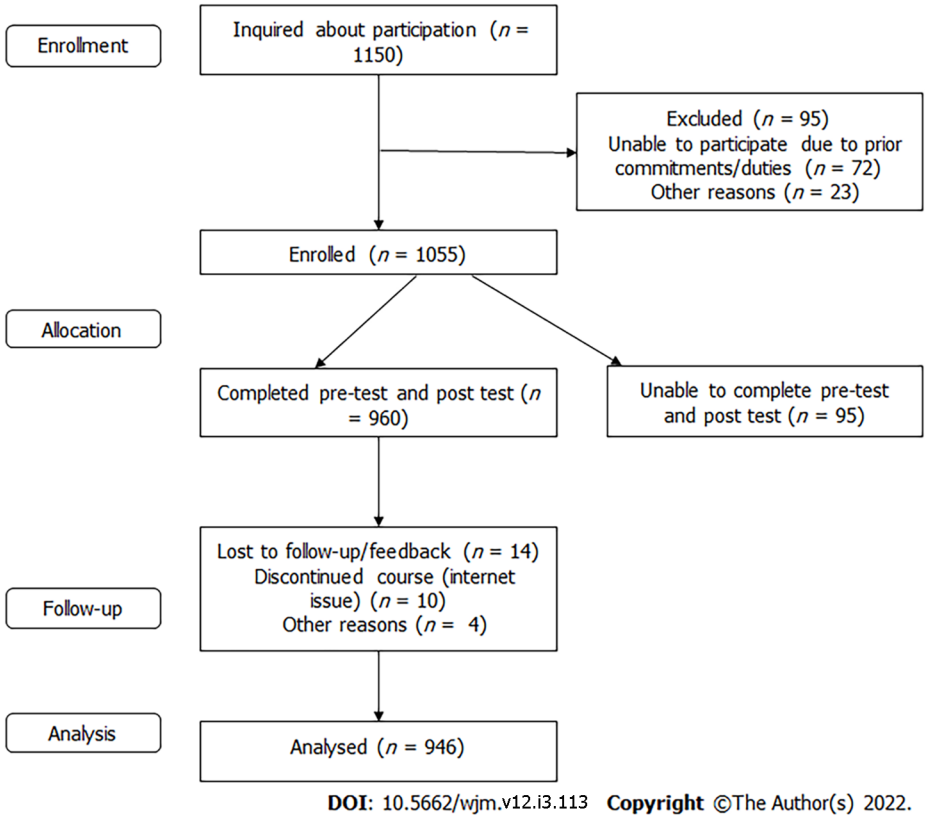

Figure 1 Flow diagram for participant enrollment and analysis.

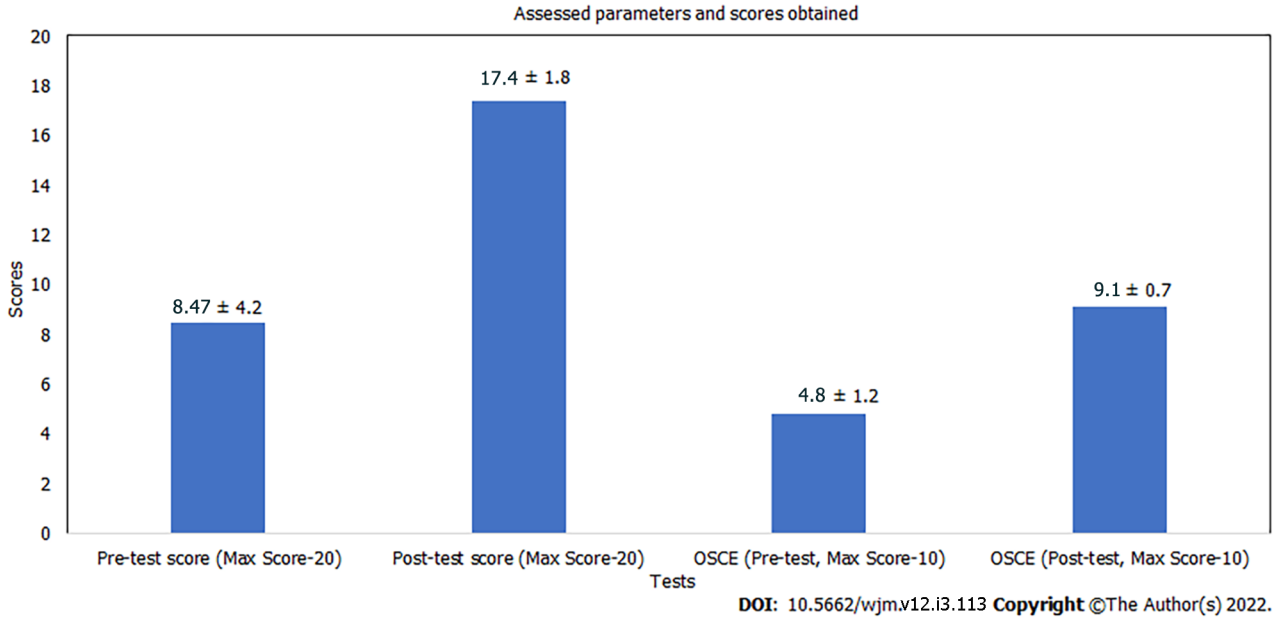

Figure 2 Scores obtained in pre-test, post-test, and objective structured clinical examinations.

also felt self-reliant in executing the roles of airway assistant (63.3%) and drug assistant (74.3%). An increase in level of self-confidence was reflected in other parameters like performing laryngoscopy, supraglottic airway device (SGA) insertion, and arrangement of necessary equipment as well (Table 1). Fear of self-infection with COVID-19 was also high, as 66% of participants feared working with the patient's airway (Table 2). This short online training module for airway management in COVID-19 patients was liked by majority of our participants and they strongly believed that it helped in improving their clinical acumen and skills.

Table 1 Level of confidence in various roles amongst study participants (scores 1 to 5, with 1 meaning strongly disagree and 5 meaning strongly agree)

S. No.	Role/procedure	Percentage of participants with a score ≥ 4	Percentage of participants with a score of 3	Percentage of participants with a score ≤ 2
A Pre-training assessment				
1	Airway assistant	35	31	34
2	Drug assistant	41	45	14
3	Laryngoscopy	9	15	76
4	SGA insertion	14	28	58
5	Familiarity with airway management plan	29	24	47
B Post-training assessment				
1	Airway assistant	63.3	21.6	15.1
2	Drug assistant	74.3	23	2.7
3	Laryngoscopy	58	13	29
4	SGA insertion	78	19	3
5	Familiarity with airway management plan	79	14	7

SGA: Supraglottic airway devices.

Table 2 Reasons for fear regarding management of coronavirus disease 2019 patients

S. No.	Reason for fear	Percentage of participants
1	Breach in PPE	49
2	High aerosol generation	34
3	Lack of airway experience	38
4	Cross-infection	57
5	Difficult airway situation	23
6	No fear	18

PPE: Personnel protective equipment.

DISCUSSION

The role of nursing staff in any health care service is indispensable. They form a pivotal part in the patient care in wards, emergency area, outpatient department (OPD), operation theatres, high dependency units, and intensive care units (ICU). They are actively involved in various tasks of airway management like preparation of airway equipment and drugs, checking for adequate resources like oxygen, airway suctioning, and basic airway management[7]. There is a high probability that the first responder to any patient with respiratory urgency is a nurse who might have to manage airway till a physician help arrives[8]. In such a scenario, the lack of knowledge and experience in airway management can not only jeopardize patient care but also result in a heightened risk of infection transmission[9]. The goals for the airway rescuer in COVID-19 patients is to rapidly secure an airway, preferably in first attempt, with clear backup contingencies, while reducing the aerosol generation and preventing redundant contamination[10-12]. European Society guidelines for management of airway in COVID-19 patients, recommend endotracheal intubation using rapid sequence intubation (RSI) for Plan A; in the advent of failure of plan A, SGA placement as Plan B; face mask ventilation as Plan C; and finally FONA as Plan D[13]. Our prime expectation from this training module was to equip the nursing officers with adequate information, clearing their queries and fears related to COVID-19 patient care such that they could efficiently work in a high performing airway rescue team without compromising personal safety. Although the pretest score was as low as 8.47 ± 2.4, by the end of our session, the respondents were clear with these features and achieved a high score of 17.4 ± 1.8. It was evidenced by

their poor performance in the pre-test questionnaire regarding airway assessment and difficult airway predictors like modified Mallampati grade and MACOCHA score. The reason for this could be accredited to the mixed population from different practice areas (general wards, OPDs, operation theatres, emergency, and ICU) and the years of experience. We succeeded in educating them about airway assessment sufficiently enough to perform significantly better in the post-session analysis with the same set of questions.

Although the regular curriculum of nursing does impart education about basic airway support, it is not emphasized enough. The proportion of respondents (58%) who were already trained in BLS had a better understanding of basic airway care as compared to those who had not completed BLS course. This was evident by their fair knowledge about identification of respiratory distress, indications for intubation, and basic equipment in airway management. The majority of participants (71%), however, showed gross deficit in information with respect to the plans for airway management, drugs required for RSI, advanced airway equipment like video laryngoscope and procedure of intubation, supraglottic airway device insertion, and FONA. This revealed the necessity to train them in both basic and advanced airway care so that in the crisis, they can play the role of competent assistants in airway management.

Continuing medical education programs, workshops, and seminars comprise an efficient approach to achieve proficient teaching and learning[14,15]. Conforming to the principle of social distancing amidst this highly infectious health emergency, simulation-based medical education has an important role in learning. Various international recommendations include airway training simulation as a part, which has shown to be beneficial with respect to behavior changing process, acquisition of skills, and trainee satisfaction[16].

A wide range of airway complications and increased viral transmission may occur if nurses who are involved in such teams have no experience in emergency airway management[17,18]. Cook *et al*[19] documented that permanent harm or death due to airway related complications was mainly due to inadequate access to properly skilled staff or equipment, inability to identify at-risk patients, poor planning, and lack of structured strategies for tackling predictable airway complications.

It is noteworthy that registered nurses, even those working in ICU, may spend a larger fraction of working hours in patient care, without the requirement to manage respiratory emergencies on an everyday basis[20]. This in itself reveals the state of experience of nurses working in non-ICU environment with respect to airway care. Kelleher *et al*[21] conducted a study to investigate the endotracheal care practices amongst critical care nurses and found a wide variety in their techniques, with non-adherence to best practice recommendations and resultant lower-quality care. Another descriptive analytic study showed that the knowledge and performance of intensive care nurses regarding endotracheal suctioning and care was good (71.6 ± 10.91) and medium (41.22 ± 7.91), respectively[22].

The key to effective airway management is proper assessment and anticipation of any associated difficulties[23]. The foundation of any high performing team is a strong understanding of team dynamics. Ranging from deploying of the scarce available resources or employing the latest evidence-based guidelines to building a firm groundwork of healthy teamwork with good and clear communication can provide a strategic lead in tackling this pandemic. Our aim was to emphasize on the clear role allocation, closed loop communication, and cross monitoring (checking for cross- contamination) while working in the airway rescue team. There was a statistically significant improvement in terms of knowledge and confidence in competent role execution as airway team members in the post survey analysis as compared to their pre-test evaluation.

The feedback submitted by the participants highlighted the truth that this global crisis has fostered fear among all healthcare workers. The majority of the participants admitted that the fear was mainly based on risk of breach in PPE, aerosol spread, lack of proper training in airway prior to actual patient handling, and fear of contracting infection and carrying the infection back home amongst others. These responses go in line with a study done in healthcare workers working with COVID-19 patients that revealed higher anxiety, depression, and apprehension due to similar factors among 71.5%, 44.6%, and 50.4% of the respondents, respectively[24,25].

This training module highlighted the need to put more emphasis on airway training of the nursing staff and contributed to fill up the lacunae in the realm of airway care while giving due weightage to occupational safety and health. We believe that by using simulation based online training program for nurses, we successfully educated them and simultaneously strengthened our workforce in airway management, if and when the need arises.

Strengths

Our study adds to the theoretical development of efficacy of online simulation-based training of health care professionals in inevitable situations like the COVID-19 pandemic. To the best of our knowledge, this is a novel study to train nurses for airway management of COVID-19 patients through an online platform and gives evidence of statistically significant improvement in knowledge, attitude, and confidence regarding the same. We took extra care to reach up to individual level participation and trained them in the language that they understood well. Free will to attend the training program as many times needed was the additional advantage of our course.

Limitations

We completely acknowledge that the chief and inevitable limitation in our study was an inability to conduct the skill station training in person. The heterogeneous study population with diverse levels of exposure to airway care was another limitation. Although the majority of participants passed the post session evaluation, 140 of them had to repeat this course once due to sub-par scoring. The infrequent issues with internet connectivity, first time online course learning, difficulty to comprehend, and language disturbance were responsible for inefficiency in understanding, leading to poor response in post-test analysis and hence the need for repetition of course.

CONCLUSION

Amidst this COVID-19 public health emergency, when the health care response systems are being persistently challenged, training of nursing staff from different subsets of practice in the safe practice of airway management can play a substantial role in ensuring access of life-saving treatment to COVID-19 patients, without compromising the safety of health care professionals. Our study in its unique aspect has the potential to pave way for further large-scale research while confirming to incorporate similar training regimes aimed at improving the preparedness and skill of various health professionals to tackle this crisis efficiently.

ARTICLE HIGHLIGHTS

Research background

The nursing officers are an integral part of medical team. They contribute in basic airway management and as an assistant in advanced airway management, which holds great significance in the coronavirus disease 2019 (COVID-19) pandemic.

Research motivation

The pandemic has resulted in over-burdened medical staff with lack of adequate skills for airway management to handle this respiratory disease pandemic.

Research objectives

The primary research objective was to create an interactive online airway course to increase the ability of nursing officers in airway management of critically sick patients.

Research methods

The training was conducted through live audio-visual lectures, case scenarios, and skill demonstrations through mannequin-based videos. The demonstrations for airway equipment, preparation of drugs, airway examination, and plans of airway management were done.

Research results

The mean score out of the total score of 20 was 8.47 ± 4.2 in the pre-test, while it was 17.4 ± 1.8 in the post-test ($P < 0.001$). After attending the program, 79% of participants felt that they were familiar with airway management techniques and protocols for COVID-19 patients. An increase in level of self-confidence was reflected in other parameters like performing laryngoscopy, Supraglottic airway insertion, and arrangement of necessary equipment as well.

Research conclusions

The training of nursing staff from different subsets of practice in the safe practice of airway management can play a substantial role in ensuring access of life-saving treatment to COVID-19 patients, without compromising the safety of health care professionals.

Research perspectives

This research has the potential to pave way for further large-scale research while confirming to incorporate similar training regimes aimed at improving the preparedness and skill of various health professionals to tackle this crisis efficiently.

ACKNOWLEDGEMENTS

We would like to acknowledge the contribution of all the coordinators, instructors, and participants of

this training program and Department of Advanced Centre of Continuous Professional Development and Department of Anesthesiology, All India Institute of Medical Sciences, Rishikesh, India.

FOOTNOTES

Author contributions: Gupta B and Jain G designed the research study and performed the research and manuscript editing and review; Pathak S and Mishra P performed the literature search, data analysis, statistical analysis, and manuscript preparation and editing; Rao S and Kumar H performed the study design, research study, and manuscript editing and review; and all authors have read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the AIIMS Institutional Review Board (study registration No. AIIMS/IEC/20/283).

Conflict-of-interest statement: The authors declare that there are no conflicts of interest to disclose.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement, and the manuscript was prepared and revised according to the STROBE Statement.

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S-Editor: Ma YJ
L-Editor: Wang TQ
P-Editor: Ma YJ

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 May 20; 12(3): 122-131

DOI: 10.5662/wjm.v12.i3.122

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Single-use duodenoscopes for the prevention of endoscopic retrograde cholangiopancreatography -related cross-infection – from bench studies to clinical evidence

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Tang J, China; Yang X, China; Yao D, China

Received: November 28, 2021
Peer-review started: November 28, 2021
First decision: January 12, 2022
Revised: January 16, 2022
Accepted: March 16, 2022
Article in press: March 16, 2022
Published online: May 20, 2022

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Abstract

BACKGROUND

Several strategies have been implemented to reduce or abolish the life-threatening risk of endoscopic retrograde cholangiopancreatography (ERCP)-related multidrug-resistant infections due to duodenoscopes contaminations; among those strategies, serial microbiologic tests, thorough reprocessing schedules, and use of removable scope cap have been adopted, but the potential cross-infection risk was not eliminated.

AIM

To review available evidence in the field of single-use duodenoscopes (SUD) use for ERCP.

METHODS

An overview on ongoing clinical studies was also performed to delineate which data will become available in the next future.

RESULTS

One bench comparative study and four clinical trials performed with EXALT model-D (Boston Scientific Corp., United States) have been identified. Of them, one is a randomized controlled trial, while the other three studies are prospective single-arm, cross-over studies. Pooled technical success rate (4 studies, 368 patients) was 92.9% [95% confidence interval (CI): 89.9-95.5; I^2: 11.8%]. Pooled serious adverse event (4 studies, 381 patients) rate was 5.9% [3.7%-8.5%; I^2: 0.0%].

CONCLUSION
Although few clinical trials are available, evidence is concordant in identifying an absolute feasibility and safety and feasibility for SUD use for ERCP. The expertise and quality of evidence in this field are going to be improved by further large clinical trials; data on cost-effectiveness and environmental impact will be needed for a worldwide spread of SUD use for ERCP.

Key Words: Multidrug; Resistance; Contamination; Infection; Reprocessing; Guidelines

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Core Tip: Endoscopic retrograde cholangiopancreatography (ERCP) has significantly changed the management and natural history of patients with biliary and pancreatic diseases. While in the past decades ERCP procedure were considered safe and bearing low-risk for exogenous pathogens transmission, the risk of duodenoscopes contaminations and related cross-infection was recently demonstrated and quantified. To overcome this issue, two different single-use duodenoscopes (SUD) have been developed and are commercially available. The sterile packaging and the disposable intent guarantee to avoid exogenous patient-to-patient cross-infections. A systematic review of all available clinical evidence on the use of SUD for ERCP was performed, demonstrating an overall pooled safety and efficacy. Although few clinical trials are available, evidence is concordant in identifying an absolute feasibility and safety and feasibility for single-use duodenoscopes (SUD) use for ERCP. Future large clinical trials are ongoing to increase the knowledge and quality of evidence in the field; data on cost-effectiveness and environmental impact will be needed for a worldwide spread of SUD use for ERCP.

Citation: Lisotti A, Fusaroli P, Napoleon B, Cominardi A, Zagari RM. Single-use duodenoscopes for the prevention of endoscopic retrograde cholangiopancreatography -related cross-infection – from bench studies to clinical evidence. *World J Methodol* 2022; 12(3): 122-131
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/122.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.122

INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) has significantly changed the management and natural history of patients with biliary and pancreatic diseases[1-3].

Millions of ERCP procedures have been performed annually and this worldwide amount is going to constantly increase due to epidemiological trends of main indications (*i.e.*, biliary stone disease, malignant biliary obstruction), aging of population, and increasing therapeutic applications[4,5].

In recent years, several outbreaks of multi-drug resistant ERCP-related infections have been reported; main risk factors for ERCP-related infections are patients' immunocompromised status and interventional procedures, such as biliary stenting for intrahepatic strictures[6,7]. Contamination of the biliary tract from endogenous gut microbiota bacteria is responsible for the vast majority of post-ERCP infections. However, several issues related to multidrug resistant infections related to duodenoscope contaminations have been reported (*i.e. P. aeruginosa* and carbapenem-resistant enterobacteriaceae)[8,9].

While in the past decades ERCP procedure were considered safe and bearing low-risk for exogenous pathogens transmission, the risk of duodenoscopes contaminations and related cross-infection was recently demonstrated and quantified[10-12].

Food and Drug Administration alerted physicians' community about duodenoscope-related infections in 2015. The peculiar design of these side-viewing instruments was identified as the potential sources of contamination. Indeed, in the tip of the scope is allocated the elevator mechanism with his dedicated cable passing through the scope body; this complex mechanism, despite adequate procedures, is difficult to accurately clean making reprocessing more challenging due to the possible formation of bacteria-containing biofilm[13].

Post-market studies conducted by main manufacturers demonstrated an unexpected higher rate of duodenoscope contamination. A recent meta-analysis tried to overcome the lack of data and quantify the risk of cross-infection in ready-to-use duodenoscopes. A pooled contamination rates up to 15% was identified and none of the available standard reprocessing protocols are able to correctly clean these instruments[14,15].

Several strategies have been proposed to overcome duodenoscope-related infections, such as deep bacterial coltures, improved protocol for reprocessing, and avoiding the use of scopes with fixed cap to allow decontamination. Unfortunately, duodenoscope contaminations could not be avoided with these

strategies[16-19].

Four reusable duodenoscopes with detachable cap are available, from three manufacturers. For a detailed focus on this field, a recent American Society for Gastrointestinal Endoscopy (ASGE) practice guideline was published[4,20].

Two different single-use duodenoscopes (SUD) are commercially available in the US. The sterile single-use package allow the avoidance of exogenous contaminations[4].

The aim of this study was to perform a systematic review of all available clinical evidence on the use of SUD for ERCP.

MATERIALS AND METHODS

Study selection

A systematic literature research was performed through MEDLINE using Pubmed, Google Scholar, and Embase interfaces at the end of November 2021. The search queries were ("duodenoscope"[all fields] OR "single-use"[all fields] OR "disposable"[all fields]) AND "ERCP"[all fields]). Institutional Review Board evaluation for this purpose was not required. Relevant studies were independently analyzed by two authors (AL, RMZ).

Inclusion criteria were: (1) Population: All adult individuals who underwent ERCP; (2) Interventions: SUD use for ERCP; (3) Objectives: Technical success (amount of successfully-completed procedures with SUD among all procedures); and (4) safety: Incidence of ERCP-related complications.

Statistical analysis

Technical success rate and other aims were pooled through a random-effects model based on DerSimonian and Laird test. Heterogeneity was estimated using I^2 tests: I^2 less than 30% was considered low, while $I^2 > 30\%$ but $< 60\%$ was considered weak. Funnel plots inspection was used to assess possible publication bias.

Main objective was the technical success, (completed ERCP using SUD among the entire amount conducted). Secondary objectives were adverse events (AEs).

Statistical analysis was performed with MedCalc package v20 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

RESULTS

Development of single use duodenoscopes

In 2017, several animal studies on porcine and canine models have been conducted with ERCP experts, to evaluate duodenoscope prototypes. Simulated ERCP procedures have been tested with the SUD prototype (Boston Scientific, United States) and a reusable duodenoscope. Involved physicians were asked to rate specific endoscopes tasks qualitatively and quantitatively. These pre-clinical tests allowed the development of the EXALT model-D by Boston Scientific[21].

Bench model comparison

In 2019, ERCP experts from United States completed the first comparative study on two simulators. Three reusable duodenoscopes from the major companies in the field (Olympus Corp., Japan, Pentax Corp., Japan and Fujifilm Holdings Corp., Japan) were compared to EXALT-D using a 0-10 score in four different tasks (and 14 sub-tasks): Guidewire locking with elevator, plastic and metal stents placement and removal, and Dormia Basket passage. The technical success rate for each task and time to achieve the completion was recorded and compared.

The results of this bench study showed that EXALT-D SUD showed similar overall performance, task completion times, tip control and guidewire locking to three different reusable duodenoscopes. Moreover, mechanical scope navigation and image quality was considered excellent (≥ 8 on a scale of 10)[21].

Clinical studies

Four studies have been conducted since the introduction of SUD (study flow diagram according to PRISMA 2009 guidelines is shown in Figure 1); studies are summarized in Table 1.

Muthusamy *et al*[22] conducted in April-May 2019 a multicenter study involving ten US centers and seven ERCP experts; 73 consecutive patients undergoing ERCP have been enrolled. Thirteen patients entered a running "first-in-men study" evaluating the feasibility of ERCP maneuvers. All these "roll-in" procedures (100%) have been successfully completed and the operators stated that they feel confident to extremely-confident in performing ERCP with the SUD.

Table 1 Characteristics of studies assessing the performance of single-use duodenoscope for endoscopic retrograde cholangiopancreatography

Ref.	Region, Study design	Population (no.); male gender (%)	Age (yr, SD)	Naïve papilla (%)	ASGE complexity 3-4 (%)	Technical success (%)	Serious AEs (%)	Note
Muthusamy *et al*[22], 2020	United States, Case-series	No. 60, Male 61.7%	64.4 ± 14.1	26.70%	45.00%	96.70%	6.70%	The study included a roll-in phase with 13 patients
Bang JY *et al* [23], 2020	United States, RCT	No. 48, Male 54.2%	67.2 ± 14.4	100%	16.70%	SUD: 95.8%; Reusable: 100%	4.20%	Primary outcome was no. attempts to achieve cannulation (SUD median 2; reusable 5; P = 0.013)
Napoléon *et al* [24], 2022	France, Prospective	No. 60, Male 43.3%	65.5 ± 13.6	53.30%	40.00%	95%	1.70%	96.7% of cases with optimal operators' satisfaction
Slivka *et al*[25], 2021	United States, Prospective	No. 200, Male 48.5%	62.6 ± 14.0	45.50%	40.50%	90.50%	6.50%	Included 14 expert and 5 "non-expert" ERCP operators with similar outcomes

ASGE: American society for gastrointestinal endoscopy; AEs: Adverse events; SD: Standard deviation; SUD: Single-use duodenoscope; RCT: Randomized controlled trial. ASGE complexity score refers to Cotton PB, Eisen G, Romagnuolo J, Vargo J, Baron T, Tarnasky P, Schutz S, Jacobson B, Bott C, Petersen B. Grading the complexity of endoscopic procedures: results of an ASGE working party. *Gastrointest Endosc* 2011; 73: 868-874 [PMID: 21377673 DOI: 10.1016/j.gie.2010.12.036].

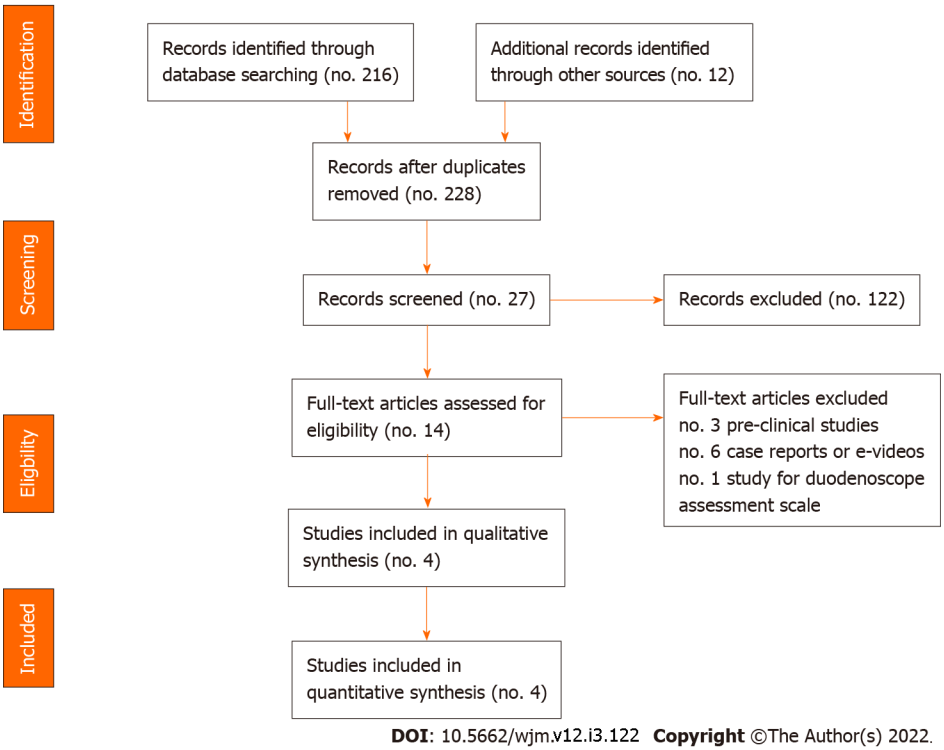

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Figure 1 Study flow diagram according to PRISMA 2009 guidelines.

Sixty consecutive patients have been subsequently enrolled in the study. Most patients (61.7%) were male with a mean age of 64.4 ± 14.1 years. Most cases (73.3%) had a medical history of previous ERCP. In two cases (3.3%), cross-over to a reusable duodenoscope was required due to ERCP technical failure. In one case (tight intrahepatic stricture dilation in a patient with sclerosing cholangitis) the use of a reusable duodenoscope allowed a successful ERCP completion; in one case, papilla showed neoplastic infiltration.

Between January and March 2020, Bang *et al*[23] randomized 98 patients to underwent ERCP with a reusable duodenoscope (TJF-180, Olympus America Inc., United States) or a SUD (EXALT-D, Boston Scientific, United States). Forty-eight patients (54.2% male, 67.2 ± 14.1-year-old) were allocated to the SUD arm and compared to 50 patients (46.0% male, 60.8 ± 18.2-year-old) of the reusable duodenoscope arm; no patient had previous ERCP or bilio-pancreatic intervention. The Authors observed comparable selective cannulation rate (95.8% *vs* 100%), with similar time to reach the papilla (20 *vs* 20 sec); the authors observed that the number of attempt (2 *vs* 5) and time to achieve selective biliary cannulation (35 *vs* 99 sec) were significantly lower in the SUD group.

Napoléon *et al*[24] have recently published the first study conducted outside the US. In this French multicenter study involving six centres, 60 patients (43.3% male, median 65.5 [55-76] year-old) were prospectively enrolled. 95% of the procedures were successfully completed with the SUD, while in three cases the Authors switched to a reusable duodenoscope. In these 3 cases, ERCP could not be completed even with the use of a reusable duodenoscope because of a complete duodenal stricture, a neoplastic infiltration of the ampullary region and a complete biliary stricture; these patients were treated with surgery, EUS- hepaticogastrostomy and percutaneous trans-hepatic drainage, respectively. In this study, 46.7% of patients had previous ERCP. Among the remaining cases, selective biliary cannulation was achieved in 93.8% of cases, after a median of 1 minute and 1.5 guidewire attempts[24].

The results of a large prospective study, conducted in United States, have been published by Slivka *et al*[25]. The Authors enrolled 200 patients undergoing ERCP for various indications; in fact, 40.5% of ERCP procedures presented high complexity (ASGE 3-4). The Authors reported an overall 90.5% technical success rate. Interestingly, this is the first study that included not only expert operators (defined as > 2000 lifetime ERCP performed), but also five "non-expert" operators. The Authors observed that the crossover to a reusable duodenoscope rate (2.5% *vs* 11.3%), the ERCP completion rate (97.5% *vs* 96.3%) and procedure time (28.5 *vs* 25.0 min) were similar among expert and non-expert groups[25].

Safety profile

Muthusamy reported a case of post-ERCP pancreatitis in 1 out of 13 patients involved in the roll-in study. Moreover, they reported 2 post-ERCP pancreatitis (3.3%), one post-sphincterotomy bleeding (1.7%) and one infection of a walled-off pancreatic necrosis in the 60 patients included in the main study. The overall serious adverse event rate was 6.7%[22].

No difference was observed in term of adverse event (AE) and mortality, when ERCPs performed with the SUD were compared to those performed with a reusable duodenoscope. The authors observed two adverse events in the SUD arm (4.2%) compared to 8% adverse event rate in the control group. The Authors reported an ERCP-related mortality of 2.1% and 2%in the two groups, respectively[23].

Napoléon *et al*[24] reported 3 ERCP-related adverse events (5.0%). Of them, two cases were mild (biliary pain and one mild pancreatitis), while one patient (1.7%) presented worsening of underlying condition due to pancreatic cancer and died one week after the procedure[24].

Slivka *et al*[25] reported 13 serious adverse events (6.5%); of them, 5 bleeding, 3 post-ERCP pancreatitis and 2 cholangitis. The incidence of adverse events was similar in expert and non-expert groups (5.0% *vs* 6.9%) and was independent by ASGE complexity grade (low – ASGE 1-2: 7.0% *vs* high – ASGE 3-4: 6.2%).

All studies reported no SUD-related adverse event.

No data on specific SUD contamination after ERCP has been provided in the included studies.

Operators' satisfaction.

Muthusamy reported a median overall satisfaction with the SUD of 9 (range, 1-10). In 4 cases (6.7%) the Authors observed a poor satisfaction (4 or less), due to difficulty of stent insertion, low image quality, and technical issue with the device (turning off during the procedure)[22].

Bang *et al*[23] observed that the SUD present lower image quality and stability comparing to a reusable duodenoscope. From a mechanical point of view, the Authors reported a lower ease to pass into the stomach and frequent dysfunction of air-water valve.

Napoléon *et al*[24] reported a median overall satisfaction of 9 on a scale of 10. In two cases (3.3%), the operator reported a low satisfaction (less than 5) due to malfunction of the insufflation valve leading to irrigation water in the lumen, limiting the visibility. Among 22 different tasks, the authors considered the SUD clinically-satisfactory in 100% and comparable to a reusable duodenoscope in 97.9%of cases.

The recently published study by Slivka *et al*[25] confirmed an optimal overall satisfaction with the SUD [median 8 (range VAS 1-10)]. Among 23 evaluated maneuvers, all obtained a median of at least 4 (range 1 to 5).

Pooled safety ed efficacy

Our study group recently conducted a meta-analysis including all clinical studies assessing the safety and efficacy of SUD use for ERCP, identifying 4 studies (368 patients) [26]. We observed a 92.9% [89.9 – 95.5; I^2 11.8%] overall success rate and 5.9% [3.7 – 8.5; I^2 0%] overall incidence of serious AEs. Overall incidence of pancreatitis (2.5%), infections (1.8%) and bleeding (1.8%) was very low, in line with

suggested threshold and confirming the optimal safety[26].

Study pipeline

Five clinical studies on the use of SUD for ERCP are ongoing (no. 2) or ready to start recruitment (no. 3); these studies and contact information are summarized in Table 2.

Two of them are planned to be conducted in US, while the remaining two studies in Europe (Italy and UK) and one in China.

Following the feasibility and safety studies performed in high-volume centers by extremely experienced operators, one study (NCT04103749) will include large real-life experience with operators with various expertise.

Interestingly, an Italian study is going to assess the performance of SUD in combination with single-use digital cholangioscope in a tertiary referral center.

Finally, a large multicenter study is testing the performance of another SUD, namely the aScope™ Duodeno, manufactured by Ambu A/S (Denmark) on 550 patients undergoing ERCP. In less than 12 mo, the knowledge and quality of evidence in the field of SUD use for ERCP is going to be strongly expanded. The introduction of a validated tool for duodenoscope assessment will allow physician to utilize a reproducible and reliable tool for the assessment of technical performance of duodenoscopes [27].

Cost-effectiveness

A recently published study, based on a "Montecarlo model" assessed the cost-effectiveness of different approaches adopted for the reduction of duodenoscope-related cross-infections[28]. The cost for each ERCP procedure, based on United States data, performed with SUD has been estimated in $2991. The analysis, based on an estimated < 1% risk of duodenoscope-related cross-infections did not identified routinely SUD use as a cost-effective strategy. The Authors acknowledged that these results should be contextualized based on duodenoscope-related cross-infection rate, local ERCP volume, quality adjusted life years, post-ERCP lifespan and environmental costs[28,29].

Limitations

The lack of a reliable quantification of the impact of duodenoscope contamination-related infections does not allow to correctly evaluate the benefit of the systematic use of a SUD.

Indeed, all the published studies have been designed to compare SUD to standard reusable duodenoscopes with a non-inferiority purpose, in terms of technical and clinical success rate. Since the estimated rate of duodenoscope-related cross-infection was < 8% published studies are underpowered to detect any clinical difference.

Environmental sustainability

Another point of critical discussion will be the ecological impact of production and wasting of a single-use endoscope.

A recent international named "Green Endoscopy" (Twitter account @GreenEndoscopy) wrote an inspiring editorial on this issue. The Authors estimated a mean 1.5 kg of waste for each single endoscopic procedure, with very-low amount of recyclable materials.

The disposal SUD is equivalent up to 400 g of household waste and this weight should be added to this waste. The Authors considered "unthinkable" that each ERCP could be performed with SUD based both on cost and environmental burdens.

A comparative study on two different approaches adopted with bronchoscopes [http://ambu.co.uk/pulmonology/environmental-impact] has reported that single-use endoscopy does not much differ since the cost of disposing plastic endoscopes should be balanced with sterilization process, disinfecting equipment and consumable costs.

On the other hand, SUDs are made from recycled plastic and are claimed to be recyclable through third party companies, even if material from these duodenoscopes will not be used for production of medical devices[29].

DISCUSSION

In conclusion, the recent identification of several cluster of exogenous multidrug-resistant bacterial infection caused by duodenoscope cross-contamination necessitated the implementation of various strategies for at least prevention or abolition of that life-threatening risk. Among those strategies, the introduction of sterile, disposable duodenoscopes is able to completely abolish the contamination and cross-transmission of bacteria.

Although there are only few clinical trials available, evidence is concordant in identifying an absolute safety and feasibility. Indeed, no SUD-related adverse event is still reported, and overall risk of adverse events and mortality is comparable to ERCP performed with reusable duodenoscopes. Moreover, the pooled technical success rate in expert hands stands at optimal values, with no significant heterogeneity

Table 2 Summary of ongoing registered studies assessing the performance of single-use duodenoscope for American society for gastrointestinal endoscopy, registered on clinicaltrial.gov portal

Title, reference	Region	Investigators	Design, population, Duodenoscope	Primary outcome	Status
Single Use ERCP -SURE Study (SURE). NCT04671095	Nottingham, United Kingdom	Dr. Suresh Vasan Venkatachalapathy; suresh.venkatachalapathy@nuh.nhs.uk	Prospective, 50 patients, EXALT-D[1]	Technical success (ERCP completion)	Not yet recruiting
International Study to Evaluate Outcomes and Safety of Patients Undergoing ERCP Using a Single-use Cholangioscope and Single-use Duodenoscope (MESE). NCT04712253	Rozzano (MI), Italy	Prof. Alessandro Repici alessandro.repici@hunimed.eu; Dr. Andrea Anderloni andrea.anderloni@humanitas.it	Retrospective, 50 patients, EXALT-D[1]	Technical success, clinical outcomes	Recruiting
Global Prospective Case Series Using a Single-Use Duodenoscope. NCT04103749	United States	Gregory Tirrell; gregory.tirrell@bsci.com; Pooja Goswamy; pooja.goswamy@bsci.com	Prospective, 1000 patients, EXALT-D[1]	Technical success (ERCP completion)	Not yet recruiting
Exalt D Single-use Duodenoscope in ERCP Procedures in China (ExaltDScope). NCT04687774	China	Zhiwei Guzhiwei.gu@bsci.com; Jingjing Gu	Observational, 30 patients, EXALT-D[1]	Technical success (ERCP completion)	Not yet recruiting
A Single-Use Duodenoscope in a Real-World Setting. NCT04628949	United States	Elizabeth Smith; elsm@ambu.comTrine; Højgaard Tølbøll; trht@ambu.com	Prospective, 550 patients, aScope	Technical success (ERCP completion)	Recruiting

[1]EXALT-D is manufactured by Boston Scientific (MA, United States). The aScope™ Duodeno is manufactured by Ambu A/S (Denmark). ASGE: American Society for Gastrointestinal Endoscopy.

among studies.

Future studies will deepen the knowledge in this field; data on cost-effectiveness and environmental impact will be needed for a worldwide spread of SUD use for ERCP.

CONCLUSION

In conclusion, the recent identification of several cluster of exogenous multidrug-resistant bacterial infection caused by duodenoscope cross-contamination necessitated the implementation of various strategies for at least prevention or abolition of that life-threatening risk. Among those strategies, the introduction of sterile, disposable duodenoscopes is able to completely abolish the contamination and cross-transmission of bacteria.

Although there are only few clinical trials available, evidence is concordant in identifying an absolute safety and feasibility. Indeed, no SUD-related adverse event is still reported, and overall risk of adverse events and mortality is comparable to ERCP performed with reusable duodenoscopes. Moreover, the pooled technical success rate in expert hands stands at optimal values, with no significant heterogeneity among studies.

However, further studies are needed to provide high-quality data, in terms of cost-effectiveness and environmental impact, potentially allowing a worldwide spread of SUD use for ERCP.

ARTICLE HIGHLIGHTS

Research background

Single-use duodenoscope use has been proposed as an effective strategy to avoid the risk of duodenoscope-related cross-infections in patients undergoing endoscopic retrograde cholangiopancreatography (ERCP).

Research motivation

Recentaly, several manuscript have been published reporting the ouctomes of clinical studies on single-use duodenoscope use for ERCP.

Research objectives

To perform a systematic review of the literature and report qualitative and quantitative results in terms

of technical success rate, clinical success, and safety.

Research methods
Systematic review and quantitative analysis.

Research results
Five original articles have been identified. One bench comparative study and four clinical trials performed with EXALT model-D (Boston Scientific Corp., United States) have been identified. Of them, one is a randomized controlled trial, while the other three studies are prospective single-arm, cross-over studies. Pooled technical success rate (4 studies, 368 patients) was 92.9% [95% confidence interval (CI): 89.9-95.5; I^2: 11.8%]. Pooled serious adverse event (4 studies, 381 patients) rate was 5.9% [3.7%-8.5%; I^2: 0.0%].

Research conclusions
Although few clinical trials are available, evidence is concordant in identifying an absolute feasibility and safety and feasibility for single-use duodenoscopes (SUD) use for ERCP. Data on cost-effectiveness and environmental impact will be needed for a worldwide spread of SUD use for ERCP.

Research perspectives
Future perspective and study pipelines should assess the use of other models of single-use duodenoscope, cost-effectiveness of single-use duodenoscope use for ERCP and environmental sustainability.

FOOTNOTES

Author contributions: Lisotti A and Fusaroli P wrote the paper; Napoléon B, Cominardi A and Zagari RM performed data collection for systematic review; all authors reviewed manuscript for pivotal intellectual content.

Conflict-of-interest statement: Authors declare no conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 May 20; 12(3): 132-147

DOI: 10.5662/wjm.v12.i3.132

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Nature and mechanism of immune boosting by Ayurvedic medicine: A systematic review of randomized controlled trials

B N Vallish, Dimple Dang, Amit Dang

Specialty type: Immunology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Maghazachi AA, United Arab Emirates; Peterfalvi A, Hungary

Received: December 3, 2021
Peer-review started: December 3, 2021
First decision: February 8, 2022
Revised: February 16, 2022
Accepted: April 3, 2022
Article in press: April 3, 2022
Published online: May 20, 2022

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Abstract

BACKGROUND

Many Ayurvedic preparations are claimed to have immune-boosting properties, as suggested in various published randomized clinical trials (RCTs)

AIM

To compile evidence on the nature and mechanism of immune system enhancement by Ayurvedic preparations in healthy and sick individuals.

METHODS

After prospectively registering study protocol with PROSPERO, we searched PubMed, DOAJ, Google Scholar, three dedicated Ayurveda research portals, two specialty Ayurveda journals, and reference lists for relevant records published until February 6, 2021 using appropriate search strategies. Baseline features and data pertaining to the nature and mechanism of immune system function were extracted from all eligible records. Methodological quality was assessed using the Cochrane RoB-2 tool.

RESULTS

Of 12554 articles screened, 19 studies reporting 20 RCTs (17 parallel group design, three crossover design) with 1661 unique patients were included; 11/19 studies had Indian first authors. Healthy population was included in nine studies, of which one study included pregnant women and two included pediatric population; remaining studies included patients with different health conditions, including one study with coronavirus disease 2019 patients. A total of 21 Ayurvedic interventions were studied, out of which five were composite

mixtures. The predominant route of administration was oral; dose and frequency of administration of the intervention varied across the studies. The results reported with five RCTs exploring five Ayurvedic interventions were incomplete, ambiguous, or confusing. Of the remaining 16 interventions, indirect evidence of immune enhancement was reported with four interventions, while lack of the same was reported with two interventions. Enhancement of T helper cells and natural killer cells was reported with three and four interventions, respectively, while the pooled results did not clearly point toward enhancement of other components of the immune system, including cytotoxic T cells, B lymphocytes, immunoglobulins, cytokines, complement components, leucocyte counts, and other components. Nine of the 20 RCTs had a high risk of bias, and the remaining 11 RCTs had some concerns according to RoB-2.

CONCLUSION

Various Ayurvedic preparations appear to enhance the immune system, particularly *via* enhancements in natural killer cells and T helper cells.

Key Words: Immune enhancement; Ayurveda; Immune system; Healthy volunteers; Composite preparations; NK cells

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Core Tip: Ayurvedic preparations have been anecdotally associated with immune boosting effect in both healthy and sick individuals. Through this systematic review, we explored the nature and mechanism behind this effect by scrutinizing 20 randomized controlled trials reported in 19 articles. While we could find indirect evidence for immune enhancement (by means of reduced illness duration and severity) with some Ayurvedic preparations, the evidence was insufficient to conclude about the exact mechanisms contributing to this phenomenon, although available evidence suggests that enhancements in natural killer cells and T helper cell number and function might contribute.

Citation: Vallish BN, Dang D, Dang A. Nature and mechanism of immune boosting by Ayurvedic medicine: A systematic review of randomized controlled trials. *World J Methodol* 2022; 12(3): 132-147
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/132.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.132

INTRODUCTION

As of September 2021, the ongoing coronavirus disease 2019 (COVID-19) pandemic has seen at least two waves in almost all regions of the world, including India, with many predictions hinting at a global third wave due to new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants[1]. The remarkable trail of destruction left by the pandemic, coupled with the non-availability of a drug-of-choice to treat the condition[2], has resulted in an almost complete dependence on the development of safe and efficacious vaccines that can protect individuals against existing and potential virus variants. Despite developments in vaccinology, it is common knowledge that a complete protection against all future variants of the virus cannot be absolutely guaranteed by even the most advanced vaccines. At the same time, non-neutralizing antibodies that cause antibody-dependent enhancement of the immune system are reported to exacerbate paradoxically SARS-CoV-2 infection, leading to worsened organ damage[3]. This has led to the realization that therapies that produce a sufficiently strong, but not hyperactivated, immune system can lead to a healthier society and can contribute significantly to the fight of mankind against not only the COVID-19 pandemic but also future healthcare challenges of infectious disease origin. Consequently, healthcare systems in many parts of the world, especially India, have turned their attention toward potential therapies that can produce a non-specific and unaided enhancement of the immune system, as a complementary step to vaccine development[4,5].

Ayurveda is the Indian traditional system of medicine and has a history since the 2nd century BC. The core pathophysiological principles of Ayurveda surround the concept of loss of balance with respect to the three humors (*Tridoshas*), five elements (*Pancha mahabhootas*), and seven tissues (*Saptadhatus*) of the human body. Various Ayurvedic treatments are described for diseases affecting different systems of the body, and most Ayurvedic medicinal items are sourced from natural ingredients. Classical texts of Ayurveda also have mentions about management of epidemics[6,7] and has defined 'immunity (*Bala*)' as the 'ability of the body to prevent and arrest the progression of disease for maintaining homeostasis' [4]. Few facts, such as the classification of immune system in Ayurveda into *Sahaja* (innate), *Kalaja*

(chronobiologic), and *Yuktikrut* (acquired) closely resembling the modern medical classification of immune system[4] and Ayurvedic immune-boosting preparations such as *cyavanaprasa* having stood the test of time, are testaments to the near-accurate understanding of our body's immune system by the ancient Ayurveda pioneers. Thus, it is not surprising that the Ministry of AYUSH (Ayurveda, Yoga, Unani, Siddha, and Homoeopathy) of the Indian Government released an advisory recommending various natural therapies to develop immunity against COVID-19[8]. Many components named in the said recommendation have been reported to produce immune enhancement through modulation of multiple immune system pathways[8] and also through psychoneuroimmunological mechanisms[5]. However, since most of these observations are through pre-clinical and non-human studies, we were interested to know if these pre-clinical observations hold good when the Ayurvedic preparations are studied after human administration.

With this background, we performed the present systematic literature review (SLR) with an objective for gathering evidence towards the nature and mechanism of enhancement of human immune system by the administration of Ayurvedic preparations, from published randomized clinical trials (RCTs). The research question that we were looking forward to find an answer through this SLR was: 'what is known from published RCTs about the nature and mechanism of the impact of consuming ayurvedic medicine on enhancing the immune system activity of healthy or sick humans?'

MATERIALS AND METHODS

Study protocol

The SLR protocol was drafted following the guidelines given in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement and was subsequently refined through internal discussion. The final version of the protocol was prospectively registered with PROSPERO on July 10, 2020, with ID CRD42020191289.

Eligibility criteria

To identify potentially relevant articles that answered our research question, the following eligibility criteria were drafted: 'Population' was all studies involving healthy or sick adults with any form of illness (acute or chronic). The protocol was subsequently amended to include humans of all age groups, including pediatric and geriatric individuals. We did not restrict the population with respect to the age or gender of the individuals, presence of any form of disease, presence of comorbidities, or presence of special situations such as pregnancy. 'Intervention' included any type of Ayurvedic medicine, given as a combination or single drug. Studies that did not explicitly mention whether the intervention was an 'Ayurvedic medicine' or not were included in the review if the intervention was found in the work 'Indian Medicinal Plants: an Illustrated Dictionary' by Khare[9]. We also used the same resource to compile the Ayurvedic name of various interventions. Papers that included interventions only belonging to other systems of medicine (including modern/Western medicine, traditional medicines of other regions/countries, and other systems of medicine including Unani, Siddha, Homoeopathy, Yoga, Naturopathy, Osteopathy, and Chiropractic) were excluded. We did not restrict studies based on the 'Comparator' and included all studies regardless of whether or not there was a comparator. 'Outcomes' of interest included any description of enhancement, augmentation, stimulation, increasing, or strengthening of the immune system, either directly (through observation on the effect of component/s of the immune system) or indirectly (through observation of effect of said enhancement of immune system, by means of surrogate markers such as relief from illness, quickness of recovery, *etc.*). Studies that described immunomodulation instead of immune enhancement and studies that did not describe any immune system effect were excluded. 'Study design' included only RCTs. Papers not describing primary data (such as narrative or systematic reviews, letters to editors, opinion pieces, commentaries, editorials, brief communications, news items, *etc.*), case studies, case reports, and studies describing non-human experiments (including *in vitro* studies, *in vivo* studies, *in silico* experimentation, *etc*), and studies not in English language were excluded from our review.

Literature search, data extraction, and quality assessment

Following the eligibility criteria described above, a PubMed literature search strategy was drafted and modified through internal discussion. Using the refined search strategy, a systematic literature search was performed in PubMed/MEDLINE, from their inception till June 2, 2021. Recognizing that many research articles on Ayurveda are published in journals not indexed in PubMed, and following the recommendations by Aggithaya *et al*[10] (2015), we performed an additional literature search in Directory of Open Access Journals[11], AYUSH research portal[12], Digital Helpline for Ayurveda Research Articles (DHARA)[13], Annotated Bibliography of Indian Medicine (ABIM)[14], and Google Scholar. Furthermore, we also performed targeted journal search in two Ayurveda specialty journals (Ayu and Journal of Ayurveda and Integrative Medicine) using a combination of search terms and Boolean operators such as "Ayurveda", "Ayurvedic medicine", "Immune", "Immune stimulating", "Immune enhancing", "Immunomodulatory", and "Randomized clinical trials". The detailed PubMed

search strategy is presented as Supplementary Table 1, and a brief account of searches performed in each of the portals mentioned above is provided in Supplementary Table 2. Finally, we also scanned reference lists of relevant studies to identify potentially eligible records that were missed through database search.

After pooling all the eligible records, we identified the most eligible articles for our review and extracted relevant data from these papers after thoroughly studying the full texts of each article. Extracted data, such as data related to the study details (year of publication, country of the first author, study design, and details of the intervention and comparator), participant details (participant profile, number, age, and sex), details of the intervention and comparator (Ayurvedic, commercial, and scientific names, composition, dose, frequency, duration, and route of administration), and outcome details in terms of improvement from baseline, were entered into a predefined data entry grid. All outcomes related to the immune system (or *Ojas*) were considered for this study and included direct variables [such as leukocyte subtypes including T lymphocytes, B lymphocytes, natural killer (NK) cells, and various types of myelocytes, immunoglobulins, complement components, and cytokines] and indirect variables (such as absenteeism, number of healthy days and sick days, number of doctor visits, number of symptoms, *Ojas* score, *etc*.). The methodological quality of the included studies was assessed using the Cochrane RoB-2 tool[15]. We used the official Microsoft Excel tool provided by the Cochrane Foundation for implementing RoB-2, and we used separate tools for parallel group RCTs and crossover RCTs[16].

Subsequent to executing the literature search, two authors (VBN and AD) independently screened the pooled articles for their inclusion in the study, extracted data, and assessed the articles for risk of bias; any disagreements were resolved through discussion and reconciliation that was moderated by another author (DD).

Statistical analysis and data availability

Study selection and data extraction were done electronically in Microsoft Excel. To assess the inter-rater reliability (IRR) of the study inclusion and the methodological quality assessment of the included articles, Cohen's kappa value was calculated using SPSS version 20 (Armonk, NY, United States). The cut-off points for the kappa statistic were interpreted as ≤ 0.20 = slight agreement; 0.21-0.40 = fair agreement; 0.41-0.60 = moderate agreement; 0.61-0.80 = substantial agreement; 0.81-0.99 = near-perfect agreement; and 1.00 = perfect agreement[17]. Statistical review of the study was performed by an author who is a biomedical statistician (VBN). All datasets used to derive conclusions in this study are available with the corresponding author on reasonable request.

RESULTS

Study selection, baseline characteristics

From an initial pool of 12554 potentially eligible records, 19 studies were included for data extraction and review. Figure 1 depicts the study selection process.

The 19 included studies were published from 2005 to 2021. A total of 20 RCTs were reported in the 19 included papers: 16 articles described parallel group RCTs, two described crossover trials, and one article reported two RCTs: One parallel group RCT, and one crossover RCT. The first authors of the 19 papers were from six different countries, with India being the most frequent country of affiliation (11 studies), followed by Japan and United States (two studies each); there was one study each with first authors from Germany, Iran, Romania, and Turkey. The participant profile ranged from healthy participants (six studies, healthy adults; two studies, healthy pediatric population; one study, healthy pregnant women), patients with cancer (three studies, gastrointestinal cancer; one study, head and neck cancer), patients with diabetes and human immunodeficiency virus (HIV) (two studies each), and patients with COVID-19 and allergic rhinitis (one study each). Among the 17 studies that included adult participants, two included only females, one study included only males, and the remaining 14 studies included participants of either gender. The sample size of the studies ranged from 5 to 627, with a total of 1661 unique participants across the 19 articles. The complete baseline characteristics of the included studies are summarized in Table 1.

Two of the included studies explored two interventions, and the remaining 17 studies explored one intervention each, leading to a total of 21 interventions. The predominant route of administration of the intervention was oral (n = 17), with one study each delivering the intervention through subcutaneous injection, intravenous infusion, and oil dripping on the forehead; details of route of administration of the intervention were missing in one study. Among the 17 orally administered interventions, the formulations were capsules in five studies, and *Rasayana* in three studies; one study each used other formulations including tablet, tea, *Cyavanaprasa*, *Kasaya*, *Bhasma*, *Ghrita*, *Churna*/*Kalka*, and tincture, and one study did not provide information about formulation. Details of dosing and duration of administration of the intervention varied across the studies. Placebo was used as the comparator in six studies. The complete details of the interventions and comparators among the included studies are summarized in Table 2. The detailed composition of the different composite preparations used as interventions in

Table 1 Baseline study characteristics, and summary of risk of bias assessment

No	Ref.	Country of 1st author	Type of randomized controlled trial	Patient profile	Intervention[1]			Comparator[1]			Overall risk of bias as per RoB-2 tool[2]
					Sample size	Mean age	Male (N, %)	Sample size	Mean age	Male (N, %)	
1	Enesel *et al* [18], 2005	Romania	Parallel group	Patients undergoing surgery for GIT cancer	40	62 (range 37-87)	23 (57.5)	30	NA	NA	-
2	Ishikawa *et al* [21], 2006	Japan	Parallel group	Patients with inoperable colon/liver/pancreatic cancer	25	63.6 ± 8.3	21 (84)	25	65.8 ± 6.3	18 (72%)	?
3	Brush *et al* [22], 2006	United States	Parallel group	Healthy adult volunteers	3	NA	NA	2	NA	NA	-
4	Schink *et al* [27], 2007	Germany	Parallel group	Primary/locally relapsed colorectal carcinoma patients undergoing open (complete/partial) tumour resection	11	72 ± 8.2	7 (63.6)	11	69 ± 10.4	5 (45.5)	?
5	Purandare *et al*[33], 2007	India	Parallel group	Patients with diabetic foot ulcer	23	56.26	17 (73.9)	22	56.32	19 (86.4)	?
6	Uebaba *et al* [29], 2008	Japan	Crossover	Healthy adult female volunteers	16	39 ± 9	0 (0)	16	39 ± 9	0 (0)	?
7	Bhat *et al* [28], 2010	India	Parallel group (I)	Healthy adult volunteers	13	NA	NA	13	NA	NA	?
			Crossover (II)	Healthy adult volunteers	110	NA	NA	110	NA	NA	-
8	Işik *et al*[23], 2010	Turkey	Parallel group	Allergic rhinitis patients with house dust mite sensitivity	12	NA	NA	12	NA	NA	-
9	Kianbakht *et al*[30], 2011	Iran	Parallel group	Healthy adult male volunteers	45	22.5 ± 0.6	45 (100)	44	21.1 ± 0.5	44 (100)	?
10	Mondal *et al* [24], 2011	India	Crossover	Healthy adult volunteers	22	NA	NA	22	NA	NA	?
11	Nantz *et al* [26], 2012	United States	Parallel group	Healthy adult volunteers	56	25.4 ± 5.7	23 (41.1)	56	26.9 ± 7.1	26 (46.4)	?
12	Suprabha *et al*[32], 2017	India	Parallel group	Uncomplicated pregnant women in 20-24 wk of pregnancy	15	NA	NA	15	NA	NA	?
13	Gupta *et al* [34], 2017	India	Parallel group	Healthy children aged 5-12 yr	313	7.3 ± 1.8	161 (51.4)	314	7.4 ± 1.8	164 (52.2)	?
14	Rais *et al*[36], 2021	India	Parallel group	25-60 yr, asymptomatic/ uncomplicated COVID-19 RTPCR +ve, mild symptoms	80	NA	57 (71.3)	40	NA	30 (75)	-
15	Bhaskaran *et al*[31], 2019	India	Parallel group	Healthy full-term infants (< 12 mo age), > 2.5 kg birth weight, with normal growth and development	47	NA	NA	34	NA	NA	-
16	Kumar *et al* [35], 2014	India	Parallel group	Adult patients with T2DM of any stage	56	NA	NA	28	NA	NA	-
17	Ravindran *et al*[25], 2014	India	Parallel group	Patients with head & neck cancer in complete remission following primary treatment	37	NA	NA	38	NA	NA	?
18	Somarathna *et al*[20], 2010	India	Parallel group	HIV +ve patients without AIDS surveillance signs as per WHO, and no concurrent illness	21	NA	NA	6	NA	NA	-
19	Gupta *et al* [19], 2010	India	Parallel group	New HIV +ve patients with CD4 count not < 150/microliter and no complications or comorbidities	12	NA	NA	8	NA	NA	-

[1]Not all studies had reported demographics of participants including age and sex.
[2]Separate versions of RoB-2 were used for RCTs with parallel group design and crossover design. Interpretation: ?: Some concerns of bias; -: High risk of bias. Detailed risk of bias assessment results: Supplementary Table 4. HIV: Human immunodeficiency virus; NA: Not available; GIT: Gastrointestinal tract; COVID-19: Coronavirus disease 2019; RTPCR: Reverse transcription polymerase chain reaction.

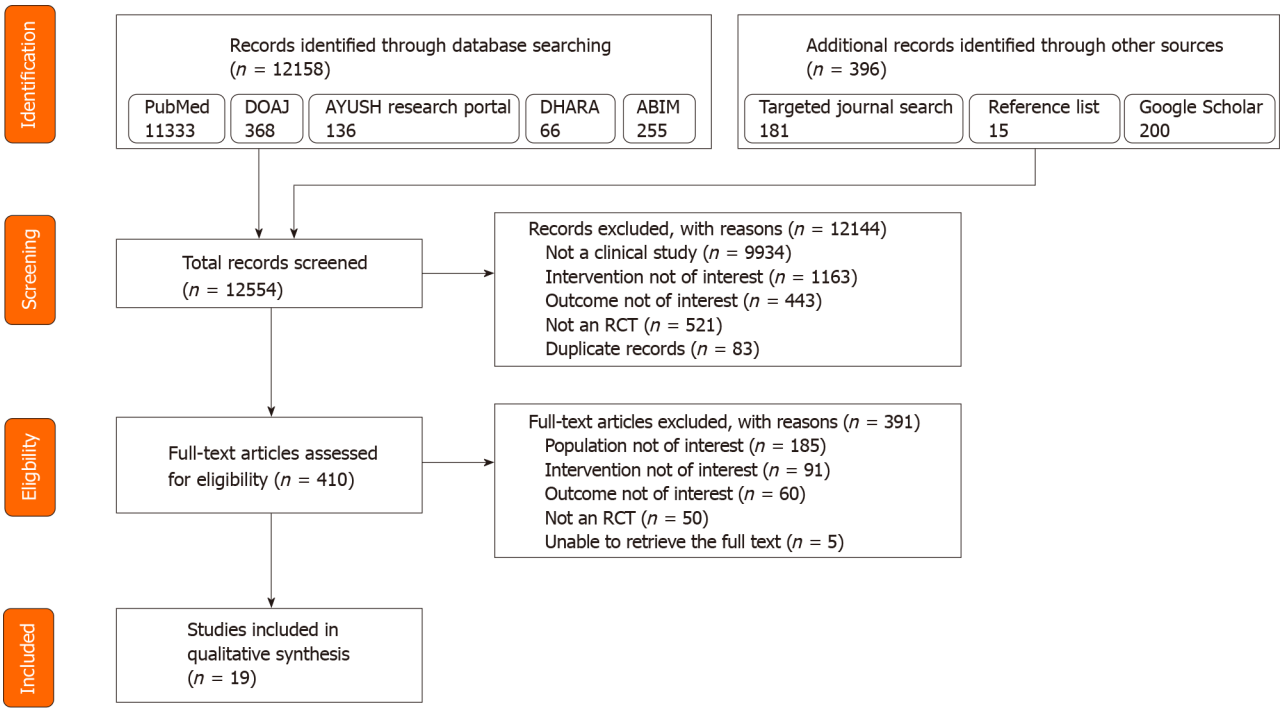

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Figure 1 Study selection process. DOAJ: Directory of Open Access Journals; AYUSH research portal: Ayurveda, Yoga, Unani, Siddha, and Homoeopathy research portal; DHARA: Digital Helpline for Ayurveda Research Articles; ABIM: Annotated Bibliography of Indian Medicine.

five of the studies is provided in Supplementary Table 3.

Of the 19 included studies, 15 studies provided information about the impact of the intervention on at least one component of the immune system (direct evidence), three studies provided information about the impact of the intervention on the overall health status of the participant (indirect evidence), and one study provided both direct and indirect evidence. The description of the results was not uniform across various studies, with different units of measurement used to express similar outcomes; this prevented us from performing a meta-analysis of the results.

Immune enhancement: Direct evidence

Impact on T lymphocyte subsets (excluding NK cells): CD4 lymphocyte counts were found to be significantly enhanced after perioperative mistletoe administration for 14 d among patients undergoing surgery for gastrointestinal (GI) cancer[18]. Among patients with HIV, CD4 lymphocyte counts were found to be enhanced after a 90-d consumption of *Shilajatu Rasayana*[19] and *Ranahamsa Rasayana*[20], as compared to standard care. However, the increase in CD4 lymphocyte count caused by the consumption of aged garlic extract for 12 wk was not statistically significant among patients with GI cancer and was less than that observed with matching placebo[21]. The results reported in three papers were ambiguous: Although *Glycyrrhizia glabra* tincture increased CD4 lymphocytes by 8.26% in 24 h in healthy individuals, the statistical significance of this finding was not reported, and the sample size was only 5 patients[22]. Significant enhancement of CD4 lymphocyte count was also not observed with *Nigella sativa* seed supplementation, and the data were not presented with clarity[23]. *Tulsi* was reported to significantly increase CD4 lymphocytes in 4 wk compared to placebo among healthy individuals, but this article did not provide any numbers to support this claim[24].

CD8 lymphocyte counts were significantly decreased with perioperative mistletoe administration for 14 d[18]. Aged garlic extract brought about a non-significant increase in CD8 lymphocyte count over 12 wk, which was also seen with matching placebo[21]. The remaining three papers presented results with less clarity: although *Glycyrrhizia glabra* tincture increased CD8 lymphocyte by 2.89% in 24 h in healthy individuals, the statistical significance of this finding was not reported, and the sample size was only 5 patients[22]. Significant CD8 lymphocyte count enhancement was observed with *Nigella sativa* seed

Table 2 Details of intervention and comparators

No	Ref.	Description	Intervention: Ayurvedic name[1]	Intervention: Scientific name	Intervention: Route; dose, frequency, duration[2]	Comparator: Description; route, dose, frequency, duration[2]
1	Enesel *et al* [18], 2005	Isorel® (Mistletoe, firtree)	Bandaaka, Suvarna-bandaaka *etc.*	*Viscum album*	Subcutaneous; 2 wk pre-operatively, 2 wk post-operatively; dose details not clear	Standard care
2	Ishikawa *et al* [21], 2006	Aged garlic extract	Lashuna, Rasona *etc.*	*Allium sativum*	Oral; 125 mg, 4 capsules daily, 12 wk	Matching placebo
3	Brush *et al* [22], 2006	*Glycyrrhizia glabra* tincture	Yashtimadhu, Madhuyashtyaahvaa *etc.*	*Glycyrrhiza glabra*	Oral; 0.44 g/7.5 mL, twice daily, 7 d	Matching placebo tincture
4	Schink *et al* [27], 2007	Standard care + Iscador® (Standardized mistletoe extract)	Bandaaka, Suvarna-bandaaka *etc.*	*Viscum album*	IV infusion; 5 mg, single dose	Standard care
5	Purandare *et al*[33], 2007	Standard care + Tinospora cordifolia	Guduuchi, Guduuchikaa *etc.*	*Tinospora cordifolia*	Details not available	Standard care + matching Placebo
6	Uebaba *et al* [29], 2008	Shirodhara oil-dripping treatment using sesame oil	Shirodhara; Tila, Snehphala	*Sesamum indicum*	Oil dripping on forehead; single sitting	Control supine position; single sitting
7	Bhat *et al* [28], 2010	Fortified tea with *Withania somnifera, Glycyrrhzia glabra, Zingiber officinale, Ocimum sanctum* & *Elettaria cardamomum*	Ashwagandha; Yashtimadhu; Aardraka, Shunthi; Tulasi; Elaa	*Withania somnifera, Glycyrrhzia glabra, Zingiber officinale, Ocimum sanctum, Elettaria cardamomum*	Oral; 2.06 g, thrice daily, 2 mo	Regular tea; Oral; 2 g, thrice daily, 2 mo
8	Işik *et al*[23], 2010	Specific immunotherapy + *Nigella sativa*	Kaalaajaaji, Kalikaa *etc.*	*Nigella sativa*	Oral; 2 g daily, 1 mo	Specific immuno-therapy alone
9	Kianbakht *et al*[30], 2011	Saffron tablet	Kumkuma, Rudhira, Kaashmiraka *etc.*	*Crocus sativus*	Oral; 100 mg daily, 6 wk	Matching placebo
10	Mondal *et al* [24], 2011	Tulsi capsules	Tulasi, Surasa, Suravalli *etc.*	*Ocimum sanctum*	Oral; 300 mg daily, 4 wk	Matching placebo
11	Nantz *et al* [26], 2012	Aged garlic extract	Lashuna, Rasona *etc.*	*Allium sativum*	Oral; 640 mg, 4 capsules daily, 90 d	Matching placebo
12	Suprabha *et al*[32], 2017	Rasayana Avaleha with milk	Composite[4]	Composite[4]	Oral; 12 g, twice daily, 2 mo	Calcium carbonate (500 mg) with ferrous sulfate (200 mg); Oral; daily, 2 mo
13	Gupta *et al* [34], 2017	Cyavanaprasa (Dabur) with milk	Composite[4]	Composite[4]	Oral; 6 g, twice daily, 6 mo	Milk; Oral, 100-200 mL, twice daily, 6 mo
14	Rais *et al*[36], 2021	**Intervention 1:** Vyaghryadi Kashaya (50 mL) + Pippali (250 mg) + Samshamani vati (500 mg)	Vyaghryadi Kashaya: Kantakari, Shunthi, Guduchi; Pippali; Samshamani vati: Guduchi	*Solanum xanthocarpum, Zingiber officinale, Tinospora cordifolia, Piper longum*	Oral; twice daily, 10 d	Vitamin C (500 mg) twice daily, Paracetamol (500 mg) as needed
		Intervention 2: Shunthi churna (2 g) + Rasona kalka (1 g)	Shunthi; Rasona, Lasuna	*Zingiber officinale, Allium sativum*	Oral; Shunti churna: Twice daily, Rasona: once daily; 10 d	
15	Bhaskaran *et al*[31], 2019	Swarna Bhasma (Calcined powder of gold), honey, ghrita	Swarna prashana, madhu, ghrita	*NA*	Oral; Swarna bhasma: 0.2-2.4 mg[3]; once daily, 4 wk	Oral Honey + ghrita; dose details not available
16	Kumar *et al* [35], 2014	**Intervention 1:** Mamajjaka capsules	Maamajjaka, Naagjhvaa *etc.*	*Enicostemma littorale*	Oral; 500 mg, twice daily, 3 mo	Control; no details available
		Intervention 2: Shilajatu capsules	Shilajatu	*Asphaltum punjabinum*	Oral; 500 mg, twice daily, 3 mo	
17	Ravindran *et al*[25], 2014	Varunadi Ghrita + Standard care	Composite[4]	Composite[4]	Oral; 5 g, twice daily, 1 y	Standard care
18	Somarathna *et al*[20], 2010	Ranahamsa Rasayana	Composite[4]	Composite[4]	Oral; 5 g, twice daily, 90 d	Standard care
19	Gupta *et al* [19], 2010	Shilajatu Rasayana	Composite[4]	Composite[4]	Oral; 95 g over first 15 d, later 6 g per day for	Standard care

| | | |

after the consumption of aged garlic extract, 'eradication of pathogens causing flu-like illnesses' could be achieved by lower levels of these cytokines[26]. By contrast, consumption of *Tulsi* capsules for 4 wk was found to increase significantly the levels of interferon gamma and interleukin-4 in healthy adult volunteers, and this 'increase in cytokine levels' was also interpreted as supporting the observation that *Tulsi* resulted in mounting 'an effective immune response'[24]. The latter paper also did not provide complete numbers to substantiate the claims.

Impact on white blood cell counts and granulocytes: Even though total white blood cell (WBC) counts were enhanced by perioperative mistletoe administration after 14 d, the enhancement seen with standard care was found to be numerically higher[18]. Total WBC counts were not found to be significantly enhanced by administration of aged garlic extract[21], saffron[30], or calcined gold powder [31].

Total lymphocyte counts were significantly enhanced by perioperative mistletoe administration among patients with GI cancer[18]. While calcined gold powder was found to enhance significantly lymphocyte counts among infants < 1 mo but not in older infants, this paper did not provide numbers to back their claims[31]. Saffron was not associated with a significant change in total lymphocyte counts as well[30].

While significant changes in absolute neutrophil counts were not reported with the use of saffron[30] and calcined gold powder[31], absolute neutrophil counts were significantly reduced by administration of *Ranahamsa Rasayana* for 90 d among patients with HIV[20]. Phagocytic function by neutrophils was found to be significantly enhanced after a 1-mo treatment with *Tinospora cordifolia* (among patients with diabetic foot ulcer)[33] and *Nigella sativa* (among patients with allergic rhinitis)[23].

No significant alterations in eosinophil or basophil levels were reported with saffron administration [30]. Absolute eosinophil count was found to decrease significantly after a 4-wk administration of calcined gold powder among infants aged < 1 mo but not among older infants; there were no significant changes in monocyte counts in either age group. However, this paper did not provide complete results to strengthen these claims[31].

Impact on other immune system variables: Perioperative subcutaneous mistletoe administration over 14 d was found to increase significantly the counts of CD2 lymphocytes (comprising of T lymphocytes and NK cells) after 14 d among GI cancer patients undergoing surgery[18]. Perioperative intravenous single-dose mistletoe infusion among GI cancer patients undergoing surgery was associated with a significant lowering of human leukocyte antigen - DR isotype expression, which was also seen among patients who received standard care[27].

Immune enhancement: Indirect evidence

Four studies reported indirect evidence of immune system enhancement following various Ayurvedic treatments.

Healthy adults who consumed capsules containing aged garlic extract for 90 d were found to have a reduced severity of cold and flu in terms of a significantly lower number of symptoms, significantly lower decrease in activity and days of reduced activity due to illness, and significantly lower number of work days missed due to illness compared to volunteers taking matched placebo capsules. However, a significant difference between these two groups was not found with respect to illness incidence, number of days with symptoms, number of symptoms per illness, and number of doctor visits. These observations were attributed to the enhancement of activities of NK cells and γδ-T cells by aged garlic extract[26].

Consumption of *Cyavanaprasa* with milk by healthy children aged 5-12 years for 6 mo was found to be associated with improved health compared to control in terms of significantly lower number of episodes of infection/allergy (overall number of episodes, and episodes of mild and moderate severity), significantly shorter duration of illness (overall duration of illness and illnesses of mild and moderate severity), significantly more children without infection/allergy, and significantly lower absenteeism due to illness, both in terms of children reporting absenteeism as well as the number of days of absenteeism due to illness. Other findings not reaching statistical significance included a lower number and shorter duration of severe episodes of infection/allergy and a lower number of children with infection/allergy [34].

The '*Ojas* score', which signifies immune function, was found to be significantly improved after 3 mo of administration of both the Ayurvedic interventions studied (*Mamajjaka* and *Shilajatu*) as well as the control treatment, and the improvement caused by both the Ayurvedic interventions was found to be significantly higher than that seen with the control treatment[35]. Finally, the number of COVID-19 patients with positive reverse transcription polymerase chain reaction results was zero, after 10 d of treatment with either *Vyagradhi Kashaya*, ginger-garlic, or Vitamin C[36].

The complete details of the main findings of all the included studies are summarized in Table 3.

Safety

Nine of the 19 included studies did not report the safety profile of the Ayurvedic intervention, and eight studies reported not finding any new safety signals of concern. Rais *et al*[36] reported that 2/40 patients

Table 3 Results from the included studies: Impact of Ayurvedic medication on components of the immune system

No	Ref.	Intervention	T Helper cells	T cytotoxic cells	T cells: Other results	NK cell count/ activity	B lympho-cyte count	Immuno-globulins	Comple-ment	Cyto-kines	WBC count/ activity	Other variables	Indirect evidence
1	Enesel *et al*, 2005	Isorel® (Mistletoe, firtree)	+	-	+	+	-	+	+	NA	+	+	NA
2	Ishikawa *et al*, 2006	Aged garlic extract	-	-	NA	+	NA	NA	NA	NA	-	NA	NA
3	Brush *et al*, 2006[1]	*Glycyrrhizia glabra* tincture	+/-	+/-	NA	+/-	+/-	NA	NA	NA	NA	NA	NA
4	Schink *et al*, 2007	Iscador® (Standardized mistletoe extract)	NA	NA	NA	+	NA	NA	NA	NA	NA	-	NA
5	Purandare *et al*, 2007	Tinospora cordifolia	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA
6	Uebaba *et al*, 2008[2]	Shirodhara oil-dripping (sesame oil)	NA	NA	NA	+/-	NA	NA	NA	NA	NA	NA	NA
7	Bhat *et al*, 2010[3]	Fortified tea with multiple Ayurvedic ingredients	NA	NA	NA	+/-	NA	NA	NA	NA	NA	NA	NA
8	Işik *et al*, 2010	Specific immunotherapy + *Nigella sativa*	+/-	+/-	+/-	+/-	+/-	NA	NA	NA	+	NA	NA
9	Kianbakht *et al*, 2011	Saffron tablet	NA	NA	NA	NA	NA	-	-	NA	-	NA	NA
10	Mondal *et al*, 2011[4]	Tulsi capsules	+/-	+/-	NA	+/-	+/-	NA	NA	+/-	NA	NA	NA
11	Nantz *et al*, 2012[5]	Aged garlic extract	NA	NA	+/-	+/-	NA	NA	NA	+/-	NA	NA	+
12	Suprabha *et al*, 2017	Rasayana Avaleha with milk	NA	NA	NA	NA	NA	-	NA	NA	NA	NA	NA
13	Gupta *et al*, 2017	Cyavanaprasa (Dabur) with milk	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+
14	Rais *et al*, 2021	**Intervention 1:** Vyaghryadi Kashaya + Pippali + Samshamani vati	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
		Intervention 2: Shunthi churna + Rasona kalka	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
15	Bhaskaran *et al*, 2019[6]	Swarna Bhasma, honey, ghrita	NA	NA	NA	NA	NA	+/-	NA	NA	+/-	NA	NA
16	Kumar *et al*, 2014	**Intervention 1:** Mamajjaka capsules	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+
		Intervention 2: Shilajatu capsules	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+

17	Ravindran *et al*, 2014	Varunadi Ghrita + Standard care	NA	NA	+	+	+	NA	NA	NA	NA	NA	NA
18	Somarathna *et al*, 2010	Ranahamsa Rasayana	+	NA	NA	NA	NA	NA	NA	NA	-	NA	NA
19	Gupta *et al*, 2010	Shilajatu Rasayana	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SUMMARY	+	3	0	2	4	1	1	1	0	3	1	4
		+/-	3	3	2	6	3	1	0	2	1	0	0
		-	1	2	0	0	1	2	1	0	3	1	2

+: Evidence suggests significant immune enhancement; +/-: Ambiguous, confusing, or incomplete results; -: Evidence does not suggest significant immune enhancement.

[1]Sample size is 5 participants; significance values of any results not reported in the paper.

[2]paper reports only significance (*P* value) without reporting the value of NK cell activity.

[3]study I (parallel group study design) reports only significance (*P* value) without reporting the value of NK cell activity, and study II (crossover design) has less than optimal washout period, and there is a difference in numbers between text and table.

[4]paper reports only significance (*P* value) without reporting the value of T lymphocytes, B lymphocytes, NK cells, and cytokines.

[5]paper reports only significance (*P* value) without reporting the value of T lymphocytes and NK cells.

[6]paper reports only significance (*P* value) and mean difference without reporting the value of immunoglobulins and total or differential white blood cell counts.

NA: Not available; NK: Natural killer; WBC: White blood cell.

receiving *Vyagradhi Kashaya* developed loose stools and subsequently discontinued the treatment, and 3/40 patients receiving ginger-garlic developed burning sensation in the abdomen and were conservatively managed. After receiving *Ranahamsa Rasayana*, 2 patients were reported to have a mild burning sensation over the body, transient mouth ulceration, and mild drowsiness, which led to discontinuation of treatment[20].

Risk of bias assessment of included studies

As per RoB-2 tool, nine of the 20 RCTs described in the 19 included studies had high risk of bias, and the remaining 11 RCTs had some concerns of bias. Only three RCTs gave adequate information about randomization process; most of the RCTs used standard methods to measure the outcomes, leading to domain 4 having the highest number of studies with low risk of bias. Since none of the studies provided information about pre-specified statistical analysis plan, all 20 RCTs received a 'some concerns' rating for domain 5, leading to a similar rating for overall risk of bias. The risk of bias summary is presented in Figure 2 and Table 1, and the complete analysis of risk of bias assessment is available in Supplementary Table 4.

IRR

The IRR for study selection between the two reviewers was substantial, with Cohen's kappa value being 0.743 [95% confidence interval (CI): 0.714-0.772] and 0.681 (95%CI: 0.652-0.710) for title-abstract screening and full-text screening, respectively. For the methodological quality assessment, an agreement between the two reviewers with respect to the methodological quality was achieved with 16/20 RCTs,

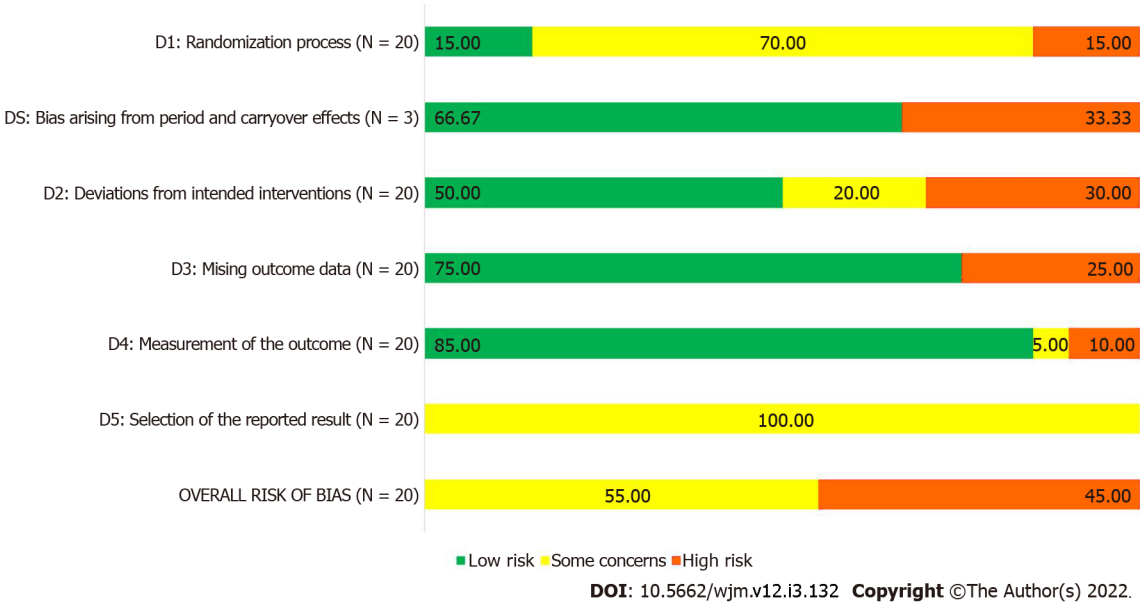

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Figure 2 Risk of bias summary of the included studies, as per RoB-2 tool.

with four papers needing mediation, leading to an inter-rater agreement of 84.21%. The Cohen's kappa value for risk of bias assessment was 0.636 (95%CI: 0.607-0.665), indicating substantial IRR.

DISCUSSION

The most prominent finding of our study is that while there is reasonably significant indirect evidence of immune system enhancement by different Ayurvedic interventions, especially *Cyavanaprasa*, the same cannot be said about direct evidence. In other words, we can say with some confidence that some Ayurvedic medicines have been shown to reduce the duration of illness and improve overall good health after long-term consumption, indirectly indicating that the immune system might have been boosted; however, the exact mechanism by which these effects occur is not yet clear. By looking at the summary of evidence in our review, it appears that enhancement of number and activity of NK cells and T helper cells might be responsible for such an enhancement, while the role of other components of immune system, including cytotoxic T cells, B lymphocytes, immunoglobulins, complement components, cytokines, and WBC counts is not clear. The high degree of inter-rater agreeability further points towards the accuracy of our observations.

We also observed some discomforting points about the included studies. Seven of the 20 interventions studied[19,20,25,28,32,34,36] were composite mixtures of a variety of Ayurvedic ingredients. By the nature of these individual studies, it is not possible to determine which component/s of these composite ingredients have prominent and contributory effect towards immune enhancement and which components have secondary roles such as excipients, flavoring and coloring agents or other non-essential functions. On the other hand, the exact mechanisms by which the remaining 13 individual Ayurvedic interventions impacted the immune system have been not adequately explored in the individual studies. Further contributing to the lack of clarity are the observations that there are some contradictory views wherein an apparent lack of effect in the results is claimed to have an effect (as in the case of cord blood IgG)[32] or contradictory correlations given for opposite directions of alterations in blood cytokine levels[24,26]. Next, as seen in Table 3, as many as six studies have confusing, ambiguous, or incomplete results for various reasons. The sample size of the included studies is not sufficiently large enough. None of the 20 RCTs described in the 19 studies included in the present review was able to score a 'low risk of bias' rating in the RoB-2 tool, had a mention of points indicative of well-conducted RCTs, such as protocol registration in a clinical trial registry, or had followed any reporting guidelines. All these factors generally tend to reduce confidence in the results presented in the studies, regardless of magnitude or statistical significance.

In the backdrop of these discouraging observations, it is apparent that, despite Ayurveda being an ancient science with an acclaimed and proven effect on immune system enhancement and despite the availability of modern medical research methodologies and technological advancements, we have not been able to know exactly how such an enhancement in immune function is brought about through the consumption of a variety of Ayurvedic medicines. The proponents of Ayurveda mostly seem to rely on the historical anecdotes, rather than using the benefits of scientific advancements to improve the general

confidence of the worldwide audience in this ancient science that has stood the test of time. While this lacuna on the part of Ayurvedic researchers has been observed and commented upon for quite some time now[37], solid steps to enhance systematic evidence generation in Ayurveda seem to be lacking. In fact, ancient Ayurvedic texts have already incorporated many of the basic tenets of modern scientific discovery, such as *Anumana* (logical questioning), *Yukti* (knowledge), *Tarka* (argument), and *Vyapti* (cause and effect relationship)[38]. It should be realized that before the advent of modern medicine, Ayurveda was the 'modern medicine' of those times. Perhaps it is time that proponents of Ayurveda and modern medicine come together with open minds to apply modern scientific methods to generate credible and reproducible evidence, so that there is rational and evidence-based integration of Ayurveda into mainstream medicine. Also, it is apt that this is done by Indian scientists, rather than waiting for the Western scientists to do it on our behalf, as in the case of turmeric, neem, *Basmati* rice[39, 40], *Pranayama*-meditation[41], and many others.

While our initial search resulted in a pool of over 12000 potentially relevant articles, we were able to identify only 19 RCTs that matched our eligibility criteria. During the process of literature screening, we observed that a large majority of research in this field is non-clinical and largely done *in vitro* or on animal models. It appears that a meaningful translation to clinical research is lacking for many Ayurvedic preparations, whose efficacy and safety were established in non-clinical models, for reasons unknown. It appears that Ayurvedic researchers are restricting themselves from entering clinical research, and the reasons for this hinderance should be sought out and resolved in order to boost clinical research in this field.

Our review should be interpreted in the backdrop of some limitations. We restricted to only papers published in English language, because of our familiarity with the language; we might have missed valid papers published in regional languages. We did not search other databases such as Embase or Scopus, since both these resources are paywalled, and our research was self-funded.

CONCLUSION

To conclude, various Ayurvedic preparations, both standalone and composite, appear to have an enhancing effect on the immune system, as evidenced indirectly through reduced illness variables, but the exact mechanism behind this enhancement is not fully established. There may be contributions from enhancement of NK cells and T helper cells, although the role of other immune system components is not clear. There were many inconsistencies and ambiguities with respect to the included studies. The numerous benefits of Ayurveda are being masked by a lack of proper research. The general need to improve the quality of research in Ayurveda is clearly visible, and the strong evidence thus generated, preferably by Indian researchers, will go a long way in fostering widespread acceptance of the immense knowledge of this ancient science by all stakeholders.

ARTICLE HIGHLIGHTS

Research background

Ayurveda is the Indian traditional system of medicine, and has a history since the 2nd century BC. Many Ayurvedic preparations have been anecdotally claimed to have immune-boosting properties and have been used for immune enhancement and general well-being. Pre-clinical research suggests that the immune enhancement is mediated through multiple immune system modulation and psychoneuroimmunological mechanisms.

Research motivation

We were interested to know the exact mechanisms of immune enhancement by Ayurvedic preparations when they are administered to healthy or sick humans.

Research objectives

The objectives of the present systematic literature review were to gather evidence towards the nature and mechanism of enhancement of human immune system by the administration of Ayurvedic preparations from published randomized clinical trials (RCTs).

Research methods

We prospectively registered the study protocol with PROSPERO. Based on predetermined eligibility criteria, search strategy was formulated and refined, and the same was used to search PubMed, DOAJ, Google Scholar, three dedicated Ayurveda research portals, two specialty Ayurveda journals, and reference lists for relevant records published until February 6, 2021. Baseline features and data pertaining to the nature and mechanism of immune system function were extracted from all eligible

records. Methodological quality was assessed using the Cochrane RoB-2 tool.

Research results

Our search strategy yielded a total of 12554 articles, and we found 19 studies reporting 20 RCTs (17 parallel group design, three crossover design) with 1661 unique patients to be eligible for inclusion. Healthy population was included in nine studies, of which one study included pregnant women and two included pediatric population; remaining studies included patients with different health conditions. A total of 21 Ayurvedic interventions were studied, out of which five were composite mixtures. Through indirect evidence, four interventions were seen to be associated with immune enhancement, and two interventions were associated with a lack of such an enhancement. The role of T helper cell and natural killer cell enhancement was reported to contribute to the enhancement of immune systems by three and four interventions, respectively. Evidence pointing to enhancement of other immune system components, including cytotoxic T cells, B lymphocytes, immunoglobulins, cytokines, complement components, leucocyte counts, and other components, was not found. Risk of bias was 'high' in 9/20 RCTs, and 'some concerns' of bias were found in the remaining 11/20 RCTs, according to RoB-2.

Research conclusions

Various Ayurvedic preparations, both standalone and composite, appear to have an enhancing effect on the immune system, as evidenced indirectly through reduced illness variables, but the exact mechanism behind this enhancement is not fully established. There may be contributions from enhancement of natural killer cells and T helper cells, although the role of other immune system components is not clear.

Research perspectives

There is a need to improve the quality of research in Ayurveda. Ayurvedic scholars should team up with experts of modern clinical research and generate credible and reproducible evidence towards immune system enhancement. This will enable widespread acceptance of the immense knowledge of Ayurveda, leading to its increased usage, and ultimately, a healthier society.

FOOTNOTES

Author contributions: Vallish BN contributed to acquisition of data, analysis and interpretation of data, drafting the article, making critical revisions related to important intellectual content of the manuscript, and final approval; Dang D contributed to acquisition of data, analysis and interpretation of data, making critical revisions related to important intellectual content of the manuscript, and final approval; Dang A contributed to conception and design of the study, acquisition of data, making critical revisions related to important intellectual content of the manuscript, and final approval.

Conflict-of-interest statement: The authors deny any conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2020 Checklist, and the manuscript was prepared and revised according to the PRISMA 2020 Checklist.

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S-Editor: Liu JH
L-Editor: Filipodia
P-Editor: Liu JH

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World Journal of Methodology

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World J Methodol 2022 May 20; 12(3): 148-163

DOI: 10.5662/wjm.v12.i3.148

ISSN 2222-0682 (online)

META-ANALYSIS

Assessment of diagnostic capacity and decision-making based on the 2015 American Thyroid Association ultrasound classification system

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Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Bhattacharya S, India; Geng J, China; Gluvic Z, Serbia

Received: December 22, 2021
Peer-review started: December 22, 2021
First decision: February 8, 2022
Revised: February 27, 2022
Accepted: April 24, 2022
Article in press: April 24, 2022
Published online: May 20, 2022

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Abstract

BACKGROUND

This study evaluates the American Thyroid Association (ATA) ultrasound (US) classification system for the initial assessment of thyroid nodules to determine if it indeed facilitates clinical decision-making.

AIM

To perform a systematic review and meta-analysis of the diagnostic value of the ATA US classification system for the initial assessment of thyroid nodules.

METHODS

In accordance with the PRISMA statement for diagnostic test accuracy, we selected articles that evaluated the 2015 ATA US pattern guidelines using a diagnostic gold standard. We analyzed these cases using traditional diagnostic parameters, as well as the threshold approach to clinical decision-making and decision curve analysis.

RESULTS

We reviewed 13 articles with 8445 thyroid nodules, which were classified according to 2015 ATA patterns. Of these, 46.62% were malignant. No cancer was found in any of the ATA benign pattern nodules. The Bayesian analysis post-test probability for cancer in each classification was: (1) Very-low suspicion, 0.85%; (2) Low, 2.6%; (3) Intermediate, 6.7%; and (4) High, 40.9%. The net benefit (NB), expressed as avoided interventions, indicated that the highest capacity to avoid unnecessary fine needle aspiration biopsy (FNAB) in the patterns that we studied

was 42, 31, 35, and 43 of every 100 FNABs. The NB calculation for a probability threshold of 11% for each of the ATA suspicion patterns studied is less than that of performing FNAB on all nodules.

CONCLUSION

These three types of analysis have shown that only the ATA high-suspicion diagnostic pattern is clinically useful, in which case, FNAB should be performed. However, the curve decision analysis has demonstrated that using the ATA US risk patterns to decide which patients need FNAB does not provide a greater benefit than performing FNAB on all thyroid nodules. Therefore, it is likely that a better way to approach the assessment of thyroid nodules would be to perform FNAB on all non-cystic nodules, as the present analysis has shown the ATA risk patterns do not provide an adequate clinical decision-making framework.

Key Words: Thyroid nodule; Thyroid cancer; Ultrasound; Bayesian analysis; Systematic review; Meta-analysis

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Core Tip: There is no analysis that evaluates the real diagnostic value of the 2015 American Thyroid Association thyroid nodule risk patterns and their usefulness for clinical decision-making; thus, we undertook this study to quantify both values.

Citation: Hurtado-Lopez LM, Carrillo-Muñoz A, Zaldivar-Ramirez FR, Basurto-Kuba EOP, Monroy-Lozano BE. Assessment of diagnostic capacity and decision-making based on the 2015 American Thyroid Association ultrasound classification system. *World J Methodol* 2022; 12(3): 148-163
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/148.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.148

INTRODUCTION

Many decisions in medicine involve trade-offs, such as weighing the balance between diagnosing patients with disease *vs* the cost of unnecessary additional testing for those who are healthy[1]. The traditional biostatistical approach to evaluating tests focuses on accuracy, evaluating calibration and discrimination, as well as using metrics such as sensitivity, specificity, or area under the curve (AUC). These methods have several advantages: They are mathematically simple, can be used for binary or continuous predictors, and are relatively easy to interpret. However, their clinical relevance is low, because there is no way to correctly discriminate between two or more diagnostic tests when there are differences in sensitivity or specificity among them. Furthermore, they do not take into consideration the consequences of the decisions made[2,3]. To address these issues, analytical methods for decision-making have been developed, which explicitly take into consideration the clinical consequences of decisions. They provide data about the clinical value of tests, including either the risks associated with an incorrect diagnosis, or the benefits of a correct diagnosis, and so can determine whether or not these tests should be used to guide decisions regarding patient care[4].

Ideally, the results of a diagnostic test should help physicians make a clear decision, meaning that, upon testing, we would either move from an epidemiological probability that a disease is present (testing threshold) to a lesser probability, and subsequent ruling out of the disease; or, on the contrary, the results could increase the probability to levels above the test-treatment threshold, and hence, point directly to treatment. However, sometimes the change in probability is higher than the testing threshold, but lower than the test-treatment threshold, in which case, the initial diagnostic test does not provide enough certainty to support decision-making regarding treatment, and additional diagnostic testing would therefore be required. This analytical process of diagnostic testing is known as "the threshold approach to clinical decision-making"[5,6] and it provides a clear, objective, and rational method to determine whether additional diagnostic testing is needed or not (Figure 1).

The sensitivity and specificity of a test cannot be used alone to estimate the probability of disease in a patient, but the two parameters when combined into one measure, called the likelihood ratio, may be used in conjunction with disease prevalence to estimate an individual patient's probability of having disease. This probability can then be transformed into a post-test probability through Bayesian analysis, and this post-test probability, when applied to the threshold approach to clinical decision-making, can then show us the true utility of a diagnostic test[7-9].

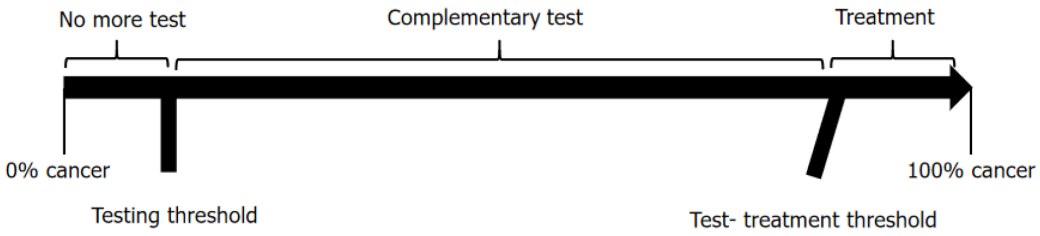

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Figure 1 The threshold approach to clinical decision-making.

Another method for clinical decision-making is decision curve analysis[2,10,11]. This method calculates a clinical "net benefit" for a diagnostic test *vs* treating all or no patients, across a range of threshold probabilities, defined as "the minimum probability of disease at which further intervention would be warranted". The net benefit, unlike accuracy metrics such as discrimination and calibration, incorporates the consequences of the decisions that were made based on the results of a diagnostic test. Therefore, if you look at the net benefit of a range of reasonable threshold probabilities (Pt) for any given intervention and, if your test has a high net benefit across the whole range, you can say that your test can help you to make an adequate decision regarding that intervention. It is clear, then, that if we use analytical methods for clinical decision-making, in addition to traditional diagnostic testing, we will have a better understanding and clinical use of the diagnostic test results.

In the context of thyroid nodule assessment, the role of ultrasound (US) has historically been very important. It was initially used only to identify the thyroid nodule and guide fine needle aspiration biopsy (FNAB), and later, it was further developed to identify nodule characteristics that would help differentiate between benign and malignant lesions, such as internal calcifications, hypoechoicity, increased central blood flow, infiltrative margins, taller than wider shape, absence of halo, solid nodule, and nodule size[12,13]. However, various meta-analyses have shown that none of these characteristics alone can differentiate with certainty between benign and malignant lesions[14-16].

The 2015 American Thyroid Association (ATA) Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer[17] reengineered these images, and, taking advantage of the practical aspects of US (non-invasive and readily accessible), described five sonographic patterns to establish the risk of malignancy: (1) Benign US features consist of purely cystic nodules, with no solid component, with an estimated risk of malignancy < 1%; (2) Very-low suspicion US features consist of spongiform or partially cystic nodules without any of the sonographic features described in low, intermediate, or high suspicion patterns, with an estimated risk of malignancy < 3%; (3) Low suspicion US features consist of isoechoic or hyperechoic solid nodule, or partially cystic nodule with eccentric solid areas, without microcalcification, irregular margin or extra thyroidal extension (ETE), or taller than wide shape, with an estimated risk of malignancy of 5%-10%; (4) Intermediate suspicion US features consist of hypoechoic solid nodule with smooth margins without microcalcifications, ETE, or taller than wide shape, with an estimated risk of malignancy of 10-20%; and (5) High suspicion US features consist of solid hypoechoic nodule or solid hypoechoic component of a partially cystic nodule with one or more of the following features: Irregular margins (infiltrative, microlobulated), microcalcifications, taller than wide shape, rim calcifications with small extrusive soft tissue component, evidence of ETE, with an estimated risk of malignancy between 70%-90%.

Based on these US descriptions and the size of the thyroid nodule, the ATA's intention is to standardize diagnostic behavior, from performing FNAB to simply keeping the thyroid nodule under observation, and the ATA patterns provide a clear and simple guideline to follow.

However, in the development of these US patterns, the origins of the percentages of malignancy suspicion assigned to each pattern is not clear, nor is the diagnostic accuracy of each pattern.

Several papers which have been published to date, both retrospective and prospective, have attempted to validate the risk patterns indicated in the ATA guidelines. These papers have shown similar findings in terms of malignancy rates in the categories of very low, low, and intermediate suspicion, although not in high suspicion, which have generally been found to be a lower percentage [18-40]. However, these studies only calculated the risk as a simple percentage and did not consider the diagnostic value in a clinical setting, which must be clearly established prior to decision-making.

Therefore, the objective of this study was to determine the real diagnostic value of the ATA classification system and to determine whether clinical decision-making based on this classification leads to an optimal management of thyroid nodules.

MATERIALS AND METHODS

Study design and data sources

We made a systematic review of the published literature related to the American Thyroid Association US classification system for the initial assessment of thyroid nodules[17] from 2016 to date.

Data extraction was performed in accordance with the PRISMA statement for Diagnostic Test Accuracy Studies[41] and by searching PubMed-Medline for all articles published in the English language with the keywords: Thyroid nodule, thyroid, ultrasound, US, ultrasonography, and 2015 ATA. Related articles suggested by PubMed were also retrieved. Bibliographies of retrieved articles were searched independently and checked for additional studies.

Study selection

Our criteria for eligibility were articles that clearly reported data related to the US patterns described in the ATA 2015 guidelines[17] and where the diagnosis of malignant or benign had been established either by histology reports, or two benign FNAB results.

Review process

Two authors (LMHL and ACM) independently reviewed the articles and established the criteria for inclusion in the pooled data analysis, with disagreements resolved through discussion. Characteristics of the included and excluded articles are presented in Figure 2.

Methodologic quality assessment

Methodologic quality was assessed using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies 2) criteria[42]. Both reviewers (LMHL and ACM) scored the 7-item tool independently and disagreements were resolved by consensus (among LMHL, ACM, and FRZR) *via* a face-to-face discussion about each disagreement (Table 1).

Data analysis

We used the Meta-DiSc, version 1.4 software (Ramon y Cajal Hospital, Madrid, Spain) in our meta-analysis[43]. The Mantel-Haenszel method of the random-effect model was used to calculate pooled sensitivity and specificity with corresponding 95% confidence intervals.

We analyzed these cases first using traditional diagnostic parameters and then the threshold approach to clinical decision-making and decision curve analysis.

To perform the threshold approach to clinical decision-making, it is important to understand that the indifference point for the choice between withholding therapy and performing a diagnostic test is a probability of disease designated here as the "testing" threshold (Tt). The indifference point for the choice between performing the diagnostic test and administering treatment is a probability of disease designated here as the "test-treatment" threshold (Ttrx).

Because the thresholds define these two indifference points, the physician can be guided by the calculated thresholds and estimated probability of disease in a given patient. As illustrated in Figure 1, the best choices are to withhold both treatment and the test if the probability of disease is smaller than the testing threshold, to administer treatment without testing if the probability of disease is greater than the test-treatment threshold, and to perform the test only if the probability of disease falls between the two thresholds.

The threshold levels were developed as follows[5]: The Tt consisted of the frequency of thyroid cancer, as reported in the 1015 ATA guidelines[17], estimated to be between 7% and 15%, making an average frequency of 11%.

For the Ttrx, we used the formula described by Pauker *et al*[5] shown in Figure 3. Using this formula, we estimated the Brx at 20% considering that the survival rate of a patient with papillary thyroid cancer is 98% with an early diagnosis, and 78% when there has been distant metastases[17,44]; Rrx at 4.2% when calculating an overall average rate of morbidity in total thyroidectomies, including permanent injury of the recurrent laryngeal nerve[45-47], injury to the external branch of the upper laryngeal nerve [48,49], hypoparathyroidism[50-53], and hematoma[54,55]; Rt at zero, as performing an US does not expose the patient to any risk; Pneg/nd at 0.98, representing true negatives, calculated based on US patterns[17] for benign and very low suspicion; and Pneg/d at 0.10 representing false negatives, as high-suspicion patterns detect 90% of cancers[17]. The resulting Ttrx was 67.2%.

To perform the decision curve analysis, the value for probability threshold (Pt), defined as the minimum probability of disease at which further intervention would be warranted[2,10], is a clinical judgement, and was calculated under five conditions: The first was set by simply identifying the general probability of cancer in a thyroid nodule, which is 11%, according to the ATA. The second was set to 28.1%, by taking an intermediate point between the Tt (11%) and the Ttrx (67.2%). The third condition was set to 3%, which is the probability of cancer in the very low pattern. The fourth condition was set to 7%, which is the lower range of probability of cancer according to the 2015 ATA guidelines. The fifth condition was set to 15%, which is the highest range of probability of cancer according to the 2015 ATA guidelines. We used these five Pt to calculate the net benefit of each ATA US risk pattern.

Table 1 Risk of bias and applicability judgments

Ref.	Risk of bias			Flow and timing	Applicability		
	Patient selection	Index test	Reference standard		Patient selection	Index test	Reference standard
Tang *et al*[25]	Low	Low	Low	Low	Low	Low	Low
Trimboli *et al*[28]	Low	Low	Low	Unclear	Low	Low	Low
Xu *et al*[29]	Low	Low	Low	Low	Low	Low	Low
Persichetti *et al*[30]	Low	Low	Low	Low	Low	Low	Low
Macedo *et al*[31]	Low	Low	Low	Low	Low	Low	Low
Chng *et al*[32]	Unclear	Unclear	Low	Low	High	Low	Low
Huang *et al*[34]	Low	Low	Low	Unclear	Low	Low	Low
Barbosa *et al*[35]	Unclear	Low	Low	Low	High	Low	Low
Hong *et al*[36]	Low	Low	Low	Low	Low	Low	Low
Valderrabano *et al*[37]	Low	Low	Low	Low	Low	Low	Low
Xiang *et al*[38]	Low	Low	Low	Low	Low	Low	Low
Gao *et al*[39]	Low	Low	Low	Low	Low	Low	Low
Shen *et al*[40]	Low	Low	Low	Low	Low	Low	Low

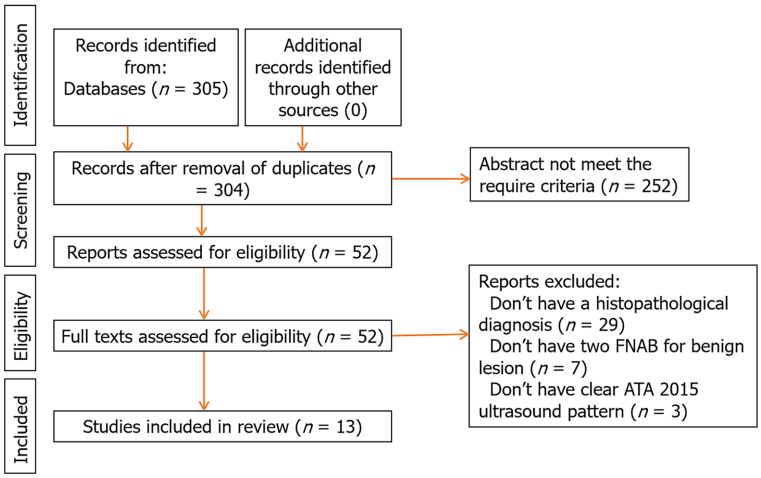

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Figure 2 Flowchart of study selection process.

$$Ttrx = \frac{(Pneg/nd)\ X\ (Rrx) - Rt}{(Pneg/nd)\ X\ (Rrx) + (Pneg/d)\ X\ (Brx)}$$

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Figure 3 Test-treatment threshold formula. Ttrx: Test-treatment threshold; Pneg/nd: Probability of a negative result in patients without disease; Rrx: Risk of treatment in patients without disease; Rt: Risk of diagnostic test; Pneg/d: Probability of a negative result in patients with disease; Brx: Benefit of treatment in patients with disease.

We calculated the net benefit, expressed as the number of unnecessary interventions avoided, in this case the number of FNABs avoided, using true negatives rather than true positives, and using the following formula: Net benefit for unnecessary interventions = (true negative count/total number of patients) – (false positive count/total number of patients) × (Pt /(1- Pt) in order to determine the number of unnecessary biopsies that had been performed, without missing any patients with cancer, in each of the ATA US patterns. We calculated these in all five possible Pt[10,11].

Once we were able to determine the best Pt by calculating the highest number of unnecessary FNABs avoided, we then calculated the net benefit for this Pt using curve decision analysis for each US pattern, using the following formula: Net benefit = (true positive count/total number of patients) – (false-positive count/total number of patients) × (Pt/1-Pt). This result would then be compared with the net benefit of performing FNAB on all thyroid nodules (Figure 4).

The resulting net benefit for each US pattern would have to be of greater value than the net benefit of performing FNAB on all thyroid nodules for it to be considered a diagnostic model that provides the correct identification of those thyroid nodules which can be safely excluded from FNAB.

We extracted the information, grouped it by author, and added all the cases together, obtaining sensitivity, specificity, and positive and negative predictive values for each of the categories. We determined the Youden's index using the following formula: Sensitivity + (specificity -1); and the positive likelihood ratio (LR) using the following formula: Sensitivity/(1-specificity). We then calculated post-test odds with Bayesian analysis using the following formula: Post-test odds = pretest odds × LR, and, in the final step, we converted the post-test odds into post-test probability.

Due to the design of this study, approval by an institutional review board was not required.

RESULTS

Our initial search retrieved 305 articles, and the summaries were reviewed by two authors (LMHL and ACM) to select those that met the required criteria. This resulted in 52 articles that we reviewed in their full text, before finally selecting 13 articles[25,28,29-32,34-40] which contained the required information in accordance with the design of the study. Nine were retrospective studies[28,29,32-37,39,40] and four were prospective studies[25,30,31,38] (Table 2 and Figure 3).

The data from 8445 thyroid nodules was obtained, of which 3937 (46.62%) were malignant and 4508 (53.38%) were benign. The average size of the tumors was 18.5 mm (5 mm to 71 mm).

When grouping the nodules into risk patterns, we found that the benign pattern was reported in only 6 of the 13 articles[30,32,34,36,39,40], for a total of 62 nodules in the category, and all of these corresponded to histopathologically benign nodules, therefore we decided to exclude this pattern from our analysis.

For the very-low suspicion pattern, there were a total of 848 cases. Of these, 832 were benign and 16 were malignant, meaning that in this pattern, the simple percentage of malignancy was 1.8%. The Youden's index was -0.18. The diagnostic value can be found in Table 3 and Figure 5.

There were 1800 nodules in the low-suspicion pattern. Of these, 1621 were benign and 179 were malignant, meaning that in this pattern, the simple percentage of malignancy was 9.4%. The Youden's index was -0.31. The diagnostic value can be found in Table 3 and Figure 6.

There were 1673 nodules in the intermediate-suspicion pattern. Of these, 1340 were benign and 333 were malignant, meaning that in this pattern, the simple percentage of malignancy was 19.9%. The Youden's index was -0.22. The diagnostic value can be found in Table 3 and Figure 7.

There were 4124 nodules in the high-suspicion pattern. Of these, 715 were benign and 3404 were malignant, meaning that in this pattern, the simple percentage of malignancy was 82.5%. The Youden's index was 0.71. The diagnostic value can be found in Table 3 and Figure 8.

After using Bayesian analysis to determine the post-test probability of cancer, our results were as follows: Very low, 0.85%; low, 2.6%; intermediate, 6.7%; and high, 40.9% (Figure 9).

The net benefit, expressed as the number of interventions (FNAB) avoided, with a Pt calculated for all five possibilities, can be seen in Figure 10.

The NB calculation for a Pt of 11% was: Very low, -0.01028; low, -0.002526; intermediate, 0.019821; and high, 0.393207. This means that the intermediate pattern was only able to identify 1.9 of every 100 patients with cancer, and the high pattern was only able to identify 39 of every 100 patients with cancer. In the very low and low pattern cases, there was no obvious interpretation for negative net benefit using this type of framework.

The true- and false-positive count for performing FNAB in all thyroid nodules is simply the number of patients with and without thyroid cancer, respectively. Calculating the net benefit for this strategy gave: (3937/8445) − (4508/8445) × (0.11/0.89) = 0.40022. The net benefit for each of the ATA suspicion patterns studied was less than that of performing FNAB on all nodules.

There was heterogeneity in the initial results, which could create a bias risk, so we made an analysis of the subgroups (Figure 10). The homogenous group was reduced to a population of 5151 thyroid nodules: 443 from the very low risk category with a 2.9% frequency of cancer, 316 cases from the low risk category with a 16.1% frequency of cancer, 946 from the intermediate risk category with a 20.7% frequency of cancer, and finally in the high risk category, 3446 nodules with an 81.5% frequency of cancer (Figure 11). After using Bayesian analysis to determine the post-test probability of cancer in the subgroup analysis, our results were: Very low, 0.7%; low, 1.8%; intermediate, 0.2%; and high, 37.4% (Figure 12).

Table 2 Studies and number of patients included

Ref.	Very low		Low		Intermediate		High	
	Cancer no	Cancer yes	Cancer no	Cancer yes	Cancer no	Cancer yes	Cancer no	Cancer yes
Tang *et al*[25]	7	1	20	5	17	7	0	8
Trimboli *et al*[28]	15	2	43	8	68	15	6	18
Xu *et al*[29]	36	2	190	21	212	59	109	277
Persichetti *et al*[30]	134	3	255	8	295	18	104	127
Macedo *et al*[31]	11	0	10	0	13	5	1	5
Chng *et al*[32]	16	1	60	10	18	12	13	27
Huang *et al*[34]	14	0	109	18	57	32	4	15
Barbosa *et al*[35]	4	1	33	10	27	9	10	46
Hong *et al*[36]	35	0	174	6	109	55	37	263
Valderrabano *et al*[37]	25	0	127	32	61	13	16	20
Xiang *et al*[38]	170	0	112	24	8	11	32	289
Gao *et al*[39]	178	0	339	20	107	55	233	1606
Shen *et al*[40]	187	6	149	17	348	42	150	708
Total	832	16	1621	179	1340	333	715	3409

Table 3 Pooled diagnostic value of ultrasound patterns studied

	Very Low	95%CI	Low	95%CI	Intermediate	95%CI	High	95%CI
Sensitivity	1%	0-1	5%	4-5	8%	8-9	87%	85-88
Specificity	82%	80-83	64%	63-65	70%	68-72	84%	83-85
Likelihood ratio (+)	0.07	0.03- 0.17	0.22	0.11-0.43	0.59	0.38-0.94	5.63	4.52-7.01

DISCUSSION

This study demonstrates how complicated it can be to interpret diagnostic tests and use them for clinical decision-making.

It is important to recognize that when we analyze decision-making based on diagnostic tests, there is no one "perfect test" that can either rule out or diagnose a disease with a 100% accuracy (in this discussion, thyroid cancer). Therefore, for every patient who undergoes a diagnostic test to help guide decision-making, there will always be three possible paths; the first is to keep the nodule under observation, the second is to do additional diagnostic testing, and the third is to proceed with treatment.

The results found in this study show an overall frequency of cancer of 46.6%. This frequency is higher than the 7% to 15% reported by the ATA because the data was obtained from reference center hospitals with a high volume of thyroid cancer cases. Although this could be considered as selection bias, it is important to emphasize that the diagnostic parameters which we used in this analysis, in particular sensitivity and specificity, and the decision-making based on these diagnostic tests, are not affected by the frequency of this disease.

We must also keep in mind that while indeed cancer was most frequent in the high-suspicion pattern, 15.5% of the cases were found among the very low, low, and intermediate-suspicion patterns.

It is also important to note that we did not include the benign pattern, as it was only reported in six of the studies analyzed, and of the 62 nodules reported with this pattern, all were confirmed to be benign. Therefore, it is reasonable to assume that any thyroid nodule that is purely cystic, regardless of size, is benign, and as such, we did not consider it necessary to include them in this study.

Another potential bias is that we had to exclude the size of the thyroid nodules, as detailed information for each nodule was not available in the articles that we reviewed. However, we do not consider this as selection bias, because the average of sizes reported in the articles that we reviewed was 18.5 mm (ranging from 5 mm to 71 mm), which is within the typical range found in most nodules in the day-to-day medical practice. We therefore consider that this variable is not of particular importance in deciding which patients will need FNAB. Nodule size has always generated controversy in terms of how it might affect the diagnosis of malignancy[56-59].

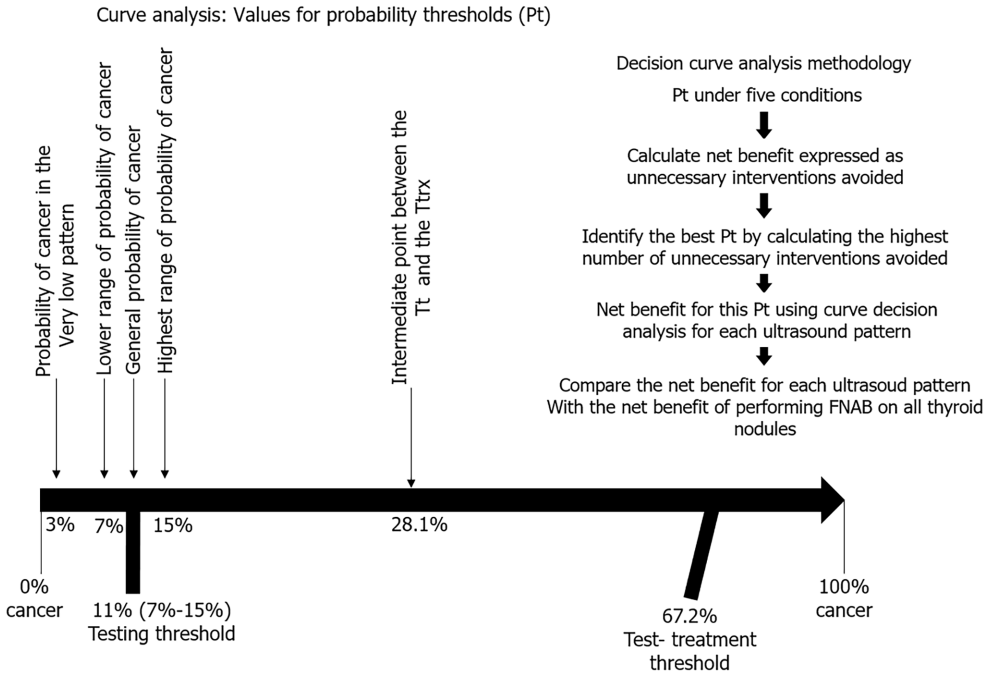

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Figure 4 Decision curve analysis methodology.

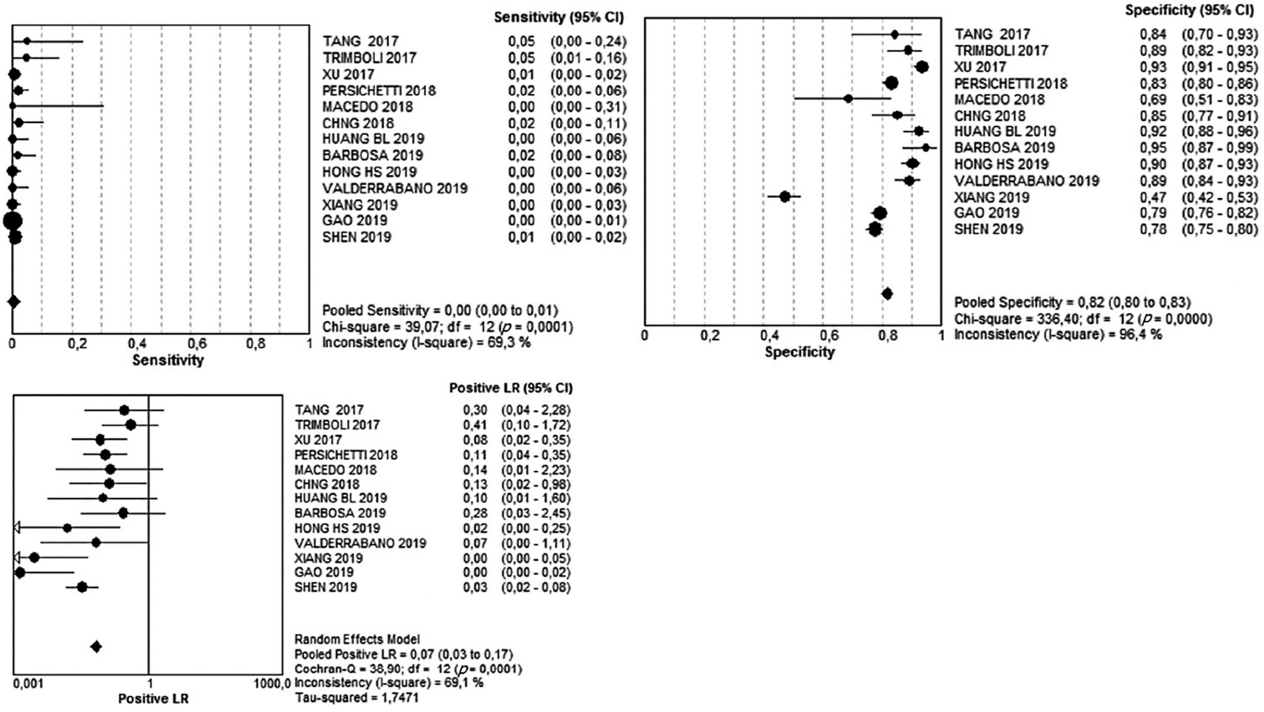

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Figure 5 Pooled diagnostic value of very low risk pattern.

The final limitation in our study is that most of the US images, and consequently their ATA US classifications, were performed retrospectively. However, this is unlikely to be significant, as the cases came from high-volume thyroid disease reference centers, and were interpreted by highly qualified US experts[60,61].

Our results show that, if we were to assume that the simple percentage for the presence of cancer in each US pattern was a reliable diagnostic tool, they would coincide perfectly with the ranges published by the 2015 ATA guidelines[17], in that the very low pattern had a 1.8% malignancy rate and the ATA reports < 3%; in the low pattern, a 9.4% malignancy rate and the ATA reports 5% to 10%; in the

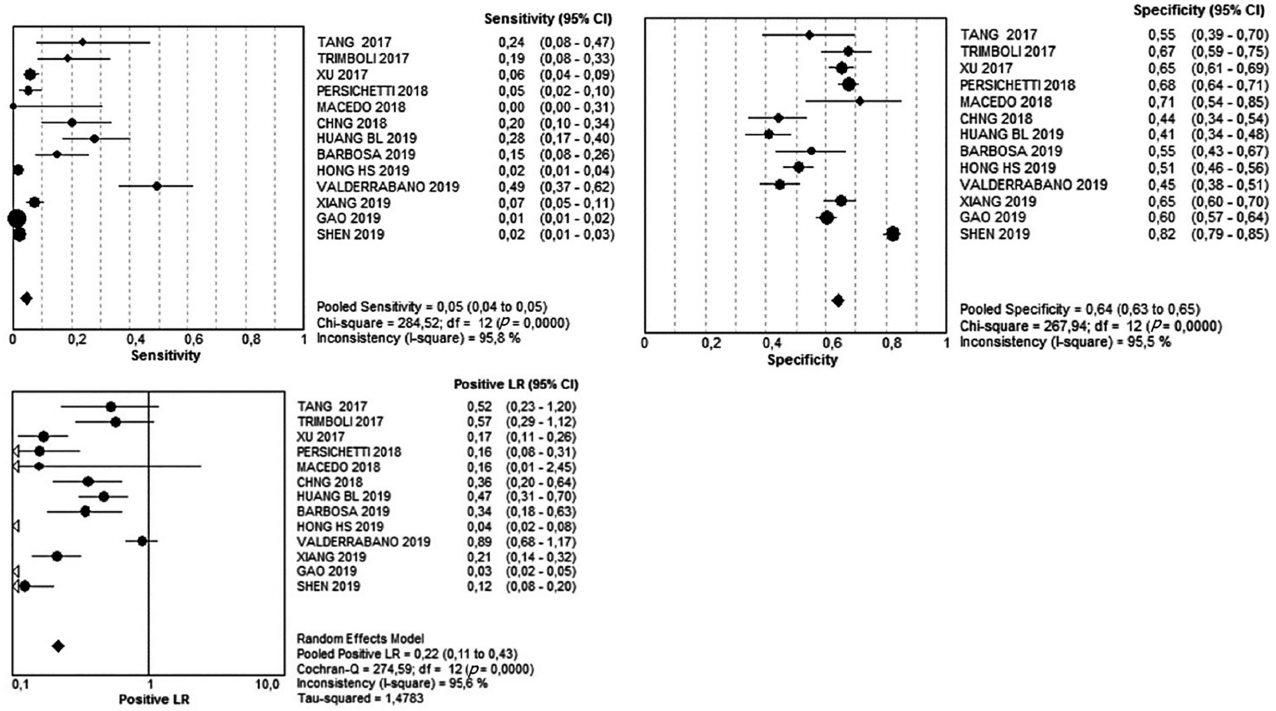

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Figure 6 Pooled diagnostic value of low risk pattern.

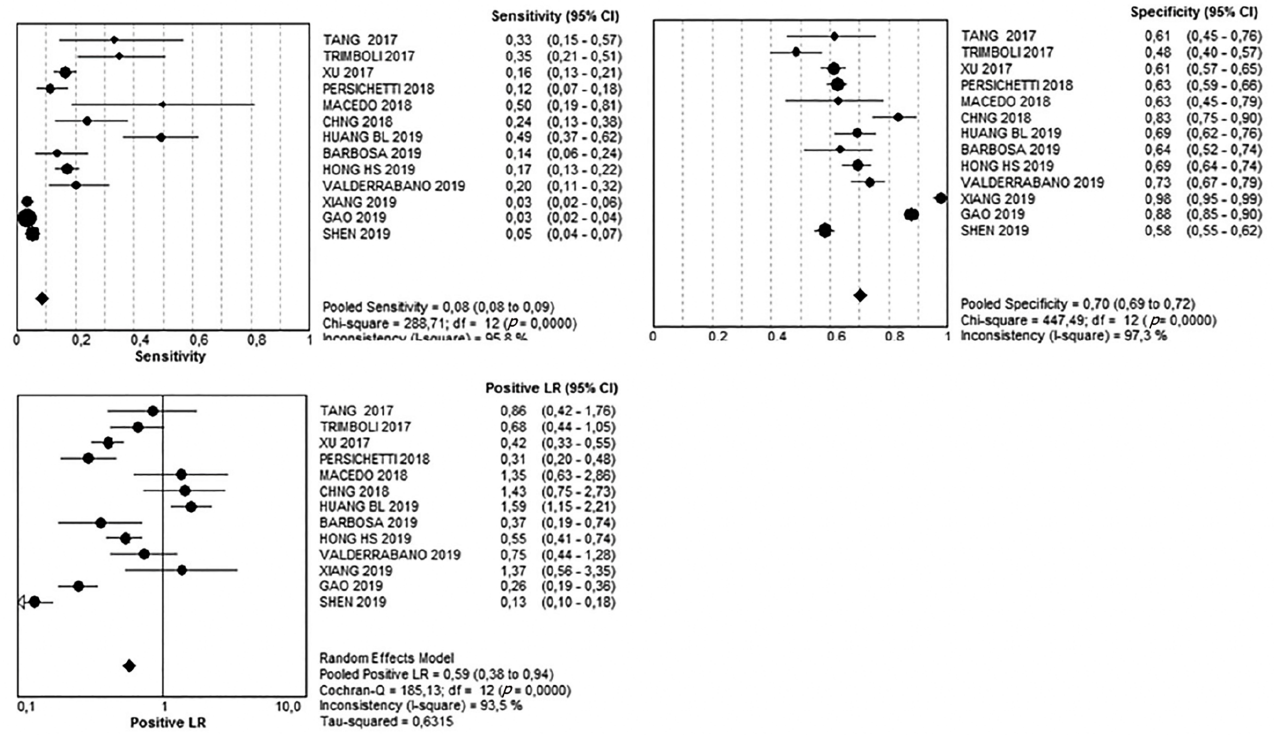

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Figure 7 Pooled diagnostic value of intermediate risk pattern.

intermediate pattern, a 19.9% malignancy rate and the ATA reports 10% to 20%; and finally, in the high pattern, an 82.5% malignancy rate and the ATA reports between 70% and 90%.

However, when we analyzed the results of the traditional biostatistical approach to evaluating tests and Youden's index, together with the threshold approach to clinical decision-making and curve decision analysis, we can see that these US patterns were no longer as clear, or as practical.

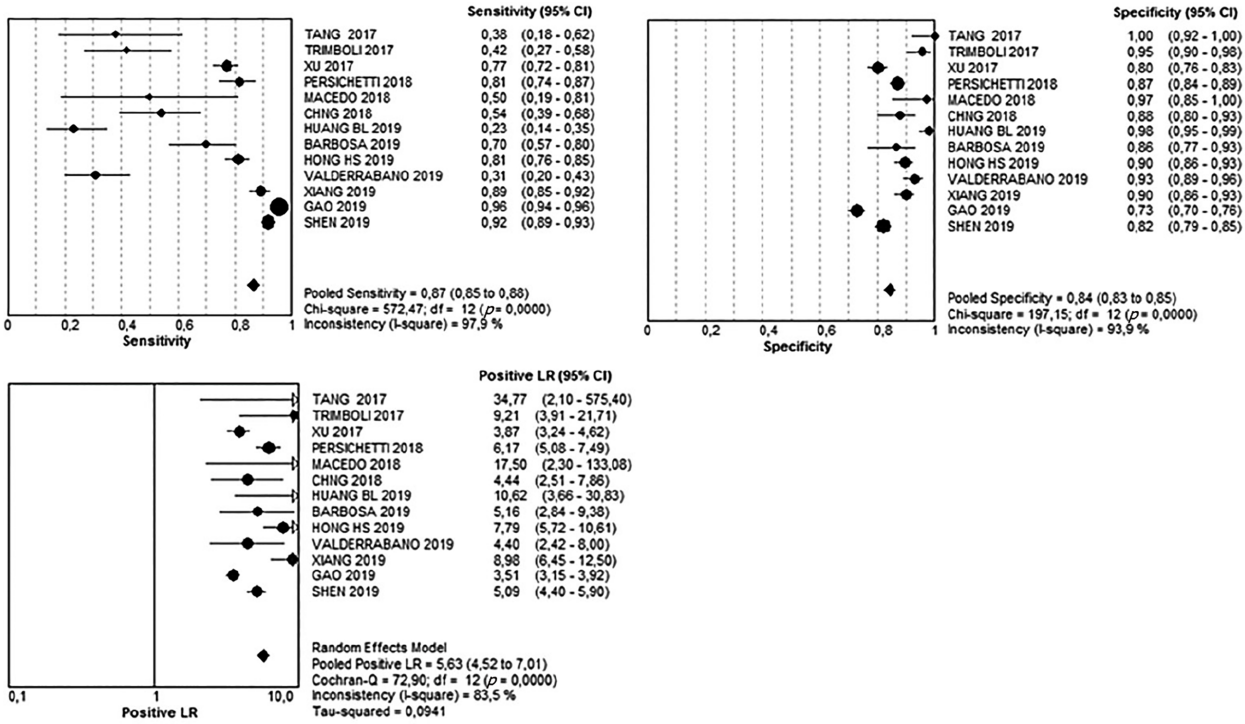

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Figure 8 Pooled diagnostic value of high risk pattern.

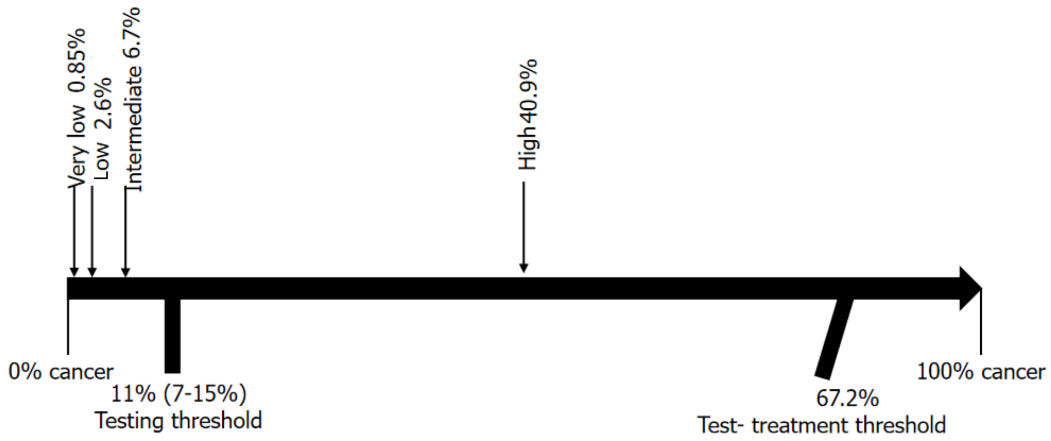

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Figure 9 Results by Bayesian analysis and the threshold approach to clinical decision-making.

When we analyzed a traditional diagnostic parameter, we can see that the very low, low, and intermediate patterns had a regular to good specificity, but a very low sensitivity, therefore they could, in theory, be used to correctly identify and rule out patients who do not have the disease, with very few false-positive results. In effect, this is the reason that the ATA has grouped thyroid nodules into patterns to guide decisions regarding whether to do further FNAB testing or not. However, our study showed that relying strictly on these patterns would have resulted in false positives: For very low, 18.1%; low, 36%; and intermediate, 29.7%, meaning that there would have been patients with cancer that went undetected using the US patterns, and, because the Youden's index for these three patterns was below 0, they would have been identified as "without diagnostic value".

On the other hand, the high pattern gave a more accurate diagnostic value with a sensitivity of 86.6%, specificity of 84.1%, and Youden´s J index of 0.7, and so, this pattern is better able to discriminate between malignant and benign cases, and therefore, can be used reliably.

When analyzing decisions based on the threshold approach to clinical decision-making, we were able to determine that the testing threshold was 11% and the test-treatment threshold was 67.2%. Once we determined the values of the US patterns using Bayesian analysis, we concluded that the very low

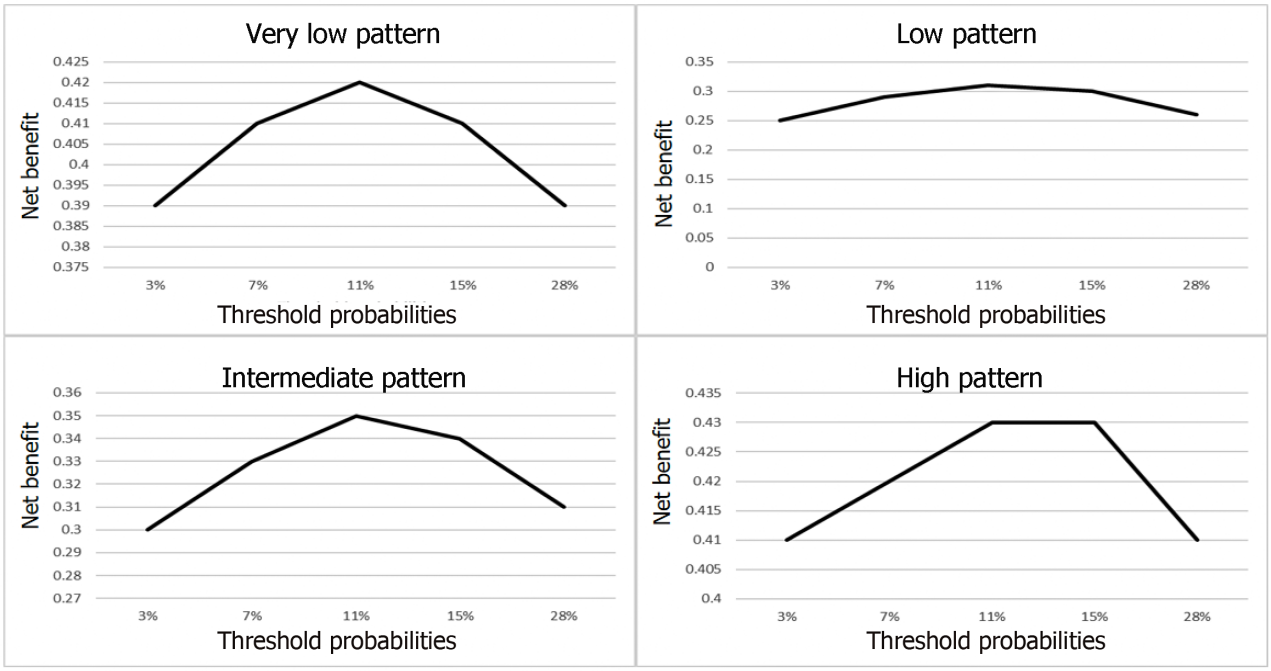

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Figure 10 Net benefit, number of interventions avoided, in study patterns.

pattern had a cancer risk of 0.3%, low of 1.5%, and intermediate of 3.4%, meaning that these three patterns would be below the testing threshold and, as a logical consequence of this method of analysis, thyroid nodules classified within these three patterns could simply be left under observation, since by definition, cancer had been ruled out, rendering further testing unnecessary. However, decisions using this type of analysis would have resulted in 15.5% of the cancers in this study going undiagnosed, troubling if we keep in mind that intermediate-risk nodules are mostly isoechoic nodules, which could be follicular carcinoma, a potentially high-risk thyroid cancer with a poor prognosis. If physicians do not perform FNAB on Bethesda IV intermediate-risk nodules, this group of potentially high-risk cancers can be missed.

The high-risk pattern, on the other hand, analyzed in the same way, raises the probability of thyroid nodule cancer to 40.3%, which is between the testing threshold (11%) and the test-treatment threshold (67.2%), therefore indicating the need to do additional FNAB testing.

With regard to heterogeneity, we made an analysis by subgroups, and it is interesting to note that the frequency of malignancy was quite similar to the initial analysis. Even more importantly, after using Bayesian analysis with the threshold approach to clinical decision-making, the results were still the same, where, in the groups of very low, low, and intermediate risk, cancer was ruled out, and the high-risk group fell in the area where additional testing would be necessary.

When analyzing decision-making based on the net benefit expressed as the number of unnecessary interventions avoided, we made our calculations using several different Pt scenarios, with a Pt of 11% being the one that most frequently matched with the test threshold defined in the analysis discussed above. We made this analysis, because the classification of US patterns was intended to determine who should undergo FNAB and who could be kept under observation, in order to reduce the number of unnecessary FNABs. When evaluating the capacity to avoid unnecessary FNABs, we found that the highest capacity for avoidance was: 42 of 100 FNABs in the very low pattern with a Pt of 11%, 31 of 100 FNAB in the low pattern with a Pt of 11%, 35 of 100 FNAB in the intermediate pattern with a Pt of 11%, and 43 of 100 FNABs in the high-risk pattern with a Pt of 11%. This means that attempting to avoid unnecessary FNABs is unadvisable, since over half of them were indeed necessary, and a cancer diagnosis would have been missed if FNAB had not been performed.

When calculating NB with a Pt of 11%, the very low and low patterns had a net benefit of less than zero, so there was no obvious interpretation for negative net benefit using this type of framework. In the case of intermediate patterns, NB could only detect slightly fewer than 2 out of every 100 patients with cancer, and in high-risk patterns, it could only detect 39 out of every 100 patients with cancer.

Further still, when comparing the net benefit of each US risk pattern studied *vs* that of systematically performing FNAB on all thyroid nodules, we found that none were higher than that of performing FNAB on all thyroid nodules. This clearly demonstrates that using these US categories to guide the decision regarding who should undergo FNAB is inferior to performing FNAB on all thyroid nodules.

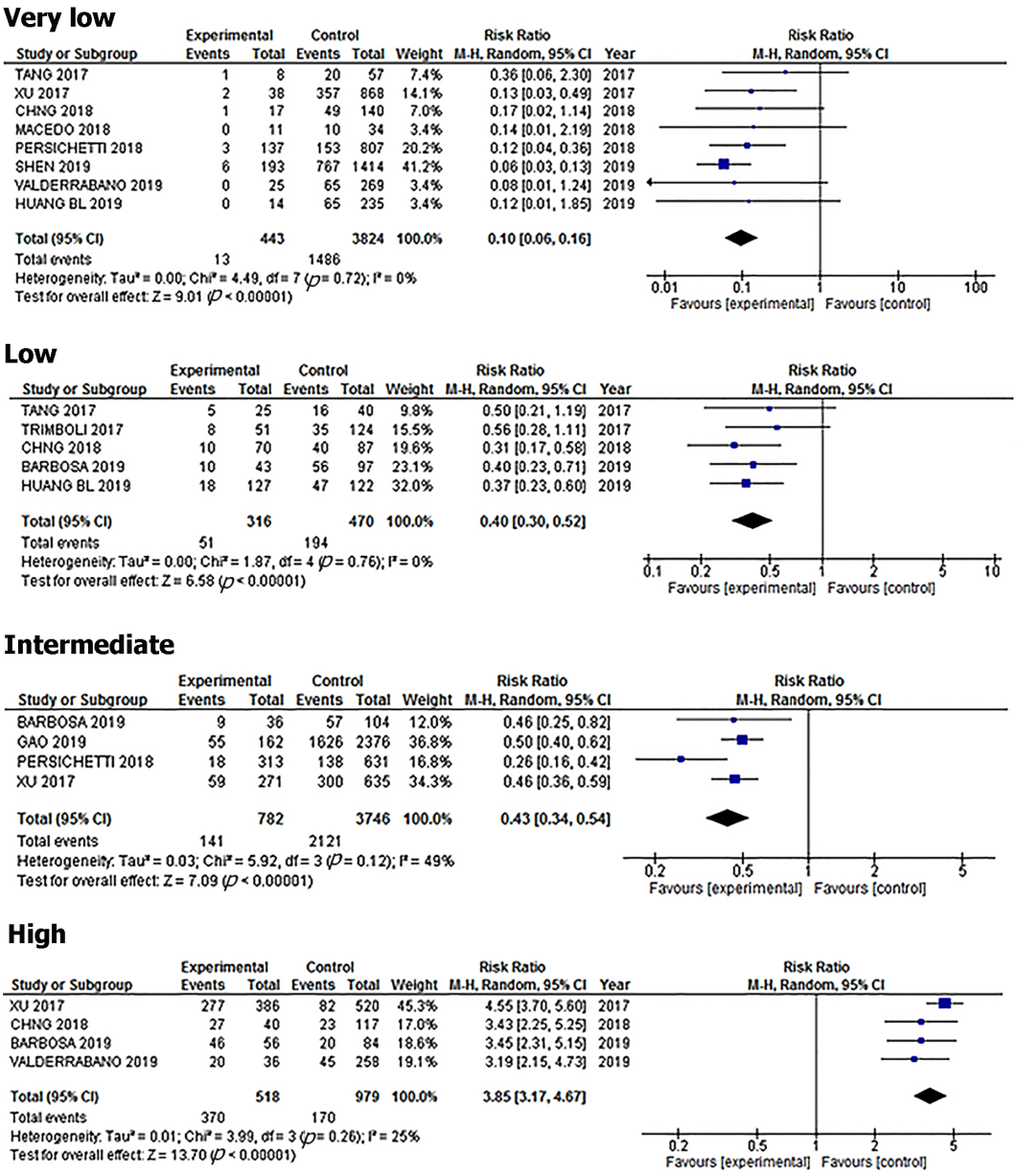

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Figure 11 Pooled subgroups analysis.

It is also important to understand that clinical decisions should not be made based on simple percentages. Instead, it is preferable to apply a rigorous study based on clinical decision models, which, although they may seem complicated to calculate, are, in reality, the only way to make an accurate professional diagnosis for the thyroid nodule patient. It is also clear that the clinically low aggressiveness of malignant thyroid cancer has allowed for a margin of error since it can be detected in a formerly undiagnosed patient at a later date. However, this diagnostic behavior would be unprofessional, as any patient who consults a physician for a thyroid nodule expects an accurate diagnosis.

From a practical standpoint, the results of this study indicate to the physician that, when evaluating thyroid nodules by US, only the high-risk and benign categories are clinically useful, indicating FNAB for high-risk cases and observation for the benign pattern. However, if the US shows a pattern of very low, low, or intermediate risk, the physician should recommend FNAB, as opposed to the current recommendations of observation only, as there is a risk of cancer of up to 15% that could go undiagnosed.

CONCLUSION

It is clear from our three types of analysis, that the only ATA diagnostic pattern that is clinically useful

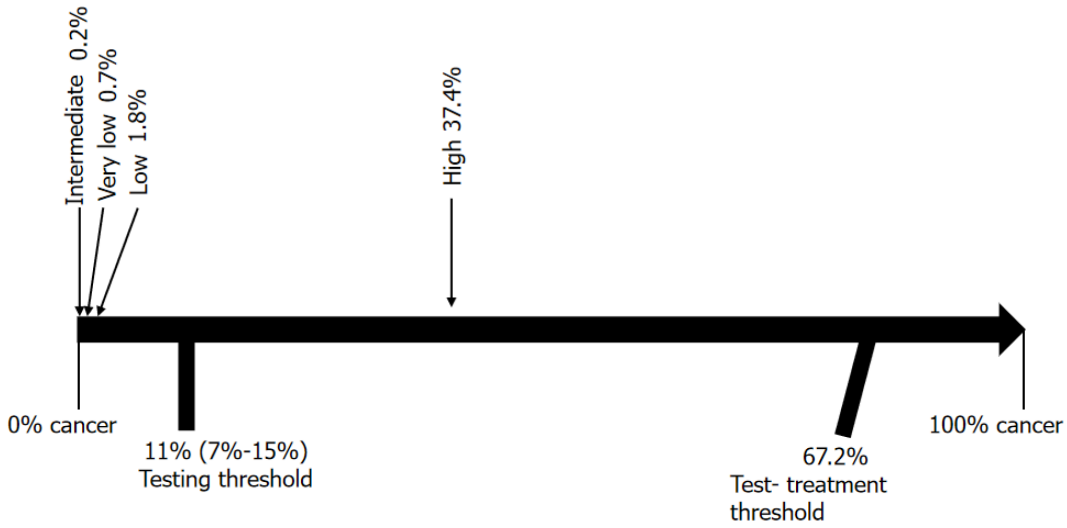

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Figure 12 Bayesian subgroups analysis and the threshold approach to clinical decision-making.

is the high-suspicion pattern, in which case, without a doubt, FNAB should be performed. However, and even more importantly, curve decision analysis has demonstrated that using these US risk patterns to decide which patients need FNAB does not provide a greater benefit than performing FNAB on all thyroid nodule patients. Therefore, we conclude that a better way to approach the assessment of thyroid nodules would be to perform FNAB on all non-cystic nodules, as the present study has shown that the ATA risk patterns do not provide an adequate clinical decision-making framework.

ARTICLE HIGHLIGHTS

Research background

It is important to make clinical decisions with the best evidence available, but the 2015 American Thyroid Association (ATA) Ultrasound (US) Guide does not yet have sufficient evidence. Therefore it should be studied and evaluated whether or not it is useful in making clinical decisions during the initial evaluation of thyroid nodules.

Research motivation

The real diagnostic value and its usefulness in clinical decision-making of the ATA 2015 US guide should be known.

Research objectives

To perform a systematic review and meta-analysis of the diagnostic value of the American Thyroid Association US system for the initial assessment of thyroid nodules.

Research methods

A meta-analysis study of the diagnostic value of the ATA 2015 ultrasonographic patterns was carried out and this diagnostic value was used to evaluate, through threshold and decision curve analysis, whether it is useful in decision-making during the initial evaluation of thyroid nodules.

Research results

The results showed that the US guided studies had no diagnostic value for decision-making in selecting which nodule should undergo or not FNAB.

Research conclusions

Physicians should continue doing FNAB to all solid or mixed thyroid nodules.

Research perspectives

An alternative diagnostic method must continue to be sought, which resolves the question of which nodule should undergo and which not FNAB.

FOOTNOTES

Author contributions: Hurtado-López LM contributed to conceptualization; and all authors contributed in methodology, investigation, original drafting, and final approval.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Ma YJ
L-Editor: Wang TQ
P-Editor: Qi WW

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World Journal of Methodology

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World J Methodol 2022 May 20; 12(3): 164-178

DOI: 10.5662/wjm.v12.i3.164

ISSN 2222-0682 (online)

META-ANALYSIS

Participant attrition and perinatal outcomes in prenatal vitamin D-supplemented gestational diabetes mellitus patients in Asia: A meta-analysis

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Specialty type: Obstetrics and gynecology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Qiong L, China; Wu QN, China

Received: December 1, 2021
Peer-review started: December 1, 2021
First decision: January 12, 2022
Revised: January 20, 2022
Accepted: March 26, 2022
Article in press: March 26, 2022
Published online: May 20, 2022

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Abstract

BACKGROUND

The role of vitamin D supplementation in gestational diabetes mellitus (GDM) patients is unclear.

AIM

To determine the burden and risk of post-randomization GDM patient attrition from vitamin D-supplemented arms of randomized controlled trials (RCTs). The auxiliary aim was to compare the effects of nutritional supplements on their fasting blood glucose (FPG) levels and perinatal outcomes.

METHODS

RCTs were searched in the PubMed, Embase, and Scopus databases. Random-effect prevalence and pairwise meta-analysis were performed for the primary objective. The auxiliary aim was to compare the effects of nutritional supplements on their fasting blood glucose (FPG) levels and perinatal outcomes. Fixed-effect network meta-analyses were undertaken for the secondary goals. All analyses were performed using Stata software, and statistical significance was determined at $P < 0.05$.

RESULTS

Thirteen RCTs from Iran and China were reviewed. The participant attrition burden in vitamin D recipients was 6% [95% confidence interval (CI): 0.03, 0.10], and its risk did not vary from non-recipients. Vitamin D and calcium co-supplementation reduced the cesarean section incidence in GDM patients [risk ratio (RR): 0.37; 95%CI: 0.18, 0.74]. The hyperbilirubinemia or hospitalization risk in their newborns decreased with vitamin D supplementation (RR: 0.47; 95%CI: 0.27, 0.83) and co-supplementation with calcium (RR: 0.35; 95%CI: 0.16, 0.77) or omega-

3 fatty acids (RR: 0.25; 95%CI: 0.08, 0.77). Vitamin D and probiotics co-supplementation decreased newborn hyperbilirubinemia risk (RR: 0.28; 95%CI: 0.09, 0.91). FPG levels and macrosomia risk did not vary across interventions.

CONCLUSION

In RCTs, vitamin D supplementation or co-supplementation in GDM patients showed a low participant attrition burden and low risk of cesarean section, newborn hyperbilirubinemia, and newborn hospitalization.

Key Words: Diabetes; Gestational; Vitamin D; Prenatal care; Nutrition therapy

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Core Tip: This meta-analysis was conducted on efficacy trials testing the effect of vitamin D in gestational diabetes mellitus (GDM) patients and/or their neonates. The post-randomization attrition burden of GDM patients from vitamin D-supplemented trial arms was low. The risk of hyperbilirubinemia and hospitalization in newborns was low with vitamin D and its omega-3 fatty acids and calcium co-supplemented forms. Vitamin D co-supplementation with calcium and probiotics reduced the risk of cesarean section and newborn hyperbilirubinemia, respectively. Compared to omega-3 fatty acids, the risk of hyperbilirubinemia and hospitalization among neonates was low when it was co-supplemented with vitamin D.

Citation: Saha S, Saha S. Participant attrition and perinatal outcomes in prenatal vitamin D-supplemented gestational diabetes mellitus patients in Asia: A meta-analysis. *World J Methodol* 2022; 12(3): 164-178
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/164.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.164

INTRODUCTION

Gestational diabetes mellitus (GDM) is a condition of glucose intolerance that is detected or diagnosed for the first time during pregnancy. The prevalence of GDM in pregnancy is between 4% and 18%, depending on the diagnostic criteria used[1]. The treatment of GDM is crucial as it can cause perinatal complications such as cesarean section (CS) in the mother and macrosomia in her newborn[2]. The benefits of standard GDM care with medical nutrition, lifestyle modification, and self-blood glucose monitoring are inconsistent across different treatment outcomes. For example, it decreases macrosomia risk but not CS occurrence compared to non-GDM care recipients[3]. Therefore, researchers have investigated the role of standard GDM care adjuncts for better perinatal outcomes. In this regard, vitamin D has drawn substantial attention due to the plausible association of its deficiency and GDM[4]. Although several randomized controlled trials (RCTs)[5] have assessed vitamin D efficacy in GDM patients, the burden and risk of post-randomization participant attrition from vitamin D-supplemented arms of these trials remain unclear. Notably, participant attrition happens even in adequately conducted RCTs[6]. Besides, the efficacy of vitamin D, its co-supplements, and other supplements included in these trials, remain unclear. Existing meta-analyses have compared how vitamin D affects the occurrence of perinatal outcomes and maternal fasting blood glucose (FPG) levels[7,8]. However, these did not distinguish how the effects of vitamin D can be differentiated from its co-supplemented forms (like with calcium) and other non-vitamin D supplements (*e.g.*, omega-3 fatty acids) included in these trials. This meta-analysis article attempted to address these underexplored areas of perinatal medicine.

Intervention description

The fat-soluble vitamin D hormone is available from the diet and nutritional supplements in the inactive D2 (ergocalciferol) and D3 (cholecalciferol) forms[9,10]. Cholecalciferol is further synthesized in the skin from sunlight. The pre-vitamin D undergoes hydroxylation in the liver and forms the albumin-bound circulatory 25-hydroxyvitamin D[9,11,12]. This active form of vitamin D causes calcium absorption by its action on the intestine and kidneys[10]. The physiologic role of vitamin D in pregnancy occurs *via* its binding to its receptors in the uteroplacental tissue[9,12]. The dietary allowance and the tolerable upper limit of vitamin D in pregnancy are 600 and 4000 IU, respectively[9].

The vitamin D supplementation effects on GDM mothers and their neonates have been assessed in several RCTs. Commonly tested oral dosages of vitamin D are 200-500 IU daily[13,14] or 50000 IU 2-3 weekly[15-18]. While some RCTs supplemented vitamin D as a mono-supplement, others co-supplemented it with zinc, calcium, and magnesium[14,16].

Objective

This review aimed to determine the burden and risk of post-randomization GDM patient attrition from vitamin D-supplemented arms of RCTs. Additionally, it determined the changes in FPG levels and risk of different perinatal outcomes (neonatal hyperbilirubinemia, newborn hospitalization, microsomia, and CS) across nutritional supplements tested in these RCTs.

MATERIALS AND METHODS

Registration and reporting

A pre-published protocol exists for this review, and it is registered in the PROSPERO (CRD42020180634) [19,20]. The preliminary findings of this review were presented at a conference[21]. This report adheres to The Preferred Reporting Items for Systematic Review and Meta-Analysis 2020 statement (Supplementary Table 1)[22].

Inclusion criteria

Trial design: Parallel arm RCTs of any duration.

Trial population: GDM patients of any age irrespective of their gestational age and previous GDM history.

Intervention arm/s: Prenatal vitamin D or its co-supplemented form with other nutrients orally.

Comparator arm: No nutritional supplements or placebo and/or prenatal nutritional supplement/s that does not contain vitamin D.

Primary outcome: GDM patients leaving the trial post-randomization during the intervention period. The participants excluded from analysis by trialists were not the outcome of interest.

Secondary outcomes (post-nutrient supplementation outcomes): Mean FPG levels and its standard deviation and CS frequency. Other outcomes of interest included macrosomia, hyperbilirubinemia, and hospitalization of newborns.

The diagnosis and management of GDM and the dosages and regimen of the nutritional supplements were accepted as per the trialists.

Exclusion criteria

Study designs other than that stated above (*e.g.*, crossover study, observational study). Non-GDM type of diabetes including type 1 and type 2 diabetes.

Data source

The title and abstract of the articles published in the English language were searched in the PubMed, Embase, and Scopus databases irrespective of the date of publication and geographic boundary. Additionally, the bibliographies of articles included in this review were searched. The search string used to search in the PubMed was composed of the following words and phrases: "vitamin D" OR calciferol OR "vitamin D2" OR ergocalciferol OR "vitamin D3" OR cholecalciferol AND gdm OR "gestational diabetes." Identical search strings were used in the remaining databases. The complete search string with their electronic links, when available, are presented in Supplementary Table 2.

Study selection and data abstraction

After uploading the retrieved citations to a reference handling software, the title and abstract of the articles were skimmed against the above eligibility criteria. Full-text reading transpired when articles appeared eligible or dubious for inclusion in this review. Figure 1 depicts the reasons for the elimination of articles read in full text. Salient detail abstraction about the trials (including its registration number and country of conduct), participants, interventions tested in respective treatment arms, and the outcomes of interest transpired.

Risk of bias evaluation

Using the Cochrane risk of bias (RoB) tool for RCTs, the following RoB components of the reviewed trials were evaluated[23]. The randomization method and successive allocation concealment method of interventions to different treatment arm participants were used to judge the selection bias. Utilizing the blinding mechanism used for trial personnel and participants and that of outcome assessors, performance and detection bias evaluation occurred, respectively. The attrition bias risk evaluation was assessed by comparing the frequency and reason of missing outcome data across intervention arms. By comparing trial findings with the pre-stated intentions of trialists, the risk of reporting bias was assessed. Any other bias besides those mentioned above was classified as miscellaneous bias.

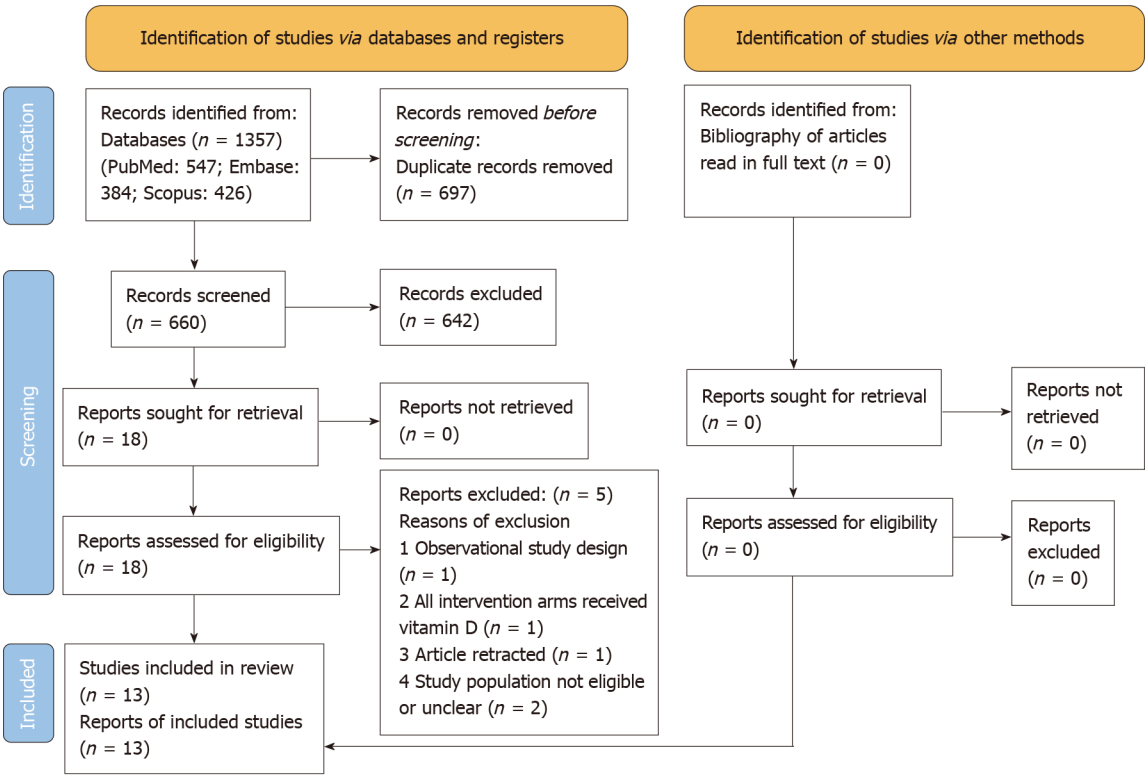

Figure 1 Preferred Reporting Items for Systematic Review and Meta-Analysis flow chart. Citation: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021; 372: n71. For more information, visit: http://www.prisma-statement.org/.

Review authors' role

The review authors performed the database search, study selection, data abstraction, and RoB assessment independently and resolved any conflict in an opinion by discourse. A third-party opinion or contact with the trialists was not required.

Analysis prevalence meta-analysis

The overall prevalence of post-randomization participant attrition from the vitamin D-supplemented arms was estimated using random-effect (DerSimonian and Laird) prevalence meta-analysis (exact binomial method). Trials with zero numerators, when all participants followed up until the end of the trial period, did not get included in the analysis.

Pairwise meta-analysis

A random effect pairwise meta-analysis model (DerSimonian and Laird) contrasted the participant attrition risk between vitamin D recipients and non-recipients and determined the summary effect in the risk ratio (RR). When any cell of the 2 × 2 table had no event, 0.5 got added to all cells. Forest plots were used to present the results of prevalence and pairwise meta-analysis.

Statistical heterogeneity evaluation

Heterogeneity was determined using χ^2 statistics (statistical significance determined at $P < 0.1$) and was successively quantified using I^2 statistics (at values 25%, 50%, and 75% heterogeneity were classified as low, moderate, and high, respectively)[24].

Supplementary analysis (network meta-analysis)

A frequentist method network meta-analysis (NMA) ensued for each outcome to determine the relative efficacy across various supplements tested in the reviewed trials. For FPG, the weighted mean difference was estimated, and its values were included in mg/dL (FPG values in mmol/L got converted into mg/dL). A fixed-effect NMA ensued for categorical outcomes (effect size estimated in RR) due to the absence of freedom for heterogeneity in respective models. An augmentation method was used when these binomial outcomes had zero events.

Transitivity
The NMA models did not include open-label trials to minimize the intransitivity risk. Local and global inconsistency models were used to assess inconsistency.

Network map
Utilizing network maps, a visual conceptualization of the relationship across various nutritional supplements tested in the trials transpired for each outcome. The nodes represent the intervention types received, and it enlarges with the increase in sample size receiving these. The node connectors represent the trials testing the interventions represented by the nodes, and it thickens as the no of trials increases.

League tables and intervention ranking
The effect sizes and their corresponding confidence interval (CI) are presented in league tables. The diagonal cells of these tables represented the interventions compared. The surface under the cumulative ranking curve values got utilized to predict the best supplement for outcomes with statistically significant effect sizes.

Subgroup analysis
Subgroup analysis and meta-regression were not applicable, as the heterogeneity was not high in the prevalence and pairwise meta-analysis.

Publication bias
Small study effect assessment for the pairwise meta-analysis ensued using funnel plot and Egger's test. The RoB across studies included in the NMA models occurred by identifying any selective reporting that deviates from the pre-stated notions[25].

Sensitivity analysis
The prevalence and pairwise meta-analysis iteration happened by dropping a study (every time the analysis was repeated) and by a fixed-effect model, respectively.

Certainty assessment
For statistically significant meta-analysis results, the Grading of Recommendations Assessment, Development, and Evaluation approach[26] was used to determine the evidence quality.

Analytic tools
The metaprop, meta, and network packages of Stata statistical software (version 16) were used for the prevalence, pairwise, and network meta-analysis, respectively. The statistical significance was determined at $P < 0.05$ and 95%CI.

RESULTS

Scope of this review
The database search retrieved 1357 citations (PubMed: 547; Embase: 384; Scopus: 426) (Figure 1). The last date of the search was July 4, 2021. Five articles read in full text were excluded[18,27-30]. Additional searches did not produce new articles. The review included 13 publications with 1109 GDM patients' data from Iran[14-17,31-37] and China[13,38]. The salient features of these trials are presented in Table 1.

RoB evaluation
The trials were primarily at low RoB except one at high RoB (due to lack of blinding of study personnel and participants) (Table 2)[34].

Meta-analysis
Prevalence and pairwise meta-analysis: The pooled prevalence of participant attrition among vitamin D recipients was 6% (95%CI: 0.03, 0.10, I^2: 38.04%) (Figure 2), and its risk did not vary from non-vitamin D recipients (Figure 3). Although the funnel plot (Figure 4) appeared somewhat asymmetrical, Egger's test did not suggest any small study effect ($P = 0.6602$).

***NMA*:** Figure 5 depicts the network maps. The maps revealed a lack of direct comparison between any supplement and following nutrients co-supplemented with vitamin D- calcium or magnesium-zinc-calcium combination or evening prime rose oil. The global and local inconsistency tests for any of the outcomes were not suggestive of any inconsistency. The league tables are shown in Tables 3 and 4. Vitamin D (RR: 0.47; 95%CI: 0.27, 0.83) and its co-supplementation with probiotic (RR: 0.28; 95%CI: 0.09, 0.91), omega-3 fatty acids (RR: 0.25; 95%CI: 0.08, 0.77), and calcium (RR: 0.35; 95%CI: 0.16, 0.77)

Table 1 Salient features of the reviewed trials

Ref.	Design	Participants	Interventions	Outcomes
Jamilian *et al*[37], 2016	Randomized, double-blind, placebo-controlled clinical trial; Intervention arms: Two; Single-centered trialTrial duration: 6 wk. Trial conducted in: Iran; Obtained ethical clearance and participant consent. Funding information provided. Clinical trial registration number: IRCT201509115623N52	Participants diagnosed with GDM (used ADA criteria); 60 participants randomized into different treatment arms (vitamin D3 and evening primrose oil: n = 30, placebo: n = 30); Mean age of participants: -Vitamin D3 and evening primrose oil receiving group: 28.4 ± 6.2 yr; -Placebo receiving group: 29.6 ± 4.3 yr	Two intervention arms: (1) 1000 IU of vitamin D and 1000 mg of evening primrose oil daily for 6 wk; and (2) Placebo	Attrition from vitamin D supplemented arm: n = 3; Other outcomes reported: Fasting plasma glucose
Jamilian *et al*[17], 2017	Randomized, double blinded, placebo-controlled clinical trial; Intervention arms: four; Single centered trial; Trial duration: 6 wk. Trial conducted in: Iran; Obtained ethical clearance and participant consent. Funding information provided. Clinical trial registration number: IRCT201605135623N78	Participants diagnosed with GDM (used ADA criteria); 140 participants randomized into different treatment arms (vitamin D and omega-3 fatty acid receiving group: n = 35, vitamin D receiving arm: n = 35, omeag-3 fatty acid receiving arm: n = 35, placebo receiving arm: n = 35); Mean age of participants: -Vitamin D and omega-3 fatty acid receiving group: 31.2 ± 4.3 yr; -Vitamin D receiving group: 31.5 ± 7.0 yr; -Omega-3 receiving group: 30.7 ± 3.5 yr; -Placebo receiving group: 30.7 ± 4.1 yr	Four intervention arms: (1) Vitamin D and omega-3 fatty acid: 50000 IU of vitamin D two weekly and 1000 mg omega-3 fatty acid twice daily; (2) Vitamin D: 50000 IU vitamin D every 2 wk; (3) Omega-3 fatty acid: 1000 mg omega-3 fatty acids two times a day; and (4) Placebo	No attrition from vitamin D supplemented arm; Other outcomes reported: Fasting plasma glucose
Jamilian *et al*[33], 2019a	Randomized, double-blind, placebo-controlled; Intervention arms: 3; Trial conducted in: Iran; Single centered trial; Trial duration: 6 wk; Obtained ethical clearance and participant consent. Funding information provided. Trial ID: IRCT201706075623N119	Participants diagnosed with GDM (used ADA criteria); 90 participants randomized into different treatment arms (probiotic arm: n = 30, vitamin D and probiotic arm: n = 30, placebo arm: n = 30); Mean age of participants: -Probiotic arm: 31.2 ± 5.9 yr; -Vitamin D and probiotic arm: 28.9 ± 6.1 yr; -Placebo arm: 29.9 ± 3.7 yr	Three intervention arms: (1) Probiotic: 8 × 109 CFU/g; (2) Vitamin D3 (50,000 IU) every 2 wk plus 8 × 109 CFU/g probiotic; Placebo	No attrition from vitamin D supplemented arm; Other outcomes reported: (1) Newborn hyperbiliru-binemia; (2) Newborn hospitalization; (3) Macrosomia; and (4) Cesarean section. Fasting plasma glucose
Jamilian *et al*[36], 2019b	Randomized, double-blind, placebo-controlled. Intervention arms: 2; Trial conducted in: IranSingle centered trialTrial duration: 6 wkObtained ethical clearance and participant consent. Funding information provided.Trial ID: IRCT201704225623N109	Participants diagnosed with GDM (used ADA criteria); 60 participants randomized into different treatment arms (vitamin D-magnesium-zinc-calcium arm: n = 30, placebo arm: n = 30). Mean age of participants: -Vitamin D-magnesium-zinc-calcium arm: 27.7 ± 4.0 yr; -Placebo arm: 29.1 ± 4.1 yr	Two intervention arms: (1) Vitamin D (200 IU) along with 100 mg magnesium, 4 mg zinc, 400 mg calcium twice daily; and (2) Placebo	No attrition from vitamin D supplemented armOther outcomes reported: (1) Newborn hyperbiliru-binemia; (2) Newborn hospitalization; (3) Macrosomia; and (4) Cesarean section. Fasting plasma glucose
Asemi *et al* [31], 2014a	Randomized, double-blind, placebo-controlled trial. Intervention arms: 2; Trial conducted in: Iran; Single centered trial; Trial duration: 6 wk; Obtained ethical clearance and participant consent. Funding information provided.Trial ID: IRCT201305115623N7	Participants diagnosed with GDM (used ADA criteria); 50 participants randomized into different treatment arms (vitamin D arm: n = 25, placebo arm: n = 25). Mean age of participants: -Vitamin D arm: 31.1 ± 5.5 yr; -Placebo arm: 30.8 ± 6.2 yr	Two intervention arms: (1) Vitamin D: 50,000 IU vitamin D3 pearl two times during the trial period (at baseline and day 21); and (2) Placebo	Attrition from vitamin D supplemented arm: n = 3; Other outcomes reported: (1) Newborn hyperbiliru-binemia; (2) Newborn hospitalization; (3) Macrosomia; and (4) Cesarean section
Asemi *et al* [16], 2014b	Randomized, placebo-controlled clinical trial. Intervention arms: TwoMulti-centric trial. Trial duration: 6 wk. Trial conducted in: IranOb-tained ethical clearance and participant consent. Funding information provided. Clinical trial registration number: IRCT201311205623N11	Participants diagnosed with GDM (used ADA criteria); 56 participants randomized into different treatment arms (vitamin D and calcium: n = 28, placebo receiving group: n = 28). Mean age of participants: -Vitamin D and calcium receiving arm: 28.7 ± 6.0 yr; -Placebo receiving arm: 30.8 ± 6.6 yr	Two intervention arms: (1) 1000 mg calcium carbonate daily and 50000 U vitamin D3 at the baseline and day 21 of the study; and (2) Placebo	Attrition from vitamin D supplemented arm: n = 3. Other outcomes reported: Fasting plasma glucose
Karamali *et al*[32], 2016	Randomized, double-blind, placebo-controlled trial; Intervention arms: 2; Trial conducted in: Iran; Multicentric trialTrial duration: 6 wk; Obtained ethical clearance and participant consent. Funding information provided. Trial ID: IRCT201407115623N23	Participants diagnosed with GDM (used ADA criteria); 60 participants randomized into different treatment arms (vitamin D and calcium arm: n = 30; placebo arm: n = 30). Mean age of participants: -Vitamin D and calcium arm: 28·7 ± 6·1 yr; -Placebo arm: 31·6 ± 6·3 yr	Two intervention arms: (1) Vitamin D3 (50000 IU) at baseline and day 21 along with 1000 mg calcium carbonate daily; and (2) Placebo	No attrition from vitamin D supplemented arm; Other outcomes reported: (1) Newborn hyperbiliru-binemia; (2) Newborn hospitalization; (3) Macrosomia; and (4) Cesarean section
Karamali *et al*[14], 2018	Randomized, double-blind, placebo-controlled trial; Intervention arms: 2; Single centered trial. Trial duration: 6 wk; Trial conducted in: Iran; Obtained ethical clearance	Participants diagnosed with GDM (used ADA criteria); 60 participants randomized into different treatment arms; (Magnesium, zinc, calcium and vitamin D supplements arm: n = 30;	Two intervention arms: (1) 100 mg magnesium, 4 mg zinc, 400 mg calcium and 200 IU vitamin D two times a day for 6 wk; and (2) Placebo	No attrition from vitamin D supplemented arm; Other outcomes reported: Fasting plasma glucose

	(participant consent information unclear). Funding information provided. Trial registration details: Unclear	Placebo arm: *n* = 30); Mean age of participants: -Magnesium, zinc, calcium and vitamin D: 30.0 ± 4.5 yr; -Placebo arm: 31.1 ± 4.2 yr		
Razavi *et al*[35], 2017	Randomized, double-blind, placebo-controlled, Intervention arms: 4; Trial conducted in: Iran. Single centered trial. Trial duration: 6 wk; Obtained ethical clearance and participant consent. Funding information provided. Trial ID: IRCT201701305623N106	Participants diagnosed with GDM (used ADA criteria); 120 participants randomized into different treatment arms (vitamin D and omega-3 arm: *n* = 30; omega-3 arm: *n* = 30; vitamin D arm: *n* = 30; placebo: *n* = 30); Mean age of participants: -Vitamin D and omega-3 arm: 29.9 ± 4.0 yr; -Omega-3 arm: 29.7 ± 3.6 yr; -Vitamin D arm: 29.9 ± 5.0 yr; -Placebo: 29.2 ± 3.4 yr	Four intervention arms: (1) Vitamin D (50000 IU): Two weekly two times a day; (2) Vitamin D (50000 IU) two weekly plus 1000 mg omega-3 fatty acids two times a day; (3) 1000 mg omega-3 fatty acids two times a day; and (4) Placebo	No attrition from vitamin D supplemented arm; Other outcomes reported: (1) Newborn hyperbilirubinemia; (2) Newborn hospitalization; (3) Macrosomia; and (4) Cesarean section
Valizadeh *et al*[34], 2016	Randomized controlled trial. Investigators and patients were not blinded. Intervention arms: 2; Single centered trial; Trial conducted in: Iran; Trial duration: Until delivery; Obtained ethical clearance and participant consent. Funding information provided. Trial ID: IRCT2012101611144N1	Participants diagnosed with GDM (used ADA criteria); 96 participants randomized into different treatment arms (vitamin D arm: *n* = 48; no supplement arm: *n* = 48); Mean age of participants: -Vitamin D arm: 32.0 ± 5.5 yr; -No supplement arm: 32.4 ± 4.7 yr	Two intervention arms: (1) 700000 IU vitamin D3 in total (regimen differed by gestational age of GDM patients); and (2) Comparison group did not receive any supplementation	Attrition from vitamin D supplemented arm: *n* = 4; Other outcomes reported: (1) Newborn hyperbilirubinemia; (2) Macrosomia; (3) Cesarean section; and (4) Fasting plasma glucose
Yazdchi *et al*[15], 2016	Randomized, double-blinded placebo-controlled clinical trial; Intervention arms: 2; Multi-center trial; Trial duration: 8 wk. Trial conducted in: Iran; Obtained ethical clearance and participant consent. Funding information provided. Clinical trial registration number: IRCT201306253140N11	Participants diagnosed with GDM (used International Association of Diabetes and Pregnancy Study Groups criteria); 76 participants randomized into different treatment arms: Vitamin D arm: *n* = 38; placebo arm: *n* = 38; Mean age of participants: -Vitamin D arm: 31.64 ± 4.40 yr; -Placebo arm: 32.11 ± 3.61 yr	Two intervention arms:(1) 50000 IU vitamin D3 oral capsules two weekly for 8 wk; and (2) Placebo	Attrition from vitamin D supplemented arm: *n* = 4; Other outcomes reported: Fasting plasma glucose
Zhang *et al*[38], 2016	Randomized, double-blind, placebo-controlled trial. Intervention arms: 4; Single centered trial. Trial duration: 24-28 wk of pregnancy to delivery; Trial conducted in: China; Obtained ethical clearance and participant consent. Funding information provided. Clinical trial registration details: Unclear	Participants diagnosed with GDM (criteria unclear). 133 participants randomized into different treatment arms (low dose vitamin D: *n* = 38; medium dose vitamin D: *n* = 38; high dose vitamin D: *n* = 37; placebo: *n* = 23); Mean age of participants: -Placebo arm: 29.8 ± 4.7; -Low dose vitamin D arm: 30.3 ± 5.1; -Medium dose vitamin D arm: 29.4 ± 4.9; -High dose vitamin D arm: 30.1 ± 4.5	Four intervention arms: (1) Low dose vitamin D: 200 IU daily; (2) Medium dose vitamin D: 2000 IU monthly; and (3) High dose vitamin D: 50000 IU every 2 wk. Placebo	Attrition from vitamin D supplemented arm: *n* = 4
Li and Xing[13], 2016	Randomized, double-blinded clinical trial. Intervention arms: 2. Multi-centric trial. Trial duration: 16 wk. Trial conducted in: China; Obtained ethical clearance and participant consent. Funding information provided. Clinical trial registration details: Unclear	Participants diagnosed with GDM (used ADA criteria)103 participants randomized into different treatment arms (yoghurt with vitamin D: *n* = 52, plain yoghurt: *n* = 51); Mean age of participants: -Yoghurt supplemented with vitamin D receiving arm: 29.0 ± 5.3 yr; -Plain yoghurt arm: 28.3 ± 4.1 yr	Two intervention arms: (1) Yoghurt was supplemented with 500 IU of vitamin D3 twice daily for 16 wk; and (2) plain yoghurt: Twice daily for 16 wk	Attrition from vitamin D supplemented arm: *n* = 4. Other outcomes reported: Fasting plasma glucose

ADA: American diabetes association.

decreased the risk of newborn hyperbilirubinemia. Vitamin D (RR: 0.47; 95%CI: 0.27, 0.83) and its co-supplementation with omega-3 fatty acids (RR: 0.25; 95%CI: 0.08, 0.77) and calcium (RR: 0.35; 95%CI: 0.16, 0.77) reduced the risk of newborn hospitalization. The incidence of CS in GDM patients was lower with vitamin D and calcium co-supplementation (RR: 0.37; 95%CI: 0.18, 0.74). Vitamin D and omega-3 fatty acid co-supplementation in GDM patients decreased the risk of hyperbilirubinemia (RR: 0.30; 95%CI: 0.09, 0.98) and hospitalization (RR: 0.30; 95%CI: 0.09, 0.98) in their newborns compared to omega-3 supplementation alone.

The surface under the cumulative ranking curve values suggested vitamin D and calcium co-supplementation in GDM patients as the best supplement for reducing the CS requirement, and vitamin D and omega-3 fatty acid co-supplementation as the best supplement for reducing the risk of hospitalization and hyperbilirubinemia in their newborns (Table 5). The macrosomia risk and FPG levels (league table not shown) did not vary among the interventions.

***RoB* across studies:** Evaluation of *RoB* across studies suggests that the trials primarily adhered to their pre-stated analytic notions.

Table 2 Risk of bias assessment of respective trial included in the review[23]

Ref.	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias); All outcomes	Blinding of outcome assessment (detection bias); All outcomes	Incomplete outcome data (attrition bias); All outcomes	Selective reporting (reporting bias)	Other bias
Jamilian *et al* [37], 2016	Low	Unclear; Comment: Precise mechanism unclear	Unclear; Comment: Precise mechanism unclear	Low	Low	Low	Low
Jamilian *et al* [17], 2017	Low	Unclear; Comment: Precise mechanism unclear	Unclear; Comment: Precise mechanism unclear	Unclear	Low	Low	Low
Jamilian *et al* [33], 2019a	Low risk	Unclear risk; Comment: Precise mechanism unclear	Low risk	Low risk	Low risk	Low risk	Low risk
Jamilian *et al* [36], 2019b	Low risk	Unclear risk; Comment: Precise mechanism unclear	Low risk	Low risk	Low risk	Low risk	Low risk
Asemi *et al* [31], 2014a	Low risk	Unclear risk; Comment: Precise mechanism unclear	Low risk	Low risk	Low risk	Low risk	Low risk
Asemi *et al* [16], 2014b	Low	Low	Low	Low	Low	Low	Low
Karamali *et al* [32], 2016	Low risk	Unclear risk; Comment: Precise mechanism unclear	Low risk	Low risk	Low risk	Low risk	Low risk
Karamali *et al* [14], 2018	Low	Unclear; Comment: Precise mechanism unclear	Unclear; Comment: Precise mechanism unclear	Low	Low	Low	Low
Razavi *et al* [35], 2017	Low risk	Unclear risk; Comment: It's unclear if the bottles were sequentially numbered and identical in appearance	Low risk	Low risk	Low risk	Low risk	Low risk
Valizadeh *et al* [34], 2016	Low risk	Unclear risk	High risk; Comment: Both investigators and participants were not blinded		Low risk	Low risk	Low risk
Yazdchi *et al* [15], 2016	Low	Unclear	Unclear	Low	Low	Low	Low
Zhang *et al* [38], 2016	Low	Unclear; Comment: Precise mechanism unclear	Unclear; Comment: Precise mechanism unclear	Unclear; Comment: Precise mechanism unclear	Low	Low	Low
Li and Xing [13], 2016	Low	Unclear; Comment: Precise mechanism unclear	Unclear; Comment: Precise mechanism unclear	Low	Low	Low	Low

Sensitivity analysis: On repeating the prevalence meta-analysis by dropping one study each time, the prevalence ranged between 5% and 8%. The pairwise meta-analysis findings were identical to the preliminary model when a fixed-effect model-based iteration occurred.

Table 3 League table. Outcomes: cesarean section (left lower triangle) and newborn hyperbilirubinemia (right upper triangle). Interventions of interest: represented in diagonal cells

Interventions and effect sizes							
Vitamin D and probiotic	1.14 (0.22, 5.92)[1]	0.79 (0.19, 3.27)	0.59 (0.16, 2.20)	0.73 (0.18, 2.96)	**0.28 (0.09, 0.91)**[2]	0.34 (0.09, 1.32)	0.42 (0.10, 1.69)
0.76 (0.27, 2.19)	Vitamin D and omega-3 fatty acid	0.70 (0.17, 2.79)	0.52 (0.16, 1.75)	0.64 (0.14, 2.99)	**0.25 (0.08, 0.77)**	**0.30 (0.09, 0.98)**	0.37 (0.09, 1.44)
1.48 (0.52, 4.21)	1.94 (0.71, 5.28)	Vitamin D and calcium	0.75 (0.29, 1.96)	0.91 (0.25, 3.35)	**0.35 (0.16, 0.77)**	0.43 (0.16, 1.19)	0.53 (0.18, 1.55)
0.69 (0.29, 1.68)	0.91 (0.44, 1.90)	0.47 (0.21, 1.07)	Vitamin D	1.22 (0.37, 3.96)	**0.47 (0.27, 0.83)**	0.57 (0.27, 1.22)	0.71 (0.28, 1.78)
0.68 (0.30, 1.54)	0.89 (0.34, 2.35)	0.46 (0.17, 1.20)	0.97 (0.45, 2.13)	Probiotic	0.39 (0.14, 1.09)	0.47 (0.14, 1.60)	0.58 (0.16, 2.07)
0.54 (0.25, 1.18)	0.71 (0.35, 1.46)	**0.37 (0.18, 0.74)**	0.78 (0.52, 1.19)	0.80 (0.42, 1.56)	Placebo	1.22 (0.64, 2.33)	1.50 (0.72, 3.14)
0.68 (0.24, 1.90)	0.89 (0.40, 1.99)	0.46 (0.17, 1.22)	0.98 (0.49, 1.96)	1.00 (0.39, 2.58)	1.24 (0.63, 2.45)	Omega-3 fatty acid	1.23 (0.46, 3.28)
1.23 (0.33, 4.57)	1.61 (0.45, 5.79)	0.83 (0.23, 2.97)	1.76 (0.56, 5.53)	1.81 (0.52, 6.33)	2.25 (0.78, 6.52)	1.81 (0.51, 6.37)	Magnesium, zinc, calcium, and vitamin D

[1]Effect sizes in risk ratio with its 95% confidence interval in parenthesis.
[2]Cells with bold-faced values depict a statistically significant decrease in effect size.
In the right upper and the left lower triangle, the columns and rows depict the reference treatment, respectively.

Table 4 League table: Outcomes: Macrosomia (left lower triangle) and newborn hospitalization (right upper triangle). Interventions of interest: Represented in diagonal cells

Interventions and effect sizes							
Vitamin D and probiotic	1.27 (0.24, 6.66)[1]	0.88 (0.21, 3.69)	0.66 (0.18, 2.49)	0.97 (0.21, 4.41)	0.31 (0.09, 1.03)	0.38 (0.10, 1.49)	0.47 (0.11, 1.91)
0.94 (0.11, 8.45)	Vitamin D and omega-3 fatty acid	0.70 (0.17, 2.79)	0.52 (0.16, 1.75)	0.76 (0.15, 4.02)	**0.25 (0.08, 0.77)**[2]	**0.30 (0.09, 0.98)**	0.37 (0.09, 1.44)
3.36 (0.13, 88.67)	3.56 (0.14, 93.17)	Vitamin D and calcium	0.75 (0.29, 1.96)	1.10 (0.26, 4.59)	**0.35 (0.16, 0.77)**	0.43 (0.16, 1.19)	0.53 (0.18, 1.55)
0.98 (0.13, 7.29)	1.03 (0.18, 6.09)	0.29 (0.01, 6.77)	Vitamin D	1.46 (0.39, 5.50)	**0.47 (0.27, 0.83)**	0.57 (0.27, 1.22)	0.71 (0.28, 1.78)
1.93 (0.19, 20.18)	2.05 (0.15, 27.32)	0.58 (0.02, 20.11)	1.98 (0.17, 22.68)	Probiotic	0.32 (0.10, 1.07)	0.39 (0.10, 1.53)	0.48 (0.12, 1.97)
0.37 (0.08, 1.77)	0.40 (0.08, 1.85)	0.11 (0.01, 1.98)	0.38 (0.11, 1.36)	0.19 (0.02, 1.55)	Placebo	1.22 (0.64, 2.33)	1.50 (0.72, 3.14)
0.63 (0.08, 4.84)	0.67 (0.12, 3.71)	0.19 (0.01, 4.45)	0.64 (0.13, 3.13)	0.33 (0.03, 3.83)	1.69 (0.45, 6.30)	Omega-3 fatty acid	1.23 (0.46, 3.28)
1.87 (0.14, 25.22)	1.98 (0.15, 26.45)	0.56 (0.02, 19.45)	1.91 (0.17, 21.96)	0.97 (0.05, 18.42)	5.00 (0.62, 40.28)	2.96 (0.25, 34.96)	Magnesium, zinc, calcium, and vitamin D

[1]Effect sizes in risk ratio with its 95% confidence interval in parenthesis.
[2]Cells with bold-faced values depict a statistically significant decrease in effect size.
In the right upper and the left lower triangle, the columns and rows depict the reference treatment, respectively.

DISCUSSION

Overall, this review included 13 publications sourcing data from 1109 GDM patients from Iran and China. The RoB across the trials was primarily low except for one with a high RoB component. The burden of attrition of GDM patients from the vitamin D supplemented arms post-randomization was 6%, and this risk did not vary from GDM patients who did not receive the supplement. Vitamin D and calcium co-supplementation benefited the GDM patients (decreased the CS incidence) and their neonates (decreased hyperbilirubinemia and hospitalization risk). Vitamin D alone and its omega-3 fatty

Table 5 The surface under the cumulative ranking curve values. Outcomes: Newborn hyperbilirubinemia, newborn hospitalization, and cesarean section

Intervention	Outcomes					
	Newborn hyperbilirubinemia		Newborn hospitalization		Cesarean section	
	SUCRA	Mean rank	SUCRA	Mean rank	SUCRA	Mean Rank
Vitamin D and omega-3 fatty acid	81.8	2.3[1]	81.1	2.3[1]	46.4	4.8
Vitamin D and probiotic	76.2	2.7	70.7	3.0	66.3	3.4
Probiotic	62.2	3.6	69.5	3.1	36.6	5.4
Vitamin D and calcium	67.9	3.3	67.2	3.3	87.6	1.9[1]
Vitamin D	52.8	4.3	52.4	4.3	39.0	5.3
Magnesium, zinc, calcium, and vitamin D	32.4	5.7	32.2	5.7	73.8	2.8

[1]The best rank corresponding to the highest SUCRA value. SUCRA: Surface under the cumulative ranking curve.

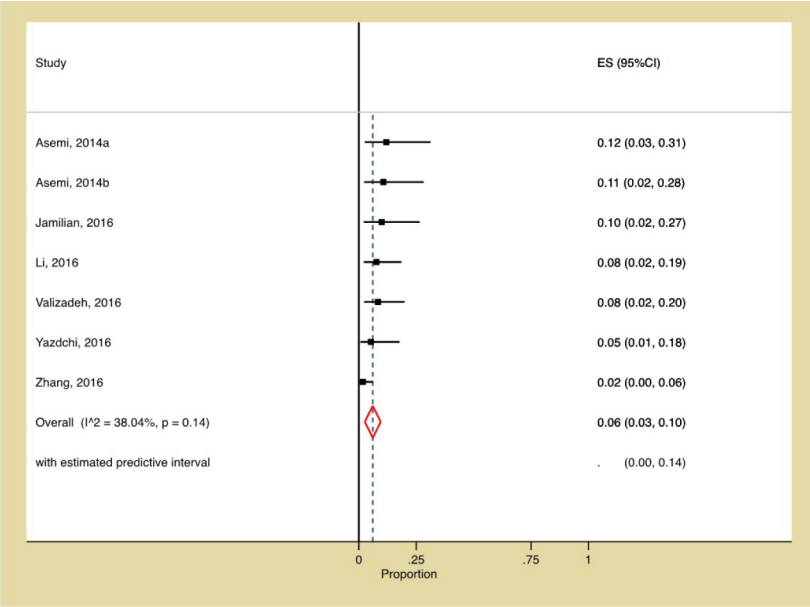

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Figure 2 Forest plot showing the overall weighted prevalence of post-randomization participant attrition from vitamin D supplementation trials in gestational diabetes mellitus patients. The diamond centers on the summary of the prevalence estimate, and the width indicates the corresponding 95% confidence interval. Articles with identical author names and years are suffixed with alphabets: Asemi *et al*[31], 2014, Asemi *et al*[16], 2014. CI: Confidence interval; ES: Effect size.

acid added form both reduced the newborn's risk of hyperbilirubinemia and hospitalization. For these outcomes, co-supplementation of vitamin D and omega-3 fatty acids was superior to omega-3 fatty acids alone. Combining vitamin D with probiotics was effective in reducing the risk of newborn hyperbilirubinemia.

Quality of evidence

Using the Grading of Recommendations Assessment, Development, and Evaluation approach[26], the NMA-generated evidence was double downgraded to low quality. This decision stood on the fact that the statistically significant findings were unlikely to be generalizable as study participants were mostly from Iran; thus, a fixed-effect model NMA was used for the categorical outcomes, and the trials had few unclear RoB components.

Comparison with existing literature

Regarding the prevalence of participant attrition, to the best of our knowledge, no literature is available to contrast with the findings of this review, perhaps due to its conceptual novelty. Concerning the perinatal outcomes, existing reviews suggested that vitamin D supplementation decreases the risk of

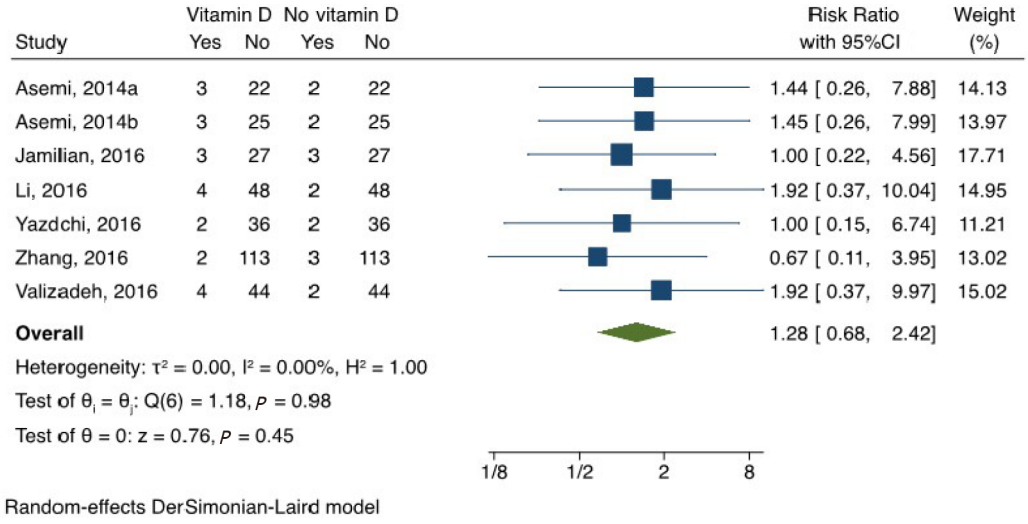

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Figure 3 Forest plot (pairwise meta-analysis; random-effect model) comparing missing outcome data between vitamin D recipients and non-recipients. Articles with identical author names and years are suffixed with alphabets: Asemi, 2014a[31], Asemi, 2014b[16].

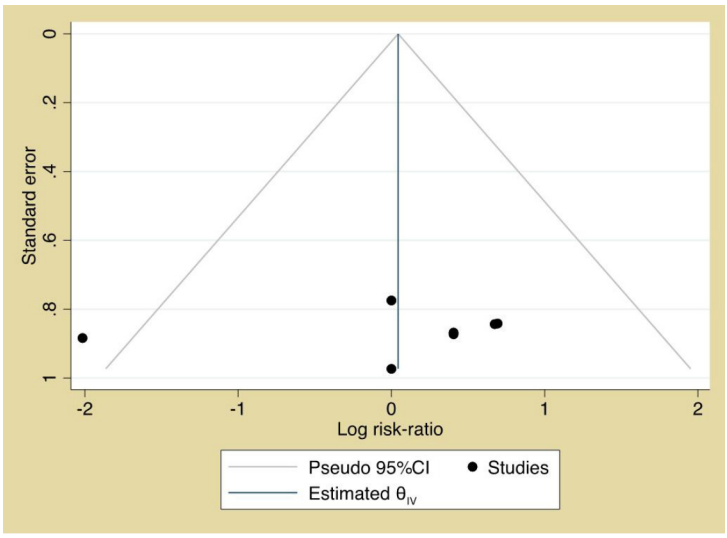

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Figure 4 Funnel plot for pairwise meta-analysis. Outcome: Post-randomization participant attrition from vitamin D-supplemented treatment arm/s.

CS, macrosomia, neonatal hyperbilirubinemia, and newborn hospitalization[8,39]. However, unlike this paper's findings, these reviews[8,39] did not sort out how perinatal outcomes vary across vitamin D, its co-supplemented forms, and other (non-vitamin D) supplements tested in these trials.

Strengths and weaknesses

The key strength of this review is its incorporation of RCTs only, the highest level of epidemiological evidence. The intransitivity risk in the NMA models is perhaps low due to the exclusion of the trial at a high RoB component. Furthermore, beyond reviewing post-randomization GDM patients' attrition burden from vitamin D-supplemented trial arm/s and its risk, this is plausibly the first study that attempted to distinguish the efficacy between vitamin D and its co-supplemented forms in GDM patients.

Despite these strengths, this study also had a few limitations. This review could not incorporate non-English language publications (if any) as the review authors are competent in handling publications in the English language only. The anticipated generalizability of the evidence generated in this study was low due to the homogenous nature of the study population. Although the prevalence meta-analysis estimate appeared weak due to its inclusion of a trial with a high RoB component, the sensitivity analysis did not observe any fluctuation upon excluding the trial from the model.

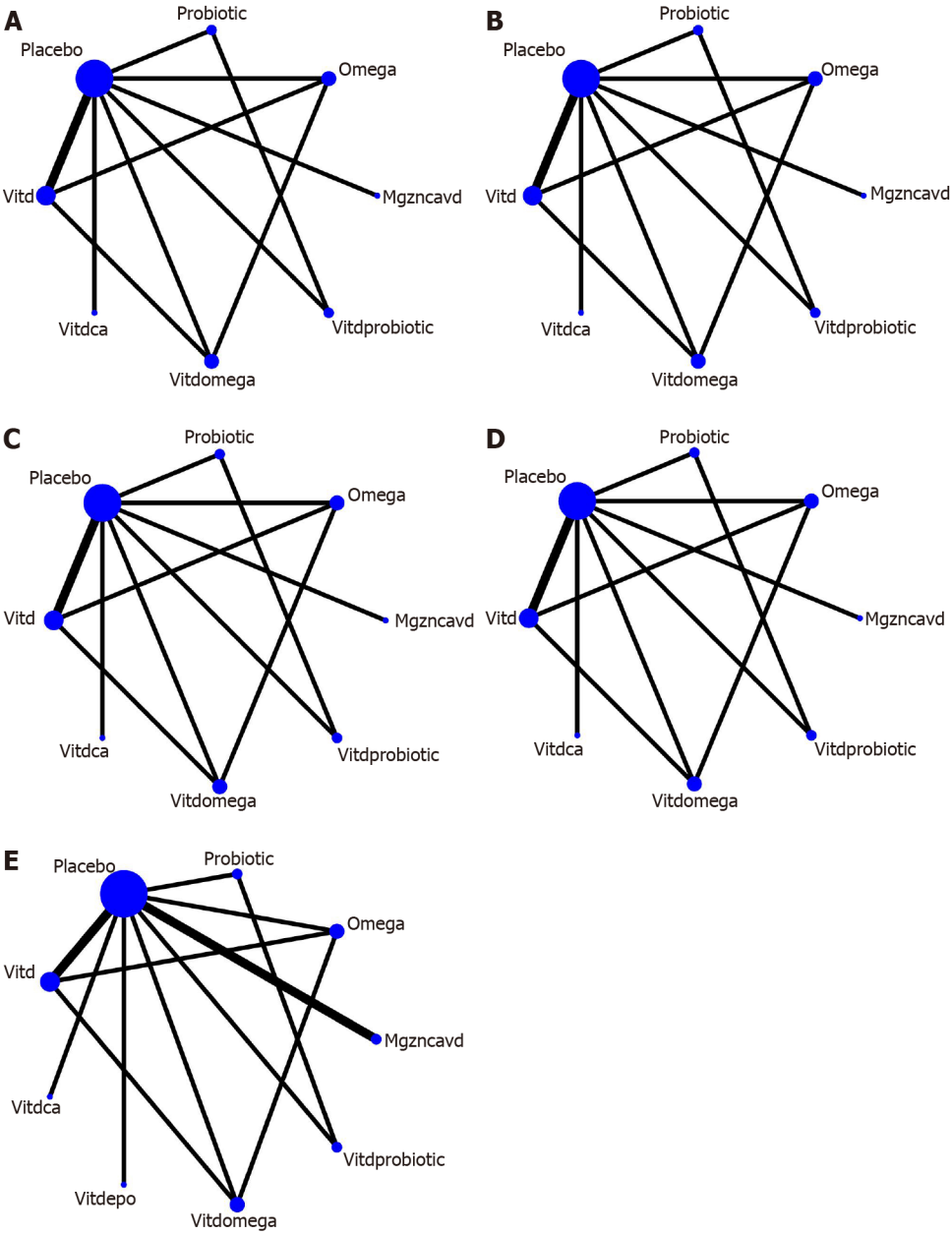

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Figure 5 Network map. A: Outcome: Newborn hyperbilirubinemia; B: Outcome: Newborn hospitalization; C: Outcome: Macrosomia; D: Outcome: Cesarean section; E: Outcome: Fasting plasma glucose. Interventions in the model: Placebo, probiotic, omega-3 fatty acids (omega), magnesium-zinc-calcium and vitamin D (mgzncavd), vitamin D and probiotic (vitdprobiotic), vitamin D and omega-3 fatty acids (vitdomega), vitamin D and calcium (vitdca), and vitamin D (vitd).

Implications

The low prevalence of post-randomization attrition of GDM patients from the vitamin D-supplemented intervention arms in RCTs suggests good adherence to the supplement and might encourage trialists across the globe to conduct identical efficacy trials. Given the substantial burden of vitamin D deficiency and insufficiency in Iranian pregnant females[40] (and most trials included in this review were from Iran), from a public health point of view, this study's findings might help the local health authority in reviewing the scope of routine prenatal supplementation of vitamin D and its co-supplemented forms with calcium, omega-3 fatty acids, and probiotics in GDM patients.

CONCLUSION

In RCTs testing the efficacy of vitamin D supplementation, the post-randomization attrition burden in vitamin D-supplemented GDM patients was low. Prenatal vitamin D and its co-supplemented form with calcium, omega-3 fatty acids, and probiotics each can curb certain perinatal complications' risks in

GDM patients and their neonates.

ARTICLE HIGHLIGHTS

Research background

The role of vitamin D in gestational diabetes mellitus (GDM) is not established. Several randomized controlled trials (RCT) have tested it.

Research motivation

The burden and risk of participant attrition from vitamin D receiving treatment arm/s of these trials are unclear. Also, the effect of vitamin D and its co-supplemented forms and other supplements on the mother's glycemic control and perinatal outcomes remains unclear.

Research objectives

This study aimed to address these issues.

Research methods

Eligible clinical trials were retrieved by searching the PubMed, Embase, and Scopus databases. The burden and risk of participant attrition got determined by random-effect prevalence and pairwise meta-analysis, respectively. The effect of different nutritional supplements on the perinatal outcomes got estimated by fixed-effect network meta-analysis. All analysis ensued in Stata statistical software (v16).

Research results

The database search produced 13 RCTs conducted in Iran and China. The participant attrition from vitamin D treated arms was 6% (95% confidence interval [CI]: 0.03, 0.10), and this risk did not vary from its non-recipient arms. The cesarean section risk decreased with the combined supplementation of vitamin D and calcium [risk ratio (RR): 0.37; 95%CI: 0.18, 0.74]. The vitamin D alone and its co-supplemented forms with calcium and omega-3 fatty acids decreased the risk of newborn- hyperbilirubinemia or hospitalization. The probiotics co-supplemented form of vitamin D decreased newborn hyperbilirubinemia risk (RR: 0.28; 95%CI: 0.09, 0.91). The fasting plasma glucose levels didn't vary across the compared interventions.

Research conclusions

This study suggests that vitamin D supplementation is a relatively well-tolerated intervention in GDM patients resulting in relatively low participant attrition from RCTs testing it. Also, this study suggests that some nutritional supplements can be beneficial in reducing perinatal outcomes.

Research perspectives

Given the low burden of participant attrition from the vitamin-supplemented arms of RCTs, future trialists may find the conduct of RCTs with a larger sample size reasonable to produce rigorous results.

FOOTNOTES

Author contributions: Sumanta Saha conceptualized, designed, analyzed, and drafted the first and final versions of the manuscript; all authors contributed to the study selection, data abstraction, and risk of bias assessment of this manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors declare there are no conflicts of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2020 statement Checklist, and the manuscript was prepared and revised according to the PRISMA 2020 Checklist.

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S-Editor: Liu JH
L-Editor: Filipodia
P-Editor: Liu JH

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World J Methodol 2022 May 20; 12(3): 179-190

DOI: 10.5662/wjm.v12.i3.179

ISSN 2222-0682 (online)

META-ANALYSIS

Global prevalence of occult hepatitis C virus: A systematic review and meta-analysis

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Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Biswas S, India; Hedayati-Moghaddam MR, Iran

Received: December 9, 2021
Peer-review started: December 9, 2021
First decision: February 15, 2022
Revised: March 3, 2022
Accepted: April 29, 2022
Article in press: April 29, 2022
Published online: May 20, 2022

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Abstract

BACKGROUND

Occult hepatitis C infection (OCI) is characterized by the presence of hepatitis C virus (HCV) RNA in the liver, peripheral blood mononuclear cells (PBMC) and/or ultracentrifuged serum in the absence of detectable HCV-RNA in serum. OCI has been described in several categories of populations including hemodialysis patients, patients with a sustained virological response, immunocompromised individuals, patients with abnormal hepatic function, and apparently healthy subjects.

AIM

To highlight the global prevalence of OCI.

METHODS

We performed a systematic and comprehensive literature search in the following 4 electronic databases PubMed, EMBASE, Global Index Medicus, and Web of Science up to 6th May 2021 to retrieve relevant studies published in the field. Included studies were unrestricted population categories with known RNA status in serum, PBMC, liver tissue and/or ultracentrifuged serum. Data were extracted independently by each author and the Hoy *et al* tool was used to assess the quality of the included studies. We used the random-effect meta-analysis model to estimate the proportions of OCI and their 95% confidence intervals (95%CI). The Cochran's *Q*-test and the I^2 test statistics were used to assess heterogeneity between studies. Funnel plot and Egger test were used to examine publication bias. R software version 4.1.0 was used for all analyses.

RESULTS

The electronic search resulted in 3950 articles. We obtained 102 prevalence data from 85 included studies. The pooled prevalence of seronegative OCI was estimated to be 9.61% (95%CI: 6.84-12.73) with substantial heterogeneity [I^2 = 94.7% (95%CI: 93.8%-95.4%), P < 0.0001]. Seropositive OCI prevalence was estimated to be 13.39% (95%CI: 7.85-19.99) with substantial heterogeneity [I^2 = 93.0% (90.8%-94.7%)]. Higher seronegative OCI prevalence was found in Southern Europe and Northern Africa, and in patients with abnormal liver function, hematological disorders, and kidney diseases. Higher seropositive OCI prevalence was found in Southern Europe, Northern America, and Northern Africa.

CONCLUSION

In conclusion, in the present study, it appears that the burden of OCI is high and variable across the different regions and population categories. Further studies on OCI are needed to assess the transmissibility, clinical significance, long-term outcome, and need for treatment.

Key Words: Occult hepatitis C virus infection; Prevalence; Worldwide; Peripheral blood mononuclear cells; Hepatitis C virus

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Core Tip: This study showed that the burden of seropositive and seronegative occult hepatitis C infections (OCIs) is high and variable in different regions and population categories. Patients with hematological disorders, kidney diseases, and abnormal liver function showed the highest OCI prevalence.

Citation: Mbaga DS, Kenmoe S, Njiki Bikoï J, Takuissu GR, Amougou-Atsama M, Atenguena Okobalemba E, Ebogo-Belobo JT, Bowo-Ngandji A, Oyono MG, Magoudjou-Pekam JN, Kame-Ngasse GI, Nka AD, Feudjio AF, Zemnou-Tepap C, Adamou Velhima E, Ndzie Ondigui JL, Nayang-Mundo RA, Touangnou-Chamda SA, Kamtchueng Takeu Y, Taya-Fokou JB, Mbongue Mikangue CA, Kenfack-Momo R, Kengne-Ndé C, Sake CS, Esemu SN, Njouom R, Ndip L, Riwom Essama SH. Global prevalence of occult hepatitis C virus: A systematic review and meta-analysis. *World J Methodol* 2022; 12(3): 179-190
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/179.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.179

INTRODUCTION

In 2019, the World Health Organization (WHO) estimated that 58 million people are living with hepatitis C virus (HCV)[1], making HCV infection a major global public health problem[2,3]. Each year, more than 1.5 million people around the world are newly infected with HCV[4] and more than 290000 people die from it[5]. HCV infection is increasingly affecting healthcare particularly in highly endemic areas[3]. The prevalence of HCV varies greatly between regions and ranges from 0.2% to 20% in the general population[6]. HCV infection can lead to liver cirrhosis (10%-20% of cases) and hepatocellular carcinoma (HCC) (1%-5% of cases)[7].

The principal multiplication site for HCV is hepatocytes, but evidence of HCV replication has been reported in peripheral blood mononuclear cells (PBMC) and other extrahepatic organs[8,9].

Occult hepatitis C infection (OCI) was first described by Castillo *et al*[10] in 2004. This new form of hepatitis is defined as the absence of RNA in serum and its presence in hepatocytes, PBMC or ultracentrifuged serum[3,11-13]. OCI is further classified as seronegative OCI in subjects who are anti-HCV negative and seropositive OCI in those who are anti-HCV positive[14]. Seropositive OCI individuals represent those chronically infected with HCV who have recovered (absence of RNA in serum) either spontaneously or following treatment. There are asymptomatic carriers of OCI with normal liver enzyme levels and some with abnormal liver function[10,15-23]. OCI can also lead to hepatic attacks including cases of liver cirrhosis and even HCC in high-risk groups[24]. The first syntheses performed at the global level and in the Middle East and the Eastern Mediterranean showed highly variable OCI prevalence (ranging from 0%-89%) according to the population groups including apparently healthy individuals, patients with hematological disorders, chronic liver disease, HIV, patients who have achieved a sustained virological response (SVR), and transplant recipients[20,25,26]. The review conducted in the Middle East and Eastern Mediterranean revealed that high frequencies of OCI were recorded in patients with chronic liver disease, HIV, and injecting drug users[20]. In the review by Hedayati-Moghaddam *et al*[20], no statistically significant difference was observed in the variability of OCI prevalence across countries, patient anti-HCV status, and HCV detection method. OCI highlights multiple concerns including the potential for transmission of this form of infection through blood transfusion or hemodialysis[27]. To date, there is no global data synthesis on the prevalence of OCI in different population categories. To eradicate HCV infection by 2030 as recommended by WHO, making data available on the burden of OCI is crucial[28,29]. The objective of this systematic review and meta-analysis is to determine the global prevalence of OCI and evaluate the potential factors resulting in heterogeneity between the population groups and regions. Findings from this review may help prioritize population groups and regions most at risk for OCI screening and managing programs.

MATERIALS AND METHODS

Study design

We used the preferred reporting items for systematic reviews and meta–analyses (PRISMA) checklist to design this systematic review (Supplementary Table 1)[30]. The systematic review was declared in the PROSPERO international database under the number CRD42021252763.

Inclusion criteria

We included all studies without time restriction, published in peer-reviewed journals in English or in French and which fulfilled the following criteria: having a cross-sectional or case-control study design and for cohorts and clinical trials, only the baseline data were considered. We considered studies with patients of all ages tested for seropositive OCI (anti-HCV positive) and for seronegative OCI (anti-HCV negative). One study could contribute to several prevalence data that we called effect ratings. We included studies that detected HCV RNA by molecular methods in PBMCs, hepatocytes or ultracentrifuged serum[11,15,20,31]. To strengthen the robustness of our estimates, we considered only studies with at least 10 participants.

Exclusion criteria

We excluded all studies that did not provide an opportunity to extract data on OCI prevalence, and studies with no baseline data for longitudinal study. Case reports, studies selecting participants with an already known OCI result, comments on an article, reviews, editorials, duplicates and studies for which the full article or abstract could not be found were also excluded.

Search strategy

We performed a systematic and comprehensive literature search in 4 electronic databases: PubMed, EMBASE, Global Index Medicus, and Web of Science from inception until 6th May 2021 to retrieve relevant studies published in the field. The electronic search strategy conducted in PubMed covered the key words of OCI (Occult Hepatitis C OR Occult Viral hepatitis C OR Occult Hepatitis C Virus OR Occult HCV) and was adapted to other databases. We also manually searched all included studies and previous systematic reviews on the topic to identify additional references. The references cited in this article were checked in the Reference Citation Analysis website (https://www.referencecitation-analysis.com/).

Study selection

The duplicate articles found in the databases were removed using EndNote software. Two investigators (JTEB and SK) independently selected articles on the basis of title and abstract using Rayyan review platform. The full texts of selected articles were then read by 22 authors on the basis of the eligibility criteria. Disagreements were resolved through discussion and consensus.

Data extraction

Data were extracted independently by each author *via* the Google Forms for articles that met the inclusion criteria. The data extracted were as follows; the name of the first author, the date of publication, the period of recruitment of the participants, the design of the study, the sampling method, the number of study sites, the time of collection of the data, country, United Nations Statistics Division (UNSD) region, type of population studied, patient demographic details such as gender, age, and location of recruitment, OCI type (seronegative or seropositive), risk of bias assessment, detection test, target detected, type of sample used, number of samples tested, and number of samples positive for OCI. All disagreements regarding eligibility and data collected were resolved by discussion and consensus.

Appraisal of the methodological quality of the included studies and risk of bias

We used the Hoy *et al*[32] tool to assess the quality of the included studies (Supplementary Table 2). This tool takes into account 10 elements to assess the internal and external validity of prevalence studies. For each item, a score of 1 is assigned to a "yes" response and a score of 0 is assigned to the other responses ("no", "not clear", "not applicable"). Basically, a study was considered to be low risk, moderate risk, or high risk of bias if the total score was 0-3, 4-6, and 7-10, respectively.

Data synthesis and analysis

To estimate proportions of OCI and their 95% confidence intervals (95%CI), we chose the random-effect meta-analysis model due to the heterogeneity expected for observational studies. The I^2 statistics and Cochran's Q-test were used to assess heterogeneity between studies[33]. The I^2 cut-offs > 50% indicate substantial heterogeneity. Potential sources of heterogeneity were explored by subgroup analyses and meta-regression including covariates: study design, sampling, setting, timing of samples collection, countries, WHO region, UNSD region, country income level, age range, population categories, OCI diagnostic method, and sample types. We used Funnel plot and Egger test to examine publication bias [34]. R software version 4.1.0 was used for all analyses[35,36].

RESULTS

Study selection and characteristics

The electronic search identified 3950 articles (EMBASE (2025), Web of Science (1183), PubMed (706), and Global Index Medicus (36) (Figure 1). The eligibility review of 179 articles resulted in the exclusion of 94 and the inclusion of 85. The excluded articles and the individual reasons for exclusion are presented in Supplementary Table 3, while the included articles are indicated in Supplementary Text 1.

Characteristics of the included studies

Overall, we obtained 102 prevalence data from the 85 included studies (75 seronegative OCI, 24 seropositive OCI, and 3 seropositive OCI and/or seronegative OCI (Supplementary Tables 4 and 5). The prevalence data were published from 1995 to 2021 and for studies with data reported, the participants were recruited from 2002 to 2019. The majority of the prevalence data were cross-sectional design (94

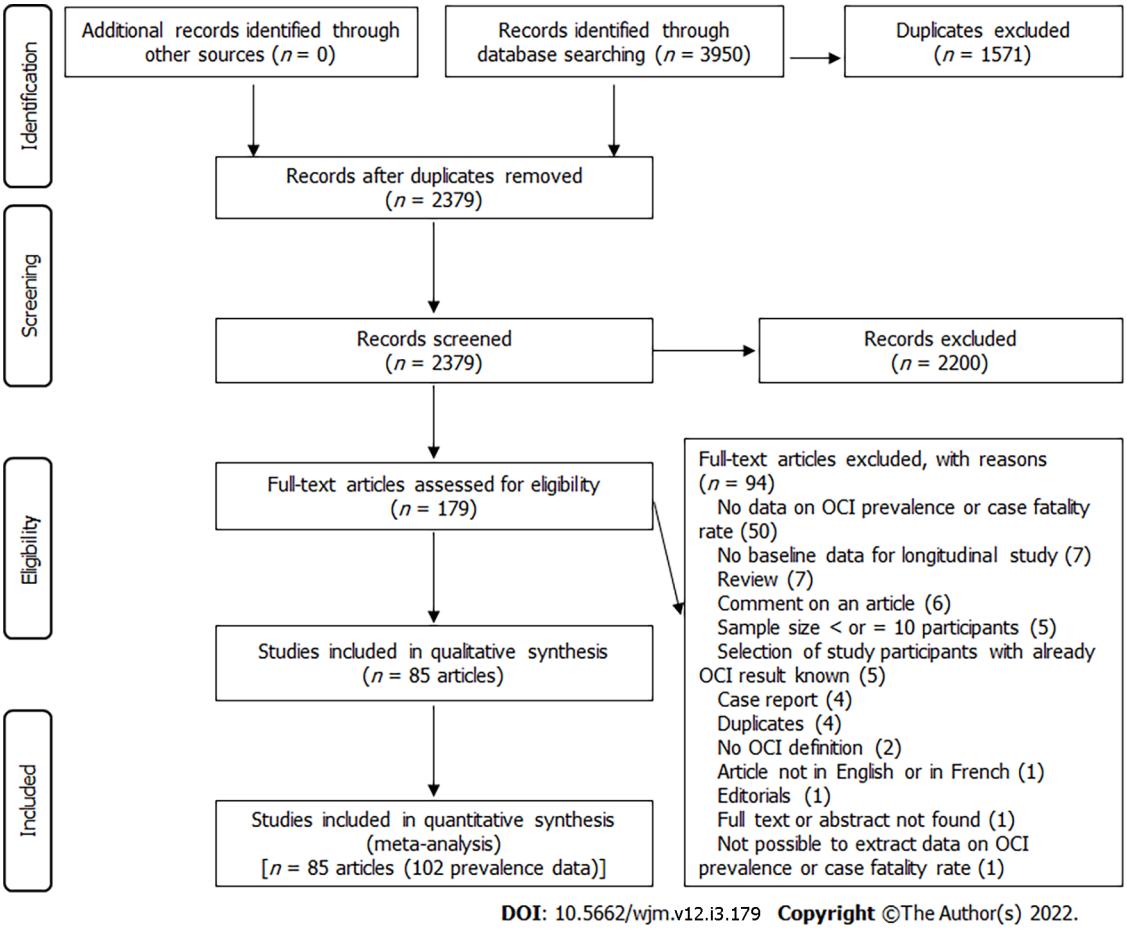

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Figure 1 PRISMA flow-chart of studies selected for the meta-analysis.

out of 102) with non-random sampling (97 out of 102) and consecutive sampling methods (95 out of 102). The setting of the study was hospital-based (98 out of 102) and monocentric (83 out of 102). Prevalence data were reported predominantly in the Eastern Mediterranean (51 out of 102) and in European (41 out of 102) WHO regions. The highest numbers of prevalence data were from high-income countries (40 out of 102) and upper-middle-income countries (35 out of 102). Prevalence data predominantly involved adults (33 out of 102), patients on hemodialysis (25 out of 102), and patients who achieved a SVR (15 out of 102). The most used sample type was PBMC (86 out of 102). The OCI diagnosis was performed using classical RT-PCR (49 out of 102) or real-time RT-PCR (44 out of 102). In most prevalence data, the risk of bias was moderate (64 out of 102) (Supplementary Table 6).

Prevalence of seronegative occult C infection

A total of 75 prevalence data reporting seronegative OCI were conducted across 4 WHO regions: America, Eastern Mediterranean, Europe, and Western Pacific (Figure 2). The pooled OCI prevalence was estimated to be 9.6% (95%CI: 6.8-12.7) in samples of 8535 participants with high heterogeneity [I^2 = 94.7% (95%CI: 93.8%-95.4%), P < 0.0001] (Figure 3A, Table 1, and Supplementary Figure 1). There was significant publication bias (Supplementary Figure 2, P = 0.006). Trim-and-fill adjusted analysis indicated a lower prevalence of 5.3% (95%CI: 2.9-8.2) with an addition of 10 studies.

Prevalence of seropositive occult C infection

Prevalence data reporting seropositive OCI were conducted in 4 WHO regions including America, Eastern Mediterranean, Europe, and Western Pacific (Figure 2). Overall, seropositive OCI prevalence was estimated to be 13.3% (95%CI: 7.8-19.9) with a total of 2642 participants from 24 prevalence data (Figure 3B). High heterogeneity was observed in the overall estimate of the prevalence of seropositive OCI [I^2 = 93.0% (90.8%-94.7%), P < 0.0001]. There was a significant publication bias (Supplementary Figure 3, P = 0.017). Trim-and-fill adjusted analysis indicated a lower prevalence of 5.3% (95%CI: 1.4-10.7) with an addition of 8 studies.

Seronegative and/or seropositive occult C infection

Prevalence data reporting seronegative and/or seropositive OCI were conducted in 2 WHO regions

Table 1 Summary of meta-analysis results for the global prevalence of occult hepatitis C virus infection

	Prevalence % (95%CI)	95% prediction interval	Studies (*n*)	Participants (*n*)	[1]H (95%CI)	[2]I^2 (95%CI)	*P* heterogeneity
Seronegative OCI							
Overall	9.6 (6.8-12.7)	(0-44.1)	75	8535	4.3 (4-4.6)	94.7 (93.8-95.4)	< 0.001
Trim-and-fill adjusted analysis	5.3 (2.9-8.2)	(0.0-45.1)	85	NA	5.1 (4.8-5.4)	96.2 (95.7-96.6)	< 0.001
Cross-sectional	9.3 (6.5-12.6)	(0-43.6)	68	8250	4.4 (4.1-4.8)	94.9 (94.1-95.6)	< 0.001
Low risk of bias	10.2 (5.9-15.5)	(0-45.9)	28	3372	4.3 (3.8-4.8)	94.6 (93.1-95.7)	< 0.001
Seropositive OCI							
Overall	13.4 (7.9-20)	(0-52)	24	2642	3.8 (3.3-4.3)	93 (90.8-94.7)	< 0.001
Trim-and-fill adjusted analysis	5.3 (1.4-10.7)	(0.0-49.9)	32	NA	4.5 (4.0-5.0)	95.1 (93.9-96.0)	< 0.001
Cross-sectional	12.5 (7.2-18.7)	(0-48.5)	23	2530	3.5 (3.1-4.1)	92 (89.3-94)	< 0.001
Low risk of bias	12.8 (4.6-23.6)	(0-57.6)	9	1659	3.6 (2.8-4.6)	92.3 (87.5-95.2)	< 0.001

[1]H is a measure of the extent of heterogeneity, a value of H =1 indicates homogeneity of effects and a value of H >1 indicates potential heterogeneity of effects.
[2]I^2 describes the proportion of total variation in study estimates due to heterogeneity, a value > 50% indicates the presence of heterogeneity.
OCI: Occult hepatitis C virus infection; *n*: Number; 95%CI: 95% confidence interval; NA: Not applicable.

(Eastern Mediterranean and Europe). Overall, seronegative and/or seropositive OCI prevalence was estimated to be 12.6% (95%CI: 1.2-32.2) with a total of 285 participants from 3 prevalence data. High heterogeneity was observed in the overall estimate of the prevalence of seronegative and/or seropositive OCI [I^2 = 93.0% (83.0%-97.1%), P < 0.0001].

Subgroup analyses and meta-regression

Seronegative OCI: Higher proportions of seronegative OCI were estimated for studies which selected participants by non-probabilistic sampling (P = 0.001), conducted in Spain and Egypt (P < 0.001), in Southern Europe and Northern Africa (P < 0.001), or in countries with lower-middle income economies (P = 0.045), investigated children (P = 0.01) or patients with abnormal liver function, hematological disorders, and kidney diseases (P < 0.001), and detected OCI cases by real-time RT-PCR (P < 0.001) or by examining liver tissue (P < 0.001) (Supplementary Table 7). The heterogeneity of the prevalence of seronegative OCI was explained at 84.0% (R^2 = 84.0%) (Supplementary Table 8).

Seropositive OCI: Higher proportions of seropositive OCI were estimated for studies performed as case controls (P < 0.001), conducted in Italy, United States of America, and Egypt (P < 0.001), in Southern Europe, Northern America, and Northern Africa (P = 0.001), or by examining liver tissue and PBMC (P = 0.023). The heterogeneity of the prevalence of seropositive OCI was explained at 46.2% (R^2 = 46.2%).

DISCUSSION

This systematic review summarized the prevalence of seronegative and seropositive OCI in relevant articles published between 1995 and 2021 in 17 countries across 4 WHO regions: America, Europe, Eastern Mediterranean, and Western Pacific. Overall, we found a high prevalence of seronegative OCI (9.61%) and seropositive OCI (13.39%), respectively. Higher seronegative OCI prevalence was found in Southern Europe and Northern Africa and in patients with abnormal liver function, hematological disorders, and kidney diseases. Higher seropositive OCI prevalence was found in Southern Europe, Northern America, and Northern Africa.

Many studies have previously shown that multiple transfused subjects are at high risk of HCV infection[20,25,37-39]. Seronegative OCIs aligned well with classical HCVs and were very predominant in subjects with hematological disorders and renal diseases in this study. It is therefore important to implement screening measures for OCI in blood transfusion banks, dialysis and/or transplant units[40].

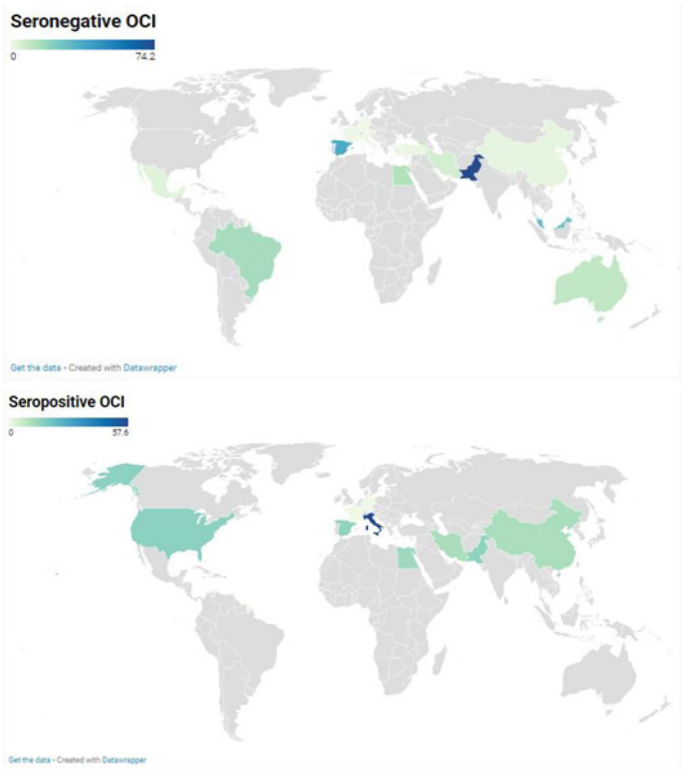

Figure 2 Global prevalence of seronegative and seropositive occult hepatitis C virus infection.

As in the present review, it has also been shown previously that patients with abnormal liver functions are at high risk of OCI[26]. There is, however, a significant residual heterogeneity in our estimates that could be related to the different types of chronic liver disease that we did not take into account. North Africa and particularly Egypt is the country with the highest prevalence of HCV in the world[41,42]. The findings of the present study corroborate this fact and show higher seronegative and seropositive OCI prevalence in North Africa (Egypt). However, it should be noted that Southern Europe and North America also showed high prevalence of OCI in this study, while most other regions were absent or poorly represented in the estimates. HIV patients, people who inject drugs and men who have sex with men are groups known to be at high risk of HCV infection and were poorly represented in this review. Additional studies characterizing the epidemiology of HCV in these groups are awaited to fully explain the global epidemiology of OCI and more specifically in the WHO regions of Africa and South-East Asia. In a first global review without meta-analysis conducted in 2017, Dolatimehr *et al*[25] reported OCI prevalence of 0%-45% and 0%-2% in hemodialysis patients (10 studies) and kidney transplant recipients (2 studies), respectively. In a conference abstract, Fu *et al*[26] reported a highly variable prevalence of OCI in different population groups ranging from 0% in patients with autoimmune hepatitis, 9% in patients with cryptogenic liver disease, 22% in patients with chronic liver disease who achieved a SVR, 33% in patients with long-standing abnormal liver-enzyme levels to 89% in patients with abnormal levels of serum aminotransferases. More recently, Hedayati-Moghaddam *et al*[20] reported the prevalence of OCI in the Middle East and Eastern Mediterranean in several population categories. This last systematic review also revealed a significant variability in OCI prevalence according to the category of the population with 19% for patients with hematological disorders, 12% for HIV-infected patients, 12% for patients with chronic liver diseases, 9% for hemodialysis patients, 8% for multitransfused patients, and 4% for apparently healthy populations. Similar to the reviews mentioned above, our study also noted a statistically significant difference in the prevalence of seronegative OCI according to population categories. However, it should be mentioned that the above reviews only included participants tested for PBMC unlike our work which also considered liver biopsies and ultracentrifuged serum. The strong heterogeneity recorded in our work could also be explained by the differences in HCV prevalence according to the regions with the areas of high HCV endemicity which should also be the areas of high prevalence of OCI[11]. As the different risk factors and the different approaches for controlling HCV infection also vary widely between studies, regions and populations, this could also potentially represent a considerable source of the variability observed in our work. We should also cite the examples of a history of accidental exposure to infected needle sticks, history of blood transfusion, history of surgery, history of endoscopy, history of unsafe sexual intercourse, history of liver disease, the length and frequency of dialysis sessions, immunodepression, injecting drugs, tattoos or imprisonment. Other potential sources of heterogeneity in our estimates may also include gender of

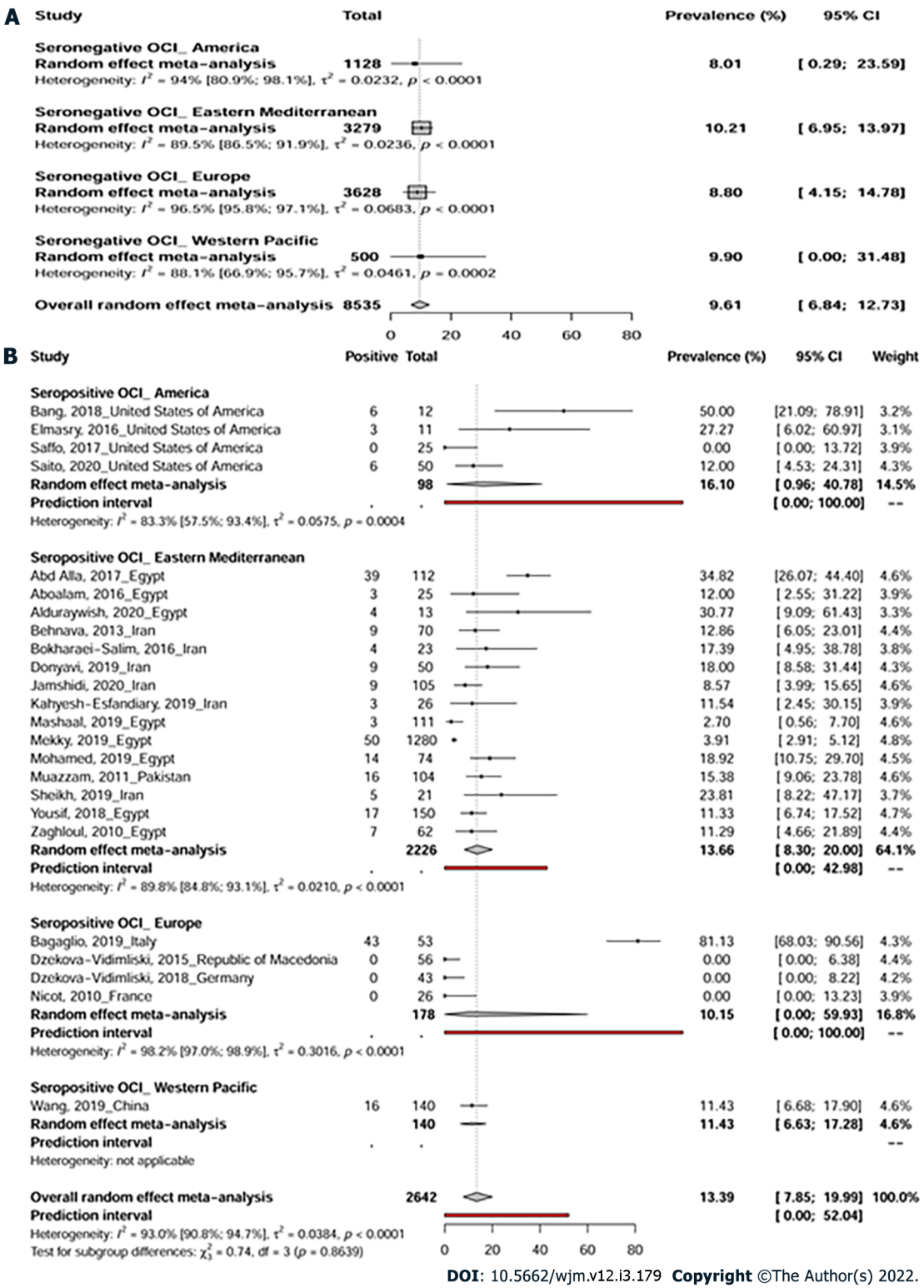

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Figure 3 The pooled global prevalence of seronegative (A) and seropositive (B) occult hepatitis C virus infection.

participants, sample size, and year of participant recruitment. Our study revealed that although PBMC are an excellent non-invasive sampling approach for diagnosing OCI, liver tissue exhibits superior sensitivity for seronegative OCI. It was also found that the ultracentrifuged serum obtained was not an insignificant fraction in patients positive for OCI. These results suggest that it is potentially insufficient to test for OCI in one type of sample. We also observed that real-time RT-PCR was significantly more sensitive for the detection of seronegative OCI. This suggests a further improvement in the sensitivity of molecular techniques for OCI detection.

Our study is limited due to the included studies where the WHO Africa and South-East Asia regions are not represented. Our OCI prevalence could therefore be over- or underestimated. Substantial

residual statistical heterogeneity in prevalence measures was identified in all aggregate and subgroup meta-analyses. Despite these limitations, the main strength of our study is that we identified a very large number of studies, which covered multiple categories of symptomatic, apparently healthy populations and those at high risk of HCV infection. We also accounted for the variability of the prevalence according to the anti-HCV serostatus.

Our results suggest that the implementation of screening programs for OCI in high-risk populations, especially patients with hematologic complications, hemodialysis patients, and patients with chronic liver disease should be initiated. More studies are needed to assess the transmissibility, clinical significance, long-term outcome, and need for OCI treatment.

CONCLUSION

In conclusion, it appears that the burden of seronegative and seropositive OCI is high and very variable according to regions and categories of populations.

ARTICLE HIGHLIGHTS

Research background

In 2004, Castillo *et al* first described an unknown form of hepatitis C in humans that was different from the common chronic hepatitis C and was called occult hepatitis C Infection (OCI).

Research motivation

To eradicate hepatitis C virus (HCV) by 2030, as recommended by the WHO, it is crucial to determine the burden of OCI across the different regions of the world and in different population categories.

Research objectives

Highlight the global prevalence of seronegative and seropositive OCI according to population categories and regions of the world.

Research methods

The authors searched PubMed, EMBASE, Global Index Medicus, and Web of Science databases from inception to May 6, 2021. Data were extracted independently by each author and the Hoy *et al* tool was used to assess the quality of included studies. Prevalence and 95% confidence intervals were determined using random-effect meta-analysis.

Research results

The authors included 85 articles out of the 3950 identified by the electronic search. The combined prevalence of seronegative OCI was 9.61% (95%CI: 6.84-12.73) and the prevalence of seropositive OCI was 13.39% (95%CI: 7.85-19.99). For variations by region, seropositive OCI prevalence was higher in Southern Europe, Northern America, and Northern Africa, and seronegative OCI prevalence was higher in Southern Europe and Northern Africa. For variations by population categories, seronegative OCI prevalence was higher in patients with abnormal liver function, hematological disorders, and kidney diseases.

Research conclusions

The burden of OCI is high and greatly variable according to world regions and population categories.

Research perspectives

Consideration should be given to the implementation of screening programs for OCI in high-risk populations such as patients with hematologic disorders, kidney disease, and those with abnormal liver function.

FOOTNOTES

Author contributions: Kenmoe S, Mbaga DS, and Riwom Essama SH were responsible for conception and design of the study as well as project administration; Mbaga DS, Kenmoe S, Njiki Bikoï J, Takuissu GR, Amougou Atsama M, Atenguena Okobalemba E, Ebogo-Belobo JT, Bowo-Ngandji A, Oyono MG, Magoudjou-Pekam JN, Kame-Ngasse GI, Nka AD, Feudjio AF, Zemnou-Tepap C, Velhima EA, Ndzie Ondigui JL, Nayang Mundo RA, Touangnou-Chamda SA, Kamtchueng Takeu Y, Taya-Fokou JB, Mbongue Mikangue CA, Kenfack-Momo R, and Kengne-Nde C were responsible for the data curation and interpretation of results; Kengne-Nde C and Kenmoe S were responsible for

statistical analysis; Kenmoe S, Mbaga DS, and Riwom Essama SH were responsible for the project supervision; Kenmoe S and Mbaga DS wrote the original draft; All authors critically reviewed the first draft and approved the final version of the paper for submission, and have read and approved the final manuscript.

Supported by the European Union (EDCTP2 programme), No. TMA2019PF-2705.

Conflict-of-interest statement: The authors deny any conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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Corresponding Author's Membership in Professional Societies: International Society of Global Health, No. 00768.

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World Journal of Methodology

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World J Methodol 2022 May 20; 12(3): 191-192

DOI: 10.5662/wjm.v12.i3.191

ISSN 2222-0682 (online)

LETTER TO THE EDITOR

Severe acute respiratory syndrome coronavirus 2 pandemic and surgical diseases: Correspondence

Pathum Sookaromdee, Viroj Wiwanitkit

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Jin X, China; Malekzadegan A, Iran; Mallineni SK, Saudi Arabia

Received: January 18, 2022
Peer-review started: January 18, 2022
First decision: March 16, 2022
Revised: March 16, 2022
Accepted: April 27, 2022
Article in press: April 27, 2022
Published online: May 20, 2022

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Abstract

This letter to editor discussing on the publication on severe acute respiratory syndrome coronavirus 2 pandemic and surgical diseases. Concerns on procedures are raised and discussed.

Key Words: Pediatric; Surgery; COVID-19

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Core Tip: This letter to editor discussing on the publication on severe acute respiratory syndrome coronavirus 2 pandemic and surgical diseases: Concerns on study techniques and clinical implication are raised and discussed.

Citation: Sookaromdee P, Wiwanitkit V. Severe acute respiratory syndrome coronavirus 2 pandemic and surgical diseases: Correspondence. *World J Methodol* 2022; 12(3): 191-192
URL: https://www.wjgnet.com/2222-0682/full/v12/i3/191.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i3.191

TO THE EDITOR

We read with interest a case report on "Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic related morbidity and mortality in patients with pediatric surgical diseases: A concerning challenge" by Vaos and Zavras[1]. We would like to share ideas on this report. Basically, the adaptation of medicine to the coronavirus disease 2019 (COVID-19) is necessary. For surgery, to save lives while maintaining excellent surgical standards, dynamic prioritizing of SARS-CoV-2 infected and surgical patient groups is critical[2]. In emergency departments, non-intensive care wards, and

operating rooms, strict segregation of patient groups inhibits virus spread, while appropriately training and carefully selecting hospital staff allows them to confidently and successfully perform their respective clinical roles[2]. How to find a solution in surgery need a good systematic study.

In this report, a literature retrospective review is done. However, there is no clear information on searching technique and extracting of data. There is no interrelationship network analysis of recruited literatures and it does not follow standard meta-analysis technique, bioinformatics interrelationship analysis and bibliometric analysis. The summarization is based on crude summary on surgical cases, without adjustment to the background condition of the cases (age, underlying disease, surgical intervention, *etc.*). Also, there is no study on the correlation with the stages of COVID-19 background in different recruited publication. It should not possible to recommend the new guidelines for management of pediatric surgical cases. For pediatric surgery, a meta-analysis on each specific condition with specific aim or target for study, such as comparison of surgical approach, should be the best method to find out the solution during the current COVID-19 crisis. Good example of the studies in this kind are reports by Chan *et al*[3,4].

FOOTNOTES

Author contributions: Sookaomdee P and Wiwanitkit gave ideas, analyzed the data and wrote the manuscript; all authors have read and approve the final manuscript.

Conflict-of-interest statement: Authors declare for no conflict of interest.

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S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

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7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

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ISSN 2222-0682 (online)

World Journal of *Methodology*

World J Methodol 2022 July 20; 12(4): 193-330

Published by Baishideng Publishing Group Inc

World Journal of Methodology

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INDEXING/ABSTRACTING

The *WJM* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Wen-Wen Qi*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Ji-Hong Liu*.

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Bimonthly

EDITORS-IN-CHIEF
Bruno Megarbane

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2222-0682/editorialboard.htm

PUBLICATION DATE
July 20, 2022

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World J Methodol 2022 July 20; 12(4): 193-199

DOI: 10.5662/wjm.v12.i4.193

ISSN 2222-0682 (online)

OPINION REVIEW

Laparoscopic bilateral inguinal hernia repair: Should it be the preferred technique?

Christos Doudakmanis, Christina Kolla, Konstantinos Bouliaris, Matthaios Efthimiou, Georgios D Koukoulis

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Cornett EM, United States; Hamza M, United States

Received: January 29, 2022
Peer-review started: January 29, 2022
First decision: March 25, 2022
Revised: April 3, 2022
Accepted: May 14, 2022
Article in press: May 14, 2022
Published online: July 20, 2022

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Abstract

Inguinal hernias are amongst the most common conditions requiring general surgical intervention. For decades, the preferred approach was the open repair. As laparoscopy became more popular and available and more surgeons became familiarized with this modality, laparoscopic inguinal hernia repair became an alternative. The aim of this study is to assess the effectiveness of laparoscopic inguinal repair, with a focus on bilateral inguinal hernias. Initial reports have shown promising clinical outcomes compared to those of conventional repair of bilateral hernias. However, there are only a few studies concerning laparoscopic repair of bilateral hernias. It is yet to be proven that laparoscopy is the "gold standard" in the treatment of bilateral inguinal hernias. So far, the choice of an inguinal hernia repair technique has been up to each surgeon, depending on their expertise and available resources after taking into consideration each patient's needs.

Key Words: Bilateral inguinal hernia; Laparoscopic repair; Open repair: Gold standard; Chronic pain; Recurrence

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Core Tip: Laparoscopic repair of bilateral inguinal hernias has become a common procedure over the past few years. It is associated with less pain and faster return to daily life compared to the open repair. As yet, there is little evidence to sufficiently support that it should be the preferred technique, as it depends on each surgeon to choose the repair technique that they will use.

Citation: Doudakmanis C, Kolla C, Bouliaris K, Efthimiou M, Koukoulis GD. Laparoscopic bilateral inguinal hernia repair: Should it be the preferred technique? *World J Methodol* 2022; 12(4): 193-199
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/193.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.193

INTRODUCTION

Inguinal hernias are amongst the most frequent clinical manifestations of a general surgery department. Therefore, surgical procedures, both elective and emergent, are in most cases necessary to relieve the symptoms caused by hernias. Incidence of inguinal hernias is greater in patients older than 50 years of age, although they are also common in young children and infants. The vast majority of patients are male[1,2]. Bilateral hernias represent approximately 8% to 30% of inguinal hernias[3].

Mesh repair, such as the Lichtenstein or laparoscopic mesh repair approach, should be considered first by a surgeon. When considering non-mesh techniques, the Shouldice repair should be the primary choice[4]. Currently, open mesh repair remains the most widely used technique[5]. The European Hernia Society recommends laparoscopic repair for recurrent inguinal hernias. Regarding unilateral hernias, the choice between an open or laparoscopic approach depends on each surgeon and their expertise, as a surgeon needs to perform 50 to 100 repairs to master the laparoscopic repair technique [5]. When it comes to bilateral inguinal hernias, there is no official recommendation; however the European Hernia Society highlights that laparoscopic repair of bilateral hernias is associated with better short-term results without undermining long-term results[4,6]. This is stated as "self-evident" in the 2018 HerniaSurge guidelines, as a laparoscopic operation of two inguinal hernias through the same three incisions is considered superior in terms of recovery, chronic pain and cost-effectiveness[5]. Time to recovery and postoperative pain are considered to be less in laparoscopic repair due to less surgical trauma as it promotes diminished acute inflammatory postoperative response, proven by smaller quantities of cytokines[7].

The purpose of this study was to evaluate the effectiveness of laparoscopic repair techniques in bilateral inguinal hernias and to examine whether laparoscopic repair is superior compared to open repair based on the existing literature.

SEARCH OF THE LITERATURE

We conducted a thorough search of the literature using PubMed, the Scopus Elsevier Database and Cochrane Database. The search terms we used were: "bilateral hernias", "inguinal hernias", "laparoscopic hernia repair", "laparoscopic *vs* open hernia repair", "postoperative pain", "chronic groin pain", "cost-effectiveness", "quality-of-life" and "recovery". We collected the international guidelines regarding hernia repair issued by the European Hernia Society and HerniaSurge Group in order to review the official recommendations.

As there was no official recommendation on using laparoscopic repair in bilateral inguinal hernias as the "gold standard," our main goal was to review the available literature to examine whether there is evidence supporting this assumption. We reviewed all available literature on this subject, with emphasis on prospective randomized trials. We included data from six prospective randomized studies regarding bilateral hernias (Table 1) and from one prospective randomized study, which focused on unilateral hernias but was the first to suggest beneficial results of laparoscopic repair on bilateral hernias. We also retrieved data from one prospective randomized trial that compared different techniques of laparoscopic repair. We reviewed comparative studies, meta-analysis and one large-scale retrospective study. The draft of this manuscript was written on Microsoft Word v.16 of Microsoft Corporation.

LAPAROSCOPIC HERNIA REPAIR

Since the introduction of laparoscopic repair techniques, there has been a debate regarding the superiority of laparoscopic over open inguinal hernia repair. Initial analysis has shown that laparoscopic repair is at least not inferior compared to the open approach in terms of operative time, postoperative pain, recovery and hospital stay[8]. The main factors used to compare the two approaches are immediate postoperative pain and pain following the months after surgery as well as mean postoperative recovery time to daily activities[9]. As there has been tremendous progress in laparoscopic surgery in the past decades, laparoscopic hernia repair techniques are now becoming widely available to surgeons, and there is a belief that these techniques may supersede open repair procedures.

Table 1 Prospective randomized trials regarding bilateral hernias

Ref.	Journal	Title of study	Compared techniques	Patients	Subject
Sarli *et al* [20], 2001	*Surg Laparosc Endosc Percutan Tech*	Simultaneous repair of bilateral inguinal hernias: A prospective, randomized study of open, tension-free *vs* laparoscopic approach	TAPP *vs* Lichtenstein	43 (20 *vs* 23)	Surgical procedure, postoperative pain and course, follow-up, cost analysis
Mahon *et al*[21], 2003	*Surg Endosc*	Prospective randomized trial of laparoscopic (transabdominal preperitoneal) *vs* open (mesh) repair for bilateral and recurrent inguinal hernia	TAPP *vs* Lichtenstein	120 (60 *vs* 60)	Surgical procedure, postoperative pain and course, recovery
Ielpo *et al* [22], 2018	*Am J Surg*	A prospective randomized study comparing laparoscopic TAPP *vs* Lichtenstein repair for bilateral inguinal hernias	TAPP *vs* Lichtenstein	134 (61 *vs* 73)	Surgical procedure, postoperative course, recovery, quality of life, chronic pain
Bignell *et al*[23], 2012	*Hernia*	Prospective randomized trial of laparoscopic (TAPP) *vs* open (mesh) repair for bilateral and recurrent inguinal hernia: incidence of chronic groin pain and impact on quality of life: Results of 10-yr follow-up	TAPP *vs* Lichtenstein	120 (60 *vs* 60)	Chronic groin pain, quality of life
Hynes *et al*[24], 2006	*J Am Coll Surg*	Cost effectiveness of laparoscopic *vs* open mesh hernia operation: Results of a department of veterans affairs randomized clinical trial	All laparoscopic *vs* all open	1395 (687 *vs* 708)	Quality of life, cost-effectiveness
Ielpo *et al* [25], 2018	*Ann Surg*	Cost-effectiveness of randomized study of laparoscopic *vs* open bilateral inguinal hernia repair	TAPP *vs* Lichtenstein	165 (81 *vs* 84)	Quality of life, cost-effectiveness, cost analysis

TAPP: Transabdominal preperitoneal.

LAPAROSCOPIC REPAIR TECHNIQUES

Laparoscopic inguinal hernia repair may be conducted using two different techniques the transabdominal preperitoneal procedure (TAPP) and the totally extraperitoneal procedure (TEP). These approaches may differ in terms of access but share the same concepts of laparoscopic surgery. So far, they have shown similar outcomes in terms of recovery, hospital stay, chronic pain and quality of life [10]. TAPP, although it is easier to learn and perform, has a longer operating time and greater incidence of postoperative pain, while TEP is associated with a greater incidence of seroma formation. The differences between the two approaches are not significant, thus the techniques are comparable. It is reported that the risk for seroma and hematoma formation is also comparable regarding TAPP, TEP and the open repair[11]. The cost for both laparoscopic procedures is similar[10,12].

Since the first studies regarding laparoscopic hernia repair techniques were published, these techniques have progressed. Newer lightweight meshes are associated with less pain and a lower recurrence rate, in contrast to outdated heavyweight meshes[13]. Mesh fixation techniques have also undergone changes in the past few years. Tack fixation while widely used, is associated with considerable postoperative pain due to the presence of a foreign body in the inguinal region. In recent years, titanium tacks have gradually been replaced by absorbable tacks, which cause less pain[14]. Transfacial suture fixation and fibrin glue fixation are new techniques associated with significantly less pain compared to the use of tacks[15,16]. The technique shown to cause minimal pain, both postoperatively and long-term, is the use of a lightweight mesh fixed using fibrin glue[17,18]. We must note that in the totally extraperitoneal procedure, mesh fixation is not a prerequisite, and it can be avoided without putting the effectiveness of the procedure in danger[19].

DO SHORT-TERM RESULTS INDICATE LAPAROSCOPIC REPAIR OF BILATERAL INGUINAL HERNIAS AS A BETTER OPTION?

There are three randomized prospective trials in the literature that compare laparoscopic to open repair of bilateral inguinal hernias. Sarli *et al*[20] published the first prospective randomized control trial, which included 43 patients, comparing open mesh repair to laparoscopic repair of bilateral inguinal hernias. In their study, the Lichtenstein procedure was compared to the TAPP, and factors such as operating time, complications, postoperative pain, time to recovery and cost-effectiveness were analyzed, with a follow-up of up to 3 years postoperatively. Despite a higher cost, laparoscopic repair was associated with faster recovery and less pain in the immediate postoperative period, while complications, days of hospitalization and recurrence rates were similar in both groups[20].

These first results were subsequently supported by the randomized control trial of Mahon *et al*[21]. In this study, a total of 120 patients were included. The endpoint of this study was the superiority of TAPP over the open repair for bilateral hernias, in terms of postoperative pain, days of hospitalization and time to recovery[21]. Ielpo *et al*[22] published their randomized control trial in 2018, comparing TAPP with the open repair for bilateral inguinal hernias. In their study, a total of 134 patients were included over a 2-year span. Their results supported those of prior randomized controlled trials, in terms of beneficial short-term results, such as recovery, postoperative pain and complications[22].

Clinical outcomes of laparoscopic surgery outperformed those of open repair and supported the concept of establishing laparoscopic repair in bilateral inguinal hernias as the "gold standard," regardless of the technique performed (as TAPP and TEP are associated with similar outcomes)[12,20-22].

IS THERE SUFFICIENT EVIDENCE OF LONG-TERM SUPERIORITY OF THE METHOD?

Chronic pain, quality of life and recurrence rates are the most important factors to evaluate long-term superiority. In the study of Ielpo *et al*[22], chronic pain and long-term quality of life are under investigation, and it is one of the two published randomized controlled trials regarding chronic pain, along with the 2012 study of Bignell *et al*[23]. The results of the study by Ielpo *et al*[22] indicated that patients undergoing laparoscopic repair had less postoperative pain, fewer complications and, more importantly, less chronic pain, but there was no statistically significant difference regarding the long-term quality of life.

Chronic groin pain is one of the factors indicative of long-term success of the method. The existing literature suggests that laparoscopic repair is superior in terms of short-term clinical outcomes but, so far, has failed to provide adequate evidence of superiority in the years following surgery. Incidence of chronic pain in the inguinal area is higher, but pain is milder in patients who have undergone laparoscopic repair compared to open repair. The most representative indicator of the long-term success of the procedure is quality-analyzed life years, which is presumed higher in laparoscopic repair, demonstrating the superiority of the method. However, overall quality of life as determined through questionnaires was found to be similar in laparoscopic and open repair groups[23]. This result was also supported by data derived from studies focusing on the effectiveness of the techniques. Data from these two studies underline the comparable quality of life of patients from both repair groups. There were no statistically significant differences[24,25].

Besides quality-of-life markers, recurrence rates depict the success of the procedure in the years following surgery. Available data from prospective randomized studies have shown that only a few cases of recurrence following both laparoscopic and open repair were recorded. In addition, recurrence rates are similar between laparoscopic and open repair groups[20-23,25,26]. In five studies, more cases of recurrence were recorded in the laparoscopic group as an absolute number of cases, but the two groups did not differ significantly. A statistically significant difference ($P < 0.001$) in recurrence rates was only recorded in the retrospective study of Hynes *et al*[24], with a higher recurrence in the laparoscopic repair group. This was mostly attributed to operations performed by less experienced surgeons [24,26]. It must be noted that the study of Hynes *et al*[24] refers to operations performed in the early 2000s with the techniques and consumables available at that time. This may have been a contributing factor to the difference in recurrence in this study (Table 2).

IS LAPAROSCOPY WORTH THE COST?

A critical issue about laparoscopic repair is the cost in accordance with the postoperative quality of life. Two randomized prospective trials about cost-effectiveness of laparoscopic repair were found in the literature. Early data from a randomized controlled trial published in 2006 demonstrated that laparoscopic repair had a significantly higher cost and higher quality of life. The data supported the concept of open repair being more cost-effective for bilateral inguinal hernias[24]. In contrast, Ielpo *et al*[25] analyzed clinical outcomes, such as pain, recovery, recurrence and complications, costs, quality-adjusted life years and calculated cost-effectiveness. Their study showed a significantly higher cost of laparoscopic repair. At the same time, clinical outcomes of laparoscopic repair outperformed those of open repair. This demonstrates that laparoscopic repair may be cost-effective for bilateral inguinal hernias [25].

Laparoscopy has a priori higher cost, which is even higher when consumables are included. Although laparoscopic instruments may be reusable, making their use affordable, the main factor increasing the cost is the mesh fixation technique. Newer fixation techniques, such as self-gripping meshes and fibrin glue fixation have been proposed as more cost-effective fixation techniques[27]. It is of utmost importance to investigate cost-effectiveness. It must be noted that the latest randomized controlled trial analyzing cost-effectiveness indicated that laparoscopic repair in bilateral inguinal hernias is considered cost-effective. This difference between prior studies[24] and this one[25] likely derives from the fact that

Table 2 Recurrence rates

Ref.	Patients	Laparoscopic	Open
Sarli *et al*[20], 2001	43 (20 *vs* 23)	0%	4.34%
Mahon *et al*[21], 2003	120 (60 *vs* 60)	6.7%	1.7%
Ielpo *et al*[22], 2018	134 (61 *vs* 73)	6.6%	5.5%
Bignell *et al*[23], 2012	120 (60 *vs* 60)	7%	8%
Hynes *et al*[24], 2006[1]	1395 (687 *vs* 708)	8%	4%[a]
Neumayer *et al*[26], 2004	353 (175 *vs* 178)	4.57%	2.80%
Ielpo *et al*[25], 2018	165 (81 *vs* 84)	7.4%	4.8%

[1]Recurrence rates for both unilateral and bilateral hernias.
[a]$P < 0.01$.

with advances in laparoscopic surgery, necessary equipment along with consumables have become more accessible and more affordable. It should be emphasized that in the past few years more patients have undergone laparoscopic repair, so more patients have been enrolled in newer studies. This evidence is considered more representative[25].

IS THERE SUFFICIENT EVIDENCE?

All of the trials supporting the superiority of laparoscopic repair of bilateral hernias included only a small number of patients[20-27]. In the literature, there is only one large-scale retrospective non-randomized study. This particular study, which included more than 2800 patients with bilateral inguinal hernias, concluded that laparoscopic repair was at least non-inferior to the open repair and that it should be considered as "gold standard"[28]. As this study is retrospective, the level of evidence is not considered sufficient to set a "gold standard," but it still provides an indication. It is more than clear that more large-scale prospective randomized trials are needed to prove this point. The first studies regarding bilateral hernias were published in the late 1990s and early 2000s. Until recently, and for approximately 15 years, there were only a few studies published underlining the fact that there is research progress to be made to define laparoscopic repair of bilateral inguinal hernias as the "gold standard." The wide range of techniques used explains the diversity of the results of the existing trials. Uniformity of future studies is an issue that should be addressed. A consensus on the methods used between different study groups should be determined if significant results are to be extracted. In existing studies, study design depends mostly on each researcher and their clinical practice. Another issue is that some studies investigated laparoscopic repair in both unilateral and bilateral hernias. Newer studies have greater uniformity as they compare TAPP *vs* open repairs, but they lag behind in terms of patients enrolled[20-23].

WHICH TECHNIQUE SHOULD A SURGEON USE?

Laparoscopic techniques in hernia repair surgery have progressed over the past decades. Clinical outcomes of laparoscopic repair in bilateral hernias are very promising, as they outperform those of open repair in terms of pain in the immediate postoperative period and recovery. Over the years, these techniques have become more cost-effective. There is a shortage of evidence supporting the long-term superiority of these surgical procedures regarding quality of life as well as chronic groin pain. So far the results are controversial. To this day, it is still not possible to recommend a specific repair technique for bilateral hernias.

Available evidence is in favor of laparoscopic repair, but there is a lack of solid data. Future prospective studies are needed to compare the use of different techniques and surgical instruments as well as different meshes and fixation techniques. As existing evidence supports short-term superiority of the laparoscopic repair and suggests that it is a safe procedure when performed by a suitably trained surgeon, alongside the diminishing cost, it is promising to await future studies focusing on the long-term results of this method.

The answer to a surgeon's question "which technique should I use" is multifactorial. First, as there is still progress to be made in laparoscopy in order to establish it as the "gold standard" procedure, willingness of patients to undergo laparoscopic repair must be taken into consideration. It is crucial to

explain to them that a laparoscopic repair requires general anesthesia, whereas an open repair may be conducted in most cases under spinal anesthesia. In addition, we should not undermine the expertise of surgeons. As laparoscopic repair has a prolonged learning curve, it is more than clear that reforms in surgical training alongside special training programs are required to train surgeons, in order to familiarize them with these techniques. Only when these procedures are widely available and can be done safely, can we conclude that laparoscopic repair is the "gold standard" technique for the treatment of bilateral inguinal hernias.

CONCLUSION

Laparoscopic repair of bilateral inguinal hernias is associated with less postoperative pain and faster return to daily life compared to the open repair, but we do not have solid evidence supporting the long-term superiority of laparoscopic procedures over open repair regarding quality of life as well as chronic groin pain.

FOOTNOTES

Author contributions: Doudakmanis C and Koukoulis G conceived the idea for the manuscript; Doudakmanis C, Koukoulis G, Bouliaris K and Kolla C reviewed the literature and drafted the manuscript; Efthimiou M critically revised the manuscript for important intellectual content; all authors read and approved the final version of the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interests.

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S-Editor: Wang LL
L-Editor: Filipodia
P-Editor: Wang LL

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World J Methodol 2022 July 20; 12(4): 200-223

DOI: 10.5662/wjm.v12.i4.200

ISSN 2222-0682 (online)

REVIEW

COVID-19 disease and autoimmune disorders: A mutual pathway

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Specialty type: Rheumatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Naswhan AJ, Qatar; Wang MZ, China

Received: March 21, 2022
Peer-review started: March 21, 2022
First decision: June 16, 2022
Revised: June 17, 2022
Accepted: July 6, 2022
Article in press: July 6, 2022
Published online: July 20, 2022

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Abstract

Coronavirus disease 2019 (COVID-19) is a real challenge for humanity with high morbidity and mortality. Despite being primarily a respiratory illness, COVID-19 can affect nearly every human body tissue, causing many diseases. After viral infection, the immune system can recognize the viral antigens presented by the immune cells. This immune response is usually controlled and terminated once the infection is aborted. Nevertheless, in some patients, the immune reaction becomes out of control with the development of autoimmune diseases. Several human tissue antigens showed a strong response with antibodies directed against many severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins, such as SARS-CoV-2 S, N, and autoimmune target proteins. The immunogenic effects of SARS-CoV-2 are due to the sizeable viral RNA molecules with interrupted transcription increasing the pool of epitopes with increased chances of molecular mimicry and interaction with the host immune system, the overlap between some viral and human peptides, the viral induced-tissue damage, and the robust and complex binding between sACE-2 and SARS-CoV-2 S protein. Consequently, COVID-19 and its vaccine may trigger the development of many

autoimmune diseases in a predisposed patient. This review discusses the mutual relation between COVID-19 and autoimmune diseases, their interactive effects on each other, the role of the COVID-19 vaccine in triggering autoimmune diseases, the factors affecting the severity of COVID-19 in patients suffering from autoimmune diseases, and the different ways to minimize the risk of COVID-19 in patients with autoimmune diseases.

Key Words: COVID-19; SARS-CoV-2; Autoimmune Diseases; Vaccines

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Core Tip: There is a mutual relation between coronavirus disease 2019 (COVID-19) and autoimmune diseases. Patients with immune deficiencies or autoimmune disorders are at a higher risk for infection with COVID-19, as they are frequently treated with anti-cytokine, glucocorticoids, and immunosuppressive drugs. Meanwhile, COVID-19 and its vaccine could trigger the development of autoimmune diseases. Therefore, a multi-purpose comprehensive social and family program with exercise and psychological support is highly needed for patients with autoimmune disorders to lessen the harmful effects of social isolation impeded during the COVID-19.

Citation: Al-Beltagi M, Saeed NK, Bediwy AS. COVID-19 disease and autoimmune disorders: A mutual pathway. *World J Methodol* 2022; 12(4): 200-223
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/200.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.200

INTRODUCTION

The coronavirus disease 2019 (COVID-19), with its global pandemic, which started with the first reported case in December 2019, is a real challenge for humanity. These challenges are due to the uncertainty about the origin of the virus, its rapid transmission, the difference in racial susceptibility, the wide variety of clinical presentations, the conflict in diagnosis, the rapid mutations that continuously elaborate, the disparity of the treatment regimens in the different parts of the world, and the high morbidity and mortality rates[1,2]. The virus that causes COVID-19, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a member of the beta coronaviruses group. This group is a part of a Coronaviridae family with spherical, positive-sense, non-segmented, single-stranded, and large (100–160 nm) RNA viruses[1].

SARS-CoV-2 is a single-stranded RNA virus with a positive sense and a unique pleomorphic or spherical, non-segmented envelope with distinctive crown-shaped peplomers or spikes[3]. It has four main structural proteins: spike protein (S), small envelope glycoprotein (E), membrane glycoprotein (M), and nucleocapsid protein (N). Other several accessory proteins are present and have particular functions. S protein is a sizeable trimeric glycoprotein that facilitates viral binding to host cells through binding to angiotensin-converting enzyme II receptors with its two non-covalently associated subunits. The first subunit (S1) binds to angiotensin-converting enzyme II (ACE2) with its subunit receptor-binding domain (RBD). The second subunit (S2 subunit) controls the fusogenic ability of the virus-cell membrane and fixes the S protein to the cell membrane[4]. The E protein is a small envelope glycoprotein of three variants, participates in viral assembly and virion release, and plays a critical role in the virus pathogenesis. M glycoprotein shapes the viral envelope and is accountable for transmembrane nutrient transporting and bud release. N protein is formed from the matrix protein and is present near the viral nucleic acid material within a capsid to help pack the viral RNA genome inside the viral envelope. This process is a fundamental component of the self-assembly and replication of the virus[5,6]. The virus's genome encodes for the four essential structural proteins (S, M, E, and N), hemagglutinin esterase, and another six accessory genes occupying the main part of the viral genome (about two-thirds). There are three well-defined variants of SARS-CoV-2: A, B, and C, according to their genomic differences[7].

PATHOGENESIS AND IMMUNOGENICITY OF SARS-COV-2

Despite being primarily a respiratory illness, COVID-19 can affect nearly every human body tissue, causing a broad range of illnesses. Similar to SARS-CoV, SARS-CoV-2 uses ACE2 receptors to enter the host cells. It uses the surface S glycoprotein with its two domains (S1 and S2) to bind at the RBD with

the ACE2 receptor and fuse the viral envelope membrane with the cell membrane. However, SARS-CoV-2 S proteins bind stronger with human ACE2 receptors than SARS[8]. These spike proteins have high antigenicity, as indicated by the elevated plasma anti-S neutralizing antibodies levels in convalescent patients[9].

The body localization and expression of ACE2 receptors could determine the potential target organs of SARS-CoV-2 infection and outline the disease progression and clinical consequences. ACE2 receptors were first reported in the heart, kidneys, and type-I and II alveolar cells. Then, recently high expression of ACE2 receptors was reported in the brain, eyes, nasal and oral mucosa, thyroid, esophageal epithelium, gastric mucosa, liver, cholangiocytes, pancreas, the smooth muscle cells, and enterocyte from the small intestine to the colon, skin, testis, ovary, uterus, vagina, and urinary bladder[10,11]. All these organs should be considered as a potential target for SARS-CoV-2 infection. ACE2 receptor expression may be absent in the bone marrow, lymph nodes, thymus, spleen, and numerous immune system cells[12]. The patients' differences in ACE2 receptor distribution and ACE2-SARS-CoV-2 mutual interactions could affect the disease pathophysiology, progression, and consequences. Many factors could affect ACE2 receptors distribution, including heritability, patient demographics, lifestyle, comorbidities, and drugs[13]. Meanwhile, soluble ACE-2 (sACE-2) is found in the serum or plasma due to shedding from the cell surface. These sACE-2 strongly interact with SARS-CoV-2 S protein and vasopressin to initiate receptor-mediated endocytosis, enabling SARS-CoV-2 to enter the host cells[14].

After viral entry into the body, it attaches to the mucosal cell, entering the cell either at the plasma membrane or the endosome through receptor-triggered endocytosis[15,16]. The receptor-dependent endocytosis starts by latching the RBD part of the S1 protein of the viral envelope to a pocket in the ACE2 receptor, fixing the virus to the cell membrane. Then, the transmembrane protease serine 2 present near ACE2 receptors cleaves a protein between the S1 and S2 units in a specific location, with the help of the Furin enzyme, which enables the viral entry into the cell after binding. The enzymatically induced cutting of S protein exposes previously hidden parts of the S protein, which undergo a series of remarkable conformational changes and more fixation into the cell membrane. Once inserted, S proteins pull back on themselves, pulling the membranes of the cell and the virus together to fuse. When the viral envelope starts to merge with the host cell membrane, it creates a fusion pore that allows the virus to release its genetic material into the cell cytoplasm of the infected cell[17,18].

After receptor engagement and viral replication inside the affected epithelial cells of the nasal cavity, there will be an initial asymptomatic phase for one to two days. During this phase, the virus continues to replicate and multiply without significant resistance by the innate cellular immunity. After this initial stage, the symptoms appear from 2-14 d. Once the SARS CoV-2 virus spreads to the lower respiratory tract, it stimulates a vigorous innate immune response with a more significant pro-inflammatory response that may progress to viral sepsis and other consequences of acute respiratory distress syndrome that may end with multisystem organ failures and even death[19].

Viral antigen presentation

After viral infection, T lymphocytes can recognize the viral antigens presented by major histocompatibility complex (MHC) class I on the surface of all the nucleated human cells and the platelets. This step is crucial for cytokine release and promotes CD8+ T cells cytotoxic activity. However, MHC class II can occasionally present the viral epitopes to CD4+ T cells[20,21]. The human leukocyte antigens (HLA) association is not very well-identified for SARS-CoV-2 infection, which could be crucial for preventing and treating COVID-19. However, a study by Tomita *et al*[22] showed that patients with HLA genotypes (HLA-A*11:01 or HLA-A*24:02) might efficiently produce T-cell-mediated immune responses to SARS-CoV-2 than patients with HLA-A*02:01. At the same time, reports documented the ability of SARS-CoV-2 to inhibit the expression of HLA-antigens. Giamarellos-Bourboulis *et al*[23] showed that the plasma-derived from patients with severe SARS-CoV-2 infection could inhibit the expression of HLA-DR on CD14+ monocytes, which could partially be reversed by Tocilizumab (IL-6 blocker), indicating the role of hyper-inflammation and the sustained cytokine production in inducing this immune dysregulation.

The innate immunity

The immune responses to SARS-CoV-2 start with the innate immune response by the interferon (IFN)-mediated pathways and the adaptive cellular and humeral immunity through the T lymphocyte and the antibody-mediated pathways. However, SARS CoV-2 can antagonize the IFN-mediated antiviral responses, allowing viral replication with a high early viral load and transmissibility[24]. The innate immune response to SARS-CoV-2 infection in the respiratory tract is mediated through alveolar macrophages and dendritic cells, inducing a cascade of inflammation to restrict virus replication effectively. This cascade of inflammation arises from the release of pro-inflammatory cytokines, particularly IL-18 and IL-1β, which explains the distinguished characteristic of neutrophilia and leukopenia commonly observed in patients with severe COVID-19[25]. The released inflammatory mediators recruit T lymphocytes and monocytes primarily, but not neutrophils, to the site of infection, which explains the lymphopenia and the raised neutrophil-lymphocyte ratio observed in most patients with COVID-19 [26]. However, this induced inflammatory cascade plays a significant role in the pathogenesis of severe organ injury and adverse disease outcomes[27]. The differences in patients' susceptibility to coronavirus

infection may be related to the differences in the Mannose-binding lectin (MBL) protein, which has a significant role in pattern-recognition molecules, one of the first-line host defense mechanisms against SARS-CoV-2 infections[28]. MBL pathway activates the complement pathway that promotes thrombosis and coagulopathy in severe COVID-19[29]. The viral RNA also activates Toll-Like Receptor 3, 7, 8, and 9, which accordingly activates the pathway including Nuclear Factor kappa B (NF-κB)[30].

This immune response is usually controlled and terminated once the infection is aborted. Nevertheless, in some patients, the immune reaction becomes out of control with excessive unwanted response ending with a cytokine storm with a low level of IFN in early-stage and high levels in late-stage, an excessive increase of interleukin (IL)-6, IL-2, IL-7, and IL-10, massive increase of granulocyte-macrophage colony-stimulating factor (GM-CSF), Macrophage Inflammatory Protein 1α (MIP-1α), Tumor Necrosis Factor-alpha (TNF-α), plasma-induced protein 10 (IP-10), monocyte chemotactic protein-1 (MCP-1), and Inflammatory Protein 1α (MIP-1α)[31-33]. This deviated immune response's exact mechanism is unknown but may be related to the antagonistic effects of viral N protein on the interferon signaling pathway. Interferons-mediated innate immunity is the first defense mechanism against viral infections, including COVID-19, through activating macrophages and natural killer (NK) cells, which destroy the virus-infected cells[26]. Interferon deficiency causes an elevation in pro-inflammatory cytokines, an inadequate antiviral response, ACE2 receptor upregulation, high viral load, and subsequent excessive inflammatory response[34,35]. Complement activity is essential in immunity modulation and can predict the clinical outcome of SARS-CoV-2 infection. Complement protein C3 activation occurs early in the course of COVID-19. It plays a significant role in enhancing prothrombotic and pro-inflammatory conditions with immune complex deposition in different organs that may proceed to extensive endothelial damage, acute respiratory distress syndrome, and even end-organ damage observed in severe cases of COVID-19[36,37]. Consumption of the complement proteins in the immune complexes explains the low levels of C3 and C4 observed in instances of severe COVID-19. Detection of low levels of C3 and C4 can be a warning sign of the need for additional management in patients admitted with COVID-19[38]. Immune complexes depositions induced-vascular injury and antibody-dependent enhancement increase viral replication in Fc-receptor expressing cells[39].

The adaptive immunity

The three main types of lymphocytes, B cells, T cells, and natural killer cells, play a vital role in clearing infections once they begin. Their plasma numbers correlate well with better survival. Lower leukocyte and lymphocyte numbers help the virus avoid the host immune response with a high viral load and transmission rate[40]. Once activated by SARS-CoV-2, natural killer T cells can prevent viral spread from the upper airways to the rest of the body and, consequently, determine the severity of the symptoms, the viral load, transmission to the community, and the disease outcome[41].

Although T and B lymphocytes do not express ACE2 receptors, some of them can still be infected by the SARS-CoV-2 virus, which indicates the presence of other receptors participating in the viral entry in some lymphocytes. After a few days from SARS-CoV-2 infection, naïve lymphocytes differentiate into Th2 and produce Th2 cell serum cytokines. The higher the levels of Th2 cell serum cytokines are, the worse the outcome is[42]. Some memory T cells can be primed by a previous animal or human coronavirus infections, so they can recognize some of the viral proteins, help clear SARS-CoV-2 and produce asymptomatic infections in many patients even in the absence of antibodies in their serum[43]. SARS-CoV-2 induces a direct cytotoxic effect on the lymphocytes to evade the immune system, resulting in lymphopenia, preventing cytokine storm, and diminishing the innate immune responses[44]. SARS-CoV-2 also upregulates many apoptosis-involved genes, including P53, which helps develop lymphopenia. This SARS-CoV-2-induced lymphopenia is prevalent in patients with old age or other comorbidities such as obesity, hypertension, or diabetes mellitus[45]. Lymphopenia could also result from increased leukocyte adhesion and extravasation due to SARS-CoV-2-induced endothelial dysfunction, particularly in old age and with comorbidities, augmenting the problem of lymphopenia [46]. Effector T cells are the leading players driving immune responses to achieve immune functions. These cells have both promoting and inhibitory regulatory functions of innate immunity. The maturation and differentiation of naïve-T cells to mature fully functioning effector cells are controlled by cytokines produced by activated cells of the innate and adaptive immune systems. SARS-CoV-2 induces enhanced inhibitory receptor expression on the surface of T cells due to cytokine activity or reduction of the regulatory T-cells. These inhibitory effects negatively exhaust the effector T cells and reduce the defense against SARS-CoV-2[47]. CD4+ T cells, CD8+ T cells, and B cells have a crucial protective role against SARS-CoV-2 infections. A decrease in CD4+ T cell number and function causes cytokine, neutralizing antibody production reduction, and reduced lymphocyte recruitment to lung tissue. These effects cause an increased risk of interstitial pneumonitis and delay the clearance of infection from the lungs. However, depletion of CD8+ T cells at the beginning of SARS-CoV-2 infection does not affect the viral clearance or replication[48].

B lymphocytes represent 15% of peripheral white blood cells and are responsible for the humoral immunity and protection against various pathogens through various immunologic functions, including antibody production. Specific immunoglobulin M (IgM) anti-SARS antibodies appear within two weeks after infection, reaching the peak in the third week, to gradually disappear until the end of the third month[49]. Immunoglobulin G (IgG) started to appear by the end of the second week, reaching the peak

by the end of the fourth week, and persisted for longer but not for a long time [in SARS-CoV-1, Ig G lasts for about two years][50]. Consequently, antibody levels can be used to determine the stage of SARS-CoV-2 infection. The levels of anti-SARS-CoV-2 antibodies decrease by about 50% within 1-3 mo following the beginning of the infection[51]. However, some cases with agammaglobulinemia infected COVID-19 showed full recovery without functioning B-cells[52,53]. The antibody response may help inhibit viral replication through neutralization and blocking the viral entry, egress, or fusion with the host. However, enhancing antibodies may counteract the neutralizing antibodies. Antibodies can enhance viral infections and participate in COVID-19 pathogenesis *via* antibody-dependent enhancement. The level of enhancing antibodies is positively correlated with pro-inflammatory mediators levels and negatively correlated with anti-inflammatory mediators. Which has the upper hand, the neutralizing or enhancing antibodies depend on the dominant antibody type concentrations and affinity[54,55]. Abnormal B lymphocytes maturation and conversion to macrophage-like cells caused by the viral S protein impairs the immune system's humoral and cellular elements in responding to severe infection with SARS-CoV-2[56]. Table 1 shows the various factors that affect the severity of infection with COVID-19.

AUTOIMMUNITY AND CROSS REACTIVITY OF SARS-COV-2

In antigenic or molecular mimicry, common antigenic sites are shared between microorganisms and the host tissue. The microorganism-triggered immune response is directed against the microorganism and the host cells with the common antigenic determinant. This deviated autoimmune response is responsible for developing many autoimmune disorders in humans. Recently, it has been observed that several human tissue antigens showed a strong reaction with antibodies directed against SARS-Cov-2. This antigenic mimicry was observed for many SARS-CoV-2 proteins, including but not limited to SARS-CoV-2 S, N, and autoimmune target proteins[57]. These induced antibodies can react with a wide variety of human tissues and proteins such as skin, respiratory, digestive, cardiac, and nervous tissues, producing a wide array of autoimmune disorders with extensive cellular, tissue, and organ damage observed in severe COVID-19 cases[58]. The cross-reactivity of SARS-CoV-2 is not limited to the human body. SARS-CoV-2 also has cross-reactivity with SARS-CoV, as patients with COVID-19 can produce IgG and IgM antibodies able to react with SARS-CoV. This observation is fundamental as it helps understand that some patients may have mild or aggressive COVID-19. Previous infection with SARS-CoV with pre-exciting antibodies that can cross-react with SARS-CoV-2 may explain this variation in the clinical presentation in patients with COVID-19. However, recovery from SARS-CoV infection might not protect against SARS-CoV-2 and vice versa[59]. Cross-reactivity between SARS-CoV-2 and other human coronaviruses, especially beta coronaviruses (particularly SARS-CoV and MERS-CoV), may explain numerous phenomena. The increased pathogenicity and severity of SARS-CoV-2 infection in areas with common pre-existing SARS-CoV infection is due to the possible presence of enhancing cross-reactive antibodies against those common coronaviruses[60]. Enhancing cross-reactive antibodies to SARS-CoV-2 in patients previously exposed to SARS-CoV can explain the early response with higher titers in older age and the milder symptoms in the pediatric age[61,62]. However, the lower prevalence of COVID-19 in the pediatric age is multifactorial and could be related to the age-dependent immaturity of ACE2 receptors in children[63]. The tissue damage induced by the cross-reactive autoantibody induces the release of more self-antigens, activating more autoreactive T-cells, producing more self epitopes, and sparking autoimmunity[64]. Cross-reactive antibodies also raise a question about using convalescent plasma to treat patients with SARS-CoV-2 infection to neutralize SARS-CoV-2. However, convalescent plasma may lack effectiveness and, on the other hand, may induce endothelial damage due to the transmission of cross-reactive enhancing antibodies[65]. Cross-reactivity is also of paramount importance in the vaccination industry, considering SARS-CoV-2 cross-enhancing or neutralizing epitopes to minimize the vaccine side effects and vaccine-induced autoimmunity[66].

SARS-COV-2 INDUCED AUTOIMMUNE AND AUTO-INFLAMMATORY CONDITIONS

A variety of factors may trigger autoimmunity by generating a hyperstimulated immune system. The terms exposome, infectomes, and autoinfectomes are recently introduced in autoimmunity. Exposome describes all the environmental triggers (exogenous or endogenous) that the host could expose to it. Infectomes are all infectious microbes that the host can be exposed to during his/her life. In the same way, autoinfectomes are all infectious agents that can trigger autoimmunity upon exposure[67]. The ability of SARS-CoV-2 to initiate autoimmune and autoinflammatory responses is related to many factors. The SARS-CoV-2 can induce a state of the hyperstimulated immune system with changes in the circulating leukocyte and an extensive increase in the levels of the pro-inflammatory cytokines, known as "cytokine release syndrome" in patients with variable degrees of COVID-19[32]. The large RNA with 30,000 nucleotides and the complex transcriptome with the interrupted transcription and recombination activities increase the chance of interaction with the host immune system[68]. The interrupted RNA

Table 1 Factors affecting the severity of coronavirus disease 2019 infections

Factor		Example
Viral-related factors		The viral load[24]; Mutation/virulence; Previous infections with other Coronaviruses *e.g.*, SARS-CoV[43,59]
Host-related factors:	**Demographic factors**	Patients' age[61,62]
		Gender[80,182]
		Race/ethnic group
	Physiological	Pregnancy[215]; Personel differences in ACE2 receptors distribution[13]
	Pathological factors	Presence of comorbidities such as obesity, hypertension, tuberculosis, HIV, anemia, nutritional deficiencies, or diabetes mellitus[13,45,159,169,171,181]
	Immunological factors	The type of HLA-antigen[20-23]
		The plasma numbers of B cells, T cells, and natural killer lymphocytes[40,41]
		The hemoglobin and ferritin levels[216]
		The levels of C3 and C4[38]
		The differences in the MBL protein[28]
	Environmental factors	Socioeconomic status[217]
		Overcrowding[218]
		Smocking[205]
		Alcohol consumption[204]
		Particular occupations: Occupations that involve a higher degree of physical proximity to others over long periods [219]
	Pharmacological factors	Certain drugs increase the severity (*e.g.*, rituximab, high-dose corticosteroid)[140,187,191]. Certain drugs decrease the severity (*e.g.*, ubiquinone, ezetimibe, flecainide, rosuvastatin, artificial tears, licorice)[214]
		Vaccination status of the patients

ACE2: Angiotensin-converting enzyme II; HLA: Human leukocyte antigens; HIV: Human immunodeficiency virus; MBL: Mannose-binding lectin; SARS-CoV: Severe acute respiratory syndrome coronavirus 2.

transcription and recombination produce a wide variability of protein sequences with a powerful resource of epitopes with molecular mimicry, another reason for stimulating the immune system and inducing autoimmunity associated with COVID-19[69]. There is an overlap between some viral and human peptides, so that if altered or mutated could initiate autoimmunity. From these human peptides; cerebellum-2 (which protects against multiple sclerosis), follistatin-related protein 1 (which has anti-hypoxia-induced pulmonary hypertension), Solute carrier family 12 member 6 (responsible for electroneutral potassium-chloride cotransport), and olfactory receptor 7D4 (responsible for the sense of smell)[70]. Tissue damage may result from the viral infection causing cell death and the release of self-proteins to be identified by the host immune system as foreign material and spark the process of autoimmunity[71]. At the same time, there is a hypothesis that sACE-2, which usually binds strongly with SARS-CoV-2 S protein, forms a complex, stimulating the production of anti-ACE2 antibodies and triggering type II and III hypersensitivity reactions and Type IV cellular immune reactions against the viral particles attached to sACE-2, and autoimmunity cascade. The virus-activated T cells could injure the self-tissues by initiating an inflammatory milieu or directly damaging the cells[72]. Table 2 summarizes the causes of the increased immunogenic effect of SARS-CoV-2.

Infection with SARS-CoV-2 can serve as infectome induce a range of autoimmune and auto-inflammatory conditions such as Multisystem Inflammatory Syndrome in Adults (MIS-A), Multisystem Inflammatory Syndrome in Children (MIS-C), and various autoimmune/rheumatic manifestations with a proposed link between the autoimmune and autoinflammatory sequelae of SARS-CoV-2 infection[73]. MIS-C may include Kawasaki-like disease, toxic shock syndrome, Kawasaki disease (KD) shock syndrome, macrophage activation syndrome, and myocarditis. MIS-A, contrary to MIS-C, is not well defined with a hyperinflammatory state and inconsistent features of KD[74]. Although children usually encounter a milder COVID-19 than adults, the severe MIS-C that followed the disease in some children brought several unanswered questions to the scientific community[75].

Patients with COVID-19 may develop a wide variety of autoimmune disorders such as arthritis, antiphospholipid antibody syndrome (APS), MIS-A/C, Kawasaki and Kawasaki-like disease, antiphospholipid syndrome, systemic vasculitis, systemic lupus erythematosus (SLE), hemophagocytic lymphohistiocytosis, autoimmune blood disorders (such as idiopathic thrombocytopenic purpura, autoimmune

Table 2 Factors that increase the rate of autoimmunity in coronavirus disease 2019

The ability of the virus to infect nearly all the human body tissues
Large RNA with interrupted transcription increases the pool of epitopes with increased chances of molecular mimicry and interaction with the host immune system
The overlap between some viral and human peptides
The viral-induced tissue damage increases the chance of deviated immune system
The immunogenic effect of the robust and complex binding between sACE-2 and SARS-CoV-2 S protein

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

thrombotic thrombocytopenic purpura, and autoimmune hemolytic anemia), neurological autoimmune disorders (such as encephalitis, cranial neuropathies, Guliian Barre syndrome, myelitis, and optic neuritis, acute disseminated encephalomyelitis {ADEM}, and multiple sclerosis), interstitial lung disease, autoimmune ocular disorders (Retinal vein vasculitic occlusion), renal disorders (Crescentic glomerulonephritis, Goodpasture syndrome), inflammatory bowel disease, and autoimmune endocrine disorders (such as diabetes mellitus and subacute thyroiditis)[76].

Risk factors increasing the likelihood of autoimmune diseases in patients with COVID-19

Infections with SARS-CoV-2 increase the likelihood of autoimmune disease development as about 50% of the patients have autoantibodies in their blood, even with mild disease, and the risk increases with increasing severity. Severe disease is usually associated with a higher viral load with robust immune stimulation and higher antibody levels. There is a strong association between immune hyperactivation and excessive cytokine release in patients with severe COVID-19. However, mild COVID-19 or even asymptomatic infection may also trigger autoimmune disorders[77]. Demographic features such as female gender, old age, overweight, or obesity generally increase the risk of developing autoimmune diseases, particularly with COVID-19. Aging causes functional impairment of the immune with potentially higher autoreactive antibody levels[78]. Although females usually have milder diseases than males with higher recovery rates, they have more chance of autoimmune disorders. The risk difference of autoimmune disorders between males and females is related to sex hormone differences as androgens like testosterone are immunosuppressive, while estrogen may enhance or reduce immune response[79]. Particular ethnic populations are more genetically predisposed to have autoimmune disorders following SARS-CoV-2 infection, such as Caribbean descent, sub-Saharan, Asian, Black, and mixed ethnicity[80]. Nucleic acid vaccine administration may increase the risk of autoinflammatory and autoimmune disorders, especially in young females. In addition, a pre-existing autoimmune disorder is a risk factor for another autoimmune disorder or more severe symptoms following COVID-19[81]. Gut dysbiosis is a risk factor for both COVID-19 and autoimmune diseases. Ivermectin, a commonly used drug in managing COVID-19 in certain countries, induces significant alteration of gut microbiota, which may increase the risk of autoimmune disorders. However, more studies are needed to confirm this hypothesis[63,82].

Common autoantibodies with SARS-CoV-2 infection

Patients with COVID-19 may develop multiple categories of autoantibodies and autoimmune diseases. However, the clinical significance of these antibodies needs more elaboration. From these antibodies are anti-nuclear antibodies (ANA), antiphospholipid antibodies (as lupus anticoagulant, Anti-β2 glycoprotein 1, and anticardiolipin), anti-Interferon-gamma (Anti-IFN-ɣ) antibodies, anti-melanoma differentiation-associated gene 5 (Anti-MDA5) antibodies, and anti-ACE2 autoantibodies[83]. ANA antibodies are found in 4-50% of patients with COVID-19, especially with old age, even without autoimmune disease. Presence of ANA antibodies in patients with COVID-19 increases the incidence of neurologic and thrombotic complications and unfavorable outcomes[76]. Anti-type-I interferon (IFN) antibodies are present in 10.2% of patients presented with severe COVID-19 pneumonia[84]. Antiphospholipid antibodies (anticardiolipin and/or anti-β2 glycoprotein 1) are present in a significant portion of critically ill patients with COVID-19. These antibodies and elevated factor VIII may contribute to hypercoagulopathy in severe cases of COVID-19[85]. Anti-MDA5 antibodies are associated with the rare disease amyopathic dermatomyositis. They are also present in more than 40% of patients with severe COCID-19. Higher titers of Anti-MDA5 antibodies are associated with more severe disease and a higher risk of death[86]. Anti-ACE2 antibodies are present in many patients with COVID-19 and are associated with low plasma levels of sACE-2 and increasing angiotensin II levels, which triggers a pro-inflammatory state that causes symptoms of post-SARS-CoV-2 Acute Sequelae[87]. The SARS-CoV-2 virus causes damage to the human brain *via* complex indirect processes and stimulates autoantibody formation, predominantly against brain-based antigens (autoantibodies against contactin-associated protein 2, ganglioside GD1b, and myelin oligodendrocyte glycoprotein), inducing a wide variety of

COVID-19-triggered neurological complications[64].

COVID-19-INDUCED AUTOIMMUNE DISEASES

Multisystem inflammatory syndromes (MIS-A, MIS-C, and MIS-A/C)

Multisystem inflammatory syndrome (MIS) is a rare acute and non-chronic but seriously complicates COVID-19 in adults and children. It is currently a distinct phenomenon of severe COVID-19 due to the frequent absence of respiratory involvement. The MIS pathogenesis is unclear but primarily due to the autoimmune process. MIS-A associated with COVID-19 infection usually occurs in adults aged 35-54. Clinical recognition of MIS-A is confused with other hyperinflammatory manifestations of COVID-19, which makes MIS-A challenging to distinguish from acute biphasic COVID-19 and post-acute sequelae of SARS-CoV-2 infection[88]. To its name, MIS-A involves multiple organs and systems with at least one or more extrapulmonary organs (an average of 4-5 organs). The most affected organs are hematologic, cardiovascular (myocarditis, pericardial effusion, hypotension, cardiac dysfunction, heart failure, arterial or venous thrombosis, and cardiogenic shock), gastrointestinal tract (diarrhea), acute liver injury, respiratory system (dyspnea), skin manifestations (polymorphic rashes and other mucocutaneous manifestations), renal, and nervous system (severe mononeuritis multiplex[89-91].

It can be diagnosed according to the CDC criteria for defining MIS-A. It should occur in adults aged 21 years or older, with manifestations that need hospitalization for more than 24 h, as determined by clinical and laboratory criteria. Clinical criteria include fever (≥ 38.0 °C) for ≥ 24 h before hospitalization or within the first three hospitalization days plus three or more of the following clinical criteria; one of them at least should be from the primary criteria. Primary clinical criteria include severe cardiac involvement, skin rash, and non-purulent conjunctivitis. Secondary clinical criteria include; new-onset neurologic manifestations, non-medication-related hypotension or shock, abdominal manifestations (abdominal pain, vomiting, or diarrhea), and thrombocytopenia. Laboratory criteria include evidence of recent SARS-CoV-2 infection (positive PCR, antigen, or antibody) and elevated at least two inflammatory markers from the following: erythrocyte sedimentation rate, C-reactive protein, IL-6, ferritin, and procalcitonin[92]. MIS-A should be differentiated from meningitis, intra-abdominal sepsis, KD, drug reaction, and haemophagocytic lymphohistiocytosis. It is treated with corticosteroids, anticoagulants (*e.g.*, heparin, enoxaparin, aspirin), immune modulators such as Infliximab (TNF inhibitors), Tocilizumab (IL-6 receptor inhibitor), Anakinra (IL-1 receptor antagonist), and intravenous immunoglobulin (IVIG). Patients who develop shock/hypotension require intensive care unit admission, vasoactive medications, and respiratory support with mechanical ventilation[93].

MIS-C occurs in people younger than 21, a few weeks after infection with SARS-CoV-2. The affected children have a fever with clinical evidence of severe disease requires hospitalization and multisystem (more than two) organ involvement (heart, kidneys, respiratory, gastrointestinal, hematologic, skin, and/or nervous system) without other possible reasons explaining the manifestations, evidence of recent infection with SARS-CoV-2 (positive PCR, antigen or antibody), and presence of markers of systemic inflammation (high ferritin, fibrinogen, procalcitonin, lactic acid dehydrogenase, C-reactive protein, erythrocyte sedimentation rate, D-dimer, lactic acid dehydrogenase, or interleukin 6, reduced lymphocytes, elevated neutrophils, and low albumin)[94,95]. As MIS-C frequently affects the heart, we may need to perform B-type natriuretic peptide, cardiac enzymes and Troponin I or T, electrocardiogram, and echocardiography. According to the organs affected, other laboratory tests may be needed [96]. MIS-C is primarily treated with supportive care, fluid resuscitation, and inotropic support as a cardiogenic shock is one of the most severe presentations. Respiratory support is indicated with impending respiratory failure. Extracorporeal membranous oxygenation is rarely required. IVIG, steroids, other anti-inflammatories, and anticoagulants are frequently used. Antibiotics may be used with suspected sepsis. Aspirin is commonly prescribed due to the frequent involvement of coronary arteries[97,98].

KD-COVID-19

COVID-19 is usually milder, less frequent, and has less mortality in children than adults due to less maturity and function of ACE2 receptors. Since the early beginning of 2020, there has been an increased reporting of children presented with fever, signs, and features of systemic inflammation common with KD[99]. KD is an acute, usually self-limited systemic inflammatory disease of medium- and small-sized vessels. It mainly involves children under five years of age with higher frequency in children from Asian countries like Japan, where it was first described in 1967[100]. It is usually preceded by upper respiratory tract infections, particularly with RNA viral infection of the upper respiratory tract, as viruses were usually isolated from the mucous obtained from the bronchial epithelium[101]. Despite being a self-limited disease, hemodynamic instability and shock may occur in some cases, known as KD shock syndrome. About 20%–25% of untreated patients of KD develop changes in the coronary arteries, ranging from asymptomatic dilatation or aneurysms to massive aneurysmal dilatation of the coronary artery with thrombosis and myocardial infarction that could progress to sudden death[102].

Symptoms of COVID-19-associated MIS-C may have the standard features of KD. Therefore, it is essential to differentiate between classical KD and COVID-19 (KD-COVID-19). The table shows the differences between the classic KD and KD-COVID-19 [also known as pediatric inflammatory, multisystem syndrome temporally associated with SARS-CoV-2 infection' (PIMS-TS) in Europe and 'multisystem inflammatory syndrome in children (MIS-C)] in the United States. KD-COVID-19 usually occurs in older children, higher incidence of myocarditis and cardiac involvement, more gastrointestinal and meningeal manifestations, shock, hemodynamic failure, manifestations of macrophage activation syndrome, frequent leukopenia, significant lymphopenia, thrombocytopenia, high ferritin, procalcitonin, cardiac enzymes, and troponins than classic KD[103]. Recent or current evidence of SARS-CoV-2 infection is needed to diagnose KD-COVID-19. Patients with KD-COVID-19 have more severe diseases than those with classic KD and frequently need hospitalization and intensive care support[104]. Early diagnosis of COVID-19, recognition of KD-COVID-19, and rapid therapy initiation are vital for effective management, recovery, and prevention of end-organ damage and mortality[105]. IVIG therapy is usually effective in KD-COVID-19, but the resistance rate is more common than in children with classic KD, and steroid therapy is generally needed. In refractory cases to IVIG, pulse intravenous methylprednisolone therapy and aspirin are used, especially when a suspected cardiac injury is present[106]. Hydrogen gas inhalation treats KD-COVID-19 as a stable and efficient antioxidant that positively affects oxidative damage, improves inflammation and cell apoptosis, and antagonizes abnormal blood vessel inflammation[107]. Table 3 summarises the differences between the classic Kawasaki Disease and Kawasaki Disease -COVID-19.

APS

There is a high prevalence of venous thrombosis and embolism in patients with COVID-19, especially in severe cases (about 25% to 31% of those without thromboprophylaxis). Consequently, researchers investigated the possible underlying predisposing factors such as hypoxia, immobilization, or disseminated coagulopathy[108]. Serum antiphospholipid antibodies (aPLs), the whole mark of APS, are found in 1%-5% of the healthy population, and their titer increases with age. These rates are comparable to patients with COVID-19 (from 2.7% to 13.4%), which decreases the possibility of recognizable association with thrombosis[109,110]. Another study showed that serum antiphospholipid antibodies might be found transiently in up to 12% of young, healthy subjects, increased to 18% in older adults with chronic diseases[111]. The presence of aPL is not enough to develop APS; a second hit such as aging, critical illnesses, or infections is needed to trigger the development of APS. APS is characterized by documented thrombotic and/or pregnancy-related morbidity in the presence of persistent medium to a high titer of aPLs. To diagnose APS according to Sydney criteria, we need to have persistent high titers of lupus anticoagulant, anticardiolipin antibodies IgG or IgM, or anti-β2glycoprotein-1 IgG and/or IgM for at least 12 wk[112]. However, these criteria need to be modified to limit testing to lupus anticoagulant and anti-β2glycoprotein-1 IgG and to omit anticardiolipin antibodies and anti-β2glycoprotein-1 IgM from laboratory testing. Lupus anticoagulants and anti-β2glycoprotein-1 IgG are associated with a higher risk of thrombosis, particularly lupus anticoagulants[113].

Some studies elucidated high levels of lupus anticoagulants in patients with COVID-19. However, it is unknown whether lupus anticoagulant was newly produced with COVID-19 or increased in a previously present titer[114]. Another study by Xiao *et al*[115] showed that aPLs were present in 47% of critically ill patients due to COVID-19. They also analyzed the risk of developing cerebral infarction by the type of aPLs, with IgA anti-β2glycoprotein-1 being the aPL antibody associated with the highest infarction risk, followed by IgA anticardiolipin antibodies and IgG anti-β2glycoprotein-1. The study also showed that these antibodies need to appear five to six weeks after the disease onset, indicating that a long disease course increases the risk of developing APS and, consequently, thrombotic complications. A severe fatal form of APS (Catastrophic APS) was recorded in some patients with COVID-19. However, there is no current strong evidence of CAPS association with COVID-19. CAPS presented with acute multiorgan involvement (three or more organs, systems, and/or tissues), proof of widespread vascular occlusions, intense hypercoagulable state, and elevated titers of aPLs. Lupus anticoagulant, anticardiolipin IgG, and anticardiolipin IgM were seen in 83%, 81%, and 49% of patients with CAPS [116]. Some factors usually trigger CAPS, such as viral infections, including COVID-19, especially pulmonary infections. SARS-CoV-2 may aggravate the pathogenic effects of APS, initiating inflammatory and prothrombotic cascades. The positive tropism of SARS-CoV-2 towards the vascular endothelium may also alter the COVID-19 clinical presentation in susceptible patients and initiate flaring up of underlying vascular diseases. As CAPS has a high mortality rate, approaching 50%, timely identification and management are vital[117]. It responds to plasmapheresis or plasma exchange. However, it poorly responds to anticoagulant therapy with high mortality risk[118].

SLE

SLE is a chronic multisystem autoimmune disease with varied relapsing or remitting clinical manifestations. It is more common in females and certain ethnic groups, such as African Americans and Hispanics. Due to the aberrant immune system activity in SLE, immune complexes and autoimmune antibodies are significantly produced against cytoplasmic and nuclear antigens[119]. Few patients reports documented newly diagnosed SLE in patients with COVID-19. There is a wide variation in the

Table 3 Differences between the classic Kawasaki disease and Kawasaki disease - coronavirus disease 2019

	Classic KD	KD-COVID-19
Age	Children < 5 yr of age	Older age
General condition	Less ill than in KD-COVID-19	More severely ill
Gastrointestinal & meningeal signs	Less common	More common
CBC	Leucocytosis, anemia, & thrombocytosis. Thrombocytopenia may occur	Leukopenia with marked lymphopenia, thrombocytopenia
Ferritin	Increased	Markedly increased
Incidence of myocarditis	Subclinical myocarditis is nearly present in all patients. However, clinically evident myocarditis is uncommon.	Very high, up to 60.4% in patients with KD-like multisystemic disease.
Response to IV gamma globulins	Well-responding	Resistance to IVIG therapy is common.
Adjunct steroids	May be needed	Usually needed

COVID-19: Coronavirus disease 2019; CBC: blood cell count; KD: Kawasaki disease; IVIG: Intravenous immunoglobulin.

clinical presentation in the reported cases. It presented with manifestations of serositis (pericardial and pleural effusion), renal manifestations (nephritis, proteinuria), skin manifestations (varicella-like rash), cardiac dysfunctions (pericardial tamponade, ventricular dysfunction), secondary APS, neurological complications (neuropsychiatric symptoms, cerebral hemorrhage), hematological disorders (anemia, positive direct Coombs, hemolytic anemia, lymphopenia, thrombocytopenia,), finger vasculitis, low complement, and presence of autoantibodies (aPL, ANA, and anti- dsDNA). Patients with COVID-19-associated SLE had a high mortality rate reaching 50%. Hence appropriate and prompt diagnosis and management are highly indicated to decrease morbidity and mortality. Renal involvement carries the worst prognostic predictor with the highest mortality rate. The treatment should be individualized and may involve glucocorticoids, plasma exchange, hydroxychloroquine, anticoagulation, tocilizumab, and intravenous immunoglobulins[120-123].

Autoimmune-like neurologic disease

SARS-CoV-2-triggered inflammatory and autoimmune cascades may affect the nervous system, producing various neurological complications. About 60% of patients with COVID-19 suffer from anosmia (loss of smelling) and ageusia (loss of taste sensation), which verifies the hypothesis of its neurovirulence[48]. This high percentage of anosmia and ageusia observed with SARS-CoV-2 infection indicates the high viral neurotropism with the olfactory nerve serves as a portal of brain entry. However, anosmia and ageusia can be the first or only symptoms present in some patients with COVID-19[124]. Another portal of brain entry is through retrograde axonal transport *via* peripheral and cranial nerves. An example of this portal of entry is SARS-CoV-2-associated Guillain-Barre syndrome, an acute inflammatory, demyelinating, sensorimotor polyradiculoneuropathies frequently reported in patients with COVID-19. It results from the autoantibodies production that cross-react with myelin components gangliosides and glycolipids present in the peripheral nerves due to molecular mimicry. These autoantibodies cause peripheral nerve demyelination and axonal damage in a progressive ascending pattern[125]. It occurs primarily secondary to SARS-CoV-2-induced immune reaction, as the virus was not detected in the cerebrospinal fluid of any patient suffering from GBS[126].

Miller Fisher syndrome (MFS) and polyneuritis cranialis were rarely reported as autoimmune neurological complications of SARS-CoV-2 infection. They are other examples of the virus's neurotropism and its ability to rapidly spread to the different brain areas, including the thalamus and the brain stem. MFS is classically present with acute onset of a triad composed of external ophthalmoplegia, loss of tendon reflexes, and ataxia[127]. Polyneuritis cranialis is a rare, gradual, and slowly progressive disorder involving multiple cranial nerves (usually IV, V, VI, and VII). Viral infection often preceded these disorders, which triggered an immune-mediated mechanism. Few reported cases followed SARS-CoV-2 infection. CSF showed albuminocytological dissociation, and the patients had a significant elevation of inflammatory mediators, such as the interleukin-8. It can be successfully treated with IVIG[128]. Other reported neurological disorders related to COVID-19 aberrant immune response include acute motor-sensory axonal neuropathy, acute transverse myelitis, acute necrotizing encephalopathy, acute necrotizing myelitis, and acute disseminated encephalomyelitis[129].

Post-COVID-19 pneumonia lung fibrosis

Progressive pulmonary fibrosis following COVID-19 pneumonia is one of the severe complications of SARS-CoV-2 infections that could be associated with irreversible lung dysfunction. Post-COVID-19

pulmonary fibrosis is multifactorial, with many theories explaining the potential causes of post-COVID pulmonary fibrosis. One theory is the cytokine storm caused by an aberrant immune mechanism that triggers pulmonary fibrosis[130]. IL-6 is a pro-inflammatory cytokine with a pro-fibrotic activity that activates the neutrophils and their accumulation at the injury site. Neutrophil accumulation causes proteases and oxygen-free radical release causing pulmonary interstitial edema and acute inflammation [131]. Annexin A2 is crucial to protect against pulmonary fibrosis as it is essential to activate endogenous tissue plasminogen activator to lyse clots and promote fibrin clearance and pulmonary fibrinolysis[132]. Anti-Annexin A2 antibodies are associated with systemic thrombosis, cell death, and non-cardiogenic pulmonary edema. Annexin A2 inhibition can induce diffuse alveolar damage and pulmonary fibrosis in patients with severe COVID-19[133].

Arthritis

Arthritis was reported early in COVID-19 or lately after the resolution of the disease. Different types of arthritis were reported in patients with COVID-19; viral arthritis, reactive arthritis, chronic arthritis, and rheumatoid arthritis[134]. López-González *et al*[135] reported joint pain in some patients with COVID-19; some did not have other signs of arthritis. They also reported crystal-induced arthritis (gouty with monosodium urate and pseudogouty with calcium pyrophosphate) in some patients. Ono *et al*[136] reported the occurrence of reactive arthritis three weeks later in a patient who developed severe COVID-19 pneumonia. The patient improved with anti-Inflammatory non-steroidal drugs and intra-articular corticosteroid injection. Reactive arthritis generally develops one to three weeks after the infection. The precise mechanisms of COVID-19-induced arthritis are not entirely identified. It could be related to viral-induced macrophage activation with subsequent release of cytokines and chemokines in high amounts, sparking the inflammatory process[82]. Although viremia is expected in reactive arthritis, SARS-CoV-2 was detected only in the blood in 15% of cases with COVID-19. Consequently, molecular mimicry may explain arthritis pathogenesis[137]. Inflammatory mediators such as Interleukin 17 A are present in patients with reactive arthritis, spondyloarthritis, and COVID-19 -induced hyperinflammatory state[138].

COVID-19-induced vasculitis

SARS-COV-2 can directly infect the vascular endothelium causing endotheliopathy. Indirect damage to the vascular endothelium can also be induced by the inflammatory mediators triggered by COVID-19 [139]. Few case reports are documenting the development of COVID-19-associated vasculitis with positive anti-neutrophil cytoplasmic antibodies (ANCA). Uppal *et al*[140] described two cases of pauci-immune glomerulonephritis with high perinuclear-ANCA titer during SARS-CoV-2 infection. They clinically improved with the treatment of COVID-19 and the use of rituximab. Hussein *et al*[141] described a female patient who developed granulomatosis with polyangiitis and alveolar hemorrhage during COVID-19 infection. She was treated successfully with pulse steroid therapy, plasmapheresis, and IVIG. These reported cases clarify the importance of vascular endothelium in the pathophysiology and clinical course of COVID-19 and the need for a better understanding of the endothelial biology in patients with COVID-19[142].

Skin autoimmune disorders

Cutaneous manifestations of COVID-19 are common and may involve erythematous, maculopapular, urticarial petechial skin rashes, or diffuse disseminated erythema. The rashes may appear with the onset of the disease and may not correlate with the disease severity[143]. Pityriasis rosea-like rashes were reported in one patient with mild COVID-19[144]. Various reports described acral chilblain lesions due to vacuolar interface dermatitis with superficial and deep perivascular and periadnexal lymphohistiocytic infiltration[145,146]. Violaceous papules and digital swelling occur due to diffuse perivascular dense lymphoid infiltration of the dermis and hypodermis[147]. Desquamation of the peripheral digits may occur in younger children with severe disease or as a sign of KD-COVID-19[148]. Daneshgaran *et al* [149] showed that underlying mechanisms of skin involvement in patients with COVID-19 are related to cytokine release syndrome, coagulation and complement systems activation, or direct virus-induced skin damage with endothelial damage of the dermal vasculatures.

POST-VACCINATION AUTOIMMUNE DISORDERS

The vaccines work by provoking an immune response against specific antigens in the target organism that causes the disease with a long-lasting memory T-cell response. Vaccine adjuvants are used to enhance the immune response against the vaccine. However, these adjuvants can trigger autoimmune responses[150]. Vaccines have been involved in triggering autoimmune diseases for a long time. GBS was reported with Flu and Human Papilloma vaccines, and idiopathic thrombocytopenia occurred in some patients receiving the Measles-Mumps-Rubella vaccine[151]. COVID-19 vaccines can trigger a wide range of skin reactions; from non-specific local injection-site reactions to Type-I hypersensitivity reactions (*e.g.*, urticarial rashes, angioneurotic edema, and even anaphylaxis) to Type-IV delayed

hypersensitivity reactions (including delayed large skin lesions ("COVID arm") at the injection site, inflammatory reactions in a previous skin lesion, and more frequently erythema multiforme-like and morbilliform rashes[152]. COVID-19 vaccination-induced autoimmune skin disorders include immune thrombocytopenia, leukocytoclastic vasculitis, and lupus erythematosus[153].

Severe anaphylaxis was reported with Pfizer-BioNTech and Moderna vaccines. Consequently, the CDC recommends that prefilled epinephrine syringes be available in vaccination centers and observe the vaccinees for 15 or 30 min[154]. Delayed-type or T-cell mediated hypersensitivity adverse reactions were reported in 0.8% of the vaccinees near the injection site[155]. SARS-CoV-2 vaccination may also be complicated by autoimmune diseases that involve the skin, such as lupus erythematosus (LE), bullous pemphigoid, vitiligo, alopecia areata, and leukocytoclastic vasculitis[156,157]. Akinosoglou *et al*[159] reported bilateral elbow itchy annular granulomatous rash due to cutaneous small cell vasculitis after the first dose of the Pfizer-BioNTech vaccine. The rashes spontaneously resolved without medications within three to four days[158]. These vaccine-related adverse effects could be related to a pre-existing dysregulated immune status that could enhance polyclonal B-cell expansion with increased immune complex formation resulting in clinically significant vasculitis in genetically susceptible individuals [159].

MIS-A was reported in three patients within three to fourteen days after COVID-19 vaccination; one of them presented with shock. The three patients had underlying comorbidities such as asthma, depression, and hyperlipidemia[160]. Mild myocarditis was reported in six male patients between 16 and 49 years from Israel following BNT162b2 mRNA COVID-19 vaccination. Five presented one to three days after the second dose, while only one presented after 16 days from the first dose. All of them completely recovered within 4-8 d[161]. Autoimmune thyroid diseases (subacute thyroiditis and Graves' disease) were reported in a few persons following SARS-CoV-2 vaccinations, which could be a form of adjuvants-induced autoimmune/inflammatory syndrome (ASIA). Subacute thyroiditis and Graves' disease had developed in the reported cases within a few days following SARS-CoV-2 vaccination. ASIA was the underlying mechanism for several autoimmune endocrinopathies that developed after vaccination[150,162,163]. An *et al*[164] reported reactive arthritis in the left knee in a 23-year female; three days following the first and second doses of Sinovac-CoronaVac COVID-19 (inactivated whole virus) vaccine. She has a history of a similar condition two years before following a common cold which may indicate the genetic susceptibility of this patient.

Autoimmune hematological disorders were also observed following COVID-19 vaccination. Lee *et al* [165] reported that twenty patients between 22 and 73 years old developed immune thrombocytopenia and bleeding without thrombosis following Pfizer and Moderna SARS-CoV-2 (mRNA) vaccination. These patients tested positive for anti-platelet antibodies; some have other autoimmune conditions such as Crohn's disease or autoimmune hypothyroidism. Meanwhile, Cines *et al*[166] analyzed three independent reports describing 39 persons who developed immune thrombotic thrombocytopenia following the AstraZeneca COVID-19 vaccine (vaccine with modified recombinant adenovirus to encode SARS-CoV-2 S protein). Most patients had high antibody titer against platelet factor 4–polyanion complexes. Fourty% of the patients died from a cerebral hemorrhage, infarction, or both. Fatima *et al*[167] reported a 66-year-old woman who developed IgG-mediated autoimmune hemolytic anemia after Moderna COVID-19 (mRNA) vaccine. The patient had a history of psoriasis for five years before the vaccination.

Gaignard *et al*[168] also reported 77-year- males without previous comorbidities who developed autoimmune hemolytic anemia due to warm antibodies following Moderna COVID-19 (mRNA) vaccine. Brito *et al*[169] reported severe autoimmune hemolytic anemia in an 88-year-old Caucasian woman two days after the second dose of the COVID-19 mRNA vaccine. She had very high levels of anti-erythrocyte IgG and anti-C3d autoantibodies but without cold agglutinins. Murdych also reported severe autoimmune hemolytic anemia in an 84-year-old man with multiple comorbidities after the first dose of the Pfizer-BioNTech COVID-19 mRNA vaccine. The patient tested positive for direct antiglobulin, anti-IgG, direct antiglobulin, polyspecific antihuman globulin, and negative anti-C3[170]. There are several other reports of autoimmune hepatitis following mRNA or viral vector COVID-19 vaccines. Drug-induced hepatitis was also reported following the inactivated whole virus vaccine[171].

Neurological side effects of the COVID-19 vaccine are usually mild. However, severe adverse autoimmune neurological sequelae were reported. Waheed *et al*[172] reported GBS in an 82-year-old highly functional woman without significant comorbidities 14 d after the first shot of the Pfizer COVID-19 vaccine. She was successfully treated with IVIG. Other neurological complications such as Bell's palsy, acute transverse myelitis, acute demyelinating polyneuropathy, and transverse myelitis were reported, especially with mRNA vaccine[173]. Cerebral venous sinus thrombosis was also described in women of childbearing age, especially with adenovector-based vaccination[174]. The importance of developing vaccine-related autoimmune reactions or diseases is related to their impact on the intake of second dose vaccination and the morbidity rate. However, being cautious is preferable until reliable data and a more extended experience are established[175]. It is also essential to be highly suspicious when reporting vaccine-related side effects and rule out actual SARS-CoV-2 infection.

EFFECTS OF AUTOIMMUNE DISEASES ON THE COURSE OF COVID-19

Patients with immune deficiencies or autoimmune disorders are at a higher risk for infection with COVID-19, as they are frequently treated with anti-cytokine, glucocorticoids, and/or immunosuppressive drugs. The infection rate with COVID-19 among people with immune diseases is twice that of the general population[176]. The data derived from international registries of patients with rheumatic diseases (C19-GRA3) who encountered COVID-19 showed poor outcomes depending on their medications[177]. For example, patients treated with antitumor necrosis factor (TNF) showed decreased hospitalization risk, indicating the protective effects of anti-TNF monotherapy against severe COVID-19. Antimalarial drugs (such as hydroxychloroquine), non-steroidal anti-inflammatory drugs, and biologic therapies were not related to increasing the risk of hospitalization due to COVID-19. In contrast, patients who received moderate to high dose glucocorticoids had poor prognoses and clinical outcomes[177,178]. However, other factors may also play a role in the clinical outcome that need more studies.

Meanwhile, the study by Gianfrancesco *et al*[179] showed that most patients with autoimmune disorders who encountered COVID-19 had entirely recovered from the infection, which could help assure these patients. A meta-analysis by Akiyama *et al*[176] showed that while patients with autoimmune disorders have an increased prevalence of COVID-19, their prognosis and clinical outcome were not significantly worse than individuals without autoimmune diseases. They related the higher rate of COVID-19 in patients with an autoimmune disorder to the increased rate of glucocorticoid use. A recent study by Malek Mahdavi *et al*[180] showed that the presence of other comorbidities (female gender, obesity, hypertension, cardiac disease, diabetes mellitus, pulmonary disease, and chronic renal disease) in patients with rheumatoid arthritis in addition to treatment with prednisolone > five mg/day and TNFα inhibitors were independent predictors of COVID-19 outcome. They also observed that symptoms such as anosmia, dyspnea, and taste loss were more common than in the general population. When comparing the effects of COVID-19 with influenza on patients with autoimmune diseases, Tan *et al*[181] found that the hospitalized patients due to COVID-19 had poor outcomes and higher mortality rates than with influenza. However, this study had many limitations, so we can not generalize their findings. Both autoimmune disease and COVID-19 are known to increase the risk of venous thromboembolism. Consequently, the co-occurrence of COVID-19 in patients with autoimmune diseases may heighten this risk. D'Silva *et al*[182] found that patients with autoimmune disorders had a higher risk of venous thromboembolism when infected with SARS-CoV-2 than the general population, independent of comorbidities.

Factors affecting the severity Of COVID-19 in patients with autoimmune diseases

Table 4 summarises the factors that affect the severity of COVID-19 in patients with autoimmune disorders. The male sex and old age worsen the prognosis in patients with autoimmune diseases, similar to what is observed in the general population[183]. Freites Nuñez *et al*[184] indicated that age over fifty is an independent risk factor for hospitalization due to COVID-19 in patients with autoimmune diseases. Peach *et al*[185] found that the COVID-19-related mortality risk is higher in patients with autoimmune diseases with age equal to or higher than 35 years. They also showed that women with autoimmune diseases have a higher COVID-19-related mortality rate than men, contrary to the previous studies. The type of autoimmune disease can affect the severity of infection with SARS-CoV-2. For example, patients with SLE are at higher risk of severe COVID-19 than patients with rheumatoid arthritis. Patients with SLE may have a high rate of hypomethylation and ACE2 overexpression that may ease the viral entry into the cell[186].

On the other hand, Ayala Gutiérrez *et al*[183] found that patients with rheumatoid arthritis, polymyalgia rheumatica, vasculitis, and spondyloarthropathies had a worse prognosis; In comparison, patients with primary Sjögren syndrome and systemic sclerosis had a better prognosis. The presence of medical comorbidities (such as diabetes, hypertension, and obesity) in patients with autoimmune disorders increases the probability of hospitalization, intensive care unit (ICU) admission, and acute renal failure when they encounter SARS-CoV-2 infection[182]. The type of medication used can alleviate or worsen the course of COVID-19. Some drugs used to treat autoimmune diseases (such as Tocilizumab, Anakinra, Baricitinib, or hydroxychloroquine) might have a preventive effect in patients with severe COVID-19 infections. This finding may illustrate the underlying pathogenetic relationship between COVID-19 and autoimmune diseases[187]. Patients with autoimmune diseases treated with rituximab may be at greater risk of severe SARS-CoV-2-induced pneumonia than the general population [188]. Disruption of the medical care continuity and lack of medication adherence due to the restrictions during the pandemic may make the patient prone to flare-up and worsen the associated autoimmune disease activity[189].

Table 4 Factors that affect the severity of coronavirus disease 2019 in patients with autoimmune diseases

The age and sex of the patients
The type of the autoimmune disease
The severity of the autoimmune disease.
Presence of comorbidities
The type of medication used
Disruption of the medical care continuity
Lack of medication adherence
Other factors that increase COVID-19 severity in the general population

COVID-19: Coronavirus disease 2019.

CONCERN ABOUT COVID-19 VACCINATION IN PATIENTS WITH AUTOIMMUNE DISORDERS

The immunogenicity and safety data of COVID-19 vaccines are still limited because patients with chronic diseases, including autoimmune diseases and immunosuppressed patients, were excluded from most experimental vaccine studies. Patients with autoimmune diseases are more liable for a more severe and complicated course of COVID-19 than the general population. Hence, the vaccination benefits far outweigh the risks[190]. According to the general vaccination guidelines in patients with immune deficiency or autoimmune diseases, giving these patients non-live vaccines (including mRNA vaccines) is recommended, providing adequate cellular and humoral immune response. It is preferable to give these patients the immunization during the disease's remission and without concurrent infections[191].

Patients on high doses of corticosteroids or Rituximab may avoid the vaccination. Preferably, COVID-19 vaccinations should be given before initiating any biological disease-modifying agents. Patients may receive the COVID-19 vaccine one month before or at least six months after the last Rituximab infusion, as Rituximab impairs the antibody responses for at least six months after administration[192]. Patients with autoimmune diseases or immune deficiency should receive annual influenza and Streptococcus pneumonia vaccination. COVID-19 vaccine should be given alone and at least two weeks before or after other vaccines. Coordinating timing with dosing regimens of COVID-19 vaccines may optimize the vaccine safety and efficacy, especially in patients with autoimmune diseases[193].

WAYS TO MINIMIZE THE RISK OF COVID-19 IN PATIENTS WITH AUTOIMMUNE DISEASES

Patients with autoimmune diseases are more liable to nutritional inadequacy due to the effects of the disease itself or related to the medications used. The nutritional inadequacy may increase the susceptibility to infection, especially to COVID-19, and permit infections to be more serious, even fatal[194]. Immune-regulator micronutrients such as vitamin A, D, and zinc are essential for immune cell metabolism and may provide antibacterial or anti-viral effects. Other micronutrients such as arginine may be needed as substrates for immune-active metabolites production, such as nitric oxide, one of the most crucial players in immunity[195]. Vitamin D decreases the risk of respiratory tract infections and other respiratory disorders. It is better to be taken daily to obtain maximum effects[196,197]. Vitamin E, Vitamin C, selenium, zinc, plant polyphenols, and long-chain omega-3 fatty acids have anti-oxidative effects and protect against inflammatory stress[198]. Adequate nutrition is essential for healthy gut microbiota, which plays a fundamental role in immunity modulation[199]. Several studies showed the efficacy of probiotics in gut microbial modification, improving gastrointestinal manifestation, and reducing multiorgan inflammation in different autoimmune diseases[200,201]. Licorice is a traditional herb used as a drink in Egypt for many centuries. It has many beneficial effects, such as anti-inflammatory, antitussive, antibacterial, immunomodulatory, and detoxifying agents for many disorders, especially respiratory diseases. It has a solid potential to be an effective adjuvant to prevent and treat COVID-19 with significant anti-inflammatory, anti-ACE2, and the ability to alleviate the clinical symptoms of the disease such as dry cough, shortness of breath, and fever[63].

Gut microbiota is an essential determinant of immunity. COVID-19 causes disruption of the intestinal flora and microbiota dysbiosis, which induces Th17 cell polarization in the small intestine with excessive interleukin (IL)-17A production, recruitment of neutrophils, and more intestinal mucosal immune damage[202]. Several studies highlighted the pathogenetic prole of the microbiome in the

development of autoimmune diseases, especially in systemic lupus erythematosus. Peng *et al*[203] showed that probiotics successfully adjunctive therapy in SARS-CoV-2 infection[204]. Consequently, improving the host nutrition and general condition increases the ability to fight infection and enhances the vaccination response. Alcohol consumption and smoking should be avoided during the COVID-19 pandemic, particularly in patients with autoimmune diseases. Alcohol exacerbates intestinal inflammation, alters intestinal microbiota's composition and function, increases intestinal permeability, and disturbs intestinal immune homeostasi[205]. Cigarette smoking impairs various body functions such as cardiovascular, respiratory, and immune systems and exacerbates autoimmune diseases and allergies. Smoking impairs the nuclear factor-kappaB (NFκB), mitogen-activated protein kinases, and histone modification. It also impairs innate and adaptive immunity and makes the smoker more prone to infection[206].

Sleep hygiene has a direct impact on immunity upkeep and immunological response. Disordered Circadian rhythm, due to physical, social, or psychological disorders encountered during the COVID-19 pandemic, compromises the sleep quality and hence the immune system. Good sleep quality improves the response to vaccination and increases the resistance to infectious diseases[207]. Poor sleep quality is associated with increased pro-inflammatory interleukin levels (IL-1β, TNF-α, and IL-6)[208]. Exercise during the COVID-19 pandemic promotes health, improves host immunity, and should be encouraged. Acute and chronic exercise of moderate intensity can control excessive respiratory inflammation through multiple pathways. It also enhances and regulates the immune defense mechanism, particularly innate immunity, and improves metabolic health[209].

The enforced social isolation and stress during the pandemic negatively affect individual health, especially in children and the elderly[210,211]. Social isolation and anxiety disturb the various biological systems and the circulating stress hormones, glutamate, and immune system components[212]. Social isolation also triggers neuroinflammation and microglia overactivation and disturbs gut microbiota, inducing various neurological and autoimmune disorders. Therefore, a multi-purpose comprehensive social and family program with exercise and psychological support is highly needed for patients with autoimmune diseases to lessen the harmful effects of social isolation impeded during the COVID-19 [213-219].

CONCLUSION

Mutual relations exist between COVID-19 and autoimmune diseases. Patients with autoimmune disorders are at an increased risk for COVID-19, and COVID-19 or its vaccine can trigger autoimmune diseases. Patients with autoimmune diseases should continue their medication but could be modified according to their clinical condition. Vaccination with non-living viruses, including mRNA, is safe and could prevent serious COVID-19. However, the COVID-19 vaccination could also trigger autoimmune disease. Consequently, precautions and strict follow-up are needed for these patients.

ACKNOWLEDGEMENTS

We thank the anonymous referees and editors for their valuable suggestions.

FOOTNOTES

Author contributions: Al-Biltagi M, Saeed NK, and Bediwy AS collected the data and wrote and revised the manuscript.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

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S-Editor: Liu JH
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P-Editor: Liu JH

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 July 20; 12(4): 224-234

DOI: 10.5662/wjm.v12.i4.224

ISSN 2222-0682 (online)

MINIREVIEWS

Issues related to post-COVID-19 syndrome

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Specialty type: Infectious diseases

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ait Addi R, Morocco; Chen J, China

Received: January 31, 2022
Peer-review started: January 31, 2022
First decision: April 11, 2022
Revised: April 20, 2022
Accepted: June 22, 2022
Article in press: June 22, 2022
Published online: July 20, 2022

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Abstract

The pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in 2019-2022 leads to a multisystem illness that results in damage to numerous organ systems. In this review, our goal was to assess current research on long-term respiratory, cardiac, neurological, digestive, rheumatological, urogenital, and dermatological system complications of coronavirus disease 2019 (COVID-19). Bibliographic searches were conducted in December 2021 using PubMed and Google Scholar, retrospectively, covering all COVID-19 literature to determine the consequences of the disease. This review may help to determine the prospects for new studies and predict the upcoming aspects requiring assessment in post-COVID-19 syndrome.

Key Words: Coronavirus; COVID-19; Post-COVID-19 syndrome; Pandemic; SARS-CoV-2

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Core Tip: Coronavirus disease 2019 causes damage to multiple organ systems. Most of the current studies are based on the acute stage of illness, treatment, and vaccination. As more than two years have passed since the start of the pandemic, we should be familiar with its long-term sequelae.

Citation: Özdemir Ö, Arslan Z. Issues related to post-COVID-19 syndrome. *World J Methodol* 2022; 12(4): 224-234
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/224.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.224

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has invaded the globe. As of 8 June 2022, the cumulative number of recorded infected cases is 536.613.318, with 6.323.467 deaths[1]. Although the pathophysiologic process remains unclear, a probable hypothesis suggests that SARS-CoV-2 is an enveloped and positive-stranded RNA virus that binds to the angiotensin-converting enzyme 2 (ACE2) receptor of host cells with the structural protein spike domain S1[2]. Consequently, the novel coronavirus invades all cells that express ACE2 receptors, such as respiratory, gastrointestinal, and urinary systems[3]. Studies have indicated that the incubation period may take up to 11.2 d, and symptoms of the disease are likely to be evident on day 5.5 after infection in most cases[4]. Additionally, current studies revealed that the average incubation period in the pediatric age group is 6.5 d, which is slightly longer than that in adults[5].

SARS-CoV-2 has additional features that most other organisms may not have: (1) Ability to escape immunological response; (2) Tissue tropism which depends on ACE2 receptor consistency; and (3) Capability to reach various organs and systems[6].

Common clinical manifestations in COVID-19 patients include fever, dry cough, fatigue, dyspnea, sore throat, headache, myalgia or arthralgia, chills, nausea or vomiting, nasal congestion, diarrhea, hemoptysis, and conjunctival congestion[7]. Another study involving pediatric participants demonstrated that 61.7% had a fever, 53.2% cough, and 16.8% diarrhea or nausea[8].

The aim of this mini-review was to conduct a bibliographic search of post-COVID-19 syndrome which was carried out in December 2021 using PubMed and Google Scholar, retrospectively, and included all COVID-19 literature to determine the consequences of this disease. This review may help to determine the prospects for new studies and predict the upcoming aspects requiring assessment in post-COVID-19 syndrome.

WHAT IS POST (LONG)-COVID-19 SYNDROME?

According to the studies that were conducted to assess hospitalization and mortality data, the majority of patients have the burden of long-term morbidity complications despite 'recovery'[9,10]. A group of patients had persistent complaints, which necessitated the need to determine long-term complications of the disease. Approximately 10% of the infected patient population reported experiencing symptoms such as confusion, sleep problems, decreased exercise capacity, autonomic complaints, persistent low-grade fever, and lymphadenopathy after recovery from the acute stage[11,12]. Another large cohort study including data from patients 6 mo after recovery showed that a considerable number of patients had persistent complaints of fatigue, muscle weakness, sleep difficulties, anxiety, and depression[13]. Severely ill patients with extensive lung involvement at admission was a probable risk factor associated with pulmonary diffusion abnormality, fatigue or muscle weakness, and depression which are manifestations of a new term called 'post-COVID-19 syndrome'[14]. These manifestations are reliant on the severity of pulmonary involvement, age, muscle pain, intensive care unit (ICU) requirement, viral load, and immune response at admission[15-17]. Obesity, underlying chronic respiratory illness, abnormal radiologic findings, diminished pulmonary function on spirometry, female gender, and Black and Asian races are also reported to be potential risk factors for long-term sequelae[18].

The novel terminology of 'COVID long-haulers', 'long-COVID', or 'post-COVID-19 syndrome' covers these complaints[10]. 'Acute COVID-19' describes symptoms that extend to 4 wk after the onset of the disease. On the other hand, the definition of 'post-acute COVID-19', is symptoms present between 4 to 12 wk after onset of the disease[19,20]. Post-COVID-19 syndrome or long-COVID consists of complaints that remain beyond 12 wk and are not associated with any other disease[19,20]. A study investigating children with persistent COVID-19 symptoms found that symptoms were present for 4 to 12 wk, and could even persist for 7 to 8 mo[21]. In this review, we use the term 'post-COVID-19 syndrome'.

Studies have shown that among symptomatic patients, 21.4% had profound symptoms even 20 wk after recovery[22]. The duration of COVID-19 and comorbidities (such as unstable diabetes mellitus, and hypertension) were found to be associated with post-COVID-19 syndrome[22]. Interestingly, the age group of 1-10 years had no complaints after recovery, but patients older than 40 years had remnant findings even 20 wk after onset[22].

Although current knowledge on symptomatic patients after discharge is insufficient, in order to have a comprehensive framework, studies that investigated post-COVID-19 syndrome have been included in this review (Table 1).

Respiratory system involvement

During the course of COVID-19, an important proportion of cases suffer from severe pneumonia and tend to have long-term sequelae[23]. Ongoing fibrosis during the recovery period results in decreased diffusion capacity of the lung[24]. Studies have indicated that a large variation in respiratory morbidity may appear such as decreased exercise capacity, an increased need for continuous positive airway pressure, tracheostomy, or ventilator dependence for COVID-19 long-haulers[13,24-27].

Table 1 Involvement of organ systems in post-coronavirus disease 2019 syndrome

Systems	Findings
Respiratory system	Decreased diffusion capacity of the lung due to ongoing fibrosis
	Decreased exercise capacity, cough, and chest pain
Hematologic system	CD4+- T lymphocytes remained lower
	Mild elevation in white blood cell (WBC) count
	High levels of WBCs are driven by raised neutrophils
	Direct injury of endothelium and cytokine release causing prothrombotic tendency
	Elevation of Von Willebrand Factor antigen (VWF: Ag), VWF propeptide (VWFpp), and Factor VIII coagulation (FVIII: C) elements
Cardiovascular system	Vascular, pericardial, and myocardial tissue inflammation
	Chest pain, palpitations, dizziness, and increment in resting heart rate
	Postural orthostatic tachycardia syndrome (POTS)
Gastrointestinal system	Diarrhea, abdominal pain, and nausea
	Viral RNA could still be present in the stool after 30 d
	Weight loss and risk of malnutrition due to decreased appetite
Neurologic system	Mild headache, hyposmia, hypogeusia, fatigue, sleep disorders, pain, cognitive impairment, and rarely Guillain-Barré syndrome
	Anosmia and hypogeusia, underlying low-grade inflammation of the frontal lobe, loss of cognition, brain fog, and headache
Psychiatric issues	Social withdrawal, social isolation, economic loss due to being unable to work, increased child care and familial charges, and burden of guilt if other contacts contract the virus
	Psychological distress and post-traumatic stress disorder

The up-to-date pathophysiological process of lung fibrosis development in COVID-19 includes pulmonary consolidation, hyaline membrane formation, capillary damage and bleeding, diffuse alveolar epithelium destruction, and alveolar septal fibrous proliferation[28]. A cohort study reported that more than 50% of patients with SARS-CoV-2 pneumonia at 30 d post-infection had abnormal results for functional residual capacity, total lung capacity, and diffusing capacity of the lungs[29]. Although, pulmonary fibrosis occurs in most patients it was reversed in less than half of the patients 3 mo after onset[30].

Myall *et al*[31] conducted a cohort study that included 837 COVID-19 patients. The patients were screened *via* phone calls 4 wk after discharge. 325 patients had ongoing symptoms. Following assessment of this group using various tests [chest X-ray, 6-min walking, echocardiogram, and computed tomography (CT)], 35 (4.18%) patients were diagnosed with interstitial lung involvement, and were successfully treated with corticosteroids. The main characteristics of the group with lung involvement were being male, obese, in need of oxygen therapy, and mechanical ventilation during the acute phase.

In a study conducted to highlight long-term respiratory results, 244 patients required prolonged ICU and inpatient stay, and follow-up chest X-rays. Of these patients, 23 (9%) showed significant deterioration 2 mo after onset of the disease[32]. To evaluate the relationship between radiological involvement at admission and impaired lung function, a prospective cohort study was conducted. Patients who presented with acute respiratory distress syndrome (ARDS) during ICU stay resulting from COVID-19 were included in the study and examined *via* chest CT and pulmonary function tests 3 mo after discharge. Pulmonary function tests were abnormal in 55% of patients, with restricted diffusing capacity of the lungs[33]. In a large study of more than 4000 COVID-19 survivors, risk factors for 90-d mortality were reported as older age, immunosuppression, severe obesity, diabetes, higher renal and cardiovascular sequential organ failure assessment (SOFA) score components, lower PaO2/FiO2 ratio and a shorter time between first symptoms and ICU admission[34].

CT changes in post-COVID-19 syndrome provide information on long-term pulmonary effects. A study that included 52 subjects with COVID-19 assessed *via* CT 3 mo after diagnosis showed that 22 (42%) patients had residual findings. Problems with decreased lung capacity, cough, and chest pain were more common among patients with abnormal CT scans[35].

Cardiovascular system involvement

A history of pre-existing cardiovascular illness or hospitalization were not associated with post-acute-COVID-19 syndrome (PACS)[36]. Before the pandemic, it was hypothesized that the density of ACE2 receptors in the heart was due to myocardial injury. However, recent studies demonstrated that the

cause of type 2 myocardial infarction was increased systemic inflammation[37]. Vascular, pericardial and myocardial tissue inflammation yields typical cardiac complaints of chest pain, palpitations, dizziness, and an increment in resting heart rate[25,38].

A cohort study was performed by Puntmann *et al*[39] to determine myocardial inflammation rates in patients with a history of COVID-19 infection. The patients were analyzed 2 wk after hospital discharge by cardiac magnetic resonance (CMR) imaging to evaluate myocardial involvement. A control group was also included to investigate similar risk factors to the study group. The study group subjects were found to have significant T2 signal and late gadolinium enhancement. Another study of 148 patients with elevated troponin levels during hospitalization were followed up for 2 mo after discharge. It was reported that 26% of the patients developed a myocarditis-like pattern, while all patients had normal left ventricle function. Active myocarditis with regional elevation in T1 and T2 signals was demonstrated in 8% of patients. However, elevated troponin was not found to be predictive of myocarditis[40]. In a multicenter study, almost 20,000 athletes following recovery from COVID-19 were examined and only 3% of them were found to have possible pathology 113 d after onset of the disease [41]. It may be inferred from recent studies that myocarditis is a very rare condition, especially in asymptomatic and mild cases.

In another study, 59 patients following hospitalization due to COVID-19 were screened *via* CMR imaging. One patient's imaging data indicated pericarditis[42]. Other research demonstrated that 5% of patients were estimated to have mild pericardial effusion[43]. Although further investigations are required, it can be inferred that pericarditis after COVID-19 is rare, while effusion is a relatively more common pathology.

Postural orthostatic tachycardia syndrome (POTS) is another disorder seen in a considerable number of COVID-19 long haulers. To estimate the incidence of this condition, 28 patients with persistent cardiac complaints after COVID-19 recovery were enrolled in a study. The results of the tilt table and ten minutes-standing tests demonstrated that 20 patients (70%) had POTS[44].

Arrhythmias after COVID-19 are quite rare and investigations on this issue are scarce. An analysis of arrhythmias in 5000 patients hospitalized with COVID-19 and influenza was carried out. Similar percentages of atrial fibrillation and atrial flutter were detected in both groups[43].

Hematologic system involvement

Laboratory markers for predicting the severity of disease and mortality have been questioned. It is known that several changes occur during the course of COVID-19. A study of 1099 reverse transcriptase-polymerase chain reaction (RT-PCR) positive patients demonstrated lymphocytopenia (83.2%), thrombocytopenia (36.2%), and leukopenia (33.7%) in the initial phase of the disease[45]. A few studies have investigated hematological findings after recovery. In a study of 313 participants, 12.9% of patients had leukocytosis, which increased to 16.1% 4 wk after recovery. The percentage with neutrophilia in the initial phase was found to be 17.7%, which increased to 33.8% and lymphocytopenia decreased from 17.7% to 14.5%. Almost half of the patients had increased D-dimer levels in the acute stage, which decreased to 6.4% after 1 mo[22].

Lymphopenia is a common finding in patients with COVID-19 and represents a defective immune response to the virus[1]. Cytotoxic lymphocytes such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells have a main role in the control of infection. During the acute phase of the disease, both CTLs and NK cells decrease in number. However, after recovery, these cell numbers then increase. Hence, Zheng *et al*[46] suggested that the recovered numbers of these cells may predict convalescence.

Studies investigating the prevalence of lymphopenia in COVID-19 positive patients have provided different estimates ranging from 63% to 75%[47,48]. In patients with severe disease, a decrease in both CD4 and CD8 cells was noted. Additionally, lymphocyte count, especially CD4, may predict severity and prognosis[49]. A prospective study showed that CD8+- T lymphocytes recovered to their normal level 3 mo after onset of the disease. Another finding in this study showed that CD4+- T lymphocytes remained lower than in the healthy population even 4 wk after onset[50].

A large comprehensive meta-analysis of hematologic laboratory data demonstrated that patients with serious disease had a mild elevation in white blood cell (WBC) count. Additionally, patients who died due to COVID-19 had a significant increase in WBCs. According to this finding, WBC levels signify the severity of the disease. Despite reduced lymphocyte, monocyte, and eosinophil counts; high levels of WBCs were driven by raised neutrophils[49]. Similarly, recent research demonstrated that increased neutrophil/lymphocyte and peak thrombocyte/lymphocyte counts may help predict prognosis[51].

Thrombocytopenia in COVID-19 patients may be caused by disseminated intravascular coagulation, sepsis, or drug-induced, which was also shown to be a risk factor for increased morbidity and mortality [52]. Several studies have reported late-onset immune thrombocytopenia 4 wk after the onset of COVID-19[53].

A new description of the immune thrombotic state is termed COVID-19-induced coagulopathy[54]. A possible mechanism responsible for this prothrombotic tendency is the direct injury of endothelium and cytokine release which activates the coagulation cascade[55]. A cohort study screened 50 patients for endotheliopathy 68 d after recovering from COVID-19. This study showed that endothelial biomarkers von Willebrand Factor antigen (VWF: Ag), VWF propeptide (VWFpp), and Factor VIII coagulation (FVIII: C) elements were significantly elevated in post-acute-COVID-19 patients. Endothelial damage

may be a possible explanation for the pathogenesis of long-COVID-19 syndrome[56].

Post-discharge thromboprophylaxis has been assessed in post-COVID-19 patients. A prospective cohort study of 146 patients showed that 6 wk after discharge, while the percentage of thrombotic events was 0.7%, 30% of patients had increased D-dimer values[57]. Although there are ongoing studies to determine the rates of thrombotic events after COVID-19, routine thromboprophylaxis after discharge is not recommended. The Global COVID-19 Thrombosis Collaborative group recommends prophylaxis for selected patient groups only such as the elderly population and those with existing comorbidities [58].

Gastrointestinal system-related issues

SARS-CoV-2 mainly leads to diseases associated with the respiratory tract, but gastrointestinal disturbances can also occur. During the natural course of the disease, patients develop anorexia, nausea, vomiting, and diarrhea[47]. In contrast to early studies that suggested lower rates of diarrhea and other digestive symptoms, recent data show that almost half of patients have gastrointestinal system complaints[59,60]. A large cross-sectional study including 979 participants who recovered from COVID-19 demonstrated that almost half of the patients had diarrhea, abdominal pain, and nausea[61]. The appearance of digestive system complaints is delayed, compared to respiratory symptoms and begin at about 9.0 d[62]. Although there are numerous reports regarding gastrointestinal involvement during the acute stage, the effects of post-COVID-19 syndrome on the digestive system remain unclear.

Viral shedding from the gastrointestinal tract may be massive and continue long after the resolution of clinical signs[63]. A study on SARS-CoV-2 demonstrated that viral RNA could remain in the stool even after 30 d[64]. More than half of the patients were found to have viral RNA in their stool during the acute stage of disease, and one in five patients had positive stool samples even after viral RNA was eliminated from their airways[59]. Another investigation which assumed that SARS-CoV-2 spread *via* the stool displayed similar conclusions showing that virus shedding continued even after the convalescent phase of the disease. It was also suggested that viral RNA in feces detected by RT-PCR can be used to monitor infection[65].

Early data suggested that higher numbers of ACE2 receptors in cholangiocytes (59.7% of cells) compared to hepatocytes (2.6% of cells) show that the virus may be directly attached to ACE2-positive cholangiocytes and damage liver function[66]. Nevertheless, autopsy studies reported no viral inclusion in the liver[67]. Correspondingly, an overactive inflammatory reaction may be responsible. The underlying mechanism can be explained as follows: Typical lymphopenia detected in SARS-CoV-2 infection causes increased serum levels of interleukin-6 (IL-6), IL-10, IL-2, and interferon (IFN)-γ which may damage liver tissue[68]. Likewise, a strong association between lymphopenia and increased serum C-reactive protein level with liver injury has been proposed[69].

Studies on COVID-19 patients after remission indicate that weight loss and risk of malnutrition were highly prevalent 3 wk after recovery. Increased inflammation leads to decreased appetite. A prospective cohort study aiming to understand the long-term results of malnutrition in post-COVID-19 syndrome was carried out, and included 288 hospitalized COVID-19 patients who were followed up for 6 mo. On day 30, 136 (47.2%) patients had persistent malnutrition or sarcopenia. Gérard *et al*[70] found that the time taken to regain weight was 6 mo, but all patients generally remained 1.4 kg lighter than their weight on admission.

Urinary system involvement

An increased numbers of urinary frequency complaints have prompted the question: "Does SARS-CoV-2 infection cause viral cystitis?"[71]. The existence of viral RNA in the urine of COVID-19 sufferers showed that the urinary tract is potentially affected throughout the disease[45,72]. Ischemic and/or toxic tubular damage was detected in more than 14% of acute kidney injury (AKI) cases with COVID-19 [73]. The greater number of AKI patients with COVID-19 was related to acute tubular injury. The probable mechanism of acute tubular damage may involve volume reduction that reduces kidney perfusion. Another possible explanation is that the immune response produces cytokines that affect renal circulation[74]. There are no available data on the long-term complications of SARS-CoV-2 infection in the urinary tract.

Neurologic system involvement

Several studies have reported a large number of neurologic disorders ranging from mild headache, hyposmia, hypogeusia, and fatigue to sleep disorders, pain, cognitive impairment, and rarely Guillain-Barré syndrome[40]. To ascertain the main cause of neurological disorders, it is necessary to define the components of neuro-COVID, which tends to cause more disabling disease[6,75]. In patients with or without neurological manifestations during the acute phase of COVID-19, the cytological and biochemical study of cerebrospinal fluid, as well as neuroimaging, revealed significant alterations that represented inflammatory activity. It was also noted that during the acute phase of the disease, a consequential number of inflammatory events were demonstrated by radiological surveys of the central nervous system and both cytological and biochemical evaluations of cerebrospinal fluid[76].

To shed light on the neurological disturbances after COVID-19, it is essential to know the tropism of the virus and how it accesses the nervous system. The nasal and oral cavities provide an area for seeding of SARS-CoV-2. From the olfactory mucosa *via* retrograde neuronal transport, the virus reaches the central nervous system[77]. The inflammatory response of nasal and oral mucosa may be the reason for anosmia and hypogeusia. Moreover, as anosmia and hypogeusia have a similar mechanism, underlying low-grade inflammation of the frontal lobe might be the cause of the loss of cognition, brain fog, and headache[77]. As silent target organ damage and underdiagnosis of post-COVID syndrome results in neurological manifestations, taking precautions with regard to initial neurorehabilitation is essential[78].

There are a considerable number of reports of patients with demyelinating pathologies such as Guillain-Barre syndrome, Miller-Fisher, and other inflammatory polyneuropathies. A review of these cases showed that symptomatic neuropathy may be diagnosed 3 to 33 d after onset. The absence of SARS-CoV-2 RNA in the cerebrospinal fluid indicates that a post-infectious process is thought to be responsible rather than a para-infectious process[79]. There is another case report of status epilepticus and hippocampal atrophy due to prolonged inflammation 6 wk after SARS-CoV-2 infection[80]. Another patient with orthostatic cerebral hypoperfusion syndrome and painful small fiber neuropathy after recovery has been reported[81].

The most commonly reported neurological disturbance in COVID-19 patients is headache (18%-38%) [82,83]. Other complaints consist of peripheral neuropathy symptoms, tinnitus, memory issues, concentration, and sleep disturbances[84].

Psychiatric issues

The psychological health outcomes during COVID-19 recovery may contribute to social withdrawal, social isolation, economic loss due to being unable to work, increased child care and familial charges, and burden of guilt if other contacts contract the virus[85]. Nonetheless, patients with SARS-CoV-2 heal physically; however, they are prone to psychological distress and post-traumatic stress disorder. A study showed that more than half of patients had these mental disorders after surviving severe disease [86]. The first study on the neuropsychological findings of post-COVID-19 patients showed that the Beck Depression Inventory scores were significantly higher in post-COVID-19 patients than in healthy controls[87].

Endocrinological involvement

The impact of post-COVID syndrome on the endocrine glands cannot be underestimated. Symptoms such as tiredness, weakness, nausea, diarrhea, dizziness, and joint pain may overlap with adrenal insufficiency symptoms. For instance, Salzano *et al* reported a patient with adrenal insufficiency following recovery from SARS-CoV-2 infection[88]. Additionally, a cohort study of 453 patients was conducted and thyroid-stimulating hormone (TSH) and thyroxine (T4) levels before, during, and after SARS-CoV-2 infection were evaluated. According to this study, while most cases were found to be euthyroid, a slight decrease was reported in both TSH and T4 levels, which normalized after infection [77].

Dermatological issues

A single-center prospective study to define the skin manifestations of long COVID syndrome in 104 patients was conducted by Diotallevi *et al*[89]. Following hospital discharge, the patients were followed up at 1, 3, and 6 mo and examined by dermatologists who reported a wide spectrum of findings such as telogen effluvium, skin xerosis, diffuse folliculitis, vesicular exanthema, relapse of seborrheic dermatitis, relapse of psoriasis and pityriasis versicolor. According to the study, telogen effluvium due to interruption of the anagen phase was the most prevalent dermatological finding in patients after SARS-CoV-2 infection.

CONCLUSION

As the new coronavirus, SARS-CoV-2, involves multiple organ systems and the number of COVID-19 survivors increases every day, there is a need to develop new strategies for the systematic assessment of these patients as well as the need for rehabilitation services. Multidisciplinary post-acute COVID-19 care services should include several specialists to evaluate the consequences of the disease, and highlight some of the unrecognized disorders of COVID-19.

FOOTNOTES

Author contributions: Özdemir Ö advised, reviewed, and edited the manuscript; Arslan Z planned, researched, and outlined the manuscript; Both authors wrote the manuscript.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

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S-Editor: Liu JH
L-Editor: Webster JR
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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 July 20; 12(4): 235-245

DOI: 10.5662/wjm.v12.i4.235

ISSN 2222-0682 (online)

MINIREVIEWS

Rehabilitation in long COVID-19: A mini-review

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Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ait Addi R, Morocco; Ustun G, Turkey

Received: February 27, 2022
Peer-review started: February 27, 2022
First decision: April 13, 2022
Revised: May 7, 2022
Accepted: July 11, 2022
Article in press: July 11, 2022
Published online: July 20, 2022

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Abstract

We have been experiencing multiple waves of the coronavirus disease 2019 (COVID-19) pandemic. With these unprecedented waves, we have entered into an era of 'new normal'. This pandemic has enforced us to rethink the very basics of childhood learning: Habits, health etiquette, and hygiene. Rehabilitation has immense importance during this pandemic considering a few aspects. Multidisciplinary COVID-19 rehabilitation clinics are essential to address the demand. The equitable distribution of COVID-19 rehabilitation services for differently-abled individuals during the pandemic is an important aspect. Rehabilitation needs identification and further studies on various rehabilitation interventions are among the key unmet future research needs.

Key Words: COVID-19; Long COVID-19; Post-COVID-19 syndrome; Rehabilitation; SARS-CoV-2; Long haulers

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Core Tip: The coronavirus disease 2019 (COVID-19) pandemic has impacted negatively on multiple systems of our body, among them the pulmonary system is the most pronounced. The cardiac, nervous, and musculoskeletal systems are also involved. Post-COVID-19 especially post-intensive care unit or post mechanical ventilation and long-COVID-19 can cause significant functional loss and disability. Rehabilitation has an immense role to bring back the achievable functional status of COVID-19 patients. Multidisciplinary COVID-19 rehabilitation clinics are essential to address the demand. The equitable distribution of COVID-19 rehabilitation services for differently-abled individuals during the pandemic is an important aspect. Rehabilitation needs identification and further studies on various rehabilitation interventions are among the key unmet future research needs.

Citation: Swarnakar R, Yadav SL. Rehabilitation in long COVID-19: A mini-review. *World J Methodol* 2022; 12(4): 235-245
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/235.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.235

INTRODUCTION

We have been experiencing multiple waves of the coronavirus disease 2019 (COVID-19) pandemic. With these unprecedented waves, we have entered into an era of 'new normal'. This pandemic has enforced us to rethink the very basics of childhood learning: Habits, health etiquette, and hygiene. It taught us the importance of each stage of prevention: Primordial, primary (mask, sanitization, social distancing, and vaccine), secondary (treatment, critical care, and rehabilitation in acute care), tertiary (disability limitation and rehabilitation), and quaternary (prevent treatment side-effects) (Figure 1). On the other hand, it became a tragedy for people who lost their near and dear ones and those who lost their financial support/job. When we say rehabilitation, it must be emphasized that we should consider the person as a whole rather than the disease. Rehabilitation is defined as "a set of interventions designed to optimize functioning and reduce disability in individuals with health conditions in interaction with their environment".

Coronaviruses are single-stranded RNA viruses that cause respiratory, gastrointestinal, and neurological diseases. The first coronavirus - severe acute respiratory syndrome coronavirus (SARS-CoV) - was thought to originate in Foshan, China and resulted in the SARS-CoV pandemic (2002-2003). The second coronavirus caused Middle East respiratory syndrome, which originated from the Arabian Peninsula in 2012. SARS-CoV-2 started in Wuhan, Hubei Province, China in December 2019. On January 12, 2020, the World Health Organization (WHO) named it novel coronavirus (2019-nCoV) and officially named it 'COVID-19' on February 11 2020, and finally the International Committee on Taxonomy of Viruses officially designated the virus as SARS-CoV-2. On January 20, 2020 human-to-human transmission was confirmed. On 11 March 2020, WHO declared it as a 'pandemic'. On March 18, 2020, the WHO and partners launched the Solidarity trial, an international clinical trial aimed 'to generate robust data from around the world to find the most effective treatments for COVID-19'.

Till now, globally more than 298 million confirmed cases (in India: > 35 million) and 5.4 million (in India: > 0.4 million) deaths have been recorded.

Causative agent

COVID-19 is caused by SARS-CoV-2. SARS-CoV-2 is enveloped and spherical shaped (120 nm), having a positive-sense single-stranded RNA genome[1]. It belongs to the subfamily Orthocoronavirinae (family: Coronavirdiae; order: Nidovirales) and is classified as a beta-coronavirus [lineage B]. The name corona came from a crown-like appearance under an electron microscope ('coronam' is the Latin word for 'crown') due to the presence of spike glycoproteins on its envelope. The spike glycoprotein of SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) in humans for cellular entry. Till now, five variants of concern have been identified: Alpha (B.1.1.7); Beta (B.1.351); Gamma (P.1); Delta (B.1.617.2); Omicron (B.1.1.529).

Bat [horseshoe bats (*Rhinolophus* spp)] is the probable natural host and pangolins are considered an intermediate host[1].

Incubation period

2-14 d (mostly 5 d).

Mode of transmission

Primarily by respiratory droplets but airborne transmission is also implicated especially following aerosol-generating procedures. Fomite transmission also has been well characterized.

Clinical features

Fever (83%-99%), cough (59%-82%), and fatigue (44%-70%) are the frequent symptoms but anorexia (40%-84%), shortness of breath (31%-40%), myalgia (11%-35%), and diarrhoea (2%-38.1%) are also seen. Loss of smell (anosmia) or loss of taste (ageusia) is also reported in many cases. Most of the cases are having mild symptoms, and people with comorbidities and older age groups are particularly prone to develop severe disease.

Clinical severity

The WHO classified COVID-19 as mild, moderate (pneumonia), severe (severe pneumonia), and critical (acute respiratory distress syndrome, sepsis, and septic shock).

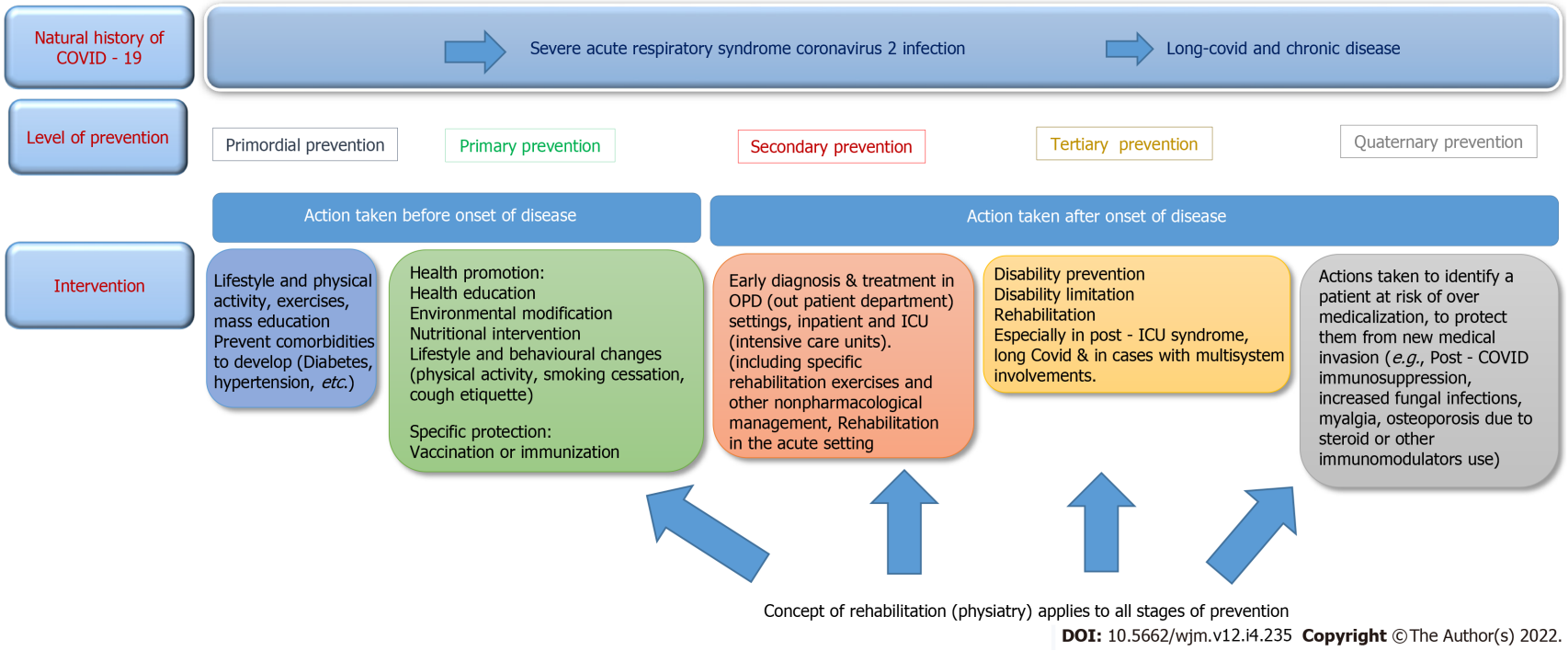

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Figure 1 Rehabilitation perspective of the coronavirus disease 2019. COVID-19: Coronavirus disease 2019; ICU: Intensive care unit.

Pathophysiology

After entry into the cell, this virus causes diffuse alveolar damage in the lungs. Excessive immune reaction to the virus, causing cytokine storm, is mainly responsible for the clinical severity of COVID-19. The predominant mechanisms of acute COVID-19 include the following: Direct viral toxicity; endothelial and microvascular damage; dysregulated immune system; hypercoagulability; and maladaptation of the ACE2 pathway.

Investigation

Confirmation is done by real-time polymerase chain reaction using the upper and lower respiratory tract samples. Faecal specimens at times can be used as a sample. SARS-CoV-2 antibody testing is not recommended for diagnosis. Lymphopenia is a cardinal feature. Chest X-ray shows bilateral infiltrates, and high resolution computed tomography (HRCT) scan of the thorax shows ground-glass appearance as the most common finding.

COVID-19 confirmed case definition (WHO)

A person with laboratory confirmation of COVID-19 infection is regarded as a COVID-19 confirmed case.

According to ICD 11, the code for the confirmed diagnosis of COVID-19 is RA01.0 and for the clinical diagnosis (suspected or probable) of COVID-19 is RA01.1; the code for post-COVID-19 condition is RA02.

LONG COVID

The Centres for Disease Control and Prevention defined 'post-COVID conditions' as "a wide range of new, returning, or ongoing health problems that people can experience four or more weeks after first being infected with the virus that causes COVID-19"[2]. It is called long COVID/long-haul COVID/post-acute COVID-19. Common long COVID-19 symptoms are fatigue, arthralgia, breathlessness, cough, loss of smell, chest pain, palpitation, anxiety/depression and sleep disturbances, difficulty in concentration, *etc.*

In the National Institute for Health and Clinical Excellence guidelines, two definitions of post-acute COVID-19 are given: (1) Ongoing symptomatic COVID-19 for persons who still have symptoms between 4 and 12 wk after the onset of acute symptoms; and (2) Individuals who still have symptoms for more than 12 wk after the onset of acute symptoms. A full blood count, liver and kidney function tests, a C-reactive protein test, and an exercise tolerance test (level of breathlessness, O_2 saturation, and heart rate) are recommended. A chest X-ray also should be advised to all patients by 12 wk after acute infection in cases of persistent respiratory symptoms[3].

Basic mechanisms leading to post-acute COVID-19 include: (1) Virus-related pathophysiologic changes; (2) Immunologic alterations and inflammatory changes in response to the acute infection; and (3) Post-critical illness sequelae. Post-intensive care syndrome is multifactorial and probably due to microvascular ischemia, immobility, and metabolic changes during critical illness. A rehabilitation physician should be well aware of this basic pathophysiology of each aspect of COVID-19 in order to address the rehabilitation need aptly and to implement rehabilitation programs accordingly.

ROLE OF REHABILITATION MEDICINE & INTERNATIONAL CLASSIFICATION OF FUNCTIONING, DISABILITY, AND HEALTH IN COVID-19

Rehabilitation has immense importance during this pandemic considering a few aspects. From the perspectives of type of patient population, there would be different rehabilitation needs for people with COVID-19/post-COVID with no comorbidities/disabilities and those with COVID-19/post-COVID with comorbidities/disabilities. On the other hand, from the perspective of COVID-19 severity, the rehabilitation needs would be different for mild, moderate, and severe/intensive care unit (ICU) admitted patients or in patients with post-intensive care syndrome. Furthermore, at an individual level, a patient may have multiple body-system involvements, in that case, rehabilitation needs would be according to the involvement and impairments. Considering the duration of the disease rehabilitation program would differ, goal setting would change (*e.g.*, acute, subacute, and chronic/post-COVID) accordingly.

At each stage, a basic outline of rehabilitation has to be followed in this sequence: Proper history and physical examination focusing on functional status, a list of problems according to International Classification of Functioning, Disability and Health (ICF) data set (body functions and structure suggesting impairments, activity limitations, participation restriction, and related environmental and personal factors), addressing each problem (management plan: Short- and long-term feasible and realistic goal setting after discussion with the patient, care-giver/partner/family), and trying to achieve each goal, encourage and make support groups to aid in better compliance in the rehabilitation program, to avoid drop-outs, and to strengthen mental health.

Impairments

Multiple system impairments occur like impaired lung function (the viral respiratory syndrome and/or pneumonia, pulmonary fibrosis, pulmonary embolism, damage to respiratory muscles, immobility, and atrophy), physical deconditioning and weakness (due to polyneuromyopathy, inflammatory storm to the muscles, and drugs such as steroids), metabolic changes (*e.g.*, hyperglycemia and malnutrition), immobility and atrophy, impaired communication, impaired swallow (post mechanical ventilation and others), delirium and cognitive impairments, anxiety depression and difficulty in managing activities of daily living (ADLs), pressure injuries, incontinence, polyneuropathy, and other disorders of the peripheral nervous system. Speech-language therapists, physiotherapists, and occupational therapists are needed in such cases.

Rehabilitation starts from acute care setting, then it includes inpatient rehabilitation, outpatient settings, telerehabilitation, and home-based and community-based setting[4].

EVIDENCE-BASED REHABILITATION

We are at the very primary stage to garner evidence for COVID-19. This is the reason why we are continuously modifying and updating guidelines and evidence-based medicine[5]. Studies with stronger evidence on the efficacy of interventions and long-term monitoring are lacking.

Self-management (hospitalized and non-hospitalized)

Objective: The objective is to increase functional independence of patients. Patients with COVID-19 should be educated on self-management regarding breathlessness and gradual activity resumption[6].

Long-COVID: Post-COVID-19 symptoms are seen in > 60% of patients infected by SARS-CoV-2. Fatigue and dyspnoea were the most common post-COVID-19 symptoms, particularly 60 and ≥ 90 d after[7]. Individualized rehabilitation programs from subacute to long-term should be provided according to patient needs. The prescription and provision of rehabilitation programs should be guided by persistent symptoms and functional limitations. Post-COVID-19 impairments like fatigue, weakness, and cognitive impairment, can impact the performance of ADLs. Providing ADL training, considering home modifications (such as grab bars in the shower and toilet, and handrails along stairs), and the provision of an assistive product (such as a mobility aid, shower chair, and over-toilet frame), as needed, are important.

Persons with COVID-19 needs supervised patient-tailored programmes that are flexible enough to adapt for patients. It should be guided by baseline oxygen needs at rest and during exercise.

Persons with physical deconditioning and weakness should start with exercises that help in recovery of daily functioning, start with active range of motion exercises, and when tolerated, proceed with progressive muscle strengthening, typically offered with resistance training.

For individuals having difficulties in memory, concentration, and problem solving, education should be provided, and advice on strategies to reduce stress and anxiety should be given. Cognitive restorative rehabilitation along with cognitive exercises like memory exercises, puzzles, games, and reading and compensation tools like prompts (*e.g.,* lists and notes) and breaking down activities are advised.

For patients with anxiety, depression, and post-traumatic stress disorder, basic mental health and psychosocial support by appropriately trained health or non-health workers should be provided.

For pain, a multidisciplinary approach for pain management is followed according to the biopsychosocial model.

For a successful rehabilitation, a multidisciplinary team approach is essential where a rehabilitation physician (physiatrist) works in collaboration with a group of physicians from multiple specialties (critical care experts, pulmonologists, neurologists, cardiologists, rheumatologists, *etc.*), nursing professionals, pharmacists, occupational therapists, physiotherapists, social workers, mental health experts, community workers, and other health care professionals

SYSTEMIC INVOLVEMENT OF COVID-19 AND THEIR SPECIFIC REHABILITATION APPROACHES

Pulmonary rehabilitation

Pulmonary rehabilitation improves functional capacity and quality of life in persons recovering from SARS-CoV-2 infection[8].

Diffuse alveolar damage, pulmonary vascular microthrombosis, and macrothrombosis, and immunological damage are responsible for pulmonary sequelae and lung fibrosis.

Pulmonary involvement is complicated with pneumonia, pulmonary embolisms, pulmonary fibrosis, and prolonged ventilation induced respiratory muscle weakness.

Based on a level 3 study, it is suggested that a large proportion of patients still present with dyspnoea at 3 wk of hospital discharge and that PaO2/FiO2 ratio and BMI at admission to the Emergency Department are the strongest independent predictors of persistent respiratory impairment and the need for follow-up in these patients[9].

Patients can try relieving breathlessness using a number of positions including standing with back support, forward lean sitting, and forward lean standing.

Because of risk of infection, lung function test using spirometers is not recommended in patients in the first 6-8 wk with COVID-19; later it can be done to test lung function when patients get non-infectious.

Increasing ventilation

Active cycle of breathing techniques (ACBTs) can be used. Sitting up in bed or chair or standing with support positions to improve ventilation can be adopted as it allows increased thoracic expansion.

Airway clearance

Airway clearance is advised when airway obstruction by sputum is suspected, as these are droplet generating techniques.

In most patients, ACBTs may be used for airway clearance. It includes deep breathing with thoracic expansion and then exhaling with a huff. Huffing is forced expiration with an open mouth (vibration frequency < 17 Hz). Huffing helps to move sputum from small airways to larger airways. A sequence of deep breaths (× 3) then 1-2 huffs and a cough can be used to clear sputum.

Bubble PEP (positive expiratory pressure) can also be used and combined with a huffing and cough sequence to help clear sputum.

Breathlessness education

It is important to learn self-management. The following self-management practices may be adopted.

Staying calm: Breathlessness leads to anxiety which may make it worse.

Using positions: There are several positions (high side-lying and forward lean sitting) that can be adopted that offer support to the body to make breathing easier.

Using breathing techniques: Different breathing techniques may be adopted in different situations. Deep breathing and paced breathing techniques are helpful. Pursed lip breathing helps to empty the lungs and can be helpful to remain calm and when doing a moderate level of activity.

Pursed lip breathing: The patient relaxes his/her shoulder and neck muscles, inhales through the nose for two counts (inhale, one, two, do not take deep breath, only normal breath), 'purses' or puckers his/her lips as if he/she is going to whistle, then lastly exhales slowly through pursed lip counting to four (exhale, one, two, three, four).

Square box breathing: The patient closes his/her eyes and inhales *via* the nose (count up to 4), holds the air inside (count up to 4), exhales slowly (count up to 4), and finally holds for count 4.

Patients should start at lower intensities, especially during the first 6 wk following acute illness.

Respiratory muscle training can improve effortless breathing. Inspiratory muscle training (IMT) can be facilitated *via* loading of inspiration, by using a breathing device (threshold IMT or power breathe). Expiratory muscle training is also helpful.

Patients should start with low-intensity exercises (≤ 3 METs or equivalent or Borg dyspnea score ≤ 3; duration: 10-15 min for first 3-4 sessions; frequency: 1-2 times/d, 3-4 times/wk) and increase slowly by 30 s to 1 min each time. Over time progression of exercises can be increased up to a Borg score of 4-5 and duration to 30-45 min for 2-3 sessions. Frequency and intensity should be individualized. Pulse oximeter is used to monitor oxygen saturation. Stop physical activities or exercises when a patient's saturation drops more than 5%-10% during exercise. Warm-up and cool-down exercises are recommended. Exercises in the home environment along with maintaining an exercise logbook are recommended[10].

At 12 wk post-discharge, all COVID-19 patients are recommended to be assessed clinically along with an evaluation with chest X-ray, for rehabilitation needs, pulmonary function tests, 6-min walking tests, sputum sampling, and echocardiogram according to clinical judgment. In cases of persistent dyspnea, high-resolution computed tomography of the chest at 6 and 12 mo is also recommended.

Cardiac rehabilitation

According to initial assessment, cardiology consultation should be taken, and further investigations are advised like blood panel, ECG, 24 h ECG, echocardiogram, cardiopulmonary exercise testing, and/or cardiac magnetic resonance imaging.

A 3-6 mo period of complete rest, based on clinical severity and duration of illness, left ventricular function at onset, and extent of inflammation on CMR, is required for patients returning to high-level sport or physically demanding occupation following myocarditis. Training and sport may resume following myocarditis, if left ventricular systolic function and serum biomarkers of myocardial injury are normal and if relevant arrhythmias are ruled out on 24 h ECG monitoring and exercise testing. Periodic assessment is needed in the first 2 years[11].

Abstinence from competitive sports or aerobic activity for 3-6 mo is recommended for competitive athletes with post-COVID cardiovascular complications and it should be until resolution of myocardial inflammation revealed by cardiac magnetic resonance imaging or troponin normalization. Serial clinical and imaging evaluation at 4-12 wk with electrocardiogram and echocardiogram may be done in those with cardiovascular complications.

Rheumatological rehabilitation (arthralgia, arthritis, and myalgia)

Early COVID-19 studies have indicated that over a quarter of mechanically ventilated patients continue to experience ICU acquired weakness at discharge from hospital[12], while half of all hospitalized patients continued to experience fatigue at 60 d post onset[13]. Physical difficulties were reported, including strength, balance, pain, exercise tolerance, and fatigue. Medical Research Council score,

handgrip test, Berg balance score, and time up and go test are important assessment measures. SPO2 during exercises and exercise tolerance test (step test or sit to stand test) should be done. Early mobilization (should begin in ICU and critical care setting if tolerable and feasible; bed mobility, bed exercises, *etc.*), education on fatigue and breathlessness, and functional mobility are to be focused. Energy conservation techniques should be considered, such as simplifying tasks, spacing activities throughout the day, and resting before and after activities. The patient should resume his/her everyday activities and exercise slowly, gradually increasing time and exertion levels, and avoid strengthening exercises until myalgia resolves. For immobile patients with profound weakness, the daily use of neuromuscular electrical stimulation is considered to address inactivity-induced atrophies in lower-limb muscles. Biceps curl, knee strengthening, squats, heel raisers, wall push up, sit-to-stand, *etc.* are strengthening exercises. The patient should start the exercise in low-moderate intensity and then gradually increase accordingly. In the initial 6 wk after discharge or illness, it is recommended to keep shortness of breath or fatigue below 4/10 on the Borg scale. Clinical signs of desaturation should be monitored using a pulse oximeter when possible. Exercise must be individually prescribed with specifying training parameters regarding frequency, intensity, duration, and type and exercises must be done in a safe environment. Walking, jogging, cycling, step-ups, and marching on the spot may be undertaken if no contraindications exist.

Post-mechanical ventilation patients or those in tracheostomy may develop speech or voice issues. Assessment of any changes in voice quality and articulation problems is needed. Even in intubated patients if conscious, means of communication like notepad, communication board, *etc.* should be provided. The use of communication boards, communication devices, computers, and smartphone apps is encouraged to augment communication. Using voice is practiced gently through singing, reading aloud, and conversation. The patient should stay hydrated and sip water throughout the day. Optimal oral hygiene is practiced to minimize the risk of infection and maximize healing of the vocal structures. Patients with a tracheostomy may be fitted with a speech valve, which enables speaking without the use of the vocal folds, a trained physician is needed to manage these things and associated caregiver education.

Post-COVID patients may also develop steroid-induced myopathy.

Neurorehabilitation like COVID-induced spinal cord injury, post-COVID stroke, and neuropathy

Hemorrhagic stroke, hypoxic-anoxic damage, posterior reversible encephalopathy syndrome, and acute disseminated myelitis are reported in post-COVID cases.

Stroke patients developing COVID-19 have a worse prognosis, with an over nine-fold increase in mortality and a higher probability of requiring rehabilitation[14].

Regarding the natural history of COVID-19, the case series concerning four severe COVID-19 patients with HO in the hips and shoulders after 30-40 d of hospital admission suggests that the global inflammation associated to COVID-19 might play a role in the pathophysiology of HO, and enhances the need for early monitoring of joint mobility and careful mobilization of patients in the acute phase.

Cognitive domains that may be affected after severe or critical illness with COVID-19 include (but are not limited to) basic functions such as attention, concentration, and memory, higher-order functions such as problem solving, decision making, and judgement, and language such as verbal and semantic memory. Nervous system involvement is due to hypoxic encephalopathy, neuroinflammation, direct viral invasion, and sepsis associated encephalopathy.

Cognitive activities: Puzzles, word and number games, memory exercises, and reading may help improve cognitive function. COVID-19 patients with cognitive function impairment should start with activities that are challenging but achievable and increase the difficulty as able. This is important for keeping the patient motivated. These patients should participate in daily activities. Compensation strategies (prompts: Lists, notes, and alerts, such as phone alarms, can be useful for patients with impaired memory, breaking down activities into individual steps can help prevent the patient from feeling overwhelmed, and relaxation exercises, including meditation, can help alleviate the stress that may be caused by and exacerbate cognitive impairment) should be followed.

Olfactory Training and Visual Stimulation Assisted by a web application for patients with persistent olfactory dysfunction after COVID-19 showed significant improvement after 28 d[15].

Olfactory training like repeated and deliberate sniffing for 20 s each of a set of odorants (commonly lemon, rose, cloves, and eucalyptus) should be conducted at least two times a day for at least 3 mo. Studies have shown improvement in olfaction in postinfectious cases after olfactory training[16]. Intranasal vitamin A and systemic omega-3 may serve as adjuvant therapies[17].

Gastrointestinal system involvement

Diarrhoea, vomiting, and nausea are common in COVID. In cases of persistent gastrointestinal issues, nutritional assessment and micronutrient blood panel may be warranted. Decreased appetite and weight loss sometimes may occur, which also needs attention. A liver function test should be advised as hepatic dysfunction is common in severe COVID-19 cases. COVID-19 has the potential to alter gut microbiome.

Renal system involvement

Acute kidney injury tends to occur in 5% of all cases and 20%-31% of critical cases. Acute tubular necrosis is a common finding along with COVID-19 associated nephropathy. A kidney function test is advised as acute kidney injury has been encountered in many severe COVID-19 cases. Renal involvement is due to sepsis, septic shock, immunoinflammatory reactions, and drug-induced or post-steroid therapy. Patients with renal dysfunction may need a changed or individualized exercise regime and hydration advice.

Haematological system involvement

Lymphopenia, anemia, and leucopenia are common and at times thrombocytopenia can occur. Patients with COVID-19 are susceptible to pulmonary embolism and venous thromboembolism. Before starting any exercises regime, a complete hemogram should be done, and in clinically suspicious cases of thromboembolism, specific investigations are warranted.

Endocrine system involvement

Due to COVID-19 itself or after steroid therapy, hyperglycemia may occur. Post COVID-19 diabetic ketoacidosis and subacute thyroiditis with clinical thyrotoxicosis have been reported. Periodic assessment is needed in such cases.

Other system involvement

Swallowing challenges associated with COVID-19 may result from intubation induced damage in the mucous membrane of the oral cavity, nasal cavities, and/or pharynx, weakening of the swallowing mechanism, diminished swallowing reflexes and muscle tone associated with inflammation, polyneuropathy, and side-effects of medications, cognitive impairment affecting safe swallowing, ventilation induced changes in viscosity of saliva, mouth breathing, medication, or dehydration.

Rehabilitation exercises addressing each of the cardiopulmonary, neurological, vestibular, musculo-skeletal, and mental/cognitive systems are recommended in a phased manner (beginning, building, and being phase). In the beginning phase, deep breathing (3 min), eye nodding, bed rolling, head nods, rocking on knees, *etc.* (3 min) can be tried. Subsequently, the patients can engage in crossbody patterns such as crawling, seating, or standing cross-crawl touches, marching, or walking (1 min). These can be followed by strength building by 'yawn to smile', biceps curls, shoulder press, heel raise, wall push-up, and mini-squats (1-3 min). Endurance building by 5-45 min walk can be done in a gradually increasing manner in all phases[18].

DISABILITY INCLUSIVE

Since the beginning of 2022, India has been facing the third wave of this pandemic. Considering the huge population and people with low socioeconomic status, rehabilitation of COVID-19 in the Indian setting can be an ideal example of other developing countries. In the Indian scenario, people with different abilities face different sorts of barriers during the pandemic. Moreover, globally people with disabilities (15% of the global population) have been affected negatively by the COVID-19 pandemic. The risk of death from COVID-19 (January 24-November 30, 2020) in England was (men: 3.1 times greater; female: 3.5 times greater) greater in people with disabilities than in those without disabilities [19]. As usual, ICU patients had more disabilities than ward patients [20]. A person with long COVID-19 will have a disability if his/her condition or any of its symptoms is causing physical or mental impairment that hinders one or more major life activities[21]. People with disabilities are susceptible to COVID-19. In India, Rights of Persons with Disabilities Act, 2016 (Section 8) guarantees the equal protection and safety for people with disabilities in such situations.

SPECIAL CLINICS AND COMMUNITY-BASED REHABILITATION

In the Indian setting, post-COVID rehabilitation clinics (adult and pediatric) are the key need currently like in the United States[22]. Community-based rehabilitation would be effective in the Indian setting to cater rehabilitation services. Falvey *et al*[23] showed how community-based therapists can help during pandemics. During the pandemic, the community health workers contribute to the COVID-19 response; it includes screening, referrals, arranging support for home care, staffing community-based isolation centres, and being involved in surveillance, contact tracing, service delivery to people with disabilities, home visit, outreach activities, and campaigns[24].

CONCLUSION

"Rehabilitation is an essential health service and crucial for achieving universal health coverage" (Rehabilitation 2030 initiative). Needless to say, globally rehabilitation for people with COVID-19 and post-COVID-19 syndrome is the key unmet need. We would like to conclude this chapter with some recent advances which can further the rehabilitation process, especially artificial intelligence (AI) and telerehabilitation. A patient-centric individualized AI system for home-based rehabilitation is beneficial [25,26]. AI can be useful to make early detection of long-COVID symptoms. Hassanien *et al*[27] showed machine learning to better understand and predict the reaction of patients to the disease and the possibilities that they may have for recovery. Furthermore, the TERECO trial showed the effectiveness of telerehabilitation in COVID-19 survivors[28]. Werneke *et al*[29] showed the importance of telerehabilitation during pandemic times in outpatient rehabilitation settings. Telemonitoring is generally recommended 1-4 times in the first-month post-discharge. Follow-up recommendation is based on the Barthel Index score: level 1 (0-39, dependent, weekly telemonitoring), level 2 (40-79, partially dependent, biweekly telemonitoring), and level 3 (80-100, independent, monthly telemonitoring). In this ultra-modern era with cutting-edge technology, at the end of the chapter we would like to emphasize that prevention against the development of disease or disability is far easier and more economical than the disease itself. All you need to do is to practice basic personal hygiene, a healthy active lifestyle, and comprehensive rehabilitation to prevent disease, disability, and death. Finally, rehabilitation need identification is an important step to evaluate long COVID-19 patients so that we can recognize them early to prevent disability[30].

Take-home message

What are the issues that individuals need to be supported in rehabilitation: Rehabilitation needs should be identified according to the ICF.

Currently, studies are going on to find out rehabilitation needs in these patients. However, physicians have to identify needs by focusing each body system and emphasizing on patient's functional aspects.

Early rehabilitation intervention even when patient is in ICU is of utmost importance apart from post-discharge rehabilitation.

How should these requirements be met: Proper rehabilitation facilities and infrastructures as mentioned in Rehabilitation Initiative 2030 (World Health Organization) should be provided.

A multidisciplinary post-COVID rehabilitation clinic at tertiary hospitals, as well as outdoor and indoor COVID-19 rehabilitation facility, is required.

Future research: Observational studies are needed to find out post-COVID rehabilitation need in able-bodied individuals and also in differently-abled individuals.

Future trials are required to find out the effectiveness of specific interventions to improve functional status of people with long COVID.

FOOTNOTES

Author contributions: Swarnakar R contributed to conception and design; Swarnakar R and Yadav SL contributed to literature search and writing.

Conflict-of-interest statement: All authors declare that they have no conflict of interest.

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Country/Territory of origin: India

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S-Editor: Wang LL
L-Editor: Wang TQ
P-Editor: Wang LL

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World Journal of Methodology

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World J Methodol 2022 July 20; 12(4): 246-257

DOI: 10.5662/wjm.v12.i4.246

ISSN 2222-0682 (online)

MINIREVIEWS

Gut microbiota interactions with anti-diabetic medications and pathogenesis of type 2 diabetes mellitus

Ravi Kant, Lakshya Chandra, Vipin Verma, Priyanshu Nain, Diego Bello, Siddharth Patel, Subash Ala, Rashmi Chandra, Mc Anto Antony

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Parker J, Australia; Sitkin S, Russia

Received: March 1, 2022
Peer-review started: March 1, 2022
First decision: April 19, 2022
Revised: May 3, 2022
Accepted: June 18, 2022
Article in press: June 18, 2022
Published online: July 20, 2022

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Abstract

Microorganisms including bacteria, viruses, protozoa, and fungi living in the gastrointestinal tract are collectively known as the gut microbiota. Dysbiosis is the imbalance in microbial composition on or inside the body relative to healthy state. Altered Firmicutes to Bacteroidetes ratio and decreased abundance of Akkermansia muciniphila are the predominant gut dysbiosis associated with the pathogenesis of type 2 diabetes mellitus (T2DM) and metabolic syndrome. Pathophysiological mechanisms linking gut dysbiosis, and metabolic diseases and their complications include altered metabolism of short-chain fatty acids and bile acids, interaction with gut hormones, increased gut microbial metabolite trimethylamine-N-oxide, bacterial translocation/Leaky gut syndrome, and

endotoxin production such as lipopolysaccharides. The association between the gut microbiota and glycemic agents, however, is much less understood and is the growing focus of research and conversation. Recent studies suggest that the gut microbiota and anti-diabetic medications are interdependent on each other, meaning that while anti-diabetic medications alter the gut microbiota, the gut microbiota also alters the efficacy of anti-diabetic medications. With increasing evidence regarding the significance of gut microbiota, it is imperative to review the role of gut microbiota in the pathogenesis of T2DM. This review also discusses the interaction between gut microbiota and the various medications used in the treatment of T2DM.

Key Words: Metabolic disease; Gut microbiota; Cardiovascular disease; Short chain fatty acid; Dysbiosis; Trimethylamine-N-oxide

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Core Tip: Gut microbiota influence the pathogenesis of type 2 diabetes mellitus (T2DM) and metabolic syndrome through multiple mechanisms. The role of dysbiosis and various pathophysiological mechanisms such as altered metabolism of short-chain fatty acids, interaction with gut hormones, increased gut microbial metabolite trimethylamine-N-oxide and bacterial translocation in the pathogenesis of T2DM and cardio-metabolic diseases have been extensively studied. With increasing evidence regarding the significance of gut microbiota, it is imperative to review the role of gut microbiota in the pathogenesis of T2DM. This review also discusses the interaction between gut microbiota and the various medications used in the treatment of T2DM.

Citation: Kant R, Chandra L, Verma V, Nain P, Bello D, Patel S, Ala S, Chandra R, Antony MA. Gut microbiota interactions with anti-diabetic medications and pathogenesis of type 2 diabetes mellitus. *World J Methodol* 2022; 12(4): 246-257

URL: https://www.wjgnet.com/2222-0682/full/v12/i4/246.htm

DOI: https://dx.doi.org/10.5662/wjm.v12.i4.246

INTRODUCTION

Diabetes mellitus is a common chronic endocrine disorder with an estimated global burden of 537 million adults worldwide and projections indicate that the number of diabetic patients worldwide, will reach 700 million by 2045[1]. Diabetes is characterized by raised blood glucose levels arising as a consequence of decreased insulin production, resistance to insulin action or both. Traditional risk factors of developing type 2 diabetes mellitus (T2DM) include family history of diabetes, advancing age, obesity, sedentary lifestyle and poor-quality diet. Over the last decade, multiple studies have indicated a possible causal role of alterations in gut microbiota with development of T2DM[2-4]. Various studies are exhaustively exploring the role of gut microbiota as a biomarker for T2DM and a possible therapeutic intervention to treat T2DM[5-9].

Microorganisms including bacteria, viruses, protozoa, and fungi living in the gastrointestinal tract (GI) are collectively known as the gut microbiota. Over 100 trillion microbes live in our gut, particularly in the large intestine[10]. Taxonomically bacteria are classified as species, genus, family, order and phylum. Human gut microbiota is primarily composed of 5 phyla namely Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Cerrucomicrobia[11]. Firmicutes (*i.e., Bacillus* spp.) and Bacteroidetes (*i.e., Bacteroides* spp.) account for 90% of the gut microbiota community[11]. Their primary physiological roles in humans include protection against pathogens, producing vitamin B and K as well as bile acids, and a very pivotal role in host metabolism and immune modulation[12,13]. The composition of gut microbiota is regulated by factors such as genes, diet, geographical factors and medication use[13-15].

The development of PCR-based techniques has shown the way for the characterization and quantification of bacterial composition *via* sequencing of bacterial genes in human fecal sample. This has enabled scientists and physicians around the world to understand the role of gut microbiota and its interplay with multiple pathological conditions. In this review article we discuss the role of gut microbiota in the development of T2DM and therapeutic action of anti-diabetic drugs.

GUT DYSBIOSIS AND ITS ROLE IN PATHOPHYSIOLOGY OF T2DM

Dysbiosis is the imbalance in microbial composition on or inside the body relative to healthy state. Dysbiosis is associated with several autoimmune and inflammatory pathological conditions including allergies, central nervous system disorders, cancers, metabolic syndrome, diabetes mellitus, polycystic ovarian syndrome and cardiovascular disease[16,17]. Altered Firmicutes to Bacteroidetes ratio and decreased abundance of Akkermansia muciniphila are the predominant gut dysbiosis associated with the pathogenesis of T2DM and metabolic syndrome (Figure 1).

The ratio of Firmicutes to Bacteroidetes is increased in obese patients and during consumption of high calorie diets[2,18-21]. The altered ratio of these two major phyla leads to impaired glucose metabolism and an increase in obesity[22]. Decreasing the amount of Firmicutes and increasing the proportion Bacteroidetes leads to weight loss and reduced inflammation. T2DM is also associated with dysbiosis and have shown decreased abundance of Bacteroides[3,19] and propionate-producing bacteria such as Akkermansia muciniphila[3,4,19,23].

Akkermansia muciniphila is an anaerobic gram-negative bacteria that has shown to affect glucose metabolism, lipid metabolism and promote intestinal immunity[24]. Akkermansia muciniphilais found in abundant quantity in gut mucosa which utilizes mucin as its energy source and produces mucin degrading enzymes[25] thereby playing a crucial role in gut barrier function. Goblet cells in the gastrointestinal tract produce a thick layer of mucus which serves as a protective barrier against pathogens. The breakdown of intestinal mucosal barrier seen in patients with diabetes can be altered by this microbiota *via* its mucus secreting action. Akkermansia-induced extracellular vesicles may help regulate gut permeability[24]. Lipopolysaccharide (LPS) is an endotoxin derived from gram negative bacteria and plays a role in increasing gut permeability and thereby promoting the inflammatory process[26]. LPS level in blood has been shown to be elevated in high fat diet (HFD) mice and mice with diabetes, which decreases following the administration of Akkermanisa[26].

Akkermansia muciniphila also upregulates the endogenous production of GLP1 thereby increasing postprandial insulin secretion[27]. In humans, levels of Akkermansia muciniphila were found to be decreased in diabetes mellitus and obesity whereas the levels of Akkermansia muciniphila increased with treatment with anti-diabetic drugs and weight loss bariatric surgery[28-30]. Akkermansia muciniphila, in fact, is considered a next generation probiotic as there is large body of evidence linking decreased abundance of Akkermansia muciniphila with development of diabetes and obesity[31,32].

Research over last couple of decades have elucidated several pathophysiological mechanisms by which gut microbiota influences the pathogenesis of T2DM and metabolic syndrome. Pathophysiological mechanisms linking gut dysbiosis, and metabolic diseases and their complications include altered metabolism of short-chain fatty acids (SCFAs), interaction with gut hormones, increased gut microbial metabolite trimethylamine-N-oxide (TMAO), bacterial translocation/Leaky gut syndrome, and endotoxin production such as LPS.

Complex sugars are metabolized into SCFA in the colon by gut microbiota, which are known to reduce inflammation and improve glucose homeostasis (Figure 2). These SCFA specifically butyrate, acetate and propionate modulate the insulin release and hunger by increasing endogenous Glucagon like peptide1 (GLP1) and Protein YY (PYY) secretion[33]. The mechanism of action of this process is based on the interaction between SCFAs and G-protein-coupled free fatty acid receptors GPR41 and GPR43. SCFAs directly bind to receptor GPR41 and GPR43 to mediate release of GLP1 and PYY from intestinal L cells[34].

GLP-1 secreted by intestinal L cells increases the secretion of glucose-induced insulin from pancreatic β-cells, decreases the secretion of glucagon and delays gastric emptying[35]. GLP1 receptor analogs are an established method of treating T2DM nowadays. Pancreatic islet-derived PYY plays an important role in controlling glucose homeostasis through the modulation of β-cell mass as well as by increasing insulin secretion[36].

Researchers and clinicians have been cautiously optimistic that gut microbiota modulation has the potential to be a novel therapeutic target for T2DM treatment. It has been noted that ingestion of fermentable dietary fibers increased SCFA concentration, whereas the high-fat diet reduced formation of SCFAs[33]. Chambers *et al*[37] showed that SCFA propionate supplementation significantly reduced weight gain in overweight adults by increasing their postprandial secretion of GLP-1 and PYY. A previous study also showed that per rectal administration of SCFA significantly raised the plasma GLP1 and PYY concentrations, thereby further suggesting the beneficial effects of gut microbiota modulation in patients with T2DM[38,39].

Dysbiosis also leads to increased inflammation and atherogenesis through the gut microbial metabolites, TMAO and its precursors. Choline is an important nutrient which is found in foods such as red meat, fish, poultry and eggs. Gut microbiota metabolizes choline into Trimethylamine (TMA) which is further transported to liver *via* portal venous circulation where TMA is oxidized into TMAO [39]. Plasma levels of TMAO are positively associated with degree of atherosclerosis in a dose dependent manner[40]. Several studies have implicated TMAO levels as a risk factor for cardiovascular disease and mortality[17,41]. However, recent studies have also shown association of higher TMAO levels with diabetes, gestational diabetes and obesity[42-44].

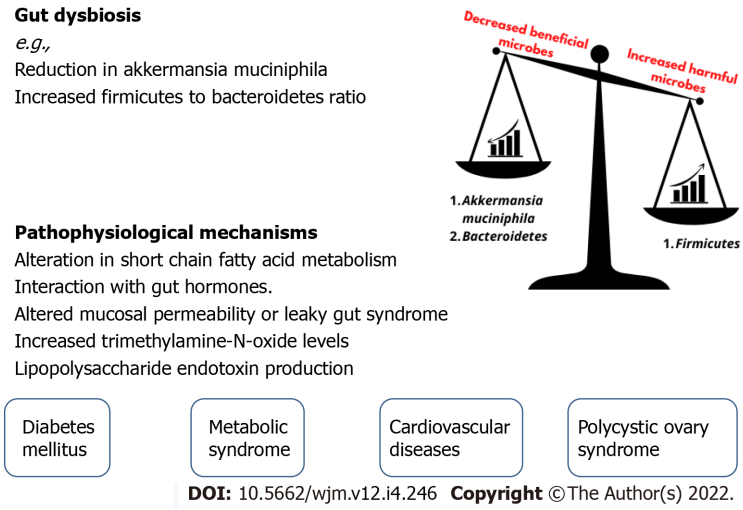

Figure 1 Gut dysbiosis and its role in pathophysiology of type 2 diabetes mellitus and cardio-metabolic diseases.

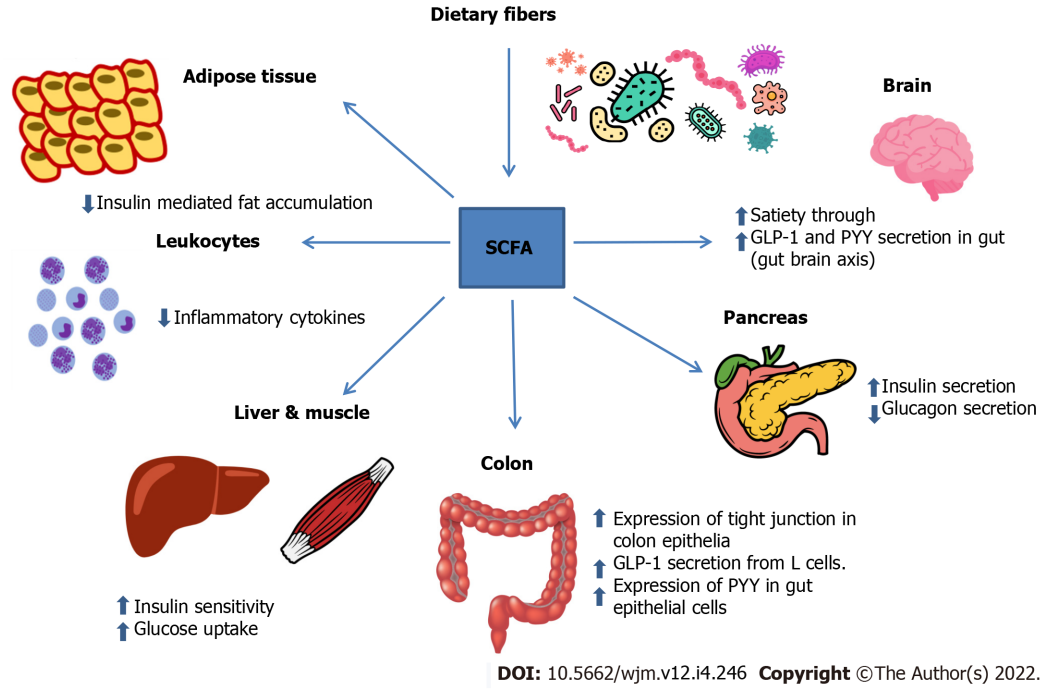

Figure 2 Role of gut microbiota and short-chain fatty acids in the pathophysiology of diabetes mellitus. SCFA: Short-chain fatty acids; GLP-1: Glucagon-like peptide-1; PYY: Peptide YY.

Dysbiosis also leads to disruption of gut epithelial barrier leading to excessive absorption of gut microbiota produced LPS. LPS is a strong endotoxin present in the outer membrane of gram-negative bacteria that can trigger an immune response associated with inflammation. Continuous absorption of LPS evokes a chronic inflammatory response and increased LPS levels are associated with diabetes and insulin resistance[45].

IMPACT OF GUT MICROBIOTA ON ANTI-DIABETIC DRUGS

The composition of an individual's gut flora is known to have an influence on metabolism and glucose homeostasis. The association between the gut microbiota and glycemic agents, however, is much less understood and is the growing focus of research and conversation. Recent studies suggest that the gut microbiota and anti-diabetic medications are interdependent on each other, meaning that while diabetic medications alter the gut microbiota, the gut microbiota also alters the efficacy of diabetic medications. Below, the results of various studies surrounding the relationship between various glycemic

medications and the gut microbiota will be reviewed.

Since the composition of gut flora is known to influence glucose homeostasis, it is vital to understand the impact of anti-diabetic medications on the gut microbiota to fully comprehend their mechanism of action (Table 1).

METFORMIN

Metformin has the strongest data regarding impact of gut microbiota on its therapeutic effects among all anti-diabetic medications. Metformin use has shown to promote the growth of various SCFA-producing healthy bacteria[5,30]. In a double-blinded randomized control trial, Wu Hao and colleagues included treatment-naive patients with T2DM to receive either 4 months (mo) of metformin or placebo[5]. Treatment with metformin for 4 mo, compared to placebo, showed an increment in the following SCFA producing bacteria such as Blautia, Bacteroides, Butyricoccus, Bifidobacterium, Prevotella, Megasphaera and Butyrivibrio, and increase in fecal concentration of lactate as well as a trend towards an increase in the fecal concentration of succinate. In the same study, metformin treatment for 2 mo, led to an increase in the microbial genera such as Proteobacteria and Firmicutes[5].

Metformin use is also associated with an increase in the mucin degrading microbiota, Akkermansia muciniphila[29,30,46]. As described in detailed earlier in this article, Akkermansia muciniphila affects glucose metabolism through regulating gut permeability, decreasing LPS and increasing postprandial insulin secretion through interaction with GLP-1[24,26,27]. A study involving community-dwelling Colombian adults showed that participants with diabetes taking metformin not only had high abundance of gut microbiota known for production of SCFAs (Butyrivibrio, Bifidobacterium bifidum, Megasphaera, and an operational taxonomic unit of Prevotella) but also had higher relative abundance of Akkermansia muciniphila, in comparison to participants without diabetes[30].

Studies in mice have shown an association between metformin treatment and an increase in the abundance of Akkermansia muciniphila in the gut flora of mice that were placed on a high fat diets[29,46]. Metformin use has also shown to have a positive effect on the gut microbiota in mice on a normal diet[46]. An abundance of microbes belonging to families such as Rikenellaceae, Ruminococcaceae, and Verrucomicrobiaceae, and an abundance of microbes belonging to species such as Alistipes, Akkermansia, and Clostridium were noted in the experimental mice with normal diet plus metformin treatment than in the control group[46,47].

There is also a suggestion that the cardiovascular protective effects of metformin may be mediated by gut microbiota. Metformin treatment in db/db mice with T2DM resulted in a twofold reduction in the concentration of TMAO and also decreased bacterial production rate of TMAO precursors[44]. Authors postulated that reduction in TMAO levels with metformin use may contribute to cardiovascular benefits of the drug.

Based on the large body of evidence summarized above, it is safe to say that metformin has consistently shown a beneficial effect towards improving the gut health and cardiovascular health.

GLP-1 RECEPTOR AGONISTS

GLP-1 is an incretin hormone secreted by the intestinal endocrine cells known as the L cells, in response to food ingestion and causes glucose-mediated insulin secretion from the beta cells of the pancreas, concomitant suppression of glucagon from the alpha cells of the pancreas and a decrease in gastric emptying[6]. GLP-1 receptor agonists (GLP-1 RAs) use in patients with T2DM not only results in improved glycemic control but has also shown to promote weight loss, favorable effects on blood pressure and cholesterol, and decreased cardiovascular morbidity and mortality[48]. Therefore, there has been a great interest in the research community to understand underlying mechanisms resulting in GLP-1 RAs therapeutic benefits.

Limited data available on impact of GLP-1 RAs on gut microbiota suggests that clinical benefits of GLP-1 RAs may be mediated by modulation of gut microbiota. Current data suggests that GLP-1 expression could be stimulated by the binding of SCFAs, which are produced by the degradation of carbohydrates by the gut bacteria, to the free fatty acid receptor 2[19]. GLP-1 RAs have shown to be associated with decreased dysbiosis particularly increase in Bacteroidetes to Firmicutes ratio, decrease in obesity-related and an increase in lean-related microbiota phenotypes, and an increase in abundance of Akkermansia[49-52].

Gut microbiota in obese people lack microbial diversity and specifically there is a decline in the Bacteroidetes population along with an abundance in the Firmicutes population resulting in decreased Bacteroidetes to Firmicutes ratio[46]. This was shown in a recent study which compared the fecal microbiota of European children (EU) and the children from Burkina Faso (BF), a rural African village where the diet is rich in fiber. There was a significant abundance in bacteroidetes and a reduction in Firmicutes in the BF children in comparison to the EU children. In one study, several mouse models that were subjected to a probiotic known as VSL#3, led to a suppres-sion of weight gain and insulin

Table 1 Impact of anti-diabetic medications on the gut microbiota

Drug	Changes in microbiota
Metformin	Increase in SCFA producing bacteria[5,30], Akkermansia muciniphila[29,30,46], Firmicutes[5] and Proteobacteria[5]; Increased fecal concentrations of lactate and succinate[5]; Decreased concentration of TMAO and its precursor metabolites[44]
Liraglutide	Increase in Bacteroidetes to Firmicutes ratio[49] and Akkermansia[50]; Increase in lean related phenotypes (Blautia and Coprococcus) [49]; Decrease in Obese related phenotypes (Romboutsia, Ruminiclostridium and Erysipelotrichaceae)[49]
Dulaglutide	Increase in Bacteroidetes to Firmicutes ratio[51]
Sitagliptin	Increase in Bacteroidetes to Firmicutes ratio[7]; Increase in SCFAs and other organic acids like succinate[54]
Saxagliptin	No change in Bacteroidetes to Firmicutes ratio[49]; Obesity related phylotype= Decrease in only one genus Candidatus Arthromitus[49]; Lean related phenotype= Increase in the family Lactobacillaceae but Decrease in genus Balutia and Coprococcus[49]
Vildagliptin	Increase in lactobacillus species and propionate[8]; Decrease in Oscillibacter species[8]
Linagliptin	Increase in Bacteroidetes and decrease in Protobacteria species[8]
Empagliflozin	Increase in Bacteroidetes to Firmicutes ratio[51]
Dapagliflozin	Increase in Bacteroidetes to Firmicutes ratio[9]; Increased Oscillospira and Akkermansia muciniphila species[9]
Canagliflozin	Increase in Bacteroidetes to Firmicutes ratio[57]; Increase in Olsenella[57], Alistipes[57], Alloprevotella[57] and Lactobacillus species[58]; Decrease in Helicobacter and Mucispirillum species[57]
PPARγ agonists	Firmicutes and Fusobacteria stimulate PPAR gamma activity[63]
Acarbose	Increase in Lactobacillus and Dialister genera[66]; Decrease in Butyricicoccus, Phascolarctobacterium, and Ruminococcus genera[66]; Increase in the ratio between primary bile acids and secondary bile acids[70]
Sulfonylureas	Glicazide have not shown any significant differences on gut microbiome in diabetic patients after 12 wk of intervention[68]

SCFA: Short chain fatty acid; TMAO: Trimethylamine N-oxide.

resistance by altering the gut microbiota. VSL#3 specifically decreased the quantity of Firmicutes and increased the quantity of Bacteriodetes, a change which was associated with an increase in Butyrate production which in turn increased the secretion of GLP-1 from the intestinal L-cells[53].

The above beneficial alteration of gut microbiota is also seen with liraglutide administration. A study showed an increase in the Bacteroidetes to Firmicutes ratio leading to weight loss regardless of the glycemic status in mice with liraglutide use[49]. This study also showed a decrease in obesity-related phylotypes such as Romboutsia, Ruminiclostridium and Erysipelotrichaceae, and an increase in lean-related phylotypes such as Blautia and Coprococcus in mice treated with liraglutide[49].

Like metformin, liraglutide has also been associated with an increased in the presence of Akkermansia[50]. In fact, one study comparing the effect of metformin *vs* liraglutide on the gut microbiota in patients with T2DM, showed higher concentrations of Akkermansia in subjects receiving Liraglutide compared to metformin[50].

Dulaglutide is another GLP-1 agonist used in the treatment of T2DM. Currently, there is limited data on the impact of dulaglutide use on gut microbiota. However, one recent study showed a decrease in the pro-inflammatory pathways and microbiota dysbiosis, specifically an increase in the Bacteroidetes to Firmicutes ratio, in non-diabetic mice with non-alcoholic steatohepatitis after treatment with either dulaglutide or empagliflozin, or both (NASH)[51].

To date, there are no studies looking at the effect of Semaglutide and Exenatide on gut microbiota.

Given the literature showing favorable modulation of gut microbiota with GLP-1 agonists use and our current understanding of role of gut microbiota in the pathophysiology of T2DM and metabolic syndrome, it is not unreasonable to hypothesize that GLP-1 agonists may exert their therapeutic benefits in patients with T2DM through alteration of gut microbiota. However, further studies are needed, particularly in human subjects, to validate these findings and improve our understanding of this topic.

DIPEPTIDYL PEPTIDASE 4 (DPP-4) INHIBITORS

Sitagliptin has shown to exert a beneficial effect on the gut microbiota. Liao X *et al*[7] analyzed the effects of Sitagliptin and acarbose on the gut microbiota in mice on high fat diet. The alteration in 24 genera induced by high fat diet were protected by sitagliptin. A total of 75% of genera increased by sitagliptin belonged to Bacteroidetes and 87.5% of genera decreased by sitagliptin belonged to Firmicutes thus resulting in increased Bacteroidetes to Firmicutes ratio[7]. This study also performed metabolomics analysis which demonstrated that DPP-4 inhibitors changed the pattern of metabolites

linked to carbohydrate, amino acid and nucleic acid metabolism. There was a trend towards an increase in SCFAs and other organic acids like succinate, both of which are already known to improve glucose tolerance and insulin sensitivity[54].

Saxagliptin was compared with liraglutide in one study to evaluate their individual effects on gut microbiota in mice[49]. Although liraglutide showed a prominent effect on the microbial diversity as mentioned in the subsection of GLP-1 RAs above, saxagliptin did not show any significant shift of the microbial composition. Among the liraglutide treated group, there was a significant reduction in all the obesity-related phylotypes whereas only one phylotype (genus Candidatus Arthromitus) decreased with saxagliptin. With regards to the lean-related phylotypes, although both medications led to a similar enrichment in the family Lactobacillaceae and the genera Lactobacillus and Turicibacter, only liraglutide caused an enrichment of the genus Balutia and the genus Coprococcus and these two were decreased in the saxagliptin group. There were also no significant changes in the phyla Firmicutes and Bacteroidetes [49].

Vildagliptin has also shown to impact the composition of the gut microbiota and its metabolic activity. In one study, male mice placed on a western diet plus vildagliptin not only showed a significant reduction in DPP-4 activity in the feces but also a reduction in Oscillibacter spp, and an increase in lactobacillus spp and propionate[8].

Linagliptin was studied along with a sulfonylurea in diabetic patients already on treatment, to evaluate their impact on human gut flora. Following 4 wk of treatment with either medication in a total of 5 patients with MODY and 19 patients with T2DM, there was no significant changes in the gut microbiota[55]. Another study evaluated the changes caused by linagliptin and a purified Peroxisome proliferator-activated receptor- alpha (PPAR-alpha) agonist (WY14643) on various GI parameters such as gut microbial composition, intestinal barrier integrity, endotoxemia, and hepatic energy metabolism in mice on a high-fructose fed diet (HFRU). The HFRU group showed glucose intolerance, endotoxemia, dysbiosis with increased Proteobacteria and a parallel decrease in Bacteroidetes, significant liver inflammation and steatosis. The Linagliptin and PPAR-alpha agonist group in comparison to the control group, had a positive impact on all the above pathological changes which included restoration in the abundance of Bacteroidetes, a significant decrease in Protobacteria species, protection of the intestinal ultrastructural damage, restoration of the intestinal permeability and improvement in hepatic steatosis *via* beta oxidation[56].

Based on the current evidence summarized above, not all DPP4 inhibitors seem to have a positive impact on gut microbiota. The limited studies involving Linagliptin may have shown a benefit due its combination with a PPAR- alpha agonist, which is known to play a role in intestinal cell metabolism, differentiation, and inflammation. Although, the studies involving Sitagliptin and Vildagliptin have shown a benefit, they were conducted in mice. Future studies in humans are awaited to see if the results from the current studies can be replicated or not.

SGLT-2 INHIBITORS

Empagliflozin has been studied along with liraglutide in non-diabetic mice with NASH, to examine their effects individually or in combination, on inflammatory pathways, hepatic steatosis and microbiome dysbiosis[51]. After placing the mice on a high-fat-high-fructose diet with cholesterol surplus for 12 wk, they were randomized to receive either empagliflozin or dulaglutide or both. Neither medication showed an effect on hepatic steatosis in the non-diabetic mice. Only dulaglutide, as a single agent and in combination with empagliflozin showed a beneficial effect on weight loss, glucose homeostasis, anti-inflammatory, and anti-fibrotic pathways. There was no beneficial effects seen with empagliflozin alone. Nevertheless, both medications, alone and in combination, showed a beneficial effect on gut microbiota with an increase of Bacteroidetes and a decrease of Firmicutis[51].

Dapagliflozin has also shown to mildly alter the gut microbiota composition in mice with T2DM. Eight wk after being randomized to receive either a standard diet *vs* a standard diet with dapagliflozin, male diabetic mice in the dapagliflozin group increased the Bacteroidetes to Firmicutes ratio, and increased Oscillospira and Akkermansia muciniphila. It also significantly lowered arterial stiffness and caused a reduction in hyperglycemia and inflammatory markers[9].

Canagliflozin was studied in male mice after inducing T2DM in them by giving a HFD for 24 wk[57]. Various cardio-metabolic parameters and changes in the colonic gut microbiota were assessed. Following treatment with canagliflozin, there were reductions in the lipid profile which was associated with lowering the index for atherogenesis and arteriosclerosis, a reduction in the vascular basement membrane thickness and markers of oxidative stress. It also altered the ratio of Firmicutes to Bacteroidetes from 230% to 98%, increased the abundance of Olsenella, Alistipes and Alloprevotella, and decreased the abundance of Helicobacter and Mucispirillum in mice with diabetic cardiovascular disease[57].

Another study assessed the effect of canagliflozin on the gut microbiota and the serum concentrations of gut-derived uremic toxins in 5/6th nephrectomized (Nx) rats[58]. Canagliflozin improved the concentration of Lactobacillus bacteria, a bacterium which is known to have the ability to maintain the

expression of tight junction proteins and thereby prevent the accumulation of uremic toxins in the serum of chronic kidney disease patients. Indeed, this study showed that canagliflozin increased the expression of the tight junctions' proteins in the ascending colon which were low in the Nx rats. Consequently, the serum concentration of gut-derived uremic toxins which were significantly elevated in the Nx rats were lowered significantly by Canagliflozin[58].

Based on the literature evidence summarized above, SGLT-2 inhibitors have a positive impact on the gut microbiota. It is well known that SGLT-2 inhibitors are effective in treating DM and in providing CV protection. Future studies are awaited to understand whether these beneficial effects are in part due to their action on the gut microbiota.

PPAR AGONISTS

PPAR gamma, a nuclear receptor is vastly present in the colon[59] where it is involved in the intestinal cell metabolism, differentiation and inflammation[60]. It is closely linked to various pathological conditions including diabetes which is linked to the gut microbiota. Evidence shows the PPAR gamma agonists can help reduce gut inflammation, colon cancer and diabetes[61,62]. PPAR-gamma activity has been shown to be induced by gut microbiota. A study in humans assessed the involvement of various gut bacterial strains belonging to the major phyla such as Firmicutes, Bacteroides, Actinobacteria and Fusobacteria on PPAR gamma activity located within the intestinal epithelial cells[63]. These bacteria were anaerobically cultured and a specific reported cell line called HT-29-PPAR gamma was used to identify the bacteria with PPAR gamma activity regulation. At the level of phyla, Firmicutes and Fusobacteria showed the strongest effect while Actinobacter showed mild to no effect. Roseburia hominis and Roseburia intestinalis within the Firmicutes phyla and Fusobacterium naviforme within the Fusobacteria phyla exhibited the strongest capacity to stimulate PPAR gamma activity.

As shown above, an agonistic effect on PPAR gamma receptors that are widely present throughout the colon, can have a positive impact on gut health. However, the current evidence is limited, and it is compounded by the infrequent use of medications belonging to this class. Hence, it will be interesting to see if future studies look more closely into the relationship between PPAR gamma receptor agonism and gut microbiota.

ALPHA GLUCOSIDASE INHIBITORS

SCFA's, including butyrate play an important role in pathophysiology of diabetes. Patients with T2DM have a decline in the abundance of butyrate-producing bacteria[64]. Acarbose has shown to increase the serum butyrate levels in patients with impaired glucose tolerance. Oral supplementation of butyrate in mice, has shown to improve insulin sensitivity and increase energy expenditure *via* mitochondrial action[65]. Zhang *et al*[66] performed a study in 52 Chinese patients with prediabetes, who were assigned randomly to receive either acarbose or placebo, to characterize the gut microbiota. The baseline gut microbiota composition in the fecal samples of these prediabetic patients showed an abundance in the genera Bacteroides (19.4%) and Faecalibacterium (8.97%), and an abundance in Firmicutes (68.53%), Bacteroidetes (27.85%), Protobacteria (1.98%) and Actinobacteria (0.98%) at the level of phyla. Acarbose treatment led to an enrichment in five genera, including Lactobacillus and Dialister and there was a corresponding decline in six genera, including Butyricicoccus, Phascolarctobacterium, and Ruminococcus[66]. The same study also showed that some species of Megasphaera thrived following acarbose treatment. This species has shown to have many beneficial effects such as conversion of carbohydrates to SCFA's, including butyrate, acetate, valerate and formate. It also utilizes lactate, a harmful end product of carbohydrate metabolism and converts it into SCFA's, including propionate, acetate and butyrate[67].

SULFONYLUREAS

The data so far, suggest a lack of positive effect on the gut microbiota by the use of sulfonylurea. In one study, type 2 diabetic patients treated with metformin were randomized to receive either gliclazide or dapagliflozin to analyze their effect on gut microbiome. At the end of 12 wk, neither treatment significantly changed the gut microbiome alpha diversity or composition[68].

Bile acid metabolism and signaling is important for maintaining metabolic health. Changes in the composition and content of plasma bile acids are seen in patients with diabetes and/or obesity[69]. Gu Y *et al*[70] assigned treatment-naïve type 2 diabetes patients to receive either acarbose or glipizide to analyze the plasma bile acids and choose the appropriate anti-diabetic medication for treatment. Acarbose, but not glipizide, led to an increase in the ratio between primary bile acids and secondary bile acids. In the same study, acarbose caused an increase in the abundance of Lactobacillus and Bifidobac-

terium in the gut microbiota[70].

The lack of an alteration in the gut microbiota by sulfonylureas may be partly due to the limited studies that have investigated its role in gut health. However, current literature has shown no organ protection action including cardiovascular protection from the use of a sulfonylurea. This poses a question about its role in gut health and future studies are needed for a better clarification.

CONCLUSION

Recent studies have remarkably improved understanding of the role of the gut microbiota in the pathophysiology of T2DM and metabolic diseases. The role of dysbiosis in the various pathophysiological mechanisms related to altered metabolism of SCFAs, interaction with gut hormones, increased gut microbial metabolite TMAO and endotoxemia in the pathogenesis of T2DM and cardio-metabolic diseases have been demonstrated in numerous studies. The impact of gut microbiota on the therapeutic effects of anti-diabetic medications is becoming increasingly recognized. Altering the gut microbiota is proposed as an attractive method to decrease inflammation and weight gain, improve glucose homeostasis, and prevent cardio-metabolic diseases. The current review has outlined the role of the microbiota in the pathophysiology of T2DM and highlighted the interplay between anti-diabetic medications, the microbiota and some of the known pathophysiological mechanisms. In future, the gut microbiota may be a novel target for new drug development to prevent and treat T2DM and metabolic diseases. However, further studies are needed prior to successful clinical application of gut microbiota modulation.

FOOTNOTES

Author contributions: Kant R, Chandra L and Antony MA designed the outline, performed the writing, prepared the figure and edited the paper; Verma V, Nain P, Bello D and Patel S performed the writing, and prepared the table and figure; Ala S and Chandra R provided the input in writing the paper, performed the writing and edited the paper.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

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S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

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World J Methodol 2022 July 20; 12(4): 258-263

DOI: 10.5662/wjm.v12.i4.258

ISSN 2222-0682 (online)

MINIREVIEWS

Reinfection, recontamination and revaccination for SARS-CoV-2

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Specialty type: Infectious diseases

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Suravajhala PN, India; Vij M, India

Received: March 7, 2022
Peer-review started: March 7, 2022
First decision: April 12, 2022
Revised: April 22, 2022
Accepted: July 11, 2022
Article in press: July 11, 2022
Published online: July 20, 2022

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Abstract

The reports on coronavirus disease 2019 (COVID-19) describe the pandemic in waves. Similar to the ocean's waves, the frequency and amplitude of the number of new cases and the number of deaths were globally quite regular; nevertheless, they showed important regional irregularities and the direction of spread has been generally rather unpredictable for COVID-19. One of the major reasons for the repeated outbreaks is the mutating capacity of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that allows the virus to infect persons who have natural immunity or have been vaccinated. Vaccination began in vast campaigns from the second year of the pandemic that was supposed to decrease the magnitude of the waves. Although it reduced the complications, the expected attenuation of the disease expansion has not yet been met. This paper provides a short overview of the most recent data on the rate of reinfection in vaccinated and non-vaccinated individuals. It points out that testing positive for a second time for SARS-CoV-2 does not necessarily mean a reinfection; it can also be interpreted as recontamination. The symptom free outcome as well as the rapid reconversion of the polymerase chain reaction test may help to determine the difference between reinfection and recontamination. Awareness of this phenomenon may be valuable in times of human resource difficulties. The available evidence may suggest that the protective value of a prior infection could be better considered for vaccine distribution in the future.

Key Words: SARS-CoV-2; COVID-19; Polymerase Chain Reaction; Immunisation; Contamination; Vaccination

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Core Tip: Reinfection: There is not enough evidence of the protective efficacy of the natural immunity induced by a primary infection with severe acute coronavirus 2 (SARS-CoV-2). Recontamination: Testing positive for a second time for SARS-CoV-2 does not necessarily mean a reinfection; it can also be interpreted as recontamination. Revaccination: The available evidence may suggest that the protective value of a prior infection could be better considered for vaccine distribution in the future.

Citation: Kullmann T, Drozgyik A. Reinfection, recontamination and revaccination for SARS-CoV-2. *World J Methodol* 2022; 12(4): 258-263
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/258.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.258

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected more than 400 million people worldwide and caused the death of over 6 million[1]. In the last two years SARS-CoV-2 has become the most common cause of death from a single infectious pathogen, preceding *Mycobacterium tuberculosis*, responsible for an estimated 1.4 million victims in 2019[2] and human immunodeficiency virus and malaria, the mortality of which was below 1 million in the last years[3].

The majority of deceased people were retired Caucasians[1]. The geography of the disease expansion may explain why coronavirus, which was most devastating in North America and Europe, received outstanding media and political attention in comparison to other infections with high mortality even if these infections affect young people as much as the elderly. Reports directly showing patients with respiratory assistance in hospital intensive care units were seen by many people for the first time. Beyond the statistical data these widely diffused images contributed to the shocking experience of the pandemic. Coronavirus disease 2019 (COVID-19) is the first pandemic in history to be broadcast live from the beginning on.

There is no efficacious treatment for COVID-19. Hospitalisation may help in those who require oxygen supplementation and in the care of some complications of the disease. Vaccines of different types have been developed to provide protection against infection. This occasion was a world first for the mRNA vaccines[4,5] and the first adenovirus-based vaccine authorized by the US Food and Drug Administration[6]. To date their efficacy in the prevention of severe complications of COVID-19 is evident but their power to reduce disease spread has not met expectations[1].

REINFECTION

The first reinfection by a different strain of SARS-CoV-2 was identified in the summer of 2020 with whole genome sequencing and comparative genome analysis in an immunocompetent person with an interval of 142 d between the two episodes[7]. In this case, the primary infection was symptomatic and the reinfection was asymptomatic. A larger analysis of several cases found that the reinfection may be either less severe, or may also have a more severe outcome as compared to the primary infection[8].

When the vaccinations started in spring 2021, follow-up of the protective effect of recovering from a primary infection became problematic, as the promotion of vaccination was so strong in most affected countries, that the majority of the people were vaccinated. Nevertheless, there are some publications available that may help elucidate this issue.

No symptomatic reinfection was detected in 1265 British health care workers who had been followed with positive anti-spike-IgG for 31 wk[9]. In the national, federated database of Qatar there were 350.000 polymerase chain reaction (PCR)-confirmed infections registered between 28 February 2020 and 28 April 2021. Among these cases 1300 reinfections were identified and these cases were matched with primary infections in a 1:5 ratio. The numbers of severe, critical and fatal cases were 158, 28 and 7 for primary infections and 4, 0 and 0 for reinfections, respectively. Vaccinated persons were excluded from the analysis. Severe outcome meant hospitalisation and critical outcome meant hospitalisation in the intensive care unit[10].

These data support the hypothesis that recovering from a primary SARS-CoV-2 infection yields natural immunity that protects from both, potential reinfection and the severe complications of a reinfection. However, vaccinations were declared to provide additional protection.

Breakthrough infections in vaccinated individuals and in those who had a prior infection were compared in the same Qatar database. The PCR cycle threshold is known to inversely correlate with viral load. Or, the cycle threshold value is 1.3 cycles higher for breakthrough infections following the BNT162b2 vaccine, 3.2 cycles higher for breakthrough infections following the mRNA-1273 vaccine, and 4.0 cycles higher for reinfections in unvaccinated individuals than at primary infection. Thus,

unvaccinated persons who recovered from a prior SARS-CoV-2 infection had the lowest viral load during a breakthrough infection as compared to their mRNA vaccinated counterparts[11]. In a Bangladesh cohort including 1644 participants, the naturally infected population was less likely to be reinfected by SARS-CoV-2 than the infection-naïve and vaccinated participants with one of the seven different vaccines authorised in this country[12]. A Danish study of 3.800 blood donors who had SARS-CoV-2 PCR positivity found no evidence of a decline in the proportion of detectable anti-SARS-CoV-2 antibodies over time up to 15 mo[13].

In contrast, in a study of 150.000 patients who had recovered from COVID-19 in Israel, those who were vaccinated had a lower risk of reinfection than those who were not vaccinated. The difference was smaller in the elderly population. The study did not report on the severity of the reinfections. The authors recognise that the lack of assessment of disease severity and hospitalisation is an important limitation of their work[14].

RECONTAMINATION

The second contact with SARS-CoV-2 is not necessarily a second infection and may only be a contamination, which means that some of the pathogen may be present on the body surface or mucus membrane. However, the invasion of adjacent tissues does not follow, as the person's defence system prevents it.

Someone contaminated with SARS-CoV-2 will have a positive test, and may possibly and transitionally transmit the virus but will remain asymptomatic. However, the duration of the positivity of a contaminated individual following primary infection or vaccination will be presumably short. In our experience, the duration of their positivity is around 5 d (unpublished data) as compared to the positivity of healthy individuals who undergo a first infection which is at least 8-20 d.

This is in reality what we may expect from the protective efficacy of vaccinations and natural immunity. They do not inhibit the virus reaching the nasal mucosa when in contact with an infected patient. Nevertheless, they provide a more reactive immunity that helps in preventing the development of the disease within the body.

The possible interpretations of a positive SARS-CoV-2 PCR test are summarised in Table 1. Under the pressure of the pandemic it may be difficult to accept that interpretation of the tests depend on the clinical situation; moreover, if the clinical context is omitted, decisions based exclusively on test results may be harmful. The importance of the correct interpretation of sustained PCR positivity at primary infection has been stressed, particularly in the case of comorbidities needing rapid treatment such as certain malignancies[15]. The authorisation of asymptomatic health care workers to return to work has become routine in many hospitals facing problems of human resources. Some other situations when a positive PCR test may be disturbing are listed in Table 2.

REVACCINATION

Initially, producers affirmed that two doses one month apart provide immunity for SARS-CoV-2, with the exception of Ad26.COV2-S with which one dose is equivalent to two doses of the other products. However, the level of protective antibodies was found to decrease with time; therefore, the potential necessity for a booster dose was discussed. It is important to note, that the waning of immunity was studied in vaccinated populations whereas for naturally immunised populations there are only observations from case series[16].

Currently, in most Western countries a booster is required 6 mo after the first vaccination for official recognition of protection. The suggestion that the booster may or should be different from the primary vaccine adds to the confusion related to the efficacy of each single vaccine. We agree with the WHO's consideration that in view of the shortage of vaccines, assuring booster doses for some populations may increase the possibility that other populations will miss even the primary vaccination[16].

In addition, the above-mentioned results[11,12,13] show that natural immunity may even be stronger and last longer than the effect of vaccination depending on both the severity of the infection and the type of vaccination. The distribution of vaccines to non-infected individuals rather than to naturally immunised individuals would probably have saved more lives and would certainly have been more equitable. This hypothetical redistribution would have concerned hundreds of millions of people.

DISCUSSION

One of the destabilising lessons of the pandemic is that scientific predictions concerning COVID-19's clinical presentation and geographical expansion were rarely correct.

Table 1 Possible meaning of a positive polymerase chain reaction test for severe acute respiratory syndrome coronavirus 2

Test result	Meaning
True positive result	Asymptomatic infection with SARS-CoV-2
True positive result	Symptomatic infection with SARS-CoV-2 (COVID-19)
Sustained positive result	Carriage of virus particles after recovering from COVID-19
False positive result	No infection with SARS-CoV-2
Repeatedly positive result	Reinfection with SARS-CoV-2
Repeatedly positive result	Recontamination with SARS-CoV-2

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Table 2 Possible situations, when the misinterpretation of contamination with severe acute respiratory syndrome coronavirus 2 may cause unfair disadvantage for the tested individual

Situations where symptom-free persons can be tested
Being a contact of an infected person
Infection control in a health care or social institution
Starting a new job
Travelling abroad
Participating at a controlled event

Measures seeming reasonable at one point may be completely useless a couple of weeks later and vice versa. For instance, the nationwide testing in Slovakia in the winter of 2020 drew international attention and the identification of a high number of asymptomatic infections gained recognition. It was assumed that containment of the detected individuals would prevent disease spread. Nevertheless, the country could not avoid the explosion of the disease and the burden on its healthcare system. In contrast, Sweden was much criticised for its liberal management of the pandemic and had a relatively high mortality rate in the first months; however, many more restrictive countries had worse outcomes one year later[1].

Decision making and observance of the prevalence are even more unpredictable than the behaviour of the virus. Decision makers are challenged with opposing expectations but miss essential references. They have to solve dilemmas such as protecting the lives of the elderly *vs* the jobs of the young or the equitable distribution of the vaccines *vs* the most rapid care of their own population. On the other hand observance supposes explanations and never meant obedience.

With the arrival of the Omicron strain there is some hope that after more than two years the disease will pass in a more controllable phase.

CONCLUSION

(1) Differentiation between recontamination and reinfection may be useful for persons testing positive for SARS-COV-2 by PCR; (2) The protective effect of prior infection should be considered before vaccination against COVID-19; and (3) Fairness in vaccine distribution should be respected at the global scale.

FOOTNOTES

Author contributions: Kullmann T and Drozgyik A contributed equally to this minireview.

Conflict-of-interest statement: All the authors declare having no conflict of interest related to the publication of this manuscript.

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S-Editor: Liu JH
L-Editor: Webster JR
P-Editor: Liu JH

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 July 20; 12(4): 264-273

DOI: 10.5662/wjm.v12.i4.264

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Prospective Study

Are we aware of radiation: A study about necessity of diagnostic X-ray exposure

Erdal Karavas, Bunyamin Ece, Sonay Aydın, Mehmet Kocak, Zeliha Cosgun, Isil Esen Bostanci, Mecit Kantarci

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Menendez-Menendez J, Spain; Yang B, China
A-Editor: Liu X, United States

Received: January 19, 2022
Peer-review started: January 19, 2022
First decision: March 24, 2022
Revised: March 27, 2022
Accepted: June 3, 2022
Article in press: June 3, 2022
Published online: July 20, 2022

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Abstract

BACKGROUND

Total exposure to ionizing radiation has nearly doubled in the last two decades. This increase is primarily due to increased computed tomography (CT) exposure. Concerns have been raised about the risks associated with patients' exposure to medical imaging radiation, which can increase a person's lifetime risk of developing cancer. Preventing unnecessary examinations becomes critical at this point. To avoid unnecessary examinations, it is necessary to understand the demanding process.

AIM

To ascertain clinicians' awareness of and reasons for requesting a CT examination.

METHODS

We developed an online questionnaire that included 20 questions about clinicians' awareness of radiation safety and their reasons for requesting a CT examination, as well as demographic information such as age, gender, and year of medical practice experience. Additionally, we asked participants the number of CT scans requested in a month, the patients' questions and approaches about the imaging

method, the effect of the patient's previous imaging history on the current imaging request, whether they believed that they had sufficient information about radiation doses, and whether they requested CT without an indication. We administered the questionnaire to clinicians from a variety of different professions in four different cities.

RESULTS

A total of 195 clinicians participated. Internal medicine specialists were the most crowded group (38/195, 19.5%). Mean age of the population was 33.66 ± 5.92 years. Mean year of experience was 9.01 ± 5.96. Mean number of requested CT scans in a month was 36.88 ± 5.86. Forty-five (23.1%) participants stated that they requested CT scans without clinical indication. The most common reasons for CT scan requests were work load, fear of malpractice, and patient demand/insistence.

CONCLUSION

CT scan requests are influenced by a variety of factors, both internal and external to the doctors and patients. Raising awareness of radiation safety and reducing fear of malpractice by limiting the number of patients per physician may result in a reduction in unnecessary CT examinations and ionizing radiation exposure.

Key Words: Ionizing radiation; Exposure; Tomography; Physicians; Knowledge; Awareness

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Core Tip: Total exposure to ionizing radiation has nearly doubled in the last two decades. This increase is primarily due to increased computed tomography (CT) exposure. Preventing unnecessary examinations becomes critical. We developed an online questionnaire about clinicians' awareness of radiation and their reasons for requesting a CT scan. The most common reasons for CT scan requests were work load, fear of malpractice, and patient demand/insistence. CT scan requests are influenced by a variety of factors. Raising awareness of radiation and reducing fear of malpractice by limiting number of patients per physician may result in a reduction in unnecessary CT examinations and radiation exposure.

Citation: Karavas E, Ece B, Aydın S, Kocak M, Cosgun Z, Bostanci IE, Kantarci M. Are we aware of radiation: A study about necessity of diagnostic X-ray exposure. *World J Methodol* 2022; 12(4): 264-273
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/264.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.264

INTRODUCTION

Normally, natural exposure to small doses of radiation is inherent in life. The average exposure is approximately 3 mSv/year. On average 2.4 mSv of the annual dose is due to radon and naturally-occurring radiation sources (natural background radiation) and 0.6 mSv is due to the man-made medical imaging and treatment methods[1].

Today, due to the development of technology and clinicians' easy access to medical imaging, ionizing radiation is one of the most used methods in diagnosis and treatment of diseases in daily practice[2-4].

Radiation is a potential carcinogen affecting many patients undergoing medical imaging worldwide. Total exposure to ionizing radiation has nearly doubled in the last decades. This increase is primarily the result of increased exposure from computed tomography (CT), nuclear medicine, and interventional fluoroscopy[5,6]. Concerns have been expressed about the risks associated with patients' exposure to medical imaging radiation[7,8]. Ionizing radiation exposure can damage DNA, increasing an individual's lifetime risk of developing cancer. The radiation doses associated with routine CT examinations are comparable to those received by individuals with a documented increased risk of cancer. For example, an increased risk of cancer has been identified in long-term survivors of the Hiroshima and Nagasaki atomic bombings who were exposed to 10 to 100 millisieverts of radiation[9,10]. A single CT scan can expose patients to an equivalent amount of radiation, and patients may undergo multiple CT scans over time[11,12]. While a single medical imaging exam with radiation does not pose a significant risk to an individual, the annual exposure to radiation from millions of imaging examinations with radiation is a significant public health problem. Additionally, accidental exposure to high doses of ionizing radiation can also result in short-term injuries, including burns and hair loss. Exposure to such doses directly in the eyes can increase the risk of developing cataracts[13,14].

In these days, the incidence of radiation exposure from medical imaging will continue to rise exponentially for several reasons. First, medical imaging technology has allowed physicians to evaluate easily and quickly both anatomy and function. Thereby, medical imaging provides benefits such as increased confidence of clinicians' decision, patient management, and protection from malpractice. In addition, patients are demanding more tests to ensure correct diagnosis and treatment[5].

Preventing unnecessary medical imaging examinations is an option to reduce total exposure to radiation. To avoid unnecessary examinations, it is necessary to understand the demanding process. At this point, concerns have also been raised that clinicians may lack important information in ordering medical imaging exams that use radiation. Clinicians may not have access to patients' medical imaging history or radiation dose history. Due to insufficient information, clinicians may unnecessarily order imaging procedures that have already been conducted. Additionally, if clinicians see a record of the total radiation dose to patients' previous medical history, such information might influence clinicians' decision to order a medical imaging test with radiation. Sometimes clinicians may be unaware or have insufficient knowledge of recommended criteria about whether medical imaging testing will be effective in their medical decision. As a result, clinicians may request unindicated medical imaging tests and unnecessarily expose patients to radiation[14,15].

In this study, we aimed to learn about the radiation awareness of clinicians and their reasons for requesting medical imaging tests with radiation through a questionnaire.

MATERIALS AND METHODS

We developed a 20-question questionnaire for clinicians to evaluate radiation awareness and the reasons for requesting radiation-containing tests. The content of the questionnaire is shown in Supplementary material.

We sent the online invitation to participate in the questionnaire to 500 clinicians from various specialties in four different cities. Of those who were invited, 195 participated in the questionnaire.

The study was designed as a descriptive cross-sectional study and local ethics committee approval was obtained for this study.

Questionnaire content: The first four questions of the 20-question survey inquired about the clinician's specializations, age, experience in medical practice, and professional title. In question 5, we inquired as to whether participants believed they had sufficient information about radiation doses. Questions 6-8 were designed to ascertain participants' level of knowledge about radiation dose. In question 9, the number of CT scans requested by clinicians in a month was asked. Questions 10-12 were designed to evaluate the patient's questions and approaches about the imaging method. Questions 13-16 were designed to investigate the effect of the patient's previous imaging history on the current imaging request. The 17th question inquired about the factors that can affect clinicians' CT request. The 18-20th questions were prepared for the purpose of analysis regarding the CT request that was made without indication Supplementary material.

Statistical analysis: Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows 20 software (IBM SPSS Inc., Chicago, IL, USA). Conformity of the data to normal distribution was assessed by the Kolmogorov-Smirnov test. Numerical variables with a normal distribution are shown as the mean ± standard deviation (SD) values, variables without a normal distribution as median (minimum-maximum) values, and categorical variables as number (*n*) and percentage (%). Chi-square test was used to analyze the difference of the answers according to gender, title, profession, and year of experience of the participants. A value of $P < 0.05$ was regarded as statistically significant.

RESULTS

A total of 195 clinicians from four different cities participated in the questionnaire. The participants' mean age was 33.6 ± 5.9 (24-56) years. Their mean years of medical practice was 9.0 ± 6.0 (1-28) years. Approximately 64.1% of the participants were specialists, 26.2% were research assistants, and 9.7% were general practitioners. The participants were from various specialties, with internal medicine doctors accounting for the highest percentage at 19.5%. Descriptive data is shown in Table 1.

One hundred and fifty-nine (81.5%) of the participants stated that they did not feel sufficient about radiation knowledge.

The answers to the questions asked to ascertain participants' level of knowledge about radiation dose are given in Table 2. According to these results, in the 6th-7th-8th questions, respectively 60.2%, 60%, and 79.5% of participants underestimated and respectively 12.8%, 22.6%, and 0% of participants overestimated the radiation dose rates of the examinations.

Mean number of requested CT scans in a month was 36.88 ± 5.86 (1-300). Among the participants, the specialties with the most CT requests per month were emergency medicine (mean, 82), general surgery (mean, 76), and neurosurgery (mean, 57).

Table 1 Some characteristics of the physicians participating in the study

Characteristic of physicians		*n* (%)
Medical department of physicians	Internal Medicine	38 (19.5)
	Emergency Medicine	35 (17.9)
	General Surgery	25 (12.8)
	Cardiology	14 (7.2)
	Anesthesiology	12 (6.2)
	Urology	11 (5.6)
	Pulmonology	10 (5.1)
	Orthopedic Surgery	10 (5.1)
	Child and adolescent psychiatry	7 (3.6)
	Neurosurgery	6 (3.1)
	Neurology	6 (3.1)
	Others	18 (9.2)
Age group, yr	24-30	68 (34.9)
	31-40	99 (50.8)
	> 40	28 (14.3)
Medical practice duration, yr	≤ 5	62 (31.8)
	6-10	69 (35.3)
	11-15	28 (14.4)
	>15	36 (18.5)
Degree of physician	Specialist doctor	125 (64.1)
	Research assistant	51 (26.2)
	General practitioner	19 (9.7)
Total		195 (100)

Others: Pediatrics, medical oncology, forensic medical specialist, otolaryngologist, family physician, gynecology and obstetrics, ophthalmology, dermatology, physical therapy, and rehabilitation.

There was no statistically significant difference between duration of medical practice experience and monthly CT requests (P = 0.385).

The proportions of the answers given to the 10-12th questions evaluating the patient's questions and approaches about the imaging method, as well as to the 13-16th questions investigating the effect of the patient's previous imaging history on the current imaging request are shown in Table 3. The most commonly mentioned causes were found to be indication, concern about failure to diagnose, and fear of malpractice (Table 4).

About 24.6% of the participants stated that they requested CT even though there was no clinical indication. The reasons for requesting CT even though there is no clinical indication are shown in Table 5. The most common reasons were the desire to complete the diagnosis quickly, the patient's demand, and fear of malpractice.

The answers given to the question of what should be done to prevent CT examinations without indication are shown in Table 6. The most frequently stated response of the participants (67.2%) was "reducing the patient density and allocating sufficient time for doctors to examine patients".

DISCUSSION

Estimating the dose rates of examinations is a frequently used technique in questionnaire studies to assess participants' knowledge and awareness of ionizing radiation. For this purpose, posteroanterior chest radiography which is frequently used in clinical practice and a daily radiation dose encountered in nature can be taken as a basis[16]. In this way, the opinions of the participants about the radiation doses

Table 2 Participants' estimates of radiation dose

Radiation dose estimation		*n* (%)
Standard CT equivalent chest X-ray	10 ×	12 (6.2)
	50 ×	30 (15.5)
	100 ×	75 (38.5)
	500 ×[a]	53 (27.2)
	1000 ×	25 (12.8)
Comparison of chest X-ray with the daily average amount of radiation in nature (cosmic rays, earth and underground sources, *etc.*)	1 d	71 (36.4)
	3 d	46 (23.6)
	7 d[a]	34 (17.4)
	15 d	44 (22.6)
Comparison of abdominal and pelvic CT with the daily average amount of radiation in nature (cosmic rays, earth and underground sources, *etc.*)	6 mo	30 (15.4)
	1 yr	60 (30.8)
	2 yr	65 (33.3)
	4 yr[a]	40 (20.5)
Total		195 (100)

[a]Correct answer. CT: Computed tomography.

Table 3 Patient questions and approach to imaging and consideration of previous computed tomography scans and radiation dose among physicians

Patient questions about radiation and physicians' consideration of previous radiation dose		*n* (%)
Informing the patient about radiation		94 (48.2)
Patients questioning radiation dose and harm		78 (40.0)
Frequency of patients asking questions about radiation dose and harm	Rarely	26 (13.3)
	Sometimes	44 (22.6)
	Mostly	8 (4.1)
Physicians checking old imaging		180 (91.8)
CT request affected if more than 10 CT scans were performed in the last 2 years		65 (33.3)
Easier CT request if less than 10 CT scans were performed in the last 2 years		64 (32.8)
Physicians affected by the last 2 yr of CT dose seen over the hospital system		130 (66.7)

CT: Computed tomography.

of the examinations used in clinical practice can be reached. The majority of participants in our study underestimated the dose rates of examinations. In the literature, in a survey study conducted with research assistants, Koçyiğit *et al*[17] found that 64.9% of participants underestimated the radiation dose associated with abdominal CT examinations and 58.8% underestimated the radiation dose associated with abdominal radiography. Ataç *et al*[18] in their questionnaire study with radiology workers, found that the majority of participants underestimated the dose value and dose rate questions. Lee *et al* in their questionnaire study among non-radiologists, found that 77% of participants underestimated the radiation dose for a chest X-ray[19]. The findings of our study and similar findings in the literature lead us to believe that participants' underestimation of the dose contents may be a factor in facilitating the request for medical imaging examinations with ionizing radiation.

In our study, we found that 48.2% of patients were informed about radiation prior to requesting an examination containing ionizing radiation. There are also studies in the literature demonstrating that the sharing of radiation risk information between clinicians and patients is rare[20-22]. One possible

Table 4 Factors affecting computed tomography request

Factors affecting CT request[a]	*n* (%)
Indication (Mandatory requirement)	192 (98.5)
Patient's age	68 (34.9)
Patient's insistence or request	22 (11.3)
Having a large number of patients	13 (6.7)
Concern about doing malpractice	70 (35.9)
Concern about not being able to diagnose	82 (42.1)

[a]A physician was able to give more than one answer. CT: Computed tomography.

Table 5 Requesting computed tomography without clinical indication

Requesting CT without clinical indication		*n* (%)
CT request without clinical indication		48 (24.6)
Causes of CT request without clinical indication (n = 48)	Patient's insist or request	21 (10.8)
	Having a large number of patients	8 (4.1)
	Worry about doing malpractice	20 (10.3)
	Concern about not being able to diagnose	16 (8.2)
	Desire to complete diagnosis quickly	23 (11.8)
	Length of US and MRI appointment times	14 (7.2)

CT: Computed tomography; US: Ultrasound; MRI: Magnetic resonance imaging.

Table 6 Measures to be taken to prevent computed tomography request without indication

Measures to be taken to prevent CT request without indication	*n* (%)
Reducing patient demand	85 (43.6)
Educating physicians about CT radiation dose	61 (31.3)
Extending the patient examination time	131 (67.2)
Shortening US and MRI appointment times	23 (11.8)

CT: Computed tomography; US: Ultrasound; MRI: Magnetic resonance imaging.

explanation for this low rate may be the high patient density which results in insufficient time to give detailed information to the patient. Additionally, there are studies in the literature showing that clinicians are uncomfortable sharing radiation risk information with patients[23]. In our study, the rate of asking questions by patients about radiation dose or potential harm in examination containing ionizing radiation was found to be as low as 40%. This result could be interpreted as the patient's low awareness of radiation exposure. Informing patients about the potential risks of radiation is left to the radiology units in many hospitals. However, after the imaging examination is requested by the clinician, the patient comes to the radiology unit to perform the desired examination, so it is not possible for the patient to think about the subject again. It is also emphasized in the FDA White Paper that informed clinical decision making together with the clinician doctor during the clinical examination will be more effective[14]. By informing patients about radiation exposure associated with imaging methods and increasing their awareness, it may be possible to reduce unindicated and unnecessary CT scans[24,25]. In the literature, it has been stated that awareness of radiation exposure has increased with the participation of patients and doctors in courses on radiation[26-28]. In addition, Sullivan *et al*[29] demonstrated that short-term and repetitive refresher training had a positive effect on raising awareness of radiation.

In our study, while the mean number of requested CT scans in a month was 36.9 ± 5.86, 81.5% of the participants stated that they did not feel sufficient about radiation knowledge. These findings are significant because they demonstrate a lack of competence about radiation information despite the frequency of CT demand as an imaging method in daily practice. In the literature, it is seen that while participants express growing concern about the risk of cancer caused by ionizing radiation, they have insufficient information about how much radiation the patient is exposed to[30,31].

In our study, it is important that a very large part of the participants (91.8%) reviewed the previous examinations before requesting a radiation-containing examination and that a significant portion (66.7%) would be affected by the high dose warning in the hospital system record. These results can be accepted as an indicator that physicians' attention can be increased with the help of assistive methods integrated into the hospital system, regarding the request for examinations containing radiation. Again, based on these results, doctors' inability to access medical imaging containing radiation performed in different health centers may be a factor in the procedure's unnecessary repetition.

The factors affecting participants' decisions to request a CT scan were examined in our study. The great majority of the participants stated the option of indication as the main factor and primary reason for requesting CT. It has been understood that options such as the concern about not being able to diagnose, the worry about doing malpractice, the high patient density and patient's insistence or request are significantly effective in requesting CT. Due to these various factors, it is inevitable that there will be an increase in CT requests, unnecessary/unindicated CT scans, and ionizing radiation exposure. It is important that the desire to make a diagnosis quickly and the concern for malpractice are frequently seen among the reasons for requesting CT even though there is no clinical indication. Additionally, it is important that the majority of the participants believe that patient density should be reduced and examination times should be extended in order to prevent non-indication CT scans. Yıldız *et al*[32] reported in their study in the emergency department that CT was frequently used in childhood head traumas, but normal imaging results were obtained in 98.5%. Additionally, they emphasized the need to prioritize clinical decision-making rules and patient follow-up for CT request. Dağlar *et al*[33] evaluated 51.2% of CT examinations performed for spine and pelvis evaluation as normal CT in their study. They emphasized that due to this high rate, precautions should be taken for unnecessary CT use. Karavas *et al* [34] stated that unnecessary CT requests may result in an increase in workload and patient density in radiology units, and related problems in reporting and an increase in diagnostic errors. We think that providing the opportunity to spend more time on clinical examination by limiting the number of patients per physician will help reduce fear of malpractice, avoid unnecessary CT examinations, and reduce ionizing radiation exposure.

According to the findings of our study, some solutions can be offered to prevent unnecessary radiation exposure. The first and most critical of these is to raise patients' and clinicians' radiation awareness and consciousness, and to schedule regular radiation training sessions. If the patient's previous radiation exposure and total dose of exposure are displayed as warnings in the patient information system in the hospital before clinicians make a request for a medical exam that includes radiation, this can help reduce unnecessary request and exam repetition. By reducing patient density, doctors can spend more time with the patient rather than rushing to a CT diagnosis, and radiation exposure can be reduced. Additionally, with detailed informed consent to the patient about the potential risks of radiation, the patient's insistence on examination with radiation is reduced, and unnecessary radiation exposure can be prevented.

Our study has some limitations, such as the low number of participants and the fact that the participating clinicians are from different specialties. However, a heterogeneous sample with diversity was created by providing participants from various cities and hospitals. There may be variations in practice based on the participants' specializations and whether they provide emergency or outpatient care. However, the study's primary objective was not to analyze these differences, but to provide an overview of ionizing radiation awareness. Additionally, the questionnaire is a test method and contains closed-ended questions, which is also a limitation of the study.

CONCLUSION

As a result of our study's findings, both patients and physicians have a low level of knowledge and awareness about ionizing radiation. While the primary consideration when requesting a radiation-containing imaging method is the indication, other considerations such as concern about not being able to diagnose, worry about doing malpractice, high patient density, and the patient's insistence also factor in. Desire to complete diagnosis quickly and fear of malpractice may be the reasons for unindicated CT demand and increase exposure to ionizing radiation. Unnecessary and unindicated ionizing radiation exposure can be reduced by reducing patient density in daily practice, extending examination times, and improving hospital systems in a way that allows for detailed documentation of the patient's previous radiation doses. Thus, potential risks to the patient associated with radiological imaging and ionizing radiation exposure can be minimized.

ARTICLE HIGHLIGHTS

Research background

Radiation-containing imaging and treatment techniques are frequently used in daily clinical practice. The advancement of technology and clinicians' increased access to radiation-containing examinations also expand the applications of radiation-containing examinations. Recently, the use of radiation-based medical exams has increased exponentially. The dangers of radiation should be highlighted, and awareness of radiation should be increased.

Research motivation

Radiation is a potential carcinogen. Ionizing radiation exposure can damage DNA, increasing an individual's lifetime risk of developing cancer. Medical exams containing radiation are sometimes unnecessary and overused. Preventing unnecessary medical imaging examinations is an option to reduce total exposure to radiation. To avoid unnecessary examinations, it is necessary to understand the demanding process.

Research objectives

To increase radiation awareness and thus reduce unnecessary radiation exposure.

Research methods

We developed a 20-question questionnaire for clinicians to evaluate radiation awareness and the reasons for requesting radiation-containing tests.

Research results

Most of the participants stated that they did not feel sufficient about radiation knowledge and the majority of participants underestimated examination dose rates. Both patients and physicians had a low level of knowledge and awareness about ionizing radiation. In our study, we found that 48.2% of patients were informed about radiation prior to requesting an examination containing ionizing radiation. A large part of the participants (91.8%) reviewed the previous examinations before requesting a radiation-containing examination and that a significant portion (66.7%) would be affected by the high dose warning in the hospital system record. Indication, concern about not being able to diagnose, worry about doing malpractice, high patient density, and the patient's insistence are various factors in requesting a radiation-containing imaging method. Desire to complete diagnosis quickly and fear of malpractice may be the reasons for unindicated computed tomography (CT) demand.

Research conclusions

According to the findings of our study, some solutions can be offered to prevent unnecessary radiation exposure. The first and most critical of these is to raise patients' and clinicians' radiation awareness and consciousness, and to schedule regular radiation training sessions. If the patient's previous radiation exposure and total dose of exposure are displayed as warnings in the patient information system in the hospital before clinicians make a request for a medical exam that includes radiation, this can help reduce unnecessary request and exam repetition. By reducing patient density, doctors can spend more time with the patient rather than rushing to a CT diagnosis, and radiation exposure can be reduced. Additionally, with detailed informed consent to the patient about the potential risks of radiation, the patient's insistence on examination with radiation is reduced, and unnecessary radiation exposure can be prevented.

Research perspectives

Following radiation awareness training for patients and clinicians and the addition of a total radiation dose warning to the hospital's patient information system, prospective studies can be conducted to determine whether the number of requests for radiation-containing examinations has decreased in certain centers.

FOOTNOTES

Author contributions: Karavas E, Ece B, and Aydin S participated in design and oversight of the study, and drafted the manuscript; Ece B and Aydin S assisted with data analysis; Kocak M, Cosgun Z, Bostanci I, and Kantarci M participated in design of the study, and was involved in data collection; all authors wrote, read, and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Erzincan University Institutional Review Board (Approval No. E-21142744-805.91-912567).

Informed consent statement: All study participants, or their legal guardian, provided written consent prior to study enrollment.

Conflict-of-interest statement: All authors declare that there is no conflict of interest related to the manuscript.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at bunyaminece@hotmail.com. Participants gave informed consent for data sharing and the presented data are anonymized.

CONSORT 2010 statement: The authors have read the CONSORT 2010 statement—checklist of items, and the manuscript was prepared and revised according to the CONSORT 2010 statement—checklist of items.

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S-Editor: Wang LL
L-Editor: Wang TQ
P-Editor: Wang LL

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World Journal of Methodology

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World J Methodol 2022 July 20; 12(4): 274-284

DOI: 10.5662/wjm.v12.i4.274

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Prospective Study

Robotic ultrasound: An initial feasibility study

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Specialty type: Medical laboratory technology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ardengh JC, Brazil; Wahome R, United States
A-Editor: Liu X, United States

Received: January 23, 2022
Peer-review started: January 23, 2022
First decision: March 25, 2022
Revised: April 8, 2022
Accepted: June 3, 2022
Article in press: June 3, 2022
Published online: July 20, 2022

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Abstract

BACKGROUND

Performing ultrasound during the current pandemic time is quite challenging. To reduce the chances of cross-infection and keep healthcare workers safe, a robotic ultrasound system was developed, which can be controlled remotely. It will also pave way for broadening the reach of ultrasound in remote distant rural areas as well.

AIM

To assess the feasibility of a robotic system in performing abdominal ultrasound and compare it with the conventional ultrasound system.

METHODS

A total of 21 healthy volunteers were recruited. Ultrasound was performed in two settings, using the robotic arm and conventional hand-held procedure. Images acquired were analyzed by separate radiologists.

RESULTS

Our study showed that the robotic arm model was feasible, and the results varied based on the organ imaged. The liver images showed no significant difference. For other organs, the need for repeat imaging was higher in the robotic arm, which could be attributed to the radiologist's learning curve and ability to control the haptic device. The doctor and volunteer surveys also showed significant comfort with acceptance of the technology and they expressed their desire to use it in the future.

CONCLUSION
This study shows that robotic ultrasound is feasible and is the need of the hour during the pandemic.

Key Words: Robotic ultrasound; Telemedicine; Ultrasonography; Haptic device; Pandemic

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Core Tip: Robotic ultrasound aims to provide remote ultrasound access through a robotic system. This system allows the radiologist to manipulate the ultrasound probe remotely from a safe distant location, in a separate enclosure, thus ensuring the safety of the sonologist and negating the need for a personal protective equipment kit each time, especially in the current coronavirus pandemic. System setup in an intensive care unit (ICU) could ensure that the sonologist can perform the ultrasound without needing to enter the ICU. Going forward, a distance transmission system may also be potentially developed so that patients can also access care at a convenient location without the need to travel long distances, further breaking the chain of transmission. This can be invaluable in a setting where healthcare is not widely available, such as in underserved rural areas.

Citation: Chandrashekhara SH, Rangarajan K, Agrawal A, Thulkar S, Gamanagatti S, Raina D, Saha SK, Arora C. Robotic ultrasound: An initial feasibility study. *World J Methodol* 2022; 12(4): 274-284
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/274.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.274

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 has infected millions of people worldwide. As of May 2021, it has infected 157973438 people worldwide and the pandemic continues to rage with a tsunami of cases continuing to be reported in India, USA, and Brazil[1,2]. Healthcare workers (HCW) face an extremely high risk of infection for themselves, a risk that also gets transmitted to their families. Many healthcare workers have been infected and succumbed to the pandemic[3-6]. Although personal protective equipment (PPE) has been effective in protecting HCW, many countries grapple with shortages, and they have immensely added to plastic waste accumulation across the globe. Often, wearing PPE is not feasible or unavailable for HCW in non-coronavirus disease 2019 (COVID-19) designated areas, where the risk of transmission remains high in these times of the pandemic[7].

Telemedicine has evolved immensely in the last few years, though the adoption of these techniques was limited in the pre-pandemic era[8]. This was primarily due to the preference of many doctors and patients to personally see and converse traditionally[9]. The pandemic, however, has brought telemedicine and many of its applications to the forefront, proving that much of the care required can be guided from a distance. Adoption of this technology has received a boost with the pandemic as it provides the much-needed solution to address the challenge of protecting oneself while treating patients adequately.

Imaging plays a vital role in medicine at various stages in terms of diagnostic aid, aiding interventions, and procedures, and in the follow-up of patients. Ultrasonography is a non-invasive, non-ionizing, cost-effective, rapid, bedside, and easily available modality with immense use in point-of-care and follow-up examinations[10]. Often point-of-care ultrasound is the first modality with which a patient is assessed as he/she walks into the casualty. Ultrasound, however, requires an operator to be in close contact with a patient. Ultrasound rooms are often small and lack adequate ventilation, making the operator vulnerable to infection during the pandemic. In addition, ultrasound is often required in intensive care unit (ICU) settings. In the setting of COVID, they may be required for assessment of the chest or screening for thrombosis in veins. This often requires the operator to don and dough the PPE multiple times, despite needing to be present only for a limited amount of time.

Robotic ultrasound aims to provide remote ultrasound access through a robotic system. This system allows the radiologist to manipulate the ultrasound probe remotely from a safe distant location, in a separate enclosure, thus ensuring the safety of the sonologist and negating the need for a PPE kit each time. Similarly, such a system setup in an ICU could ensure that the sonologist can perform the ultrasound without needing to enter the ICU. Going forward, a distance transmission system may also be potentially developed so that patients can also access care at a convenient location without the need to travel long distances, further breaking the chain of transmission. This can be invaluable in a setting where healthcare is not widely available, such as in underserved rural areas.

In this study, we assessed one such system, where the robotic arm is mounted with the probe and is fixed next to the patient couch, and the sonologist operates it with a joystick, at some distance from the patient couch, with the two separated by a glass enclosure.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Radio-diagnosis, Dr. BRA Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India between February 2021 and May 2021. Ethical approval was obtained from the institute ethics committee. Informed consent was obtained from all the volunteers. A total of 21 healthy volunteers were recruited. The sample size was one of convenience as this was a feasibility study. On each volunteer, ultrasound was performed in two settings, using the mobile robotic arm and the conventional hand-held ultrasound by the same sonologist, a few hours apart. The ultrasound examination was performed by a radiologist having 15 years of experience. The ultrasound images obtained using the mobile robotic arm and the conventional hand-held ultrasound were analyzed separately by another blinded radiologist having 20 years of experience. This study was performed to assess the feasibility of the model and its safety. All the healthy volunteers consenting to take part in the study were included. Volunteers who were < 18 years of age or those who did not give consent were excluded from the study.

Robotic ultrasound system setup

The robotic ultrasound system was co-developed by the Indian Institute of Technology, Delhi, and the All India Institute of Medical Sciences, New Delhi, in collaboration with Adverb Technologies. It consisted of a UR5e (by Universal Robots) robot arm at the patient site with the probe attached at its end using a custom-designed gripper. The doctor's site consisted of a geomatic haptic touch device (by 3D systems) used to operate the robotic arm. In addition to this haptic device, a monitor with a simple graphic user interface (GUI) was set up at the doctor's end to allow him or her to do basic control of the system. The sliding scale was provided on GUI to adjust the force exerted by the probe along with live camera feed to visualize the patient movements and responses. The systems were connected through a Wi-Fi router. Safety equipment was provided at both patient and doctor sites (Figure 1), such that the system would come to a complete standstill if pressed at either end.

Patient site

The patient site includes the robotic arm with the gripper, a USG machine, and an auxiliary staff person. The patient is made to lie on the table with its height and dimensions adjusted according to the robotic arm. The auxiliary staff applies a coupling agent (ultrasound jelly) onto the patient and positions the patient as required. The required transducer is fixed on the gripper based on the exam being performed. The patient is made to hold a safety switch to control.

In the current system, the auxiliary staff performed the ultrasound settings like gain depth and image labeling at the patient end. However, the staff may maintain a safe distance from the patient as they are not required to move the machine or position it continuously during the exam.

Doctor site

The doctor site includes the geomatic haptic device with a stylus tip that is held by the doctor and simulates the probe movements. It also provides haptic (sense of touch) feedback allowing the doctor to perceive feedback sensations of contact between the US probe and the patient body. It also has a user interface with a screen showing the ultrasound images and the patient. Since this was only a feasibility study, the doctor site was created on one end of the same room. This allows only the purpose of safe distancing to be fulfilled. However, this can evolve into a more sophisticated system by using a camera at the patient end to provide video feedback and sufficient bandwidth to prevent communication delays.

Ultrasound examination

The ultrasound machine used for the conventional and robotic ultrasound was the Sonosite M-TURBO model. Ultrasound images were acquired for each patient first using the robotic arm and subsequently by conventional hand-held ultrasound a few hours later. Time taken for each study was noted. After each study, the volunteer and the doctor were asked to fill out a satisfaction survey.

Image evaluation

All the images were transferred from the ultrasound machine to a USB drive. A google form questionnaire was created to analyze the images. Images were not annotated, and the conventional and robotic arm images were arranged randomly, each followed by the options.

The images acquired in both settings were evaluated by a reviewer with more than 10 years of experience in a blinded manner. All images were classified subjectively into either of the two groups:

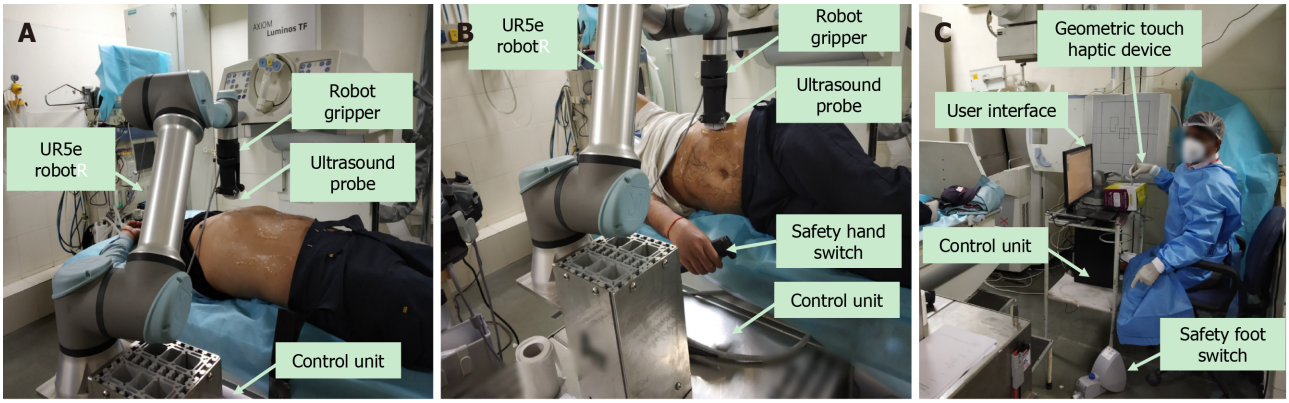

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Figure 1 Robotic ultrasound. A and B: Robotic ultrasound setup, which includes patient site; C: Doctor site.

Images adequate in resolution and for evaluation with some reservation, and those inadequate for evaluation needing repeat imaging. To compare the conventional hand-held ultrasound and robotic arm ultrasound image data, the Wilcoxon-signed rank test was used.

Satisfaction surveys

Feedback in the form of satisfaction surveys was taken from each volunteer and the radiologist after each ultrasound (Tables 1 and 2).

RESULTS

Demographics

All patients included in our study were males with a mean age of 37.09 ± 9.69 years. The average time taken to perform the conventional ultrasound was 4.05 min (range, 2-7 min), while that taken to perform an ultrasound using a robotic arm was 8.57 min (range, 4-17 min) (Table 3).

Ultrasound image evaluation

Images acquired using robotic arm ultrasound were divided into two groups (Figure 2). Image evaluation showed that 17/21 (80.9%) images of the liver were adequate with few reservations, while 4/21 (19%) required repeat imaging. Imaging of the gallbladder showed that 11/21 (52.4%) images were adequate, and 10/21 (47.6%) needed repeat imaging (Figure 3).

In the genito-urinary system, the right kidney (RK) image evaluation showed that 13/21 (61.9%) images were adequate with some reservations, and 8/21(38.1%) needed repeat imaging. For the left kidney (LK), 14/21 (66.67%) images were adequate with some reservations, and 7/21 (33.33%) needed repeat imaging. For the urinary bladder (UB), 11/21 (52.4%) images were adequate with some reservations, and 10/21 (47.6%) needed repeat imaging. Evaluation of the spleen ultrasound images showed that 13/21 (61.9%) images were adequate, and 8/21 (38.1%) needed repeat imaging.

Ultrasound images of the hepato-biliary system showed no significant difference in the need for repeat imaging of the liver between the conventional (2/21) and robotic arm (4/21) groups. GB evaluation showed a significant difference in the need for repeat imaging between the conventional (0/21) and robotic arm (10/21) groups.

In the genito-urinary system, there was a significant difference in the need for repeat imaging between the conventional (RK = 1/21, LK = 0/21, and UB = 2/21) and robotic arm (RK = 8/21, LK = 7/21, and UB = 10/21) ultrasound. Evaluation of the spleen also showed a significant difference in the need for repeat imaging in the conventional (1/21) and robotic arm (8/21) groups (Figure 4).

Doctor assessment

The radiologists performing the ultrasound using a robotic arm were asked to fill a satisfaction survey after each ultrasound examination. The radiologists somewhat disagreed with being able to use the system with ease in the initial five scans, followed by some agreement in being able to use the system easily in 15 scans. The radiologists somewhat agreed to understand the system in 18 scans. On the survey of wanting to use the robotic arm over a conventional system, the radiologist somewhat agreed in 13 scans. The radiologists reported difficulty in case of being able to handle errors with ease (somewhat disagreed in 11 cases). The radiologists showed some concern about the safety of the patients in seven scans. They found the user interface to be useful and trusted the results of the system

Table 1 Patient/volunteer satisfaction survey, *n* (%)

	Strongly disagree	Somewhat disagree	Neither disagree nor agree	Somewhat agree	Strongly agree
I was worried to undergo this procedure	0 (0)	7 (33.4)	2 (9.5)	12 (57.1)	0 (0)
I felt comfortable during the procedure	0 (0)	1 (4.8)	1 (4.8)	18 (85.6)	1 (4.8)
I felt no difference between this and conventional ultrasound	0 (0)	8 (38.1)	5 (23.8)	8 (38.1)	0 (0)
I felt comfortable knowing that doctor is controlling the robot	0 (0)	0 (0)	0 (0)	13 (61.9)	8 (38.1)
I will trust the results of this technology	0 (0)	5 (23.8)	6 (28.6)	10 (47.6)	0 (0)
I understand how the procedure took place	0 (0)	7 (33.4)	11 (52.4)	3 (14.3)	0 (0)
I felt less pressure on my body in comparison to conventional ultrasound	0 (0)	7 (33.33)	7 (33.33)	7 (33.33)	0 (0)
I would like to use this technology in future	0 (0)	2 (9.5)	4 (19.1)	12 (57.1)	3 (14.3)
I would recommend this technology to others	0 (0)	0 (0)	4 (19.1)	14 (66.6)	3 (14.3)
Overall rating					Average: 6.2

Table 2 Doctor satisfaction survey, *n* (%)

	Strongly disagree	Somewhat disagree	Neither disagree nor agree	Somewhat agree	Strongly agree
I could use the system easily	0 (0)	5 (23.8)	1 (4.8)	15 (71.4)	0 (0)
I have understanding of the working of the system	0 (0)	3 (14.3)	0 (0)	18 (85.7)	0 (0)
I could learn to use the system with more trials	0 (0)	0 (0)	0 (0)	8 (38.1)	13 (61.9)
I would like to use the system over conventional system	0 (0)	0 (0)	8(38.1)	13(61.9)	0 (0)
I feel the system is precise, safe, and effective	0 (0)	1 (4.8)	6 (28.6)	11 (52.4)	3 (14.2)
In case of errors, I was able to handle them with ease	0 (0)	11 (52.4)	4 (19)	6 (28.6)	0 (0)
I was not concerned about the safety of the patient during the procedure	0 (0)	7 (33.3)	0 (0)	13 (61.9)	1 (4.8)
I feel the user interface is useful	0 (0)	1 (4.8)	5 (23.8)	15 (71.4)	0 (0)
I trust the results of the system	0 (0)	0 (0)	4 (19.05)	13 (61.9)	4 (19.05)
Overall rating					Average: 6.38

in the majority of the cases. The overall rating of the system was between 5 and 7, with an average of 6.38 (Figure 5).

Volunteer assessment

Each volunteer was requested to fill out a satisfaction survey after the set of ultrasounds (both conventional and robotic arms).

The volunteers were somewhat worried to undergo this procedure (n = 12); however, most of them "somewhat agreed" to have felt comfortable during the procedure (n = 18). The volunteers "somewhat disagreed" to feeling no difference between robotic and conventional ultrasound. They somewhat agreed to be feeling more comfortable knowing that the doctor is controlling the robot. The volunteers were equivocal on trusting the results of the technology (n = 14 neither agreed nor disagreed, n = 4 somewhat agreed, and n = 1 somewhat disagreed). The volunteers somewhat agreed to understand how the procedure took place (n = 16). The volunteers did experience some pressure on their body with the robotic arm-loaded probe; however, they somewhat agreed (n = 19) that the pressure was equal to or less than conventional ultrasound and was not discomforting. The volunteers showed acceptance towards the technology (n = 12 somewhat agreed) and on their likelihood to use the technology in the future and recommended it to others (n = 14 somewhat agreed). The overall rating of the system was between 4 and 8, with an average of 6.2 (Figure 6). This can be attributed to the learning curve at the initial time.

Table 3 Time taken for performing ultrasound

Patient	Conventional hand-held (min)	Robotic arm (min)
1	3	17
2	5	17
3	5	17
4	3	15
6	2	10
7	4	9
8	4	8
9	7	6
10	4	9
11	4	6
12	5	5
13	6	8
14	3	8
15	4	6
16	3	5
17	4	4
18	5	4
19	3	7
20	5	6
21	3	8

DISCUSSION

There were only male volunteers in our study, which was coincidental and did not result from any deliberate selection or exclusion. The average time taken for ultrasound using a robotic arm in our study was almost double in comparison with that performed by hand-held ultrasound. Initial ultrasound exams in our study using the robotic arm took 17 min; however, as the operators became more accustomed to it, this was reduced to 4-7 min in the later ultrasound exams, which was comparable to that with handheld conventional ultrasound. This likely represents the learning curve associated with robotic ultrasound. This shows that with practice and as familiarity increases with the arm, imaging times would be very comparable to conventional ultrasound.

Image evaluation showed that most of the images acquired using the robotic arm were adequate with some reservations. In the evaluation of the liver, kidney, and spleen, the robotic arm performed well. However, evaluation of the gallbladder and UB showed that the robotic arm images needing repeat imaging were significantly more than conventional imaging. This could be attributed to fine probe angulations needed to focus on these organs.

In comparison with the conventional ultrasound images, evaluation of the liver using both modalities showed no significant difference. This could be attributed to the ability to image the liver in a supine manner without significant probe inclination. For the rest of the organs evaluated, there was a significant difference in the need for repeat imaging between conventional ultrasound and robotic arm ultrasound.

This can be attributed to the learning curve required for the radiologist to be able to control the haptic device and perform fine probe inclinations. With increasing experience later in the study, we observed that satisfactory scores for all images improved. The operators also subjectively reported better coordination and adaptation, which may help achieve better images using the robotic arm.

The radiologists performing the ultrasounds were able to use the system after an initial lag and understood the working of the system. The radiologists indicated their preference to use the system over the conventional ultrasound, which could be attributed partially to the current exposure of the healthcare workers during the pandemic. The radiologists experienced some difficulty in handling errors with the system, particularly at the beginning of the study, which needed the intervention of engineers. However, this was eventually addressed in the later part of the study, indicating again a need for dedicated training on the system before use. Regarding the safety of the patients, there was initial

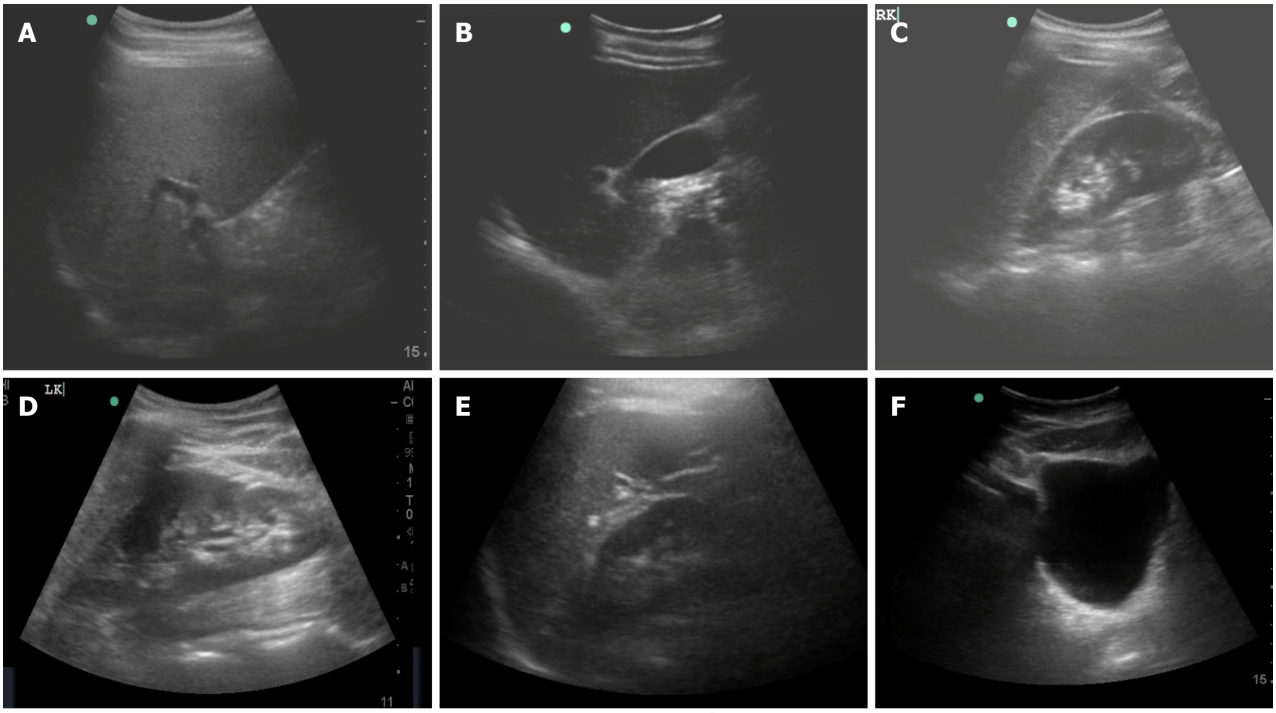

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Figure 2 Ultrasound images of abdominal organs acquired using a probe mounted on the robotic arm. A: Liver; B: Gall bladder; C: Right kidney; D: Left kidney; E: Spleen; F: Urinary bladder.

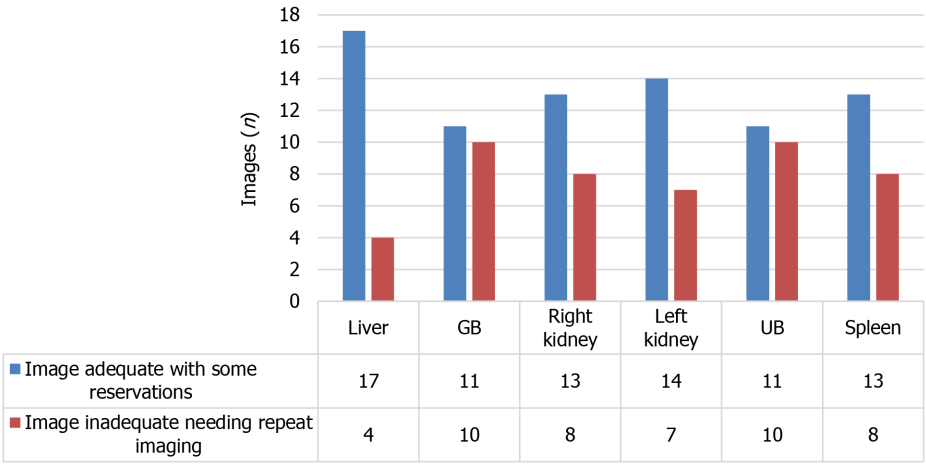

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Figure 3 Quality details of images acquired using robotic arm ultrasound. UB: Urinary bladder.

apprehension, both among radiologists and patients, concerning the landing of the robotic arm mounted with a probe on the patient's abdomen and the pressure exerted during the examination. However, assurance was provided about adequate prior testing; in addition, the interface at the doctor's end allowed force monitoring (through a slider on the computer screen) which may be used for dynamically increasing or decreasing the pressure whenever required. With controlled motions and increasing experience, the apprehension for patient safety was reduced. The radiologists found the user interface useful and were able to understand it with ease and trusted the results of the system. The radiologist performing the scan was more confident of the findings as he had scanned the entire organ compared to the single image provided for evaluation.

The volunteers were initially apprehensive to undergo the procedure as it was a first-time experience for them. Most of the volunteers were comfortable during the ultrasound. The volunteers felt that they were more comfortable with the conventional hand-held technique as it allowed more interaction with the radiologist. However, in some situations, they would be more comfortable using this technique. The awareness that the arm was being operated by a radiologist made them more comfortable and willing to

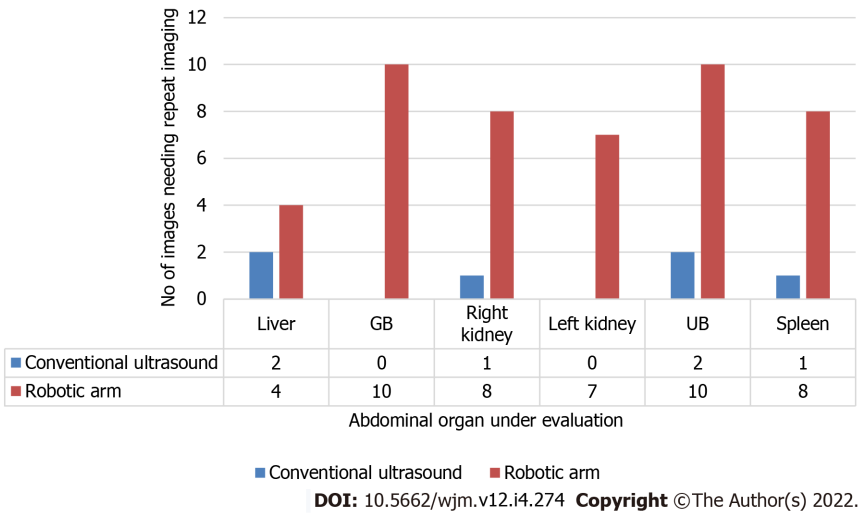

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Figure 4 Comparison between conventional and robotic ultrasound regarding the need for repeat imaging.

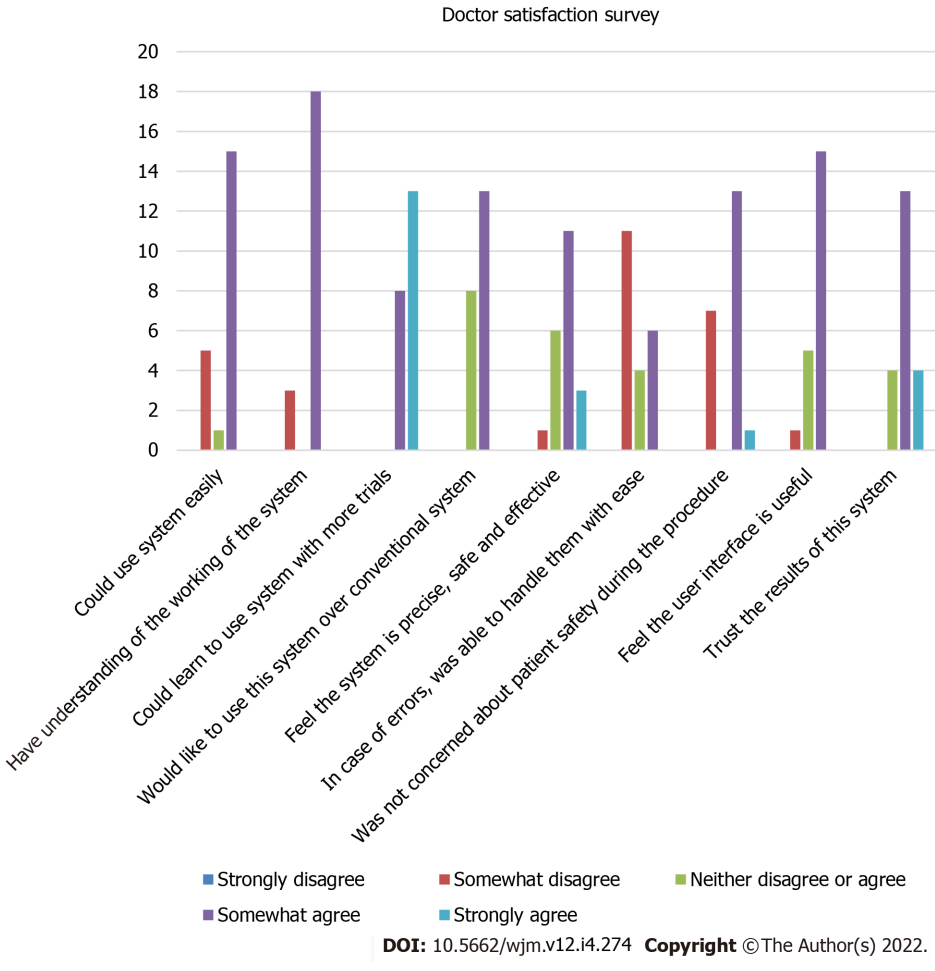

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Figure 5 Histogram showing results of the doctor satisfaction survey.

use the technology in the future. The volunteers believed that the force exerted by the robotic arm-mounted probe was almost similar to that exerted by conventional ultrasound. The volunteers also indicated that they felt secure and comfortable with the technology and were willing to use the same in the future and recommend it to others.

Limitations

The major limitation with the robotic arm is the increased setup and working cost. This cost is justified

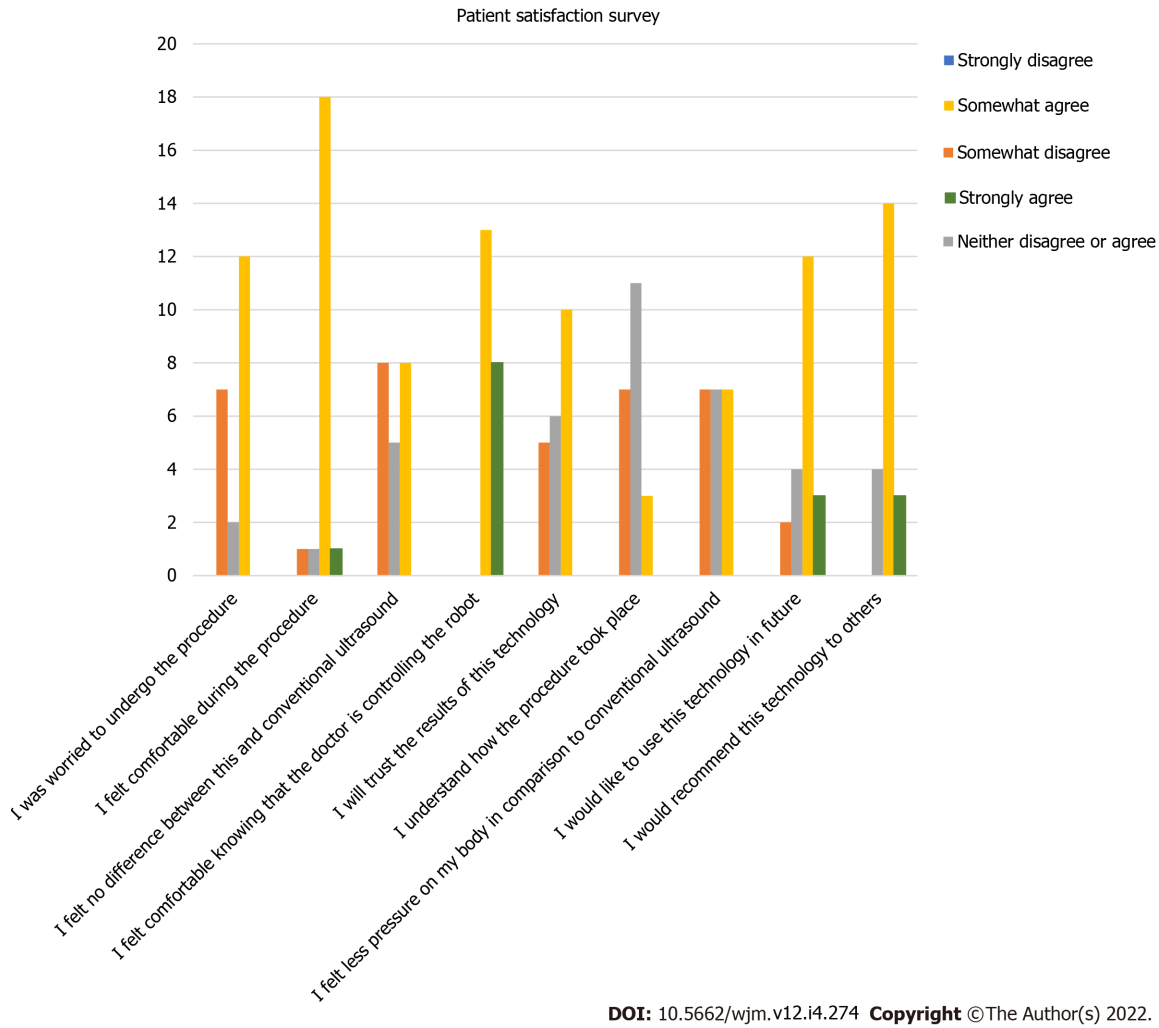

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Figure 6 Histogram showing results of the patient satisfaction survey.

in the case of pandemic situations like the current one. However, it may take some time to become a routine method of performing ultrasound.

With the current robotic system, auxiliary staff is needed at the patient end for helping with patient positioning and rotation during the examination and for the application of the coupling agent. However, the staff is not needed to remain close to the patient during the entire study and may maintain a safe distance once the patient is positioned and a coupling agent was applied. With the use of a robotic arm for gel application, this time may be further reduced.

There is a learning curve required to be able to operate the haptic device; however, it was seen that with adequate training, the initial difficulty could be mitigated. Time for comfortable ultrasound was also organ based with imaging requiring more probe angulation and inclination requiring more time for the operator to be able to coordinate the haptic device and the robotic arm's movements.

Our study had a small sample size, and recruiting more volunteers or patients would allow us to assess the system better.

CONCLUSION

Robotic ultrasound is the need of the hour, especially during this pandemic. The conventional hand-held ultrasound is the gold standard and is more cost-effective; however, in specific scenarios like the current pandemic, the robotic ultrasound is vital. Efficient use of this technology like other forms of telemedicine can help break the chain of transmission, reduce the amount of plastic waste, and provide adequate care while keeping the healthcare workers and patients safe. It will also play a role in broadening the reach of ultrasound in rural areas, thus improving the standards of health care.

ARTICLE HIGHLIGHTS

Research background

Special circumstances like the current pandemic have led to the need to exploit the utility of robotics and telecommunication systems to perform remote diagnostic ultrasound. It requires robust engineering effort to achieve high precision, flexibility, and repeatability, which can replace the conventional handheld ultrasound examination. A robotic ultrasound system was developed in this study so that ultrasound examination can be performed without having patient contact with the radiologist.

Research motivation

In the coronavirus 2019 (COVID-19) pandemic, the chances of cross-infection significantly increase among health care workers while performing ultrasound examination. There is a need to negate the need for a PPE kit each time when ultrasound examination is done, especially in COVID wards. This has motivated us to develop the robotic ultrasound system and conduct a study to validate it.

Research objectives

To perform ultrasound remotely using a mobile robotic arm on healthy volunteers to assess the feasibility and effectiveness of the system; validate the system by comparing the accuracy of the images generated through remote manipulations of probe attached to robotic arm by the radiologist; and to assess the comfort of the patient and radiologist with the robotic technology.

Research methods

This prospective study was conducted in the Department of Radio-diagnosis, All India Institute of Medical Sciences, New Delhi, India. Ethical approval was obtained from the institute ethics committee. Informed consent was taken from all the volunteers. A total of 21 healthy volunteers were recruited. On each volunteer, ultrasound was performed in two settings, using the mobile robotic arm and the conventional hand-held ultrasound by the same sonologist. The ultrasound images acquired using the mobile robotic arm and the conventional hand-held ultrasound were analyzed separately by another blinded radiologist.

Research results

Our study showed that the robotic arm model was safe and feasible, and the results varied based on the imaged abdominal organs. The liver images showed no significant difference. For other abdominal organs (such as the pancreas, spleen, kidneys, and urinary bladder), the need for repeat imaging was higher in case of robotic arm, which could be attributed to the learning curve and ability to control the haptic device. The doctor and volunteer surveys demonstrated significant comfort with acceptance of the technology and desire to use it in the future.

Research conclusions

This study shows that robotic ultrasound is safe and feasible and has potential to perform ultrasound with reliability.

Research perspectives

The scope of the developed tele-robotic ultrasound system can be expanded to perform ultrasound examinations remotely in distant rural places, emergency, trauma, and isolation wards.

FOOTNOTES

Author contributions: Chandrashekhara SH led the study in its conception, study design, statistical design, and manuscript writing and editing; all the authors have contributed in manuscript writing, study design and conduct, and statistical analysis.

Institutional review board statement: The study was reviewed and approved by the All India Institute of Medical Sciences, Delhi Institutional Review Board [Approval No. IEC-855/04.09.2020, RP-16/2020)].

Informed consent statement: All study participants, or their legal guardian, provided written consent prior to study enrollment.

Conflict-of-interest statement: There is no conflict of interest to disclose.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at drchandruradioaiims@gmail.com.

CONSORT 2010 statement: The authors have read the CONSORT 2010 statement—checklist of items, and the manuscript was prepared and revised according to the CONSORT 2010 statement—checklist of items.

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S-Editor: Wang LL
L-Editor: Wang TQ
P-Editor: Wang LL

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World Journal of Methodology

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World J Methodol 2022 July 20; 12(4): 285-292

DOI: 10.5662/wjm.v12.i4.285

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Telehealth has comparable outcomes to in-person diabetic foot care during the COVID-19 pandemic

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Specialty type: Orthopedics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Al-Ani RM, Iraq; Moreno-Gómez-Toledano R, Spain

Received: February 9, 2022
Peer-review started: February 9, 2022
First decision: April 12, 2022
Revised: May 19, 2022
Accepted: July 11, 2022
Article in press: July 11, 2022
Published online: July 20, 2022

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Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic has posed obstacles to the delivery of diabetic foot care. In response to this remote healthcare services have been deployed offering monitoring, follow-up, and referral services to patients with diabetic foot ulcers and related conditions. Although, remote diabetic foot care has been studied before the COVID-19 pandemic as an alternative to in-person care, the peculiar situation of the pandemic, which dictates that remote care would be the sole available option for healthcare practitioners and patients, necessitates an evaluation of the relevant knowledge obtained since the beginning of the severe acute respiratory syndrome coronavirus 2 outbreak.

AIM

To perform a thorough search in PubMed/Medline and Cochrane to identify original records on the topic.

METHODS

To identify relevant peer-reviewed publications and gray literature, the authors searched PubMed-MEDLINE and Cochrane Library-Cochrane Central Register of Controlled Trials starting September 27 till October 31, 2021. The reference lists of the selected sources and relevant systematic reviews were also hand–searched to identify potentially relevant resources. Otherwise, the authors searched *Reference Citation Analysis* (https://www.referencecitationanalysis.com/).

RESULTS

A number of randomized prospective studies, case series, and case reports have shown that the effectiveness of remote care is comparable to in-person care in terms of hospitalizations, amputations, and mortality. The level of satisfaction of patients' receiving this type of care was high. The cost of remote healthcare was

not significantly lower than in - person care though.

CONCLUSION
It is noteworthy that remote care during the COVID-19 pandemic appeared to be more effective and well - received than remote care in the past. Nevertheless, larger studies spanning over longer time intervals are necessary in order to validate these results and provide additional insights.

Key Words: Diabetes; Diabetic foot; Telehealth; Telemedicine; COVID-19; SARS-CoV-2

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Core Tip: Telehealth has a major potential to sustain and improve diabetic foot care during the coronavirus disease 2019 (COVID-19) pandemic. Studies reporting the experience of healthcare providers and patients around the globe are encouraging. These findings need to be validated with larger and long – term studies. In the post COVID era, the knowledge and experience obtained can serve as the standpoint of a hybrid approach of telemedicine and in-person care oriented towards delivering fast, efficient and cost-effective care to the patients.

Citation: Kamaratos-Sevdalis N, Kamaratos A, Papadakis M, Tsagkaris C. Telehealth has comparable outcomes to in-person diabetic foot care during the COVID-19 pandemic. *World J Methodol* 2022; 12(4): 285-292
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/285.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.285

INTRODUCTION

During the coronavirus disease 2019 (COVID-19) pandemic, access to healthcare has been hampered by restrictions on citizen movement applied by governments globally as well as people in vulnerable demographics avoiding or delaying visiting healthcare facilities due to health concerns. Internal hospital rearrangements in order to prioritize COVID-19-centered care, especially relevant from our experience in the Diabetes Center of Tzaneio General Hospital of Piraeus in Greece, result in debilitation of the health systems' capacity to assess patients in need in a timely manner[1]. Patients with diabetes mellitus (DM) have been greatly affected by this. In addition to being a high-risk group, they need to consult their treating physicians often to maintain DM and its complications under control[2]. This need has remained unmet on many occasions. The repercussions of this have been evident particularly with regard to diabetic foot ulcerations, where lockdown periods have been followed by an increased rate of emergency hospitalizations and limb amputations[3].

Diabetic foot (DF), as defined by the International Working Group on the Diabetic Foot, is infection, ulceration or destruction of tissues of the foot associated with neuropathy and/or peripheral artery disease in the lower extremity of a person with (a history of) diabetes mellitus[4]. On a global scale, according to Global Burden of Disease an estimate of 131 million (1.8% of the population) people had developed a diabetes related lower extremity complication, chief among them being foot ulcers[5]. DF amounts for a significant amount of healthcare spending, as it is estimated to account for one third of diabetes spending which was $237 billion in 2017 in the United States, increasing by 26% from 2012[6, 7]. As a result, this is a disease which rivals cancer cost ($80.2B in 2015)[7]. We should also take into account indirect costs which include absenteeism from work or reduced productivity and even early mortality, which accounted for $90B[8].

While DF is one of the many diabetes sequelae, it is the one responsible for the most hospitalizations [5]. All diabetic patients have been estimated to have a 25% risk of developing a DF ulcer, with type 2 diabetics having a slightly higher chance[9,10]. Almost 50% of them are expected to become infected and in moderate to severe cases of infection about 20% will require to be amputated[11]. In fact, diabetes dominates nontraumatic lower extremity amputations, accounting for 85% of these operations.

To better understand the challenges of providing appropriate care and preventing amputations in patients with DF, one should consider this condition as a culmination of vascular disease, neuropathy and oftentimes disrupted immunity, vision impairment, debilitating comorbid conditions and frailty [12]. DF care requires frequent visualization, measurement and assessment of the wound by a specialist in addition to diverse treatment strategies including the use of medications, debridement patches and surgical cleaning of the wound. Having all this in mind, we can see how limited healthcare access directly affects the care of these individuals. The potential of remote care to patients unable to access healthcare facilities to stave off this highly morbid disease has been acknowledged before the pandemic. During the pandemic, the need to decrease the DF related burden of secondary and tertiary healthcare

facilities, prevent hospitalizations and protect the patients from life-changing complications became even more evident. Although there is abundant research about remote diabetes care before and during the pandemic, there is limited evidence focusing specifically on DF care under these circumstances.

The authors summarize primary research focusing on digital health and remote care for DF, its precipitating factors and sequelae and identify relevant research gaps and fields of action.

MATERIALS AND METHODS

To identify relevant peer-reviewed publications and gray literature, the authors searched PubMed-Medline and Cochrane Library-Cochrane Central Register of Controlled Trials starting September 27 till October 31, 2021. The reference lists of the selected sources and relevant systematic reviews were also hand–searched to identify potentially relevant resources. Otherwise, the authors searched *Reference Citation Analysis* (https://www.referencecitationanalysis.com/). The search terms: ("Digital health" OR "Remote Healthcare" OR "Telemedicine") AND ("Diabetic Foot"[MeSH] OR "Diabetic Angiopathies"[MeSH] OR "Foot Ulcer [MeSH]" OR "Diabetic Neuropathies"[MeSH]) AND "COVID-19"[MeSH] were used. Studies were included if they fulfilled all the following eligibility criteria: (1) Ongoing or published clinical studies reporting on digital and remote healthcare applications in the prevention or management of DF, its risk factors and sequelae; and (2) Epidemiological analyses and reports. A study was excluded if it met at least one of the following criteria: (1) Non-English publication language; and (2) Study types: editorials, opinion articles, perspectives, letters to the editor. No sample size restriction was applied when screening for eligible studies. Disputes in the selection of relevant studies were discussed between the two primary authors and a senior author until a consensus was reached. The literature was searched and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for Scoping Reviews.

RESULTS

The initial search yielded 29 relevant publications, following the exclusion of non - primary sources from the database search and the deletion of duplicates. After screening titles and abstracts (n = 29) and excluding 12 records on the grounds of irrelevance to the topic, the full texts of 17 articles were assessed. Twelve studies were eventually included in the present review (Figure 1).

A detailed overview of the included studies' characteristics is presented in Table 1.

Eight clinical studies reported on the utilization of telehealth services during the COVID-19 pandemic in the United States, Europe, the United Kingdom, Turkey and India (2020-2021). Four clinical studies with similar design and outcomes that were conducted before the pandemic were included. These studies serve as control when compared to studies conducted during the COVID-19 pandemic. The majority of the studies presented observational data from cohorts, case series or sole case reports, fewer studies were designed as randomized clinical trials and one was based on a cross sectional survey. The existing evidence focused on the effectiveness of remote DF care and touched upon patients' experience and satisfaction and cost evaluation

Effectiveness of remote DF care

Studies regarding the effectiveness of various models of remote DF care during the COVID-19 pandemic paint a mostly positive picture. Utilizing a regime of virtual triage and consultations for a group of patients and comparing the outcomes with standard care from before the pandemic, Rastogi *et al*[13] concluded similar ulcer and limb outcomes in both groups, in a total of 1199 patients. In a randomized control trial (RCT) by Téot *et al*[14] in France that examined 173 patients, healing was insignificantly slower in the telehealth group, while both groups showed similar mortality rates. In an observational cohort study in Italy, Meloni *et al*[15] found telemedical care to be similarly as effective as outpatient care, while neutralizing healthcare setting transmission risk of COVID-19. Moving on to smaller scale studies, case report studies by Shankhdhar *et al*[16], Kavitha *et al*[17] and Ratliff *et al*[18], in India, India and United States respectively, report a positive healing outcome in an ulcer treated exclusively with telemedicine, effective assessment and follow-up of lower risk diabetic foot ulcer (DFU) cases and enhanced healing outcomes with telemedicine utilization respectively. Examining pre-pandemic literature on this topic we can derive that during recent years there has been a rise in interest in modernizing DFU care, although not without some potentially concerning findings. Interestingly studies before the pandemic report higher mortality in telehealth or inadequacy of remote care means like mobile photos - *e.g.*, Rasmussen *et al*[19]; van Netten *et al*[20]. In an RCT by Rasmussen *et al*[19] in 2015, comparing outpatient *vs* telemedical monitoring in DFU, similar healing and amputation rates were found in both groups of 401 patients, but with an inexplicable higher mortality rate in the second group. van Netten *et al*[20], while observing a cohort of 50 patients regarding the reliability of DFU ulcer using mobile phone images concluded it to be an unreliable method of remote assessment. Finally,

Table 1 Characteristics of the included studies

Ref.	Country	Study type	Objective of the study	Sample size	Key outcomes
Rastogi *et al* [13]	India, United Kingdom	Observational cohort	Virtual monitoring of DF complications during COVID-19	1199	Virtual healthcare has similar ulcer/limb outcomes as face-to-face care
Shankhdhar *et al*[16]	India	Case report	DF amputation prevention *via* telemedicine	1	Complete healing was achieved in 4 wk
Rasmussen *et al*[19]		Randomized controlled trial	Comparison between outpatient *vs* telemedical monitoring in DFU	401	Similar healing, amputation rates between both groups, higher mortality in telemedicine
Kilic *et al*[22]	Turkey	Randomized prospective	Developing and evaluating a mobile foot care application for persons with DM	88	Both groups increased knowledge (test group significantly more so), behavior, and self-efficacy
Téot *et al*[14]	France	Randomized Control Trial	Complex Wound Healing Outcomes for Outpatients Receiving Care *via* Telemedicine, Home Health, or Wound Clinic	173	Healing time marginally faster for in-person patients. Mortality comparable
Iacopi *et al* [23]	Italy	Survey	A survey on patients' perception of a telemedicine service for DF	206	Patients thought telemonitoring to be useful during and after the pandemic. Pts with complications worry more about DF than COVID-19
Kavitha *et al* [17]	India	Case Reports	Application of tele-podiatry in diabetic foot management	3	Telemedicine effective in low-risk cases of DFU and for referral of higher-risk. Also effective for follow up
Ratliff *et al* [18]	United States	Case Reports	Telehealth for Wound Management During the COVID-19 Pandemic	2	Improved healing outcomes with implemented telemedicine
Meloni *et al* [15]	Italy	Cohort	Management of DFU during COVID-19: Effectiveness of a new triage pathway	151	Effective telemedical care with negated hospital transmission
Fasterholdt *et al*[24]	Denmark	Randomized Control Trial	Cost-effectiveness of telemonitoring of diabetic foot ulcer patients	374	Telemedicine cost is €2039 less per patient treated *vs* standard care; not statistically significant. Amputation rates were similar
Smith-Strøm *et al*[21]	Norway	Cluster Randomized Control Trial	Effect of Telemedicine Follow-up Care on Diabetes-Related Foot Ulcers	182	No significant difference in healing time, deaths, number of consultations, or patient satisfaction between standard care *vs* telemedicine. TM group had significantly fewer amputations
van Netten *et al*[20]	Australia	Cohort	The validity and reliability of remote diabetic foot ulcer assessment using mobile phone images	50	Mobile phone images should not be used as a stand-alone diagnostic instrument for remote assessment of diabetic foot ulcers due to low reliability

DF: Diabetic foot; COVID-19: Coronavirus disease 2019; DFU: Diabetic foot ulcer.

standard medicine was found comparable to telemedicine in terms of outcome and patient satisfaction in a cluster RCT in Norway by Smith-Strøm *et al*[21], and notably, there were significantly less amputations in the telemedicine group.

Patients' perceptions and cost evaluation

As with any implementation in healthcare, it is of vital importance to gauge patient experience and perception. In a randomized pilot study in Turkey by Kilic *et al*[22], a novel mobile application was developed as a way for patients to submit their blood glucose measurements and potentially pictures as well. This was compared to receiving 30 min of training once by a healthcare professional. After 6 mo, patient education and behavior had improved, and overall increased self-efficacy was found. Patients reported, in their majority, that they appreciated this portal of communication with the specialists and overall thought this was an effective contribution to their DFU care. In another similar study by Iacopi *et al*[23] in Italy, 206 patients' opinions regarding their telemedicine consultations for DFU during the pandemic were assessed, as well as their anxiety regarding both COVID-19 and DFU. Patients were found to be very positive about their experience with telemedicine, finding it both very useful and a potential modality to keep using after the experiment. DFU patients seemed to be significantly more anxious regarding their existing DF disease compared to COVID-19, a result that was more apparent in the subgroup of patients with a history of ulceration, and even more prevalent in a subgroup that had undergone amputation. Regarding cost-effectiveness evaluation, in a study by Fasterholdt *et al*[24], the telemedical approach to treatment and monitoring of DFUs was not statistically significantly cheaper, although being cheaper by 2039 euros per patient. Some limitations of this study are the fact that it was

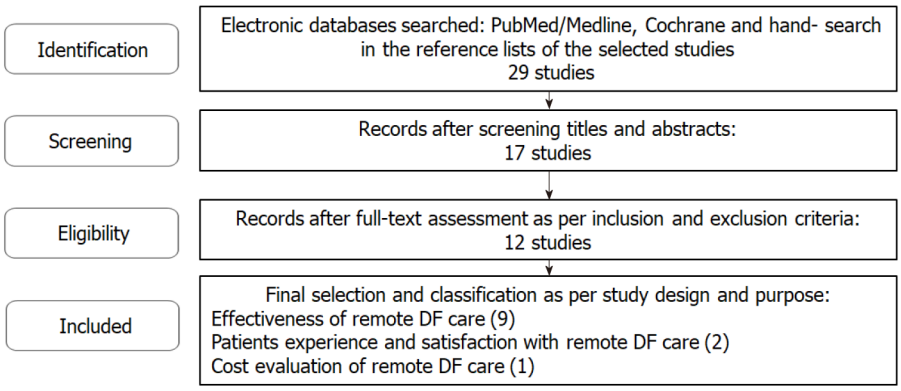

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Figure 1 Literature search flow diagram.

conducted in Denmark in a highly urban setting which reasonably translates to a smaller distance between the patient's setting and the care center in comparison to more rural areas. Furthermore, it did not take into account costs regarding personnel training and telemedicine implementation that would be required in order to apply this remote care modality.

Overall, available evidence suggests that remote DFU care has approximately similar or better outcomes to standard therapy regarding healing time and amputations. There is potential in utilizing telehealth methods in order to triage and consult patients without inconveniencing them with unnecessary and potentially hazardous trips to the physician's office. In the study from Rasmussen *et al* [19] it was concerning that mortality was statistically significantly higher in the telehealth group, but without a concrete accountable reason, more large-scale studies are needed to justify this result. Finally, patients seemed to be content with telehealth applications, can recognize their usefulness and would be open to adding a telehealth element to their treatment regime. It is unfortunate that evidence regarding patient satisfaction is scarce up to this point, but with a more patient-centered healthcare approach undertaken globally, it would be reasonable to expect additional literature in the upcoming years.

DISCUSSION

Overall, it appears that telehealth services for DF remote care during the COVID-19 pandemic have been described in a number of studies, primarily during the first months of 2020. Remote DF care had already been developed before the pandemic, but its use was limited. This can be linked to studies showing increased mortality among telehealth services recipients[19]. It seems that remote DF care during the COVID-19 pandemic became more effective than before, as shown in a study done in Australia examining the adherence to national DF guidelines and treatment efficacy using telemedicine [25]. This can be attributed to the accumulated knowledge that helped physicians to avoid mistakes of the past, to the increased familiarization of physicians, patients and caregivers with telehealth during the last two years and to the relatively short - term monitoring time of the studies in comparison with previous research. Perhaps, monitoring these patients for a longer time would still reveal adverse outcomes that have not become evident to date. This interpretation is subject to a number of factors.

Firstly, one should acknowledge the geographical variation scarcity of the literature. Studies that we reviewed come from Europe (Norway, Denmark, Italy, France, United Kingdom), United States, India and Turkey. Suffice it to say that there's a whole unknown world out there in terms of research on this subject, with large geographical regions not being represented as is. There is no literature regarding regions such as South America, Russia, Central Asia, Asia-Pacific and Africa, among others which inevitably lead to some level of bias. For example, the studies were done in countries and people that had access to remote healthcare services. This is best exemplified by the example of some developing countries, where it's estimated that about one third of the population has access to the internet, the principal foundation of telehealth in DFU. In addition, even in more developed countries there is often a shortage of tech-savvy physicians and lack of appropriate equipment. In our experience in public hospitals in Greece, for example, before the pandemic few web-cameras were available to use by the staff, a problem that thankfully was fixed on time.

There are certainly a number of knowledge gaps with regard to the matter. On top of those implied before. A considerable gap stems from the lack of cost effectiveness data in comparison to the pre-pandemic era. which necessitates further assessment, given that a non - cost effective model of remote care has lower likelihood to survive after the pandemic. Furthermore, there is no data in regard to the physician's perception of remote care, the level of physicians' digital literacy, accountability and financial compensation. Again, judging from the authors' experience, there is a lack of familiarity with

concurrent technology that's proportional to the personnel's age, mostly affecting the most senior members of the staff. In regards to the economics of telehealth, it is unclear whether state and private insurance have a homogenous stance of compensating remote care and whether they compensate at the same rate as in-person care, which, as expected, could stress medical staff. Last but not least, it is necessary to mention that the reported studies involved limited numbers of patients monitored for a number of weeks or months.

Future research needs to address the above limitations in the form of large scale and long-term studies providing - wherever necessary - head-to-head comparisons between patients treated in physical and remote settings. Studies evaluating patients and healthcare professionals' digital literacy can also help make digital health applications more relevant and improve the quality of the provided services. The latter calls for multidisciplinary research and initiatives involving digital health and network specialists apart from healthcare professionals, patients and caregivers.

CONCLUSION

Current evidence seems to favor the implementation of telehealth approaches to DF care. The encouraging results that have been reported thus far need to be monitored and reevaluated in the long term. Likewise, research needs to expand by getting more diverse and inclusive of a greater spectrum of socio-political landscapes. A good example of that is a recent study by Yunir *et al*[26] in Indonesia. We believe the conditions of the pandemic will inevitably contribute to the rapid development of the means of this method, either in the form of new software or patient and physician digital education and familiarization. This could serve as an excellent transition to the post-COVID era, as examined by Anichini *et al*[27], where a hybrid approach of telemedicine and in-person care will work best for all parties involved, delivering fast, efficient and cost-effective care to the patients.

ARTICLE HIGHLIGHTS

Research background

Diabetic foot (DF) care requires frequent visualization, measurement and assessment of the wound by a specialist in addition to diverse treatment modalities. Therefore, limited healthcare access directly affects the care of these individuals.

Research motivation

There is limited evidence focusing specifically on DF care during the pandemic.

Research objectives

To summarize the existing research focusing on digital health and remote care for DF, its precipitating factors and sequelae and identify relevant research gaps and fields of action.

Research methods

The authors searched studies published in PubMed-Medline and Cochrane Library-Cochrane Central Register of Controlled Trials from September 27 until October 31, 2021. The search terms: ("Digital health" OR "Remote Healthcare" OR "Telemedicine") AND ("Diabetic Foot"[MeSH] OR "Diabetic Angiopathies"[MeSH] OR "Foot Ulcer [MeSH]" OR "Diabetic Neuropathies"[MeSH]) AND "COVID-19"[MeSH] were used.

Research results

Remote diabetic foot ulcer care appears to be comparable to standard therapy in terms of outcomes, *i.e.*, healing time and amputation rates.

Research conclusions

The authors believe the conditions of the pandemic will inevitably contribute to the rapid development of the means of this method, either in the form of new software or patient and physician digital education and familiarization.

Research perspectives

These findings need to be validated with larger and long – term studies.

FOOTNOTES

Author contributions: Kamaratos-Sevdalis N co-performed literature search, performed the majority of the writing, and prepared the figures and tables; Kamaratos A and Papadakis M provided input in searching the literature and writing the paper; and Tsagkaris C designed the outline, co-performed literature search and coordinated the writing of the paper.

Conflict-of-interest statement: All authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Ma YJ
L-Editor: A
P-Editor: Ma YJ

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 July 20; 12(4): 293-304

DOI: 10.5662/wjm.v12.i4.293

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Prevalence of precancerous lesions and conditions in India: A systematic review and meta-analysis

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Specialty type: Dentistry, oral surgery and medicine

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Batyrbekov K, Kazakhstan; Haile D, Ethiopia

Received: February 16, 2022
Peer-review started: February 16, 2022
First decision: April 12, 2022
Revised: April 19, 2022
Accepted: July 8, 2022
Article in press: July 8, 2022
Published online: July 20, 2022

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Abstract

BACKGROUND

Smoking and chewing tobacco are associated with numerous oral mucosal lesions and conditions, often leading to cancer progression.

AIM

To investigate the prevalence of precancerous lesions and conditions among the Indian population.

METHODS

Systematic search was conducted for population or community-based observational epidemiological studies in PubMed, EMBASE, Web of Science, IndMED, Google Scholar, reports of the WHO South-East Asia Region, MOHFW India reports, Science Citation Index, WHO Index Medicus of the South-East Asian Region, *Reference Citation Analysis* (https://www.referencecitationanalysis.com/) and Open Grey from the earliest available up to 31st January 2022. The effect size was calculated for the prevalence of precancerous lesions and conditions.

RESULTS

One hundred sixty-two estimates from 130 studies yielded 52 high, 71 moderate, and seven low-quality studies from 823845. Point estimate based on cross-sectional studies for leukoplakia was 4.3% (95%CI: 4.0-4.6), oral submucous fibrosis was 2.7% (95%CI: 2.5-3.0), palatal lesions in reverse smokers and nicotine palatine were 5.8% (95%CI: 4.4-7.2), and Erythroplakia was 1.2% (95%CI: 0.7-1.7),

and lichen planus was 1.1% (95%CI: 0.9-1.2). Amongst hospital-based studies, the pooled prevalence for Leukoplakia was 6.7% (95%CI: 6.0-7.3), oral submucous fibrosis was 4.5% (95%CI: 4.2-4.9), lichen planus was 7.5% (95%CI: 5.3-9.6), and erythroplakia was 2.5% (95%CI: 0.4-4.5), and palatal lesions in reverse smokers and nicotine palatini were 11.5% (95%CI: 8.0-15.0).

CONCLUSION

Precancerous lesions and conditions are prevailing problems among the Indian population. It is mainly due to tobacco use, the smokeless form of tobacco. The meta-analysis indicates that hospital-based studies have a higher effect size of 6.7% than community-based studies. Patients who have already developed this condition may be advised to reduce their exposure to the risk factor to prevent the condition from progressing further.

Key Words: Prevalence; Pre-cancerous lesion; Pre-cancerous condition; India

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Core Tip: World Health Organization assessment estimated that by 2020 tobacco-related death may exceed 1.5 million annually or 13% of all deaths in India. Tobacco consumption and smoking are seen in different socioeconomic groups, and this adverse habit is spread over urban and rural areas, giving rise to precancerous lesions and conditions. Prevalence of various oral lesions and conditions in India are varying in different studies. Numerous studies have been conducted throughout India to determine the prevalence of precancerous lesions and conditions.

Citation: Kumbhalwar A, Shetiya SH, Kakodkar P, Mehta V, Mathur A, Porwal P. Prevalence of precancerous lesions and conditions in India: A systematic review and meta-analysis. *World J Methodol* 2022; 12(4): 293-304
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/293.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.293

INTRODUCTION

World Health Organization (WHO) assessment estimated that by 2020 tobacco-related death may exceed 1.5 million annually, or 13% of all deaths in India[1]. Most smokers living in middle-income countries are the most giant smokers globally, amounting to 68% of all smokers[2]. South-East Asia Region (SEAR) is home to over 80% of global smokeless tobacco (SLT) users, higher than smoking[3]. Prevalence of tobacco use has decreased by 6%, points from 34.6% in GATS-1 in 2009-2010 to 28.6% in GATS-2 in 2016-2017 in India[4].

In the community-based study by Kvv *et al*[5] in 2004, 46579 were examined, and the prevalence of Lichen planus was 2.02%, and Leukoplakia was 1.73%. A study done by Mehrotra *et al*[6] in 2017 amongst 453823 people showed a prevalence of 1.29% for OSMF, 1% for Leukoplakia, and 0.47% for palatal lesions. In a hospital-based study done by Hazarey *et al*[7] in 2007, amongst 266418 patients prevalence of OSMF was 0.37%, and lichen planus was 0.7%. Erythroplakia 0.2% and Leukoplakia 4.8%. In a study done by Pratik *et al*[8] in 2015, amongst 10000 patients, the prevalence of Palatal lesions was 1.96%.

Tobacco consumption and smoking are seen in different socioeconomic groups, and this adverse habit is spread over urban and rural areas, giving rise to precancerous lesions and conditions. WHO has defined precancerous lesions as "a morphologically altered tissue in which oral cancer is more likely to occur than its normal counterpart"; a precancerous condition is 'a generalized state associated with a significantly increased cancer risk[9]. Leukoplakia associated with chewing habits may possess a greater chance of malignant transformation[10]. Different studies vary the prevalence of various oral lesions and conditions in India[11,12]. Numerous studies have been conducted throughout India to determine the prevalence of precancerous lesions and diseases. Hence, a pooled estimate was synthesized, which gave the prevalence of precancerous lesions and conditions among tobacco users.

MATERIALS AND METHODS

The title and details of this selected topic have been registered in PROSPERO (Reg. No. CRD42017062434). This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[13].

Focused question

What is the prevalence of precancerous lesions and conditions among the Indian population?

Literature search

Two authors (Kumbhalwar A and Shetiya SH) independently carried out the literature search. Disagreements on study inclusion, quality assessment, and data extraction were resolved by deliberation or by the third author (Kakodkar P). We searched databases such as PubMed, EMBASE, Web of Science, IndMED, Google Scholar, reports of the WHO South-East Asia Region, CDC tobacco reports, MOHFW India reports, Science Citation Index, WHO Index Medicus of the South-East Asian Region, *Reference Citation Analysis* (https://www.referencecitationanalysis.com/) and Open Grey. The following keywords were utilized to search PubMed: "a precancerous lesion", "precancerous condition", "prevalence", and "India", various combinations of the keywords were used for each precancerous lesion and condition to search Google Scholar, and the first 50 pages were screened for relevant and non-duplicated articles.

Similarly, various combinations of the keywords were used in each of the databases, and the same process was repeated. A set of journals was identified based on their propensity to publish articles on this topic. Each journal issue's table of contents was then screened from the journal's inception till 31st January 2022 for relevant and non-duplicated articles. The cross-references of all selected papers were scanned for additional studies. Attempts were made to retrieve grey literature such as unpublished data, dissertations, and conference proceedings. To obtain publicly inaccessible data, a minimum of two email requests were sent to the corresponding author. If more than one article was published in a study, the article that provided the most updated data was selected.

Study selection

Population or community-based observational epidemiological studies were included. Hospital-based studies assessed oral health, precancerous lesions, and conditions due to risk factors like tobacco and alcohol. Epidemiological studies that provided inadequate information for calculating prevalence, prevalence mentioned in letters to the editor, short communication, and reviews were excluded. Studies that gave prevalence separately for smokers, chewers, and those with mixed habits were not included. Classification of precancerous lesions and conditions by WHO (1978)[14] was used for classifying lesions as leukoplakia, erythroplakia, palatal lesions in reverse smokers, and conditions as oral submucous fibrosis actinic keratosis, lichen planus, and discoid lupus erythematosus.

Data collection process

Data were extracted and calculated concerning the prevalence of the precancerous lesions and conditions from the various studies that met the inclusion criteria. Studies that gave a prevalence of white lesions were considered leukoplakia. Combined prevalence was taken for studies recording oral health status and treatment needs. The highest prevalence was considered for tobacco and alcohol users. The review's objective was to report the point estimate and pooled estimate of lesions and conditions. We carried out a qualitative and quantitative analysis of the observations.

Quality assessment

A total of 5 domains were assessed mainly, study characteristics (author, year of publication, study design) were collected, as well as population variables (sample size, gender, age, and related etiological factors), OPMD features (clinical diagnosis), and outcome measures (prevalence of OPMD). The maximum possible score was 8, and studies scoring 6-8 were classified as high quality, 3-5 as moderate, and less than or equal to 2 were categorized as low-quality studies. Two reviewers (Kumbhalwar A and Shetiya SH) independently conducted quality assessments with any disagreement resolved by consensus (Table 1).

Statistical analysis

The meta-analysis was performed using Open_Meta_Analyst software using the random effect method. We assumed that the estimates from various reviewed studies arose from different populations. The effect size of interest was the prevalence of the respective lesion and condition which developed. A given lesion/condition meta-analysis was conducted separately for community-based and hospital-based studies, and the pooled effect size was obtained. Sub-group analysis was performed to know the prevalence of lesion/condition before and after the Cigarette, and another Tobacco Product Act was implemented across various country regions (North, South, East, and West). Sixty-seven estimates were included from the North region, from South 170 estimates, from East 13 estimates, whereas 102 estimates were included in the review from the West region. Sensitivity analysis was also performed to know the prevalence amongst high, moderate, and low-quality studies. The community and hospital-based studies were pooled for the subgroup and sensitivity analysis. Heterogeneity was checked, and an I^2 value of > 50% was considered evidence of heterogeneity. Statistical significance was set at a P value < 0.05.

Table 1 Quality assessment

Domain	Criteria
Examination	0-Not mentioned
	1-Others (Nurse, ENT doctor, Medical officer, Health worker *etc.*)
	2-by dentist
Study settings	Community setting (field); Hospital setting.
Clinical examination	0-Not mentioned
	1-Visual screening (Tongue blade, Illumination)
	2-Mouth mirror
Sampling technique	Detailed description of the sampling strategy used, type of sampling (random or non-random) was determined.
	0-Not mentioned
	1-Non-random
	2-Random sampling
Sample size adequacy	If description of sample size calculations was not done, the relative precision was calculated (assuming simple random sampling) from the study sample size and estimated proportion.
	Relative precision was ≤ 20% of the point estimate
	0-Relative precision > 20% of the point estimate
	(*e.g.*, If the precision of a study varied from 8%-28% for different lesions and conditions in the mouth, prevalence of more than 20% was considered and score 0 was given)

RESULTS

Qualitative synthesis

A total of 493 unique records were screened by title and abstracts (Figure 1). After full-text reading, three papers were excluded. This exclusion resulted in 130 full-text studies (162 estimates) plus nine unpublished records, one record from National Oral Health Survey, India (2002-2003), and 27 studies from cross-references were included. Few studies were split into a, b, c, *etc.*, indicating the prevalence of lesion/condition within a study. For example, a. psychiatric and b. non-psychiatric inmates, a. fishermen, and b. non-fishermen *etc.* Actinic Keratosis and Discoid Lupus erythematosus considered in the review were not reported in any studies.

Age ranged from childhood to adulthood, and either gender and various states of India were considered. The prevalence of the precancerous lesions varied from 0.44%-73.8%, and the combined prevalence of oral precancerous lesions and conditions ranged from 2.79%-51.21%. One hundred sixty-two estimates from 130 community and hospital-based studies yielded 52 high, 71 moderate, and seven low-quality studies. Prevalence of lesions and conditions was estimated for various country regions, classified as North, South, East, and West.

Quantitative synthesis

A random-effect model was used for meta-analysis as the population from different states, age groups, and gender consuming varied types of smokeless tobacco and smoking were included. The point estimate for various lesions and conditions are given in Table 2.

Heterogeneity was high. Sub-group analysis provided effect size for multiple lesions and conditions before and after COTPA (2003) was enacted and different Indian regions. The studies published before 2003 showed a lower prevalence of the lesions and diseases than those carried out after 2003 (Table 3).

Prevalence of Lichen Planus was highest in the North region, whereas Leukoplakia, Erythroplakia, Palatal lesion, and Oral submucous fibrosis in the Western part of India (Table 4).

High-quality studies showed a higher prevalence of Erythroplakia, Palatal lesion in reverse smokers. OSMF except for Leukoplakia and lichen planus, seen in moderate quality studies (Table 5).

Pooled community and hospital-based studies, studies with high and moderate quality, and studies undertaken after COTPA was enacted showed similar effect sizes around 1.4%-1.6% (Figure 2). Meta-analysis showed an effect size between 8.1-9.2% amongst the moderate quality studies in the southern region, pooled studies, and after COTPA enactment (Figure 3A). Hospital-based studies, high-quality studies, and sand studies reflections from the western and northern parts showed larger effect sizes with wide confidence intervals (Figure 3B).

Table 2 Meta-Analyses for the point estimate of various pre-cancerous lesions and conditions

Precancerous lesions and conditions (Event/*n*)	No of estimates included	Point prevalence (95%CI)	*I*²(%)
LKP[1] (16828/901715)	92	4.3 (4.0-4.6)	99.47
LKP[2] (23090/653349)	46	6.7 (6.0-7.3)	99.74
LKP[3] (39918/1555064)	138	4.9 (4.7-5.2)	99.65
ERP[1] (223/20,164)	12	1.2 (0.7-1.7)	94.97
ERP[2] (1112/275674)	6	2.5 (0.4-4.5)	99.15
ERP[3] (1335/295838)	18	1.4 (1.0-1.7)	97.91
PL[1] (4353/488610)	16	5.8 (4.4-7.2)	99.49
PL[2] (8148/57951)	19	11.5 (8.0-15.0)	99.81
PL[3] (12501/546561)	35	8.9 (7.4-10.3)	99.77
OSMF[1] (9229/749768)	50	2.7 (2.5-3.0)	99.18
OSMF[2] (8160/487272)	38	4.5 (4.2-4.9)	99.58
OSMF[3] (17389/1237040)	88	3.4 (3.2-3.6)	99.43
LP[1] (2759/233782)	48	1.1 (0.9-1.2)	97.59
LP[2] (3811/50300)	25	7.5 (5.3-9.6)	99.92
LP[3] (6570/627947)	73	1.2 (1.1-1.3)	98.14

[1]Community-based studies.
[2]Hospital based studies.
[3]Pooled community and hospital based studies.
LKP: Leukoplakia; ERP: Erythroplakia; PL: Palatal lesion; OSMF: Oral Submucous fibrosis; LP: Lichen planus.

Table 3 Subgroup analyses of precancerous lesions and conditions showing pooled point prevalence before and after COTPA (2003) was enacted

Period of study	LKP (95%CI) (Estimates)	ERP (95%CI) (Estimates)	PL (95%CI) (Estimates)	OSMF (95%CI) (Estimates)	LP (95%CI) (Estimates)
≤ 2003	3.2 (2.5-4.0) (15)	No study; (0)	5.2 (-3.2-13.6); (2)	0.6 (0.4-0.7); (13)	0.6 (0.2-1.0); (4)
> 2003	5.5 (5.2-5.9); (123)	1.4 (1.0-1.7); (18)	9.2 (7.5-10.8); (33)	4.7 (4.4-5.0); (75)	1.3 (1.1-1.4); (69)

LKP: Leukoplakia; ERP: Erythroplakia; PL: Palatal lesion; OSMF: Oral Submucous fibrosis; LP: Lichen planus.

Table 4 Subgroup analyses of precancerous lesions and conditions showing pooled point prevalence in different regions of India

Regions	East (95%CI) (Estimates)	West (95%CI) (Estimates)	North (95%CI) (Estimates)	South (95%CI) (Estimates)
LKP	4.4 (1.9-6.9) (7)	8.4 (7.7-9.1) (44)	5.2 (4.6-5.8) (24)	3.4 (3.0-3.8) (63)
ERP	One study (1)	3.5 (2.1-5.0) (4)	2.9 (-1.5-7.2) (3)	1.0 (0.5-1.5) (10)
PL	No study (0)	16.9 (5.0-28.7) (5)	6.2 (2.4-10.0) (10)	8.1 (6.4-9.8) (20)
OSMF	3.4 (2.1-4.6) (2)	5.1 (4.7-5.4) (34)	1.4 (1.0-1.8) (15)	4.7 (4.2-5.3) (37)
LP	5.0 (1.2-8.7) (3)	1.2 (1.0-1.5) (15)	1.7 (1.2-2.3) (15)	1.0 (0.7-1.2) (40)

LKP: Leukoplakia; ERP: Erythroplakia; PL: Palatal lesion; OSMF: Oral Submucous fibrosis; LP: Lichen planus.

DISCUSSION

Summary of evidence

Most of the studies in the review were carried out in the Southern states of India. For those studies

Table 5 Sensitivity analyses of precancerous lesions and conditions showing pooled

Study quality (Studies)	LKP (95%CI)	ERP (95%CI)	PL (95%CI)	OSMF (95%CI)	LP (95%CI)
High (52)	4.6 (4.2-5.0)	1.6 (0.9-2.3)	11.0 (8.2-13.8)	4.0 (3.6-4.4)	1.1 (0.9-1.3)
Moderate (71)	6.6 (5.9-7.2)	1.6 (0.5-2.7)	8.2 (5.2-11.2)	3.3 (3.0-3.5)	1.3 (1.1-1.5)
Low (7)	1.4 (0.9-1.8)	One study	One study	2.8 (1.3-4.3)	No study

LKP: Leukoplakia; ERP: Erythroplakia; PL: Palatal lesion; OSMF: Oral Submucous fibrosis; LP: Lichen planus.

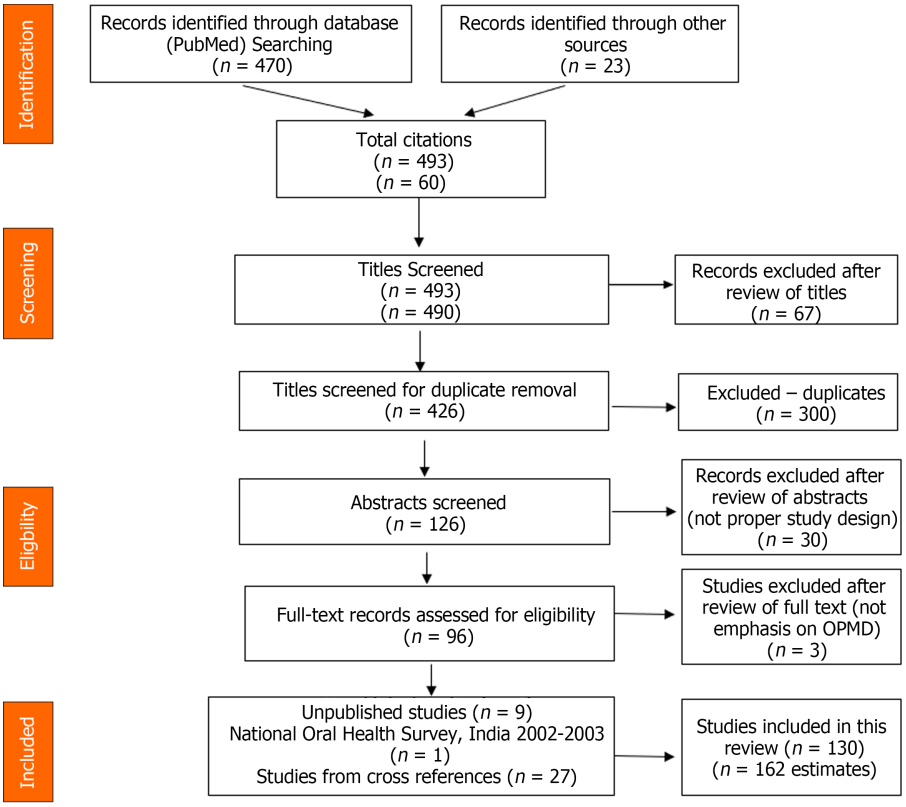

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Figure 1 Flow chart showing the literature searched.

where oral health assessment was the primary objective, the authors used WHO-Oral Health Assessment Proforma 1986, 1997, or 2013. Eighty-one field surveys were assessed, and 49 studies were exclusively done in the hospital setting, while most community and hospital-based studies were undertaken after 2003. Since there was no uniformity in the definition and classification of lesions and conditions, the chance of bias in determining the prevalence could be prevailing in the considered observational studies.

Prevalence of precancerous lesions and conditions in Population

More than 50% prevalence of all precancerous lesions and disorders was reported in specific population groups like fishermen and urban/rural populations who are tobacco consumers or slum dwellers or patients reporting to a dental college general population.

Leukoplakia

It was observed that the prevalence was higher amongst mine laborers, industrial workers, institutionalized elderly, chewers, jail inmates, fishermen, sex workers, tribes, and laborers in community-based studies. Most of the included studies have reported the prevalence of leukoplakia, unlike the other lesions and conditions.

Hospital-based studies, moderate quality records, and studies undertaken after COTPA was enacted showed a similar effect size of around 6%. Available estimates on the affordability of SLT products have indicated that they have become more affordable in India from 2001-to 2007[3]. However, the pooled estimate and the high-quality studies and those studies done in the northern region show an effect size

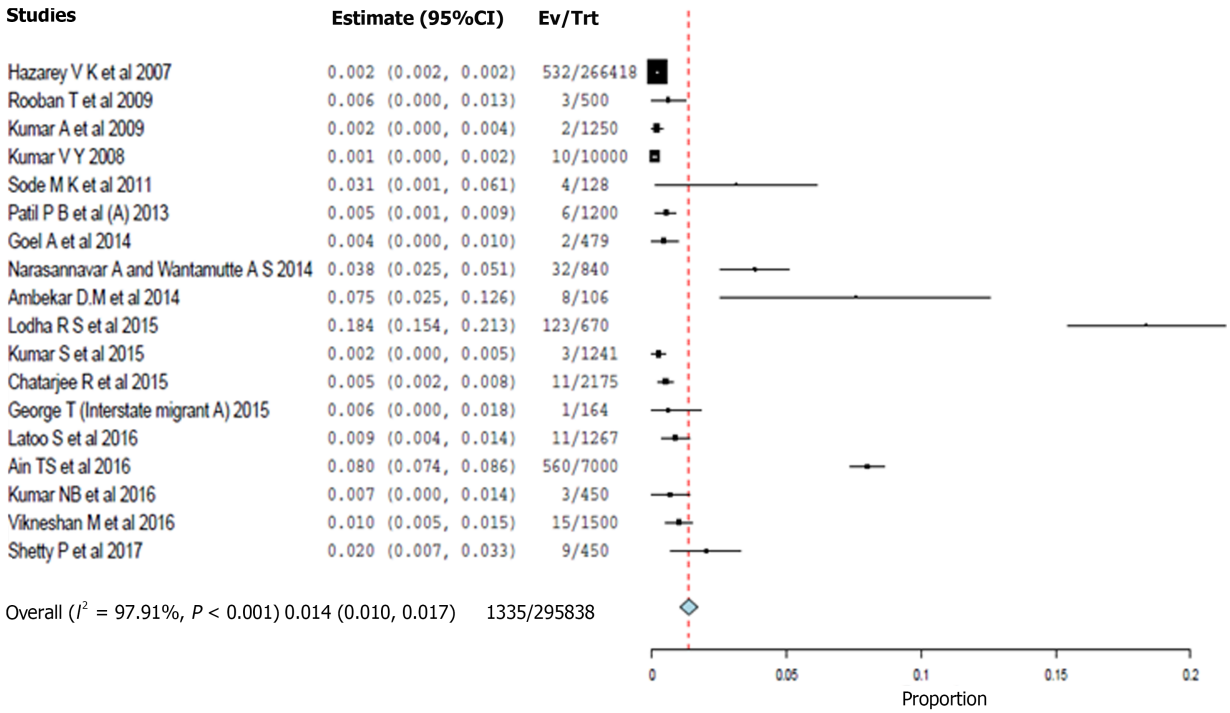

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Figure 2 Forest plot for the meta-analysis of erythroplakia prevalence (pooled community and hospital based studies).

of around 5%. This indicates that the prevalence of leukoplakia is around 5%-6%, and the effect sizes show a narrow confidence interval.

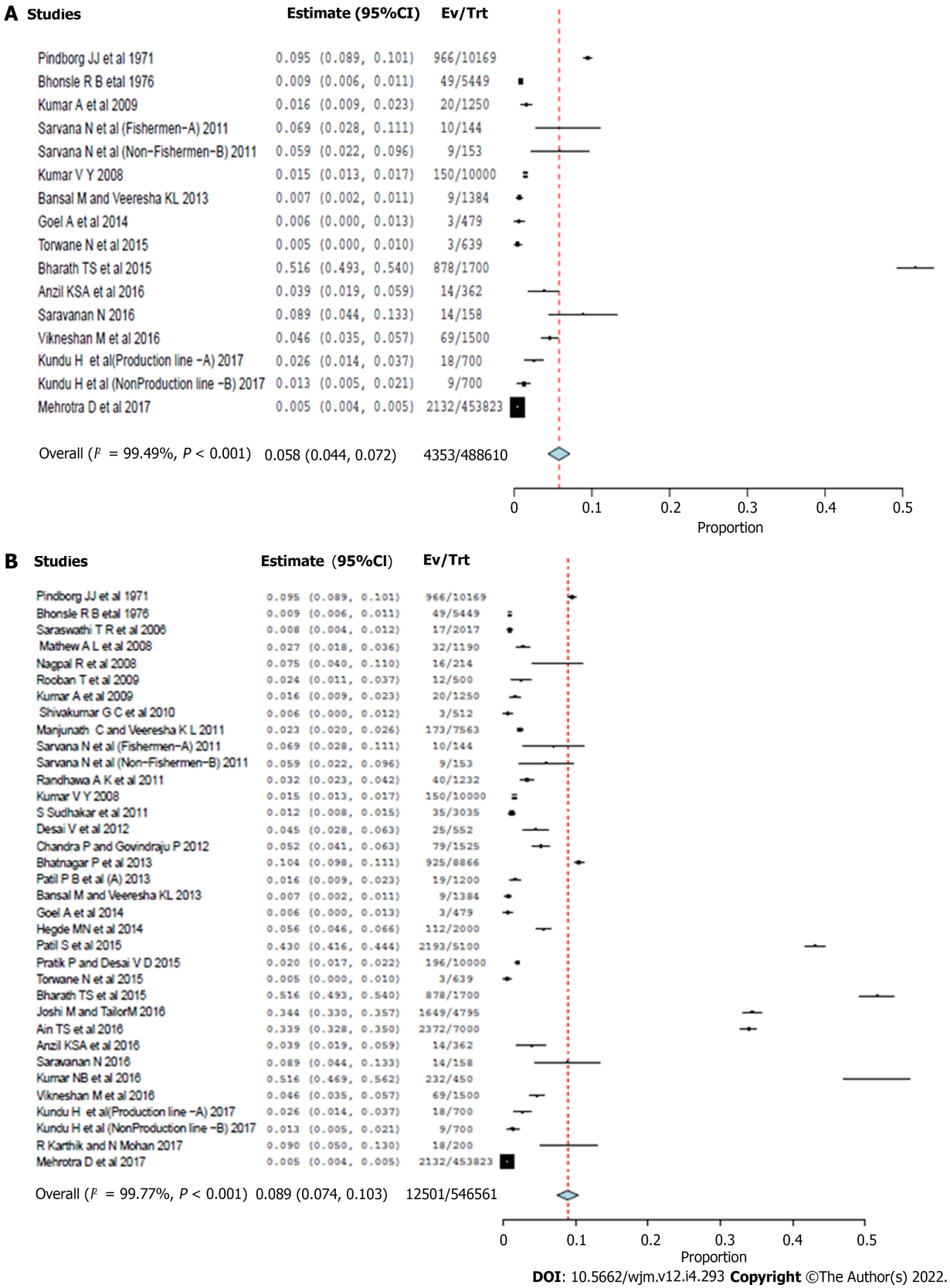
Hospital-based studies by Hazarey *et al*[7] and Kumar *et al*[15] conducted amongst 266418 and 25400 patients indicated a 4.8% and 6.16% prevalence for leukoplakia which is in concordance with this meta-analysis effect size. The risk factors that may cause oral leukoplakia include tobacco smoking (especially for localized leukoplakia), heavy alcohol consumption, and areca nut use. SLT lesions are caused by contact with tobacco-containing caustic agents. Early lesions are reversible and are usually resolved when the habit is discontinued. True leukoplakia has substantial potential to develop into cancer. It should be biopsied to rule out dysplasia[16]. Retail prices are generally lower for SLT products in low-income and low-middle-income countries and higher in high-income countries[3].

Erythroplakia

It was observed in the review that the prevalence of erythroplakia is higher in slum dwellers and prisoners. Erythroplakia showed an effect size of 1.2% for the community-based studies, which is lower than hospital-based studies. Pooled community and hospital-based studies, studies with high and moderate quality, and studies undertaken after COTPA was enacted showed similar effect sizes around 1.4%-1.6%. However, the western region's hospital-based studies estimates and analyses show an effect size of around 2.5%-3.4%. The prevalence of erythroplakia was about 1.4%-1.6%, as indicated by the narrow confidence interval. Studies carried out by Hazarey *et al*[7] and Kumar *et al*[15] amongst 266418 and 10000 study populations showed the prevalence of erythroplakia to be 0.2%-0.1% in concordance with the present meta-analysis effect size. Erythroplakia offers dysplastic features and often presents as "carcinoma in situ" at the time of biopsy. Heavy alcohol consumption and tobacco use are known to be important etiological factors[17,18]. Implementation of the ban on SLT Advertisement, Promotion, and Sponsorship status over high SLT burden Parties such as in India, is poor and exposure to SLT advertisements and promotion among adults is more elevated than smoked products[3], which is a deterrent to the cause of lesion and condition.

Lichen planus

The probable cause of lichen planus from the growing database of information about this disorder suggests specific immune responses, stress, and viral infection[19]. Though tobacco is not an etiological factor, it was part of the WHO classification and considered here. It was observed in the review that the prevalence is higher in the geriatric population. High and moderate-quality community-based studies in India's southern and western regions and those conducted after 2003 showed similar effect sizes with narrow confidence intervals. The prevalence of Lichen planus could be around 1%. Community-based studies done by Smith *et al*[20] and Kvv *et al*[5] amongst 57518 and 46579 people showed a prevalence of 0.63%-2.02%, which is in concordance with the present meta-analysis, which has indicated an effect size



DOI: 10.5662/wjm.v12.i4.293 **Copyright** ©The Author(s) 2022.

Figure 3 Forest plot for the prevalence of palatal lesion in reverse smoker's. A: Community based studies; B: Pooled community and hospital-based studies.

of 1.1 %.

Oral submucous fibrosis

The review suggests that the prevalence is higher in jail inmates. Hospital-based studies, studies with

high quality, those done in India's southern and western region, and those undertaken after COTPA was enacted showed a similar effect size, around 4-5%, with a narrow confidence interval. Community-based studies, pooled studies, and studies with moderate quality showed a similar effect size of 3%. Studies were done by Kumar *et al*[15], and Mehrotra *et al*[6] amongst 25400 and 453823 study populations showed a prevalence of 1.29%, which is not in concordance with the present meta-analysis result, whereas 3.96%, which is in concordance with the current meta-analysis effect size. In southeast Asia, SLT is often mixed with areca nut, betel leaf, slaked lime, and spices, and these preparations are strongly associated with SMF, a fibrotic precancerous condition[21].

In India, some states and union territories have been relatively successful in enforcing the ban on gutkha. However, the tobacco industry is circumventing these bans by selling pan masala and tobacco in separate pouches. Successive GATS surveys in India in 2010 and 2017 revealed a significant reduction in the prevalence of SLT use in the general adult population[3]. As of 2016-2017, there has been a 1% reduction in the percentage of the adult population using *Gutkha* in India[4].

Palatal lesions in reverse smokers

This disorder is specific to populations who smoke with the lighted end of the cigarette inside the mouth, resulting in red, white, or mixed palate lesions[14]. Few studies reported palatal lesions in reverse smokers, showing a wide variation of prevalence from 0.9%[22]. 51.77%[23] from Goa and Andhra Pradesh amongst community-based studies. Fishers of Andhra Pradesh showed a higher prevalence. Meta-analysis showed an effect size between 8.1-9.2% amongst the moderate quality studies in the southern region, pooled studies, and after COTPA enactment. However, hospital-based studies, high-quality studies, and sand studies reflections from the western and northern parts showed larger effect sizes with wide confidence intervals. Studies were done by Mehrotra *et al*[6] and Pindborg *et al*[24] amongst 453823, and 10169 study populations showed a prevalence of 0.47%, which is not by the present meta-analysis result and 9.5%, which is in concordance with the current meta-analysis effect size.

Smoking prevalence in low and middle-income countries is projected to decline slower than in high-income countries[2]. There is a possibility of worldwide tuberculosis rates falling as much as 20% if smoking is eliminated[25]. 68% and 17% of cigarette and bidi smokers purchased loose cigarettes and bidis. On average, the expenditure incurred during the last purchase was Rs 30 and Rs 12.5, respectively, making the purchase easy for a commoner[4].

Actinic keratosis and discoid lupus erythematosus

Actinic keratosis represents a potentially malignant lip condition[26], while discoid lupus erythematosus (DLE) is a chronic autoimmune disease of unknown etiology[27]. None of the reviewed studies reported on the above two conditions.

CONCLUSION

Precancerous lesions and conditions are prevailing problems among the Indian population. It is mainly due to tobacco use, the smokeless form of tobacco. The meta-analysis indicates that hospital-based studies have a higher effect size of 6.7% than community-based studies, which show an effect size of 4.3%. Based on the present meta-analysis, the prevalence of leukoplakia is around 5%-6%. The majority of erythroplakia in community-based studies is lower (1.2%) than in hospital-based studies. The prevalence of erythroplakia in the current meta-analysis is 1.4-1.6%, as indicated by the narrow confidence interval. The prevalence of lichen planus seems to be higher (7.5%) for hospital-based studies than for community-based studies. The prevalence of Lichen planus is around 1%. The prevalence of oral submucous fibrosis seems higher (4.5%) for hospital-based studies than for community-based studies. The prevalence of oral submucous fibrosis was around 4%-5%. Compared to hospital-based studies, most Palatal lesions in community-based studies are lower (5.8%). Meta-analysis showed an effect size between 8.1%-9.2% amongst the moderate quality studies. Knowing these risk factors paved the way for more effective prevention of these pre-cancerous conditions. Patients who have already developed this condition may be advised to reduce their exposure to this risk factor to prevent the disorder from progressing further. Early intervention is essential to effective prevention. Thus, necessary efforts should be implemented.

ARTICLE HIGHLIGHTS

Research background

World Health Organization (WHO) assessment estimated that by 2020 tobacco-related death may exceed 1.5 million annually or 13% of all deaths in India. Tobacco consumption and smoking are seen in different socioeconomic groups, and this adverse habit is spread over urban and rural areas, giving rise

to precancerous lesions and conditions. Prevalence of various oral lesions and conditions in India are varying in different studies. Numerous studies have been conducted throughout India to determine the prevalence of precancerous lesions and conditions.

Research motivation

Tobacco consumption and smoking are seen in different socioeconomic groups, and this adverse habit is spread over urban and rural areas, giving rise to precancerous lesions and conditions. Different studies vary the prevalence of various oral lesions and conditions in India. So we were interested in compiling the data of precancerous lesions and conditions.

Research objectives

The objective of the present systematic literature review was to investigate a pooled estimate, which gave the prevalence of precancerous lesions and conditions among tobacco users in India population.

Research methods

Systematic search was conducted for population or community-based observational epidemiological studies in PubMed, EMBASE, Web of Science, IndMED, Google Scholar, reports of the WHO South-East Asia Region, MOHFW India reports, Science Citation Index, WHO Index Medicus of the South-East Asian Region, *Reference Citation Analysis* (https://www.referencecitationanalysis.com/) and Open Grey from the earliest available up to 31st January 2022. The effect size was calculated for the prevalence of precancerous lesions and conditions.

Research results

One hundred sixty-two estimates from 130 studies yielded 52 high, 71 moderate, and seven low-quality studies from 823845. Point estimate based on cross-sectional studies for leukoplakia was 4.3% (95%CI: 4.0-4.6), oral submucous fibrosis was 2.7% (95%CI: 2.5-3.0), palatal lesions in reverse smokers and nicotine palatine were 5.8% (95%CI: 4.4-7.2), and Erythroplakia was 1.2% (95%CI: 0.7-1.7), and lichen planus was 1.1% (95%CI: 0.9-1.2). Amongst hospital-based studies, the pooled prevalence for Leukoplakia was 6.7% (95%CI: 6.0-7.3), oral submucous fibrosis was 4.5% (95%CI: 4.2-4.9), lichen planus was 7.5% (95%CI: 5.3-9.6), and erythroplakia was 2.5% (95%CI: 0.4-4.5), and palatal lesions in reverse smokers and nicotine palatini were 11.5% (95%CI: 8.0-15.0). The meta-analysis indicates that hospital-based studies have a higher effect size of 6.7% than community-based studies, which show an effect size of 4.3%. Based on the present meta-analysis, the prevalence of leukoplakia is around 5%-6%. The prevalence of erythroplakia in community-based studies is lower (1.2%) than in hospital-based studies.

Research conclusions

Precancerous lesions and conditions are prevailing problems among the Indian population. It is mainly due to tobacco use, the smokeless form of tobacco. The meta-analysis indicates that hospital-based studies have a higher effect size of 6.7% than community-based studies. Patients who have already developed this condition may be advised to reduce their exposure to the risk factor to prevent the condition from progressing further.

Research perspectives

Knowing these risk factors paved the way for more effective prevention of these pre-cancerous conditions. Patients who have already developed this condition may be advised to reduce their exposure to this risk factor to prevent the disorder from progressing further. Early intervention is essential to effective prevention. Thus, necessary efforts should be implemented.

FOOTNOTES

Author contributions: Kumbhalwar A, Shetiya SH, Kakodkar P, Mehta V, Mathur A and Porwal P contributed to acquisition of data, analysis and interpretation of data, drafting the article, making critical revisions related to important intellectual content of the manuscript, and final approval; Mehta V contributed to acquisition of data, analysis and interpretation of data, making critical revisions related to important intellectual content of the manuscript, and final approval; Shetiya SH contributed to conception and design of the study, acquisition of data, making critical revisions related to important intellectual content of the manuscript, and final approval; and All authors discussed the results and contributed to the final manuscript.

Conflict-of-interest statement: All authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by

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Country/Territory of origin: India

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S-Editor: Ma Y
L-Editor: A
P-Editor: Cai YX

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World Journal of Methodology

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World J Methodol 2022 July 20; 12(4): 305-318

DOI: 10.5662/wjm.v12.i4.305

ISSN 2222-0682 (online)

META-ANALYSIS

Prevalence of human leishmaniasis in Sudan: A systematic review and meta-analysis

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Specialty type: Infectious Diseases

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Hasabo EA, Sudan; Xiong A, China

Received: December 6, 2021
Peer-review started: December 6, 2021
First decision: January 25, 2022
Revised: January 31, 2022
Accepted: June 26, 2022
Article in press: June 26, 2022
Published online: July 20, 2022

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Abstract

BACKGROUND

There are three main forms of leishmaniasis in humans: cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis. The prevalence of human leishmaniasis varies widely in different countries and different regions of the same country. To date, there is no overall estimation of the prevalence of human leishmaniasis in Sudan.

AIM

To determine the pooled prevalence of human leishmaniasis and the disease risk factors among Sudanese citizens.

METHODS

From all articles written in English or Arabic languages conducted before the 4th of August 2021 from [Scopus, Web of Science, PubMed, and MEDLINE, African Journals Online (AJOL), ResearchGate, direct Google search, Google Scholar, and

universities websites], just 20 articles with a total of 230960 participants were eligible for this study. Data synthesis and analysis were done using STATA software, version 16. EndNote citation manager version X9.3.3 and *Reference Citation Analysis* (*RCA*) were used to remove the duplicated studies and manage the citation respectively.

RESULTS

The overall pooled prevalence of human leishmaniasis in Sudan was 21% (with confidence interval 12%-30%). CL was the most common type of leishmaniasis in Sudan, with a pooled prevalence of 26% followed by VL (18%). Nevertheless, the pooled prevalence of human leishmaniasis in Sudan was higher in males compared with females (60% *vs* 40%). The current results revealed that the people in the age group between 15 and 44 were the most affected group (60%), and central Sudan has the highest pooled prevalence of human leishmaniasis (27%) compared with other regions of Sudan. Finally, the prevalence of human leishmaniasis seems to decrease with time.

CONCLUSION

This study showed that human leishmaniasis infection is still endemic in many regions in Sudan and highly prevalent in central and eastern Sudan, and CL is the most prevalent in the country. Males and adults were more susceptible to infection compared with females and children. However, the human leishmaniasis prevalence decreased relatively over time.

Key Words: Cutaneous leishmaniasis; Human leishmaniasis; Meta-analysis; Prevalence; Sudan; Visceral leishmaniasis

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Core Tip: A comprehensive systematic review and meta-analysis study was conducted to find the pooled prevalence of leishmaniasis and its associated factors among Sudanese citizens. After applying all required quality check-ups for the individual studies, 20 studies were included in this study. The pooled prevalence of human leishmaniasis in Sudan was 21%, and cutaneous leishmaniasis was the commonest form of leishmaniasis in Sudan. Finally, the results of this study showed that human leishmaniasis infection is still endemic in many regions in Sudan.

Citation: Ahmed M, Abdulslam Abdullah A, Bello I, Hamad S, Bashir A. Prevalence of human leishmaniasis in Sudan: A systematic review and meta-analysis. *World J Methodol* 2022; 12(4): 305-318
URL: https://www.wjgnet.com/2222-0682/full/v12/i4/305.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i4.305

INTRODUCTION

Neglected tropical zoonotic diseases (NTZDs) are endemic diseases in many developing countries of Africa, Asia, and Latin America[1]. The WHO's annual report for 2021, revealed that leishmaniasis is set among the top ten NTZDs worldwide[2].

In addition to the zoonotic nature of the disease, leishmaniasis is transmitted to humans by the infected female sandflies with *Leishmania* parasite, when it feeds on the human's blood[3]. There are three main forms of the disease in humans: cutaneous leishmaniasis (CL), which mainly features skin lesions, visceral leishmaniasis (VL), or Kala-azar, which can affect the spleen, liver, and bone marrow leading to some serious symptoms, and mucocutaneous leishmaniasis (ML)[3]. Of the three leishmaniasis forms, VL is the most lethal with a fatality rate of 95% if it is left untreated, while CL is the most common form[2]. In general, the high incidence and prevalence of human leishmaniasis have been highly associated with the prevalence of conditions that leads to a weak immune response, such as AIDS or tuberculosis. Studies also found a strong association between leishmaniasis prevalence and poor household status, poverty, population displacement, and recent climate change[4-7].

Evidence showed that the annual incidence of human leishmaniasis was 700000 to 1 million new cases. Although the disease was reported in 89 countries all around the world, East Africa, Southeast Asia, and South America countries, have the highest incidence rates[8]. Nevertheless, almost all reported outbreaks of human leishmaniasis were from East African countries, namely Sudan, South Sudan, and Ethiopia[9-13].

Sudan has a long history of leishmaniasis which was firstly discovered by Neave in the early 1900s [14]. Moreover, in the late twentieth century, several leishmaniasis (CL & VL) outbreaks were reported

in the eastern and central parts of the country[15]. The geographical distribution study of human leishmaniasis in Sudan found a high relationship between disease occurrence and vector distribution[16, 17]. Reports from Sudan found that the VL is endemic in the country, especially in the savannah area in the eastern and central parts of the country, which lies between four states (White Nile State in the west, Gadarif state in the east, Blue Nile State in the south, and Kassala state in the northeast)[18]. Moreover, VL was reported outside the savannah area in some scattered foci in the western parts of the country in Darfur states and Kordofan states[19]. Furthermore, national-wide epidemiological studies, report the endemic presence of the CL, especially in the northern, central, and western parts of the country[15]. For all the above reasons, it can be said that human leishmaniasis (both CL & VL) is endemic in Sudan, and the disease represents a serious health problem that affects the whole healthcare system[20].

Despite the importance of the disease in Sudan and the many published studies across the country that described the epidemiology of human leishmaniasis, no study estimated the overall prevalence of the disease at the national level exists to date. The lack of evidence about the disease in the country may prevent the health care policymakers and stakeholders from developing and adopting a suitable prevention program. Thus, the current study aimed to investigate the pooled prevalence of human leishmaniasis (both CL and VL) in Sudan.

MATERIALS AND METHODS

Eligibility criteria

The following were the eligibility criteria of this study: (1) All human observational studies; (2) Done on the Sudanese population; (3) Published in Arabic or English; (4) Reported the prevalence of human leishmaniasis (CL and VL); and (5) The positive cases of leishmaniasis were detected using the standards' diagnostics methods (serological and molecular tests). Moreover, studies were not eligible for this study (1) If they were reviews, letters, editorials, animal studies; and (2) If the full text was not available and has been requested from the author(s) through email but no feedback was received after 2 wk.

Information sources

This meta-analysis study was conducted according to the guideline of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[21]. The relevant information was retrieved from the electronic databases sources, namely Scopus, Web of Science, PubMed, MEDLINE, African Journals Online (AJOL), ResearchGate, direct Google search, Google Scholar, and universities websites. All indicated databases were searched from their inception to the 4th of August 2021, for human studies published in English and/or Arabic.

Search strategy

To achieve the current study objectives, a research strategy was developed using the Boolean search terms (AND, OR, NOT). The final search strategy included the use of Title/Abstract related to ((human leishmaniasis) AND ((prevalence) OR (epidemiology) OR (frequency)) OR (Risk factors)) AND Sudan) taken from the study questions. In addition, a manual search was done by the investigators for the grey literature and unpublished thesis/papers.

Selection process

Initially, primary screening was done based on the inclusion and exclusion criteria. Thereafter, all retrieved studies were exported to the EndNote citation manager version X9.3.3, to remove the duplicated studies. After that, the remaining articles were screened and evaluated by two investigators (Ahmed M and Abdulslam Abdullah A) independently. The investigators carefully have read the title, abstract, and full text of each article to eliminate the unrelated studies to prior defined objectives. Furthermore, the remaining articles were considered for further quality checkups against the checklist of Joanna Briggs Institute quality assessment tools[22]. Any discrepancy in the study findings was resolved by discussion between the two authors (Ahmed M and Abdulslam Abdullah A) or by consulting Hamad S. Figure 1 shows the selection process using the PRISMA statement flow diagram. Finally, *Reference Citation Analysis* (*RCA*) were used to manage the citation.

Data collection process

Following the selection process, the relevant data were extracted using a Microsoft word 2016 data extraction template.

Two investigators (Ahmed M and Abdulslam Abdullah A) contacted the corresponding author of any study that failed to report the information required for the eligibility criteria indicated above (*via* email) to get the original data; however, if the missing data were not obtained after 2 wk, a sensitivity analysis was carried out to remove the studies with the missing information. The extraction template contains (author/s name and publication year, study period, study design, study setting, geographical location

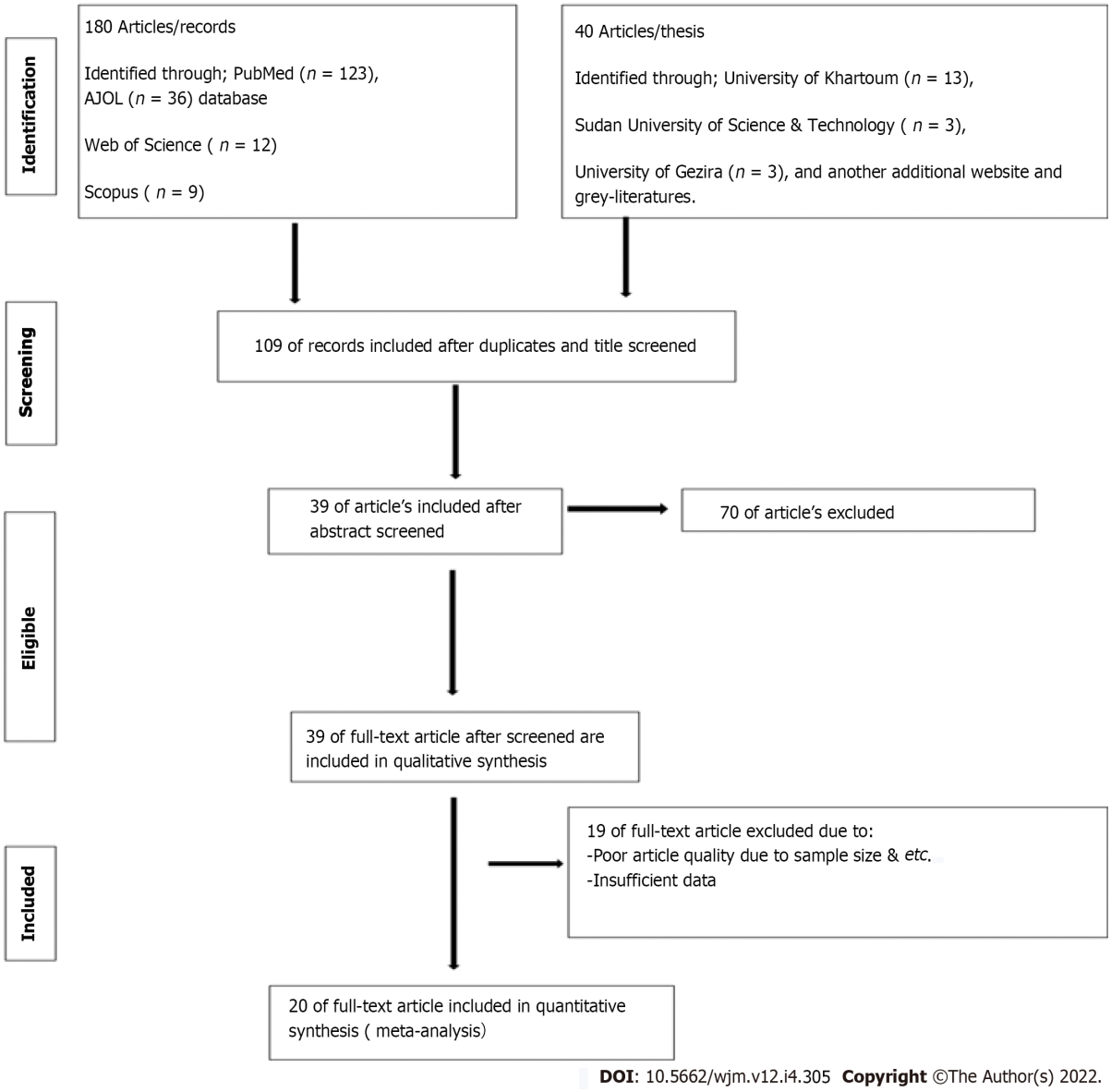

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Figure 1 Flow diagram of the studies included in this meta-analysis.

(based on state names), type of leishmaniasis (VL & CL), sample size, diagnostic method, and the prevalence of leishmaniasis in (overall and male and female) (Table 1). The accuracy of the data extraction process was verified by comparing the extraction results of 2 authors (Ahmed M and Abdulslam Abdullah A), who extracted the data independently, in a randomly- chosen set of papers (30% of the total).

Data items and effect measures

The prevalence of human leishmaniasis in Sudan was the main outcome of the current study. Moreover, the prevalence was measured from the individual studies by the direct report. To quantify the outcome, studies that reported the prevalence of VL and/or CL in their statistics were considered. Finally, the result was interpreted by the proportions of the population who tested positive for leishmaniasis compared with the total population studied.

Study risk of bias assessment

The risk of bias for this study was checked through several steps: firstly, by appraising the eligibility criteria for all retrieved articles by checking the title and abstract for each retrieved study; secondly, the full-text for each included study from step one was screened using the quality assessment criteria to identify their quality before the final selection. The quality assessment criteria used to determine if the study could be included were: (1) The presence of Leishman parasite in the patient was identified after performing the appropriate diagnostic tests; and (2) From the statistical point of view, the study sample was representative of the study population. To minimize the risk of bias two strategies were followed:

Table 1 Main characteristics of studies included in the meta-analysis

Ref.	Sample size	Method	Type of leishmaniasis	Geographical location	Study design	Study setting	Prevalence *n* (%)		
							Overall, *n* %	Male	Female
Hashim[24], 1997	126	PCR & LST	VL/CL	Central Sudan	CS	HB	43 (34.1)	NR	NR
El Dawi[25], 1994	44	DAT	VL	Central Sudan	PS	HB	19 (43.2)	NR	NR
Ibrahim[26], 2012	734	LST	CL	Central Sudan	CS	CB	73 (9.9)	NR	NR
Sharief *et al* [27], 2019	1781	DAT & LST	VL	Western Sudan	ES	CB	238 (13)	NR	NR
Osman[28], 2011	332	PCR	CL	Western Sudan	CS	CB	32 (9.6)	NR	NR
Noraldaim[29], 2012	110	DAT & ELISA	VL	Central Sudan	PS	CB	46 (41.8)	NR	NR
Mohamed *et al* [30], 2019	95	DAT	VL	Eastern Sudan	CS	CB	5 (5.3)	NR	NR
Dereure *et al* [31], 2003	79	Culture	VL	Eastern Sudan	NR	CB	23 (29.1)	NR	NR
EL-Safi *et al* [18], 2002	947	DAT & LST	VL	Eastern Sudan	CS	CB	132 (13.9)	NR	NR
El-Safi and Peters[32], 1991	9657	DAT	CL	Central Sudan	RS	HB	736 (7.6)	449 (61)	287 (39)
Atia[23], 2012	373	DAT	VL	Eastern Sudan	CS	CB	64 (17.2)	29 (45.3)	35 (54.7)
Abdallah[34], 2015	352	DAT & ELISA	VL	Eastern Sudan	PS	HB	71 (20.2)	43 (60.6)	28 (39.4)
Ebrahim[19], 2016	48972	Mixed	VL	Western Sudan	RS	HB	815 (1.7)	(62)	(38)
Awadalla[35], 2007	399	DAT	VL	Eastern Sudan	CS	CB	35 (8.8)	23 (65.7)	12 (34.3)
Muawyia *et al* [36], 2021	40	DAT	CL	Central Sudan	NR	HB	13 (32.5)	10 (76.9)	3 (23.1)
Osman *et al* [37], 2021	410	LST	CL	Northern Sudan	CS	CB	290 (70.7)	91 (31.4)	199 (68.6)
Abdullah *et al* [38], 2021	162443	Mixed	VL/CL	Western Sudan	RS	HB	7131 (4.4)	4657 (65.3)	2474 (34.7)
Ahmed[39], 2011	50	Mixed	VL	Central Sudan	CS	HB	NR	38 (76)	12 (24)
Ahmed[40], 2017	215	Mixed	VL	Eastern Sudan	R-CC	HB	NR	140 (65.1)	75 (34.9)
Collis *et al*[41], 2019	3801	LST	CL	Nationwide	RS	HB	NR	2178 (57.3)	1599 (42.1)

CB: Community-based study; CL: Cutaneous leishmaniasis; CS: Cross sectional study; DAT: Direct agglutination test; DS: Descriptive study; ELISA: Enzyme-linked immunosorbent assay; ES: Epidemiological surveys; HB: Hospital-based study; LST: Leishmania skin test; NR; Not reported; PCR: Polymerase chain reaction; PS: Prospective study; R-CC: Retrospective case-control study; RS: Retrospective study; VL: Visceral leishmaniasis.

(1) A comprehensive search for all electronic and non-electronic databases; and (2) A critical appraisal tool (Joanna Briggs Institute Quality Assessment Tool)[22] was used by two investigators (Ahmed M and Abdulslam Abdullah A) independently to critically appraise the included studies. The publication bias in the current review was checked primarily by Egger's regression test, which is a test of statistical symmetry of the funnel plot. Also, visualizing the inspection of the funnel plot was used to check the publication bias.

Registration and protocol

This review was developed based on the PRISMA guideline[21]. The review protocol has been registered by the International prospective register of systematic reviews at https://www.crd.york.ac.uk/Prospero/#recordDetails (No. CRD42021270418).

Synthesis methods

The collected study data were synthesized and analyzed using the STATA software, version 16.0 (Stata Corp LLC, 77845 Texas, United States). Statistically significance was set for *P* values < 0.05. The heterogeneity test was conducted using the degree of inconsistency (I^2), which is a percentage, and range from (0%-100%), moreover, Higgins *et al*[23] described the heterogeneity to be low, medium, and high, for the (I^2) values of 25%, 50%, and 75% respectively. Two statistical measurements were used to calculate the result of this study: effect size with a 95% confidence interval (CI) and standard error (SE). The prevalence of leishmaniasis (proportion) was considered as the effect size of this study, and the binomial distribution was used to calculate it.

The standard error was calculated using the following data: sample size (n) and the proportion of leishmaniasis positive case among the overall population (p) using the SE formula: SE = sqrt[]p (1-p) / n).

In the final meta-analysis model, the outcome of each individual study, as well as the pooled outcome of all included studies, were presented as forest plots [reported as effect size (prevalence) with a 95%CI]. The visual symmetry of the funnel plot and the result of Egger's Regression were used to check the potential publication bias; however, unlike other statistical tests reported here, the Egger's test was considered significant if the *P* values were less than 0.10.

A meta-regression test was conducted (univariate and multivariate regression) to investigate the possible relationship between study variables (study year/s, sample size, diagnostic method, type of leishmaniasis, study region, study design, and study setting) and the prevalence of human leishmaniasis. Sensitivity analysis and subgroup analysis were performed to check the potential heterogeneity among the included studies and possible sources of bias.

Finally, the findings of this study were reported according to the PRISMA guidelines[21], and the results were presented using a narrative synthesis and followed by the full meta-analysis chart.

RESULTS

Study selection

After applying the search strategies of the current study, a total of 220 articles were identified and retrieved from the major electronic databases sources. From the 220 retrieved articles, 111 of them were removed due to duplication. Meanwhile, the remaining 109 articles underwent further individual screening by title and abstract to appraise the eligibility criteria for each included study. Only 39 records were eligible for full-text quality assessment. Of the remaining 39 articles, 19 were excluded due to the article's poor quality and insufficient study data. Eventually, only 20 studies with good quality assessment scores that fulfilled the eligibility criteria were included in this review. Figure 1 showed the full process of study selection.

Study characteristics

As shown in Table 1, twenty studies with a total of 230960 participants, were included in the quantitative analysis. Of these 20 studies, 10 were community-based studies, and the remaining 10 studies were hospital-based. The overall prevalence of human leishmaniasis in Sudan was reported in 17 studies, and the association between sex and leishmaniasis was reported in 11 studies. Meanwhile, two types of human leishmaniasis were reported (CL & VL). The geographical distributions of included studies revealed that the most frequent study areas in the included studies were central and eastern Sudan (7 for each), followed by western Sudan (4), with only one study from northern Sudan, and no study from southern Sudan. From all available diagnostic tests for leishmania parasite, only five were mentioned in the included studies: (1) Direct agglutination test (DAT) - 11 times; (2) Leishmania skin test (LST) - 5 times; (3) Polymerase chain reaction (PCR) - 2 times; (4) Enzyme-linked immunosorbent assay test (ELISA) - 2 times; and (5) Culture method - 1 time.

Results of synthesis

The current comprehensive study found a wide range of human leishmaniasis prevalence in Sudan in the twenty included studies. The lowest prevalence of human leishmaniasis, 1.7 (95%CI: 1, 2.8) was reported in a study in North Darfur state[19], whereas, the highest prevalence, 70.7% (95%CI: 66, 75), was reported in a study done in Al-tragma Village, River Nile state[37]. From the included studies, the pooled prevalence of human leishmaniasis in Sudan was 21% (CI: 12%-30%), and the heterogeneity across studies was substantially high (with $P < 0.00001$; $I^2 = 98.9\%$); therefore, the random effect model (REML) was employed for the final analysis (Figure 2).

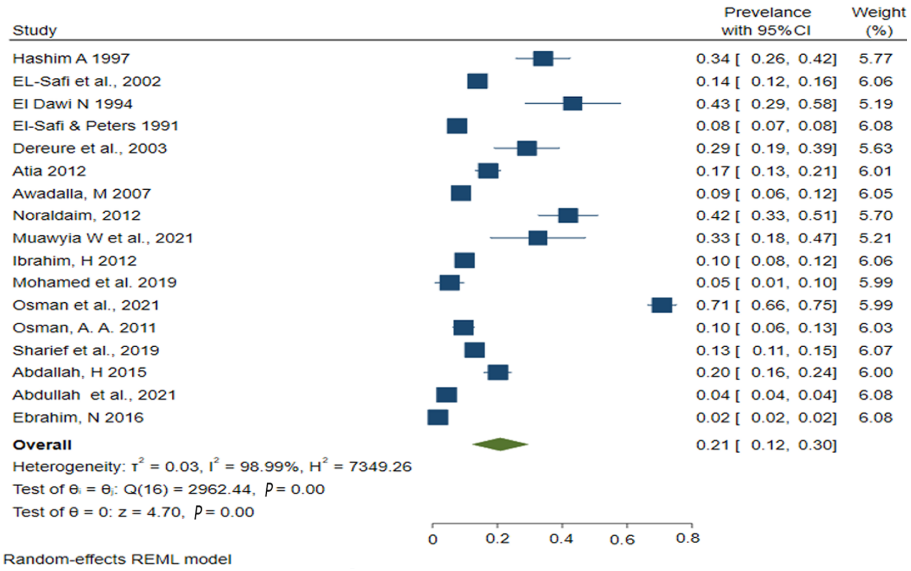

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Figure 2 Forest plot (random-effects model) for the pooled prevalence of human leishmaniasis in Sudan.

Meta-regression and sensitivity analysis

A meta-regression test was conducted (both univariate and multivariate regression) to investigate the possible relationship between study variables (study year/s, sample size, diagnostic method, type of leishmaniasis, study region, study design, and study setting) and the prevalence of human leishmaniasis. Nevertheless, all examined variables were not found to be statistically significant (Table 2), and from that, it can concluded that these study variables did not affect the heterogeneity. Alongside, the meta-regression, a sensitivity analysis was performed to identify the possible sources of the heterogeneity among the included studies. This study was done by sequentially excluding studies from the analysis model, but again the results did not find any significant difference in the analysis model. Thus, it can be concluded that the meta-analysis result of this study was stable. Furthermore, Egger's test for publication bias was statistically insignificant P = 0.128.

Subgroup analysis

Given the very high heterogeneity level presented in the analyses of human leishmaniasis, a subgroup analysis was done to find the effect of the sex, age, study year's, type of leishmaniasis, study region, study design, and study setting on the pooled prevalence of human leishmaniasis (Table 3). Using the above-mentioned factors as risk factors, the study results found that CL was the most common type of leishmaniasis in Sudan, with a pooled prevalence of 26% followed by combined infection (VL & CL) 19%, and then VL at 18%. Despite this, no data were found about ML prevalence in Sudan (Figure 3).

Nevertheless, the pooled prevalence of human leishmaniasis in Sudan was higher in males (60%) compared with females (40%) (Figure 4). In addition, the current results revealed that the people in the age group between 15 and 44 were the most affected group (60%) (Figure 5), central Sudan has the highest pooled prevalence of human leishmaniasis (27%) compared with other regions of Sudan, and the prevalence of human leishmaniasis seem to decrease over time (Table 3).

DISCUSSION

The United Nations Environment Programme 2020 annual report revealed that the majority of the Sudanese population live in the river Nile bank, forest zones, and savannah[42,43]. These areas are the natural areas for the presence of the carrier host (Sandfly)[17]. Also, the unique geographical location of Sudan, which is characterized by long staggered borders with some of leishmaniasis endemic areas on the southern and eastern sides of the country, together with the fact that the majority of the population are either nomad or farmers, make it very hard to control the disease in the country. Thus, human leishmaniasis poses an important challenge for the health and economic sectors in Sudan.

Based on a REML, the overall pooled prevalence of human leishmaniasis in Sudan was 21% (95%CI: 12%-30%). Assefa (2018), in Ethiopia, found almost the same result 21% (95%CI: 15%-27%)[44]. However, another Ethiopian study in 2021 found a lower result 9.13% (95%CI: 5-13.27)[45]. This difference between the two Ethiopian studies may be large because of the difference in the number of

Table 2 Heterogeneity related variables for the prevalence of human leishmaniasis in the current meta-analysis (based on meta regression)

Variables	Coefficient	SE	t	*P* > |t|	95%CI
Study yr/s	-0.0183371	0.0892299	-0.21	0.841	(-0.2171537, 0.1804794)
Sample size	-1.46e-06	1.10e-06	-1.33	0.204	(3.80e-06, 8.83e-07)
Diagnostic method	0.0152374	0.0500373	0.30	0.767	(-0.0962528, 0.1267275)
Type of leishmaniasis	-0.0271858	0.0653937	-0.42	0.686	(-0.172892, 0.1185204)
Study region	-0.0472426	0.0775729	-0.61	0.556	(-0.2200857, 0.1256005)
Study design	0.0029982	0.0584459	0.05	0.960	(-0.1272273, 0.1332237)
Study setting	-0.0381169	0.1762884	-0.22	0.833	(-0.4309118, 0.354678)

CI: Confidence interval.

Table 3 Subgroup analysis findings (random-effects model)

Analysis of leishmaniasis		Number of studies/pooled sample size	Pooled prevalence % (95%CI)	T^2	I^2%	H^2	*P* value
Sex	Male	10/13218	60 (52-67)	0.01	97.96	49.09	< 0.001
	Female	10/13218	40 (33-48)	0.01	97.96	49.07	< 0.001
Age group	< 5	5/8326	3 (1-6)	0.001	99.99	18316.61	< 0.001
	5-14	5/8326	22 (12-32)	0.01	97.76	44.50	< 0.001
	15-44	5/8326	60 (50-69)	0.01	95.53	22.38	< 0.001
	≥ 45	5/8326	14 (9-19)	0.001	92.09	12.63	< 0.001
Types of human leishmaniasis	VL	10/53152	18 (10-27)	0.02	99.28	138.39	< 0.001
	CL	5/11173	26 (2-50)	0.07	99.79	485.11	< 0.001
	VL/CL	2/162569	19 (10-48)	0.04	97.98	49.48	< 0.001
Study region	Central Sudan	6/10711	27 (14-40)	0.02	98.86	87.63	< 0.001
	Eastern Sudan	6/2245	15(9-21)	0.01	93.84	16.23	< 0.001
	Northern Sudan	1/410	71(66-75)	-	-	-	-
	Western Sudan	4/213528	7 (2-12)	0.00	99.97	2882.28	< 0.001
Study yr/s	Before 2000	5/10853	24 (12-37)	0.02	98.8	83.02	< 0.001
	Between 2001 to 2010	4/922	24 (9-39)	0.02	96.83	31.54	< 0.001
	After 2011	8/215119	17 (1-32)	0.05	100	24190.74	< 0.001
Study setting	Hospital-based study	8/221713	20 (10-31)	0.02	99.99	11092.03	< 0.001
	Community-based study	9/5181	21 (7-35)	0.05	99.54	218.75	< 0.001

I^2 index for the degree of heterogeneity; T^2 measure of heterogeneity; CI: Confidence interval; CL: Cutaneous leishmaniasis; VL: Visceral leishmaniasis.

included studies between them, which was 27 and 11, for Assefa[44], 2018, and Haftom *et al*[45], 2021, respectively. Although both Ethiopia and Sudan are endemic countries, the overall prevalence showed a clear discrepancy. The current findings showed variations in the pooled prevalence of human leishmaniasis between different geographical regions, age groups, sex, study settings, and years of publication, as well as between the different forms of human leishmaniasis. However, these findings showed no statistical difference in all subgroup analyses.

Two forms of human leishmaniasis were reported in Sudan, CL & VL, and between them, CL had the highest pooled prevalence of 26%, followed by mixed infection (CL & VL) (19%), and VL (18%). These results are in agreement with WHO findings[2] and Assefa's (2018) findings[44]. In contrast, Haftom and his colleagues (2021)[45] found a higher pooled prevalence of VL compared with CL in Ethiopia.

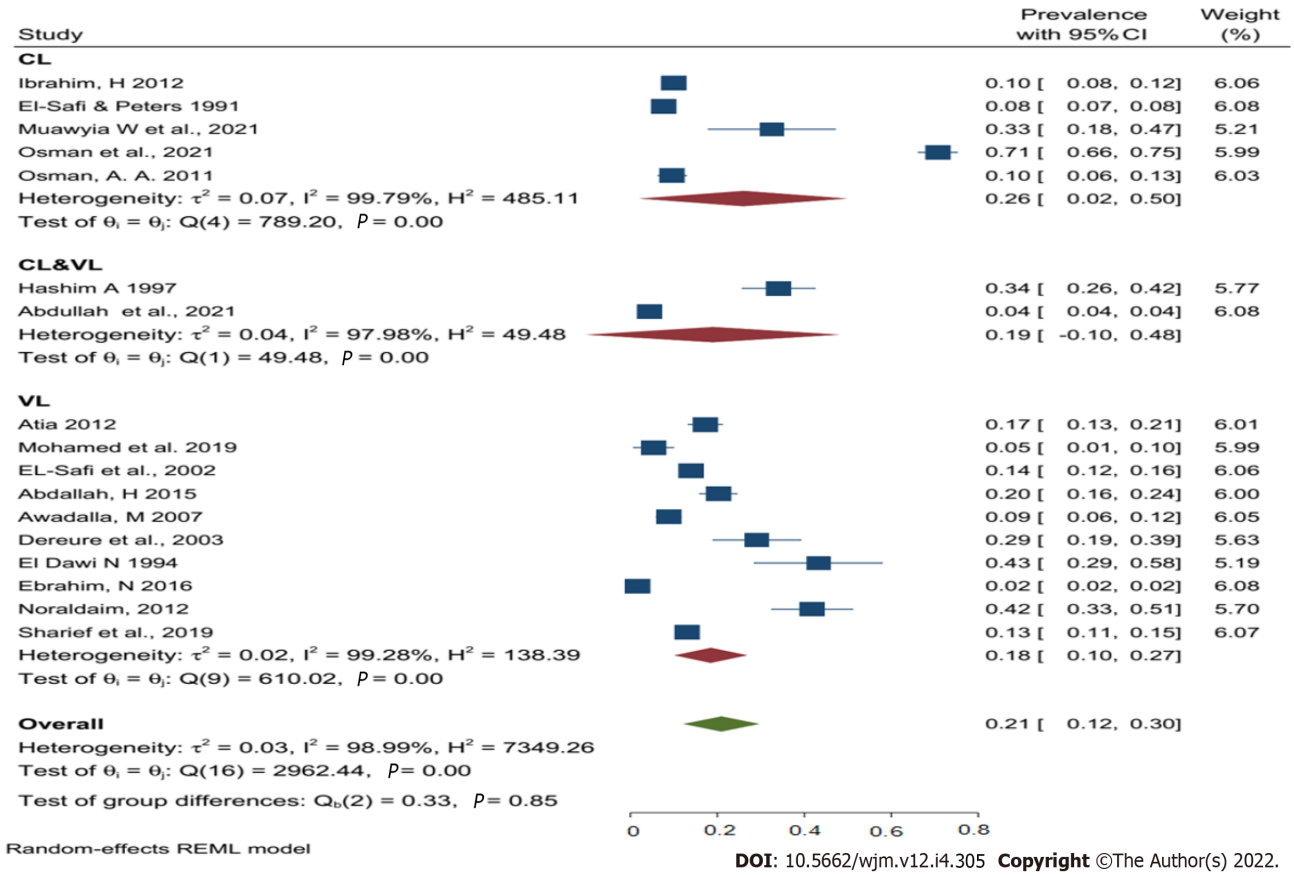

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Figure 3 Forest plot (random-effects model) for the pooled prevalence of the types of human leishmaniasis in Sudan.

Furthermore, the pooled prevalence of VL in Sudan was significantly higher than in Iran (2%)[46,47] and lower than it is in Latin America at 38.8%[48]. However, the current results seem to have one the highest reported pooled prevalence of CL worldwide, with only Sabzevari and his colleagues (2021)[49] in Iran reporting a higher pooled prevalence (45%); all other studies reported a lower pooled prevalence of CL compared with the current findings, including 22.1% in Mali[50], and 6.03%[45], and 19%[44] in Ethiopia.

The reported difference in the results between the other studies and this study may be due to differences in the climate of the study area, the study population, the absence of routine treatment or vaccinations for the definitive host, sample size, sampling procedure, and/or diagnostics method[51,52].

In Sudan usually, men work in agriculture and/or livestock sectors more than women and during the hot evenings and nights, men wear fewer clothes than women. These two main reasons may explain increased prevalence of leishmaniasis in Sudanese males compared with females (60% *vs* 40%), as these likely an increased risk of sand flies biting. These findings are in agreement with Haftom *et al*[45] (2021) in Ethiopia, Belo *et al*[53] (2013) in the Americas, and Kone *et al*[50] (2016) in Mali. However, two Iranian studies[47,49] disagreed with the current findings, with both studies reporting that the pooled prevalence of human leishmaniasis (CL & VL) was higher in females than in males. The sex-related difference in the pooled prevalence of human leishmaniasis between the current study and the Iranian studies may be due to differences in the cultural and work patterns between Sudan and Iran, Whereby, Iranian women were more involved in agricultural and livestock activities than men which would increase their risk of being bitten by sand flies[49,54].

The association between human leishmaniasis and age was reported in very few studies[26,32,35,37,38]; however, the pooled result reveals that people of workforce age had the highest pooled prevalence, followed by school-aged children and the infants. This makes sense because people who work in the agriculture and/or livestock sectors are at a higher risk of being bitten by sand flies. Similar results were found in Iran[47,49], Mali[50], and the Americas[53].

This meta-analysis study found that central Sudan has the highest reported pooled prevalence of human leishmaniasis compared with other parts of the country, and, generally, the pooled prevalence of human leishmaniasis in Sudan was decreasing over time. This result is corresponding with Al-Salem *et al*[6] (2016), who stated that "between 1985 and 2005, many epidemics of VL and CL were reported in Sudan, especially in central Sudan", and resulting from that, a high overall prevalence of human leishmaniasis in the same period of time in central Sudan. The relatively high prevalence of human leishmaniasis in Sudan may be due to the negative effects of the Sudanese civil war. Consequently, the

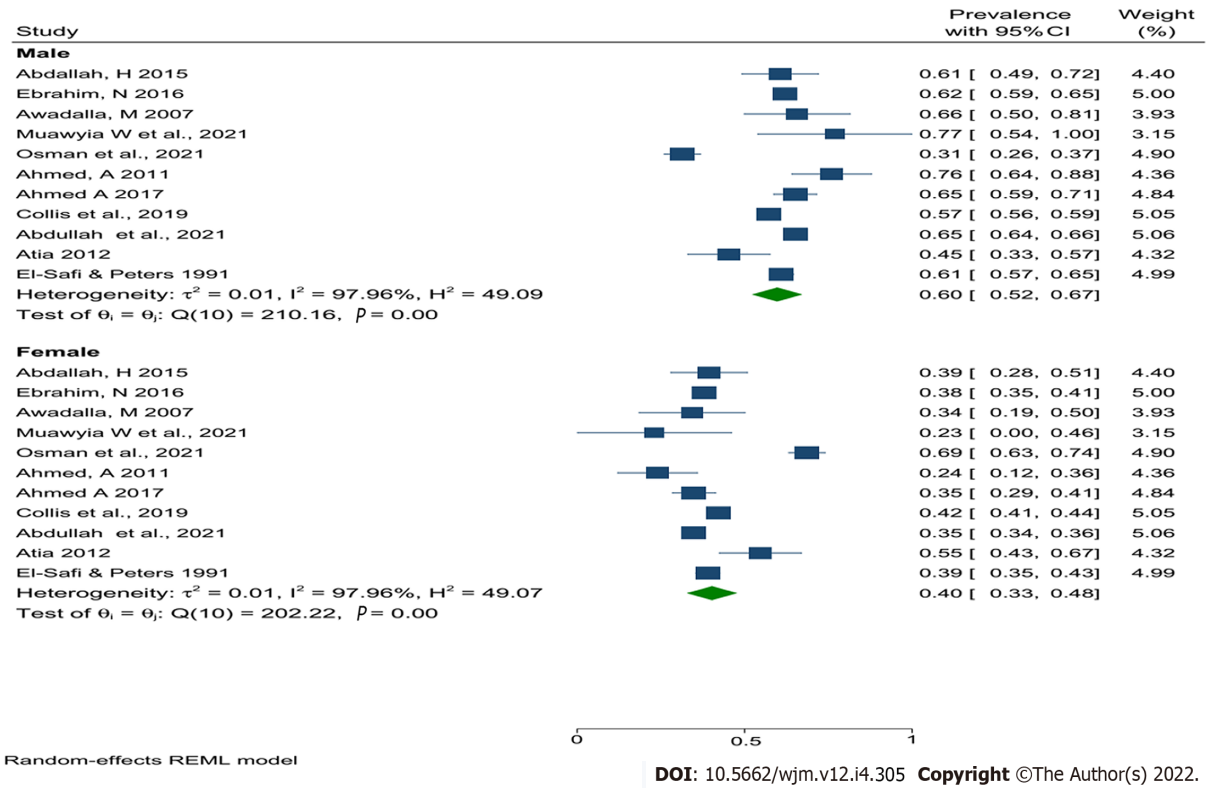

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Figure 4 Forest plot (random-effects model) for the pooled prevalence of human leishmaniasis in males and females in Sudan.

overall prevalence of human leishmaniasis and VL were significantly decreased after the leaders of the two war parties [The federal government of Sudan and the Sudan People's Liberation Army (SPLA)] signed the Comprehensive Peace Agreement on January 9, 2005 to stop the ongoing civil war[55].

Despite the seriousness of human leishmaniasis in Sudan, as presented in the current comprehensive study, no data is available about the economic impact of the disease on the livestock sector and public health sector in the country; thus, work needs to be done to cover the gap in this area. In addition, in our humble opinion, a collaborative effort and immediate action need to be taken from the policymakers and governments (federal and state government), to adopt a national wide epidemiological program to clarify the design of regional strategies and to guide the development of prevention and eradication programs in light of the one health concept during and beyond the COVID-19 pandemic.

The strengths of this study were the use of comprehensive search strategies to ensure that all published and unpublished studies related to the study objectives were included, and the use of standardized quality tools to evaluate the quality of the included studies. Finally, studies with abstracts were only included in this study.

The absence of data about patient places of residence, Leishman parasite species, and other potential risk factors in some included studies, are considered as limitations of the current study.

To the best of our knowledge, the current study is the first systematic review and meta-analysis study regarding the epidemiology of leishmaniasis in Sudanese citizens. Unluckily, there are very few published meta-analysis studies on the overall prevalence of human leishmaniasis, particularly in developing countries to compare with.

CONCLUSION

This systematic review and meta-analysis showed that human leishmaniasis infection is still endemic in many regions in Sudan and highly prevalent in central and eastern Sudan, and cutaneous leishmaniasis is the most prevalent in Sudan. Males and adults were more susceptible to infection compared with females and children. However, the human leishmaniasis prevalence decreased relatively over time. The presence of the high heterogeneity among the included studies should be considered when interpreting this study's findings. There is a lack of published research about human leishmaniasis in northern and southern regions Sudan. Research need to be updated and more research needs to be conducted in many regions in Sudan to provide adequate information.

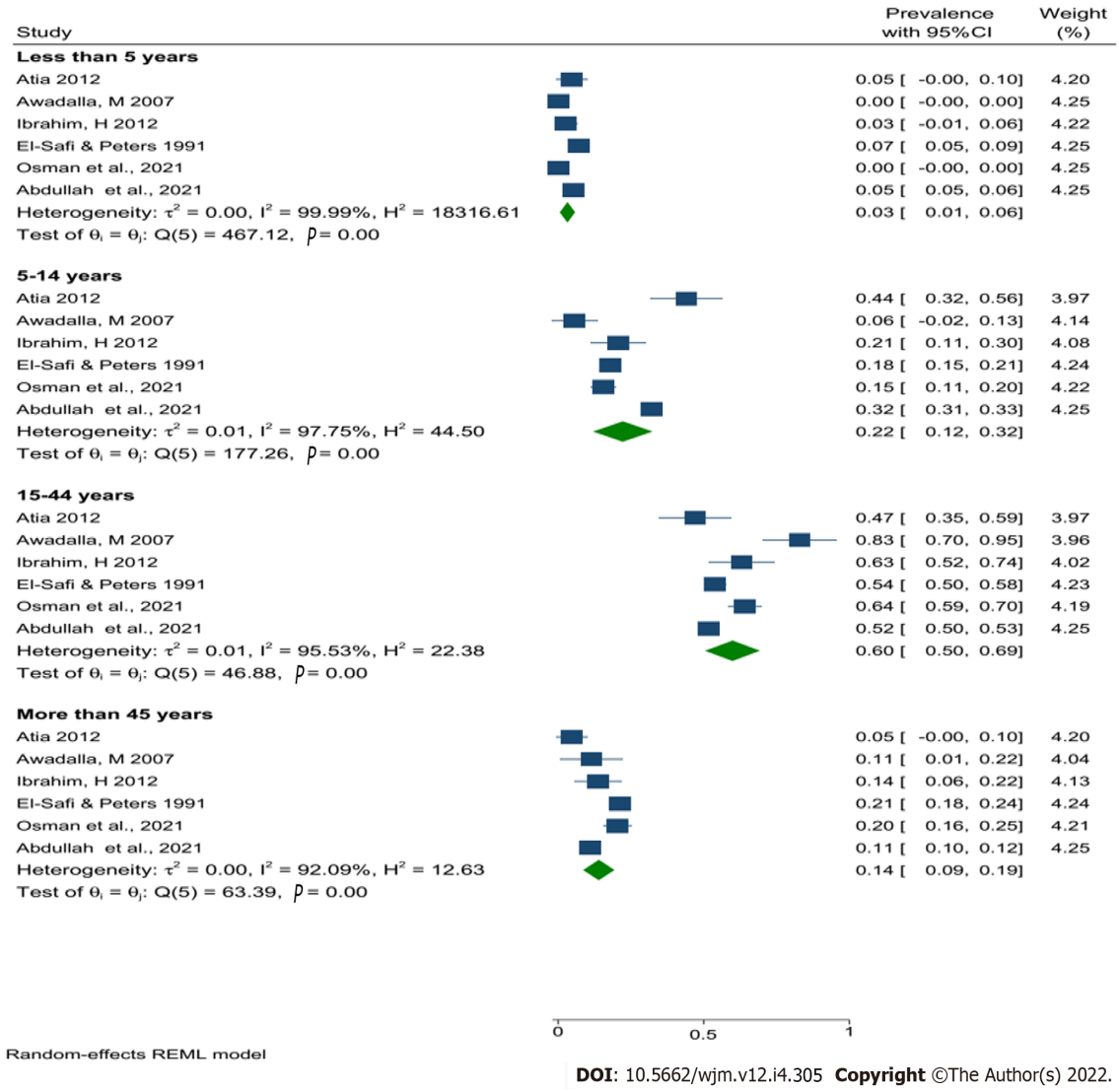

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Figure 5 Forest plot (random-effects model) for the pooled prevalence of human leishmaniasis in different age groups in Sudan.

ARTICLE HIGHLIGHTS

Research background

The prevalence of human leishmaniasis varies widely in different countries and in different regions of the same country. To date, there is no overall estimation of the prevalence of human leishmaniasis in Sudan

Research motivation

The lack of evidence about human leishmaniasis in Sudan may prevent health care policymakers and stakeholders from developing and adopting a suitable prevention program.

Research objectives

The objective of this study was to find the pooled prevalence of leishmaniasis and its associated factors among Sudanese citizens.

Research methods

A systematic literature search was conducted before the 4th of August 2021, from Scopus, Web of Science, PubMed, and MEDLINE, African Journals Online (AJOL), ResearchGate, direct Google search, Google Scholar, and universities websites.

Research results

A total of 20 articles were included in this meta-analysis after 220 articles had been subjected to full-text evaluations, and the overall pooled prevalence of human leishmaniasis in Sudan was 21% (with

confidence interval 12%-30%).

Research conclusions

Human leishmaniasis infection is still endemic in many regions in Sudan and is highly prevalent in central and eastern Sudan, and cutaneous leishmaniasis is the most prevalent in the country.

Research perspectives

More studies need to be done in Sudan to cover all epidemiological aspects of the disease in humans and animals under the umbrella of one health approach, with special emphasis on the health and economic impacts of the disease.

FOOTNOTES

Author contributions: Ahmed M, Abdulslam Abdullah A, Bello I, Hamad S, and Bashir A conceived and designed the review, developed the search strings, and rigorously reviewed the manuscript; Ahmed M, Abdulslam Abdullah A, and Hamad S carried out the draft of the manuscript; Abdulslam Abdullah A is the guarantor of the review; Ahmed M and Abdulslam Abdullah A screened and selected studies, extracted the data, evaluated the quality of the studies, and carried out analysis and interpretation.

Conflict-of-interest statement: The authors declared no potential conflict of interest concerning the research, authorship, and/or publication of this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Wu YXJ
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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 July 20; 12(4): 319-330

DOI: 10.5662/wjm.v12.i4.319

ISSN 2222-0682 (online)

META-ANALYSIS

Pain reduction and adverse effects of intravenous metoclopramide for acute migraine attack: A systematic review and meta-analysis of randomized-controlled trials

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Specialty type: Emergency medicine

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Patoulias D, Greece; Shibata Y, Japan
A-Editor: Yao QG, China

Received: February 4, 2022
Peer-review started: February 4, 2022
First decision: April 11, 2022
Revised: April 26, 2022
Accepted: July 11, 2022
Article in press: July 11, 2022
Published online: July 20, 2022

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Abstract

BACKGROUND

Metoclopramide may be used to treat people suffering from acute migraine. However, no comprehensive investigation on this issue has been recorded. This review will provide more solid evidence for the use of metoclopramide in treating acute migraine.

AIM

To compare the efficacy of intravenous metoclopramide with other therapies in migraine attack treatment in an emergency department (ED).

METHODS

We included randomized controlled trials of participants older than 18 years with acute migraine headaches, which included at least one arm that received intravenous (IV) metoclopramide at the ED. A literature search of PubMed, Web of Science, Cochrane Collaboration, and Reference Citation Analysis on December 31, 2021 retrieved other drugs or placebo-controlled studies without language limitation. The risk of bias was assessed using the Cochrane risk of bias tool. The primary endpoint was pain reduction at 60 min or closest to 1 h after treatment, as measured by the pain scale. Secondary endpoints included adverse effects or reactions resulting from metoclopramide or comparisons.

RESULTS

Fourteen trials with a total of 1661 individuals were eligible for review. The risk of bias ranged from low to intermediate. IV metoclopramide administration was not associated with higher pain reduction at 1 h (Standard mean difference [SMD] = -0.03, 95% confidence interval [CI]: -0.33-0.28, P = 0.87). However, metoclopramide

was associated with better pain reduction than placebo (SMD = 1.04, 95%CI: 0.50-1.58, P = 0.0002). In addition, side effects were not significantly different between IV metoclopramide and other drugs or placebo (odds ratio [OR] = 0.76, 95%CI: 0.48-1.19, P = 0.09 and OR = 0.92, 95%CI: 0.31-2.74, P = 0.54, respectively).

CONCLUSION

Metoclopramide is more effective than placebo in treating migraine in the ED. Despite the observed tendency of decreased side effects, its effectiveness compared to other regimens is poorly understood. More research on this area is needed to treat migraine in acute care settings effectively.

Key Words: Metoclopramide; Migraine; Efficacy; Adverse effect; Randomized controlled trials

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Core Tip: Metoclopramide may be used to treat people suffering from acute migraine. However, no comprehensive investigation on this issue has been recorded. We conducted an up-to-date systematic review and meta-analysis of the clinical efficacy of metoclopramide during an acute migraine attack. This study comprised 14 studies and found that metoclopramide was more effective than placebo in treating migraine at the emergency department. When compared to other medications, however, no substantial advantage was detected. More study is needed to enhance migraine therapy in acute care settings effectively.

Citation: Ungrungseesopon N, Wongtanasarasin W. Pain reduction and adverse effects of intravenous metoclopramide for acute migraine attack: A systematic review and meta-analysis of randomized-controlled trials. *World J Methodol* 2022; 12(4): 319-330

URL: https://www.wjgnet.com/2222-0682/full/v12/i4/319.htm

DOI: https://dx.doi.org/10.5662/wjm.v12.i4.319

INTRODUCTION

Migraine, a chronic neurological disease, is one of the most common causes that lead patients to seek medical attention[1]. Apart from regular follow-up at the outpatient department, many patients with migraine suffer from acute migraine attacks requiring an emergency department (ED) visit. There were approximately 1.2 million annual ED visits for acute migraine headaches in the United States[2]. At the same time, persons who suffer from this illness frequently encounter several other accompanying symptoms, such as nausea, vomiting, and sensitivity to light, sound, touch, or scent[3,4]. Unfortunately, its pathogenesis remains complicated and little understood. As a result, if such a problem cannot be effectively treated, it significantly impacts the health-related quality of life of individuals suffering from acute migraine[5,6].

According to the American Headache Society recommendations, several acute migraine treatments include triptans, ergotamine, non-steroidal anti-inflammatory drugs, combination analgesic, and anti-emetics[7]. Metoclopramide, an anti-emetic drug acting as a dopamine/serotonin antagonist, was initially used in migraine patients who experienced nauseating symptoms[8]. Later, it was shown to be effective in pain control of acute migraine attacks[9,10]. In the recent recommendation, metoclopramide was considered the "probably effective drug," even though several studies showed the efficacy of metoclopramide monotherapy. It has been investigated that the efficacy of metoclopramide was neither inferior to sumatriptan nor opioids[11,12].

Moreover, apart from the efficacy aspect, metoclopramide showed superiority in other aspects, such as lower adverse severe effects and lower addiction rates which are considered an essential issue in the ED as patients with migraine tend to revisit. It is undeniable that metoclopramide might not be the first choice for clinicians to use in acute migraine as its efficacy might not be outstanding compared to other drugs. As prior mentioned, the severe side effects of metoclopramide, which are extrapyramidal symptoms, such as tardive dyskinesia and akathisia, though rarely reported in short term use and less worrisome than those of triptans and opioids, should also be concerned as they might result in an irreversible and sufferable experience for the patient[11].

To comprehend the big picture of using metoclopramide in acute care for migraine, this study aimed to compare metoclopramide use with other therapy in migraine attack treatment in an acute care setting. Our study hypothesized that metoclopramide monotherapy should effectively treat acute migraine attacks in an ED.

MATERIALS AND METHODS

Protocol

We conducted this systematic review and meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement guidelines[13]. We prospectively registered our protocol with the International prospective register of systematic reviews (ID: CRD42022322609).

Search strategy and inclusion criteria

We (N.U. and W.W.) independently searched four standard databases, PubMed, Web of Science, Cochrane Collaboration, and Reference Citation Analysis, from their inception until December 31, 2021, without language restriction. The search words "metoclopramide," "Meclopran," "Plasil," "Reglan," "methoxyprocainamide," "migraine," and "headache" were the Medical Subject Headings used, in combination and with different spellings and endings. We also searched websites, organizations, relevant reviews, grey literature, and references to identify additional eligible studies. Additionally, we searched for any unpublished trials registered on the "clinicaltrials.gov" Internet site.

The selection criteria were as follows: (1) Randomized controlled trials including adults more than 18 years of age with acute migraine headaches, regardless of their types (*i.e.*, with or without aura); (2) at least one arm having received an intravenous (IV) metoclopramide during ED stay; (3) comparing of at least one agent or placebo; (4) reporting of average pain scale before the administration of each agent; and (5) reporting of at least one of the following: Pain scale at 60 or other minutes, any adverse effects, and rescue medications needed at the ED. We excluded pre-clinical studies, review articles, and studies without a control group (*e.g.*, case reports and case series). The two authors (N.U. and W.W.) independently screened the search results to identify eligible studies. Full-text articles of the retrieved studies were retrieved and independently assessed by the two authors against the pre-specified criteria (Figure 1). Any discrepancies were discussed with a third party and concluded by consensus.

Outcomes of interests

The primary endpoint was pain reduction at 60 min or closest to 1 h after treatment administration, as measured by the Visual Analog Scale (VAS) or others. Secondary endpoints included adverse effects or reactions resulting from metoclopramide or interventions. Adverse effects in this study were defined by any of the following symptoms: Upper gastrointestinal complaints (dyspepsia, heartburn, and bloating), allergic reaction, dizziness, drowsiness, nasal congestion, dry mouth, dystonic reaction, akathisia, and significant blood pressure drop.

Data extraction and assessment of risk of bias

We separately extracted the data from the included articles using a prepared data extraction form. Specifically, we extracted basic characteristics (first author, publication year, study location and setting, and number and age of participants), treatment details and interventions in the study groups, and the outcomes of interest. We sought to contact the associated author by email for incomplete or missing data or clarification. The two authors (N.U. and W.W.) independently assessed the risk of study bias using the latest version of the Cochrane Collaboration tool for assessing the trial risk of bias[14]. Any disagreements were handled through discussion with the assistance of a third independent expert.

Data synthesis and statistical analysis

The data was imported into pre-formatted record forms. We calculated individuals and pooled estimates as standard mean differences (SMDs) for continuous endpoints, with 95% confidence intervals (CIs). We calculated individuals and pooled estimates using odds ratios (ORs) with CIs for dichotomous endpoints. We estimated heterogeneity among the included studies using the I^2 statistic (the percentage of total variation across studies due to heterogeneity). We applied a fixed-effect model if the heterogeneity was minor ($I^2 \leq 50\%$). However, if there was evidence of strong heterogeneity ($I^2 > 50\%$), a random-effect model was employed instead. Visual assessment of funnel plots and Egger's test were used to assess publication bias caused by small-study effects. For statistical analyses, we applied RevMan version 5.3 (Nordic Cochrane Center, Cochrane Collaboration, 2014, Copenhagen, Denmark) [15]. All tests were two-tailed, and P values < 0.05 were considered statistically significant.

RESULTS

Study selection

Figure 1 demonstrates how the 820 retrieved articles were screened for inclusion in the review and analysis. After excluding duplicated studies, 533 remained. Of those, 470 were excluded following title and abstract screening according to the inclusion and exclusion criteria. The remaining 63 articles were retrieved and reviewed for full-text copies before including 12 studies in the data analysis. In addition, three articles were also searched by citation searching, and two articles met the pre-specified criteria.

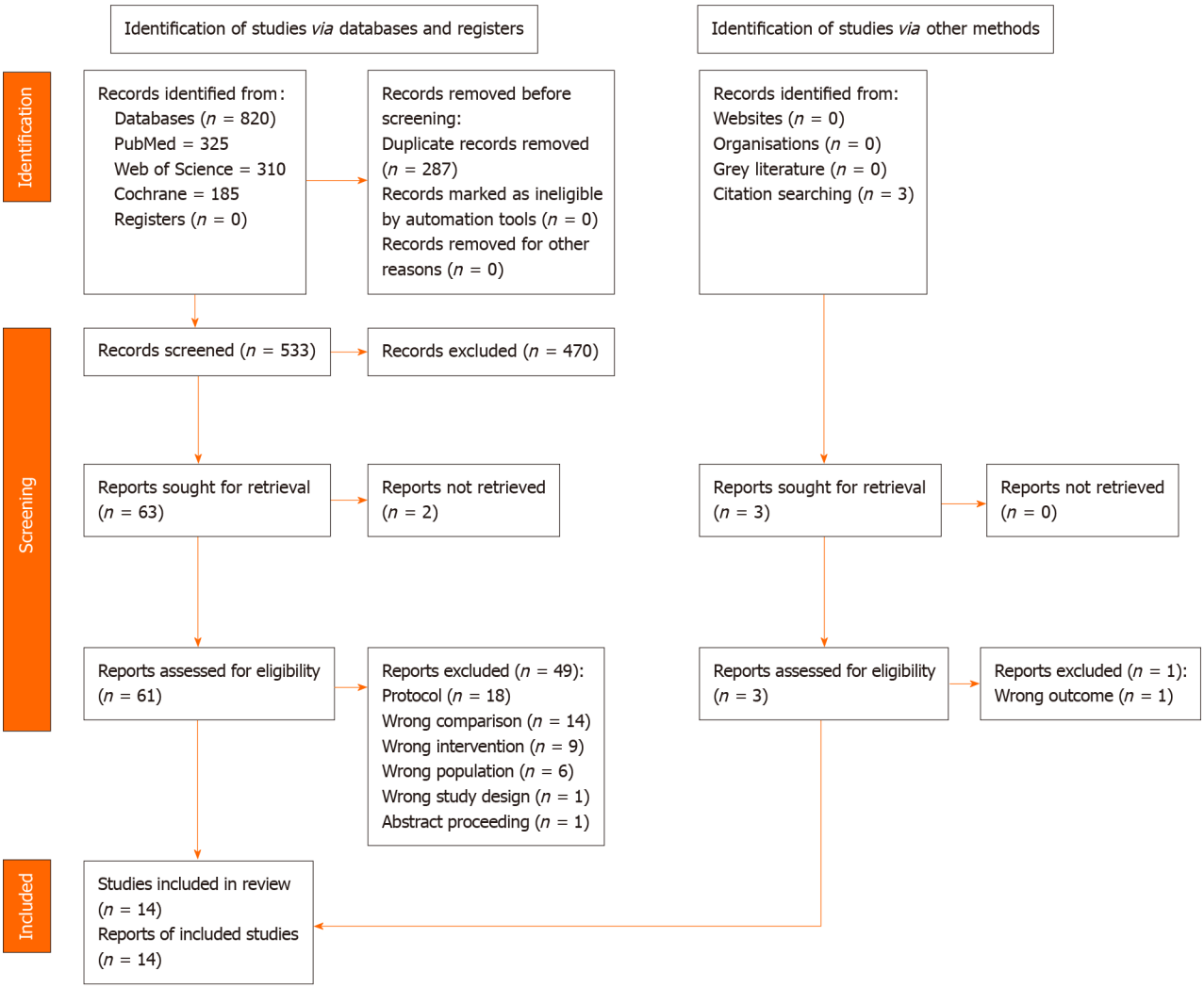

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Figure 1 PRISMA flow chart of study selection.

Finally, 14 articles[10,16-28] with 1661 participants were included in the meta-analysis.

Characteristics of included studies

Data extraction and meta-analysis were performed on 14 papers published between 1990 and 2020. The research was carried out in the United States of America (*n* = 7), Turkey (*n* = 3), and Iran (*n* = 4). The mean ages were around 34-40 years. Most studies applied 10 mg of IV metoclopramide, while three administered 20 mg of metoclopramide as interventions. Five trials investigated the efficacy of IV metoclopramide against placebo. Most studies compared more than one arm. All trials reported pain intensity at 0 and other minutes after drug administration, as VAS or other appropriate methods. Table 1 summarizes the baseline demographics and clinical characteristics of the included studies. Deviation from the intended interventions and randomization contributed to a high proportion of concerns over risk of bias. Five out of fourteen had an overall low risk of bias. The risk of bias assessment by Cochrane risk of bias assessment is illustrated in Figures 2 and 3.

Primary outcome

All 14 studies reported average pain reduction at 60 min or at the time closest to 1 h. The overall effect size showed no statistical significance with regard to the efficacy between IV metoclopramide and other drugs (SMD = -0.03, 95%CI: -0.33-0.28, P = 0.87). However, IV metoclopramide demonstrated a significant pain reduction compared with placebo (SMD = 1.04, 95%CI: 0.50-1.58, P = 0.0002). Subgroup analyses found that IV metoclopramide had a significant advantage in pain reduction compared with subcutaneous sumatriptan (SMD = 0.73, 95%CI: 0.11-1.35, P = 0.03), IV valproate (SMD = 0.27, 95%CI: 0.01-0.54, P = 0.04), and oral ibuprofen (SMD = 1.41, 95%CI: 0.41-2.41, P = 0.006). Heterogeneity was observed among the subgroups comparing IV metoclopramide and other drugs (I^2 = 81.5%, P < 0.0001; Figure 4). Figures 4 and 5 demonstrate the forest plot comparing pain reduction at 60 min between IV metoclopramide and other drugs and placebo, respectively.

Table 1 Baseline demographics and clinical characteristics of included studies

Ref.	Age, year	Intervention	Comparisons	Sample size (intervention/comparisons)	Outcomes of interest
Yavuz *et al* [16], 2020, Turkey	36.8 ± 11.4	IV metoclopramide 10 mg	1 IV dexketoprofen trometamol 50 mg; 2 IV dexketoprofen trometamol 50 mg plus IV metoclopramide 10 mg	150 (50/50/50)	VAS at 0, 15, and 30 min, adverse effects, and requirement of rescue medicine
Khazaei *et al* [17], 2019, Iran	36.8 ± 9.9	IV metoclopramide 10 mg	1 IV dexamethasone 8 mg; 2 IV ketorolac 30 mg; 3 IV chlorpromazine 25 mg	128 (32/32/32/32)	VAS at 0 min, 60 min, and 24 h, adverse effects
Doğan *et al* [18], 2019, Turkey	34 ± 13.3	IV metoclopramide 10 mg	1 Placebo	148 (74/74)	Pain intensity at 30 min, adverse effects, and requirement of rescue analgesic-Change in pain intensity, additional ED visit in 24-72 h after discharge
Amiri *et al* [19], 2017, Iran	33.5	IV metoclopramide 10 mg	1 IV granisetron 2 mg	148 (73/75)	VAS before and at 1, 2, and 4 h after drug administration, emesis episode
Friedman *et al*[20], 2014, USA	33.7 ± 13.1	IV metoclopramide 10 mg	1 IV sodium valproate 1000 mg; 2 IV ketorolac 30 mg	330 (110/110/110)	Verbal NRS and ordinal pain scale every 30 min, adverse effects, and requirement of rescue medication
Talabi *et al* [21], 2013, Iran	30.9 ± 8.0	IV metoclopramide 20 mg	1 SC sumatriptan 6 mg	124 (62/62)	VAS at 0 and 60 min
Friedman *et al*[22], 2004, Turkey	34 ± 4.4	IV metoclopramide 20 mg	1 SC sumatriptan 6 mg	78 (40/38)	NRS at 0, 2, and 24 h, and rate of pain free headache response at 2 and 24 h, rate of modified headache response, associated symptoms, satisfaction, disability score, and requirement for rescue drug
Cete *et al* [10], 2004, Iran	40 ± 12	IV metoclopramide 10 mg	1 IV magnesium sulphate 2 g; 2 Placebo	113 (37/36/40)	VAS at 0, 15, and 30 min, additional analgesic, rescue medication, adverse events in ED, and recurrence rate at 24 h
Ellis *et al* [23], 1993, USA	N/A	IV metoclopramide 10 mg	1 Oral ibuprofen 600 mg; 2 IV metoclopramide 10 mg + PO ibuprofen 600 mg; 3 Placebo	40 (10/10/10/10)	VAS and nausea scores at 0, 30, and 60 min, requirement of rescue medication
Cameron *et al*[24], 1995, USA	32.1 ± 27.0	IV metoclopramide 10 mg	1 IV chlorpromazine 0.1 mg/kg	91 (44/47)	VAS at 0 and every 15 min, requirement of rescue drug
Friedman *et al*[25], 2008, USA	36.0 ± 11.1	IV diphenhydramine 25 mg + IV metoclo-pramide 20 mg	1 IV diphenhydramine 25 mg + IV prochlorperazine 10 mg	77 (38/39)	NRS and pain intensity categorical scale at 0 and every 30 min
Coppola *et al*[26], 1995, USA	N/A	IV metoclopramide 10 mg	1 IV chlorpromazine 10 mg; 2 Placebo	70 (24/22/24)	VAS, nausea, and sedation at 0 and 30 min. Early relapse rate in 48 h
Gaffigan *et al*[27], 2015, USA	29 ± 7.9	IV diphenhydramine 25 mg + IV metoclo-pramide 10 mg	1 IV diphenhydramine 25 mg + IV haloperidol 5 mg	64 (33/31)	Pain, nausea, restlessness, and sedation at 0, 20, 40, 60, and 80 min, requirement of rescue medication, patient satisfaction, adverse events, early discharge, ED revisit, and QT interval
Tek *et al* [28], 1990, USA	N/A	IV metoclopramide 10 mg	1 Placebo	50 (24/26)	Degree of pain relief at 1 h after treatment

ED: Emergency department; IV: Intravenous; N/A: Not applicable; NRS: Numerical rating scale; SC: Subcutaneous; VAS: Visual analog scale.

Secondary outcome

Eight studies measured adverse effects across IV metoclopramide and comparisons. The pooled effect size was homogenous both compared with others (I^2 = 13.3%, P = 0.33; Figure 6) and with placebo (I^2 = 0%, P = 0.89; Figure 7). Adverse effects were not different across IV metoclopramide and other comparisons (OR = 0.76, 95%CI: 0.48-1.19, P = 0.09) or placebo (OR = 0.92, 95%CI: 0.31-2.74, P = 0.54). Subgroup analyses yielded similar results for all comparisons (Figure 6).

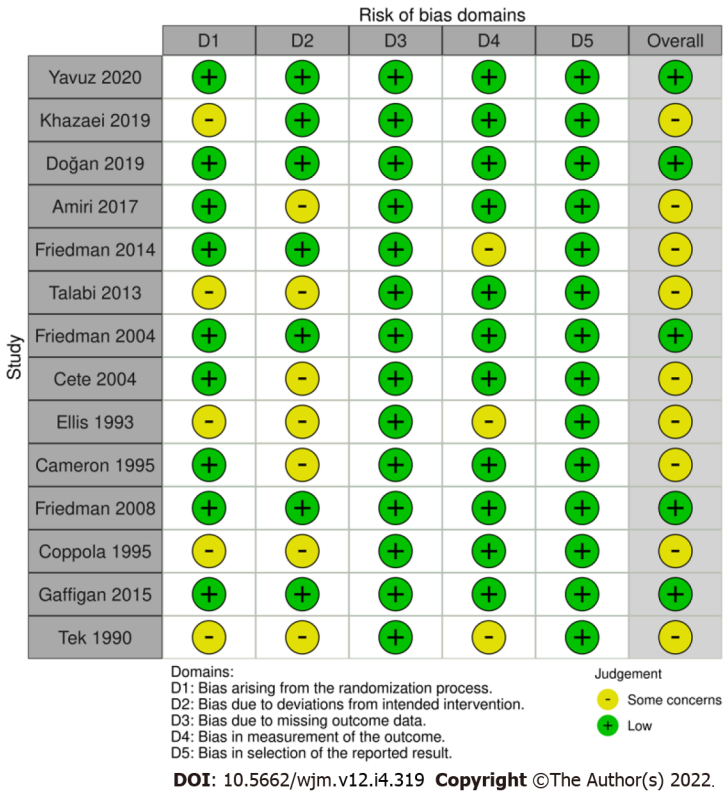

Figure 2 Cochrane risk of bias assessment of included studies.

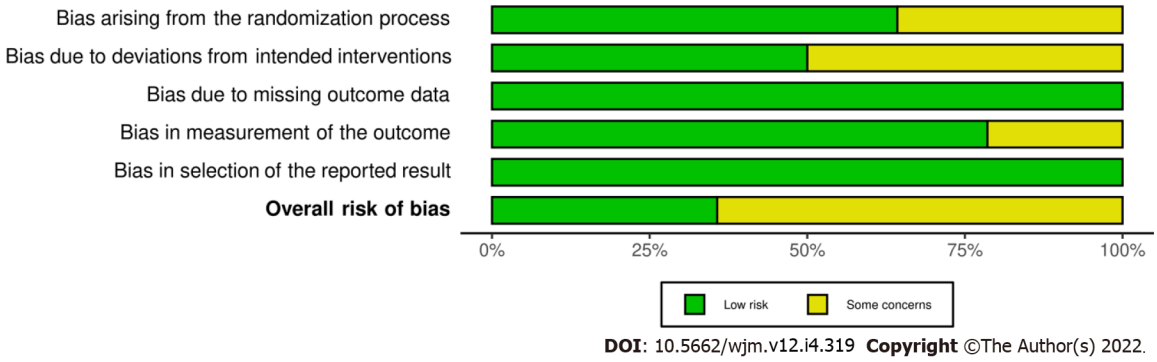

Figure 3 Details of each domain of Cochrane risk of bias assessment.

Publication bias

There was no substantial publication bias in the funnel plot for the meta-analysis of the average pain reduction between IV metoclopramide and comparisons (Figure 8). The regression-based Egger's test was performed using a random-effect model with restricted maximum-likelihood method and found that *P* value was 0.0814.

DISCUSSION

This meta-analysis investigated the clinical efficacy of IV metoclopramide for treating acute migraine attacks in the ED. This study showed that administration of IV metoclopramide was an effective treatment for migraine headache in adults, compared with placebo. However, the benefit of metoclopramide was not superior to other drugs. Our systematic review also demonstrated that IV metoclopramide tended to have fewer side effects than other interventions. The overall study risk of bias ranged from low to some concerns.

Acute migraine is a common neurovascular disorder. It is described as a moderate to severe, predominantly unilateral, and recurrent headache that lasts for several hours to a few days[3,29]. Metoclo-

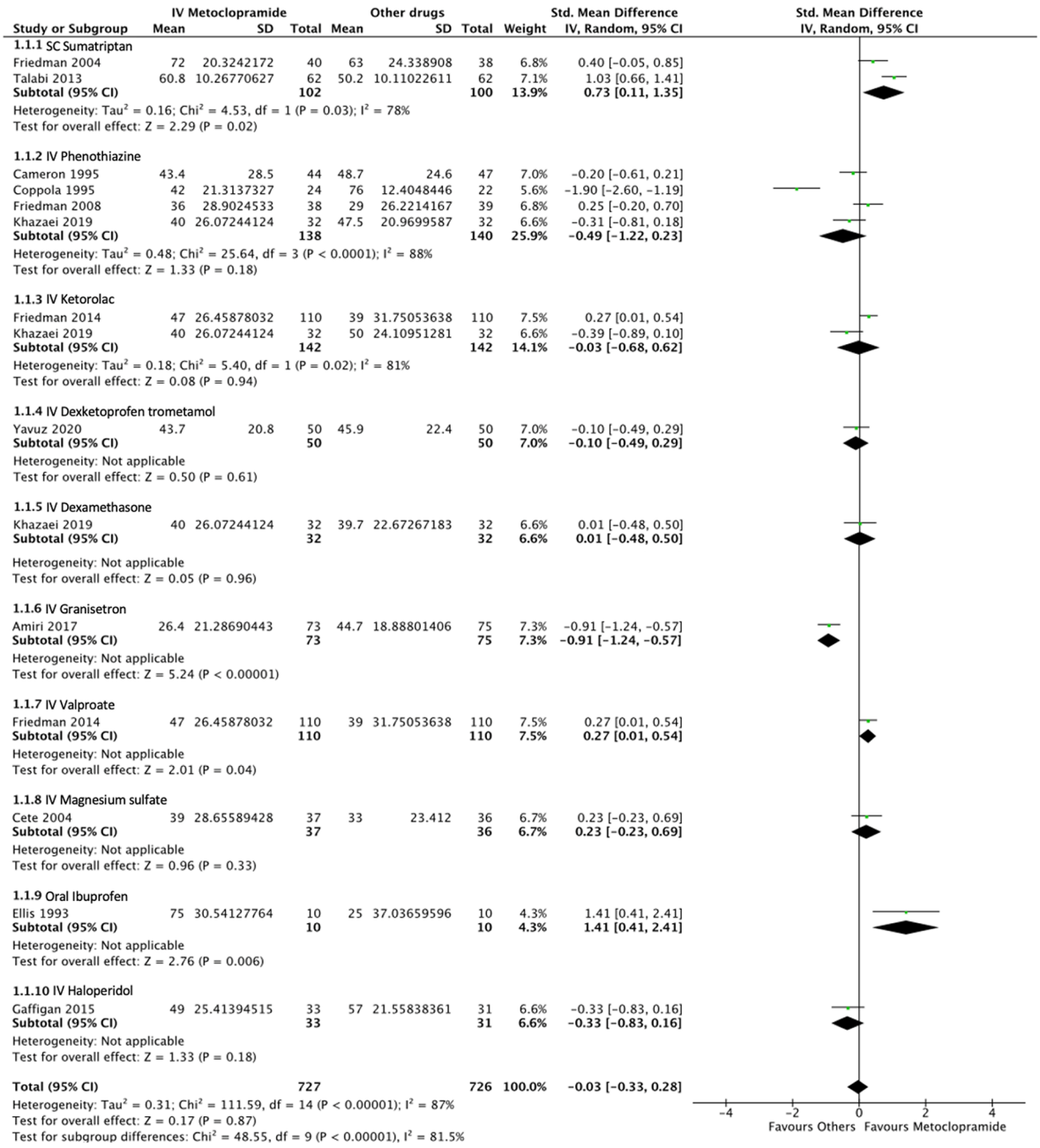

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Figure 4 Forest plot comparing pain reduction at 60 min between intravenous metoclopramide and other drugs. CI: Confidence interval; IV: Intravenous; SC: Subcutaneous.

pramide is initially used to treat acute migraine for decades[11]. A few studies over the years have highlighted that metoclopramide has substantial therapeutic effectiveness in treating acute migraine episodes[26,30]. The reason behind the use of metoclopramide could be that it antagonizes the dopamine D2 receptor, which is proposed to be one of the pathogeneses of pain in migraine[11]. A meta-analysis of pooled data illustrated that metoclopramide significantly reduced headache pain, and those patients were less likely to rescue medicines than the placebo groups[3]. However, the authors chose various inclusion and exclusion criteria for this study, which may contain data on non-migraine headaches, confounding any conclusions to be derived[3]. Furthermore, metoclopramide also had an anti-emetic effect that ameliorates migraine patients' symptoms[11]. Therefore, metoclopramide could be a first-line treatment for acute migraine episodes. Our findings are consistent with the prior research finding that metoclopramide was more effective than placebo in pain reduction[9]. In addition, metoclopramide had a higher benefit than some drugs in our analysis (subcutaneous sumatriptan, intravenous valproate, and oral ibuprofen). These findings fit with the pattern described previously by Colman *et al* [9]. However, that study selected both ED and headache clinic settings, which differed from ours. Besides, Colman and colleagues analyzed the pain using a complete relief of headache or significant reduction in headache pain. As a result, discrepancies were likely to occur across that definition. Our

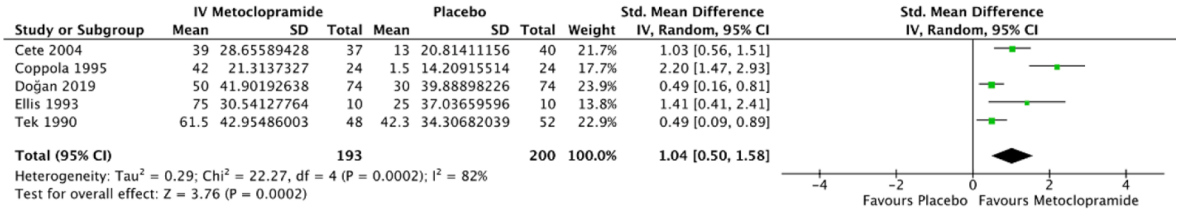

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Figure 5 Forest plot comparing pain reduction at 60 min between intravenous metoclopramide and placebo. CI: Confidence interval; IV: Intravenous.

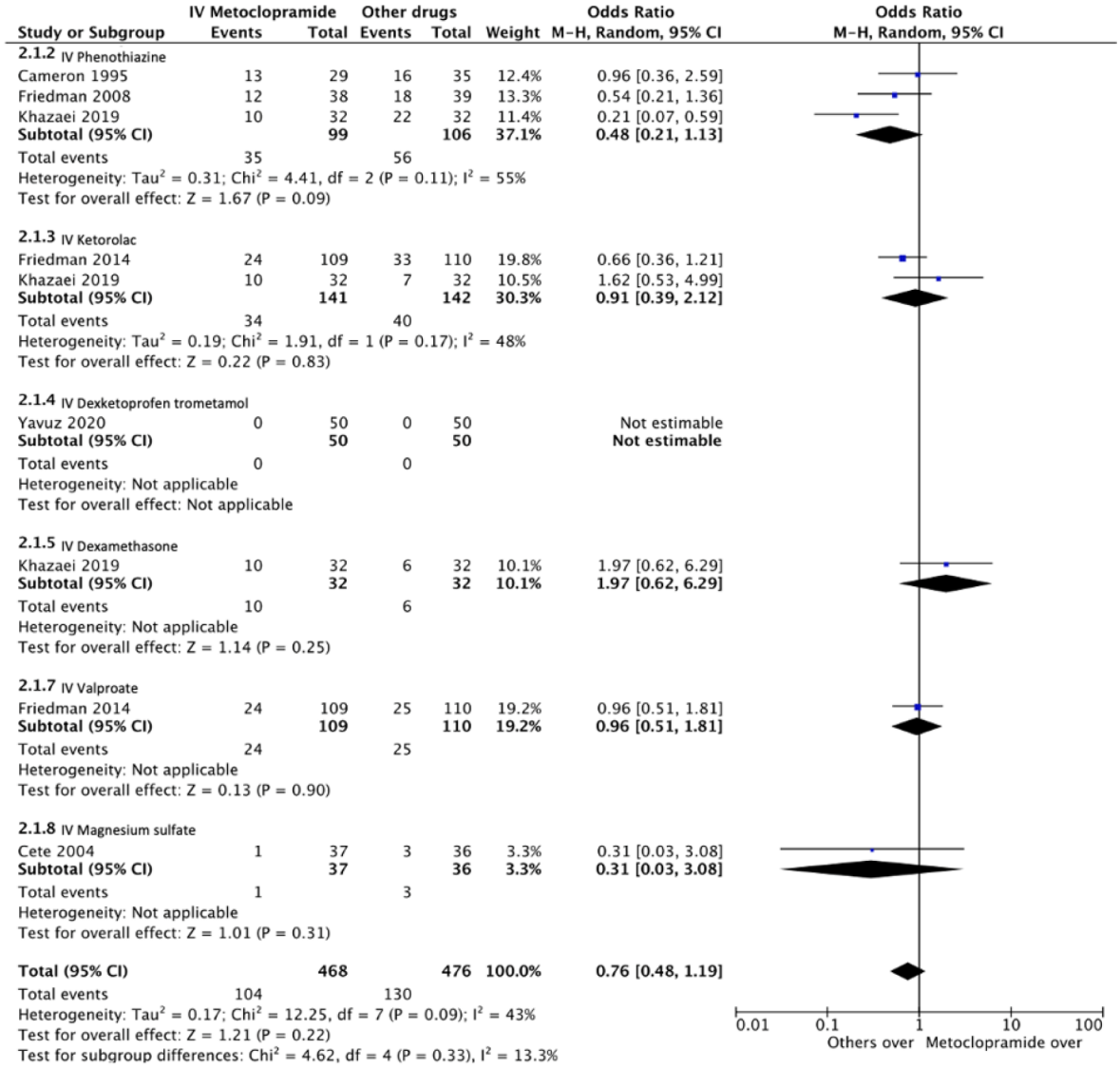

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Figure 6 Forest plot comparing odds ratios of adverse effects between intravenous metoclopramide and other drugs. CI: Confidence interval; IV: Intravenous.

study provided the difference aiming to close this gap. We compared all studies based on the pre- and post-intervention mean pain intensity in each study, which is more feasible to apply and compare.

However, the side effects of metoclopramide might be serious and irreversible, for example, tardive dyskinesia. It is characterized by the uncontrollable movement of the tongue, face, and extremities. Nonetheless, our findings reveal that the adverse effects resulting from metoclopramide were not different across the other drugs. Results obtained by Orr and colleagues[31] are consistent with our findings. Moreover, compared to other suggested therapies, metoclopramide's adverse effect profile is less concerning than triptans, which are commonly utilized in ED situations[32,33].

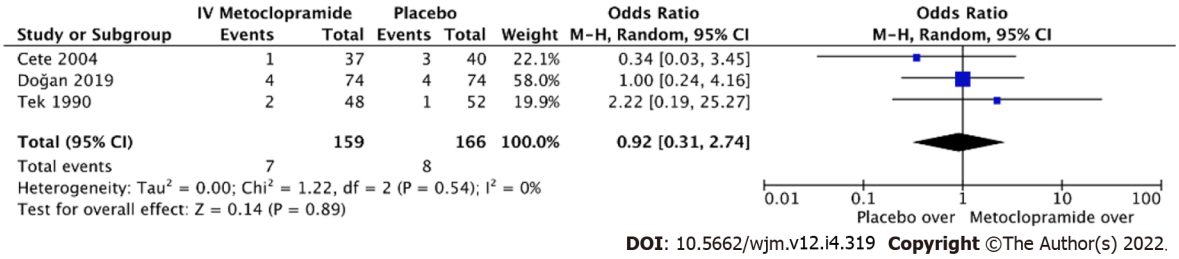

Figure 7 Forest plot comparing odds ratios of adverse effects between intravenous metoclopramide and placebo. CI: Confidence interval; IV: Intravenous.

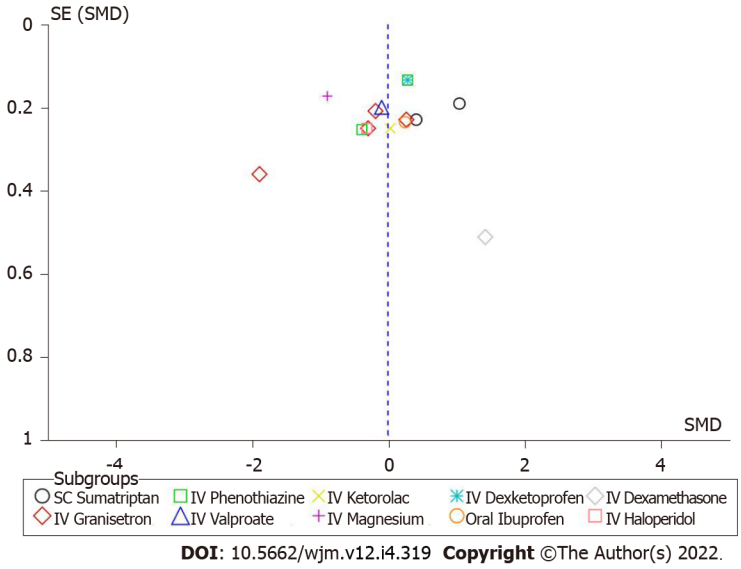

Figure 8 Funnel plot of pain reduction at 60 min between intravenous metoclopramide and other drugs. IV: Intravenous; SC: Subcutaneous; SE: Standard error; SMD: Standard mean difference.

Limitation

This review contains some limitations. First, all included studies were conducted in only three countries, including Iran, United States, and Turkey, which possibly resulted in the generalizability bias. Secondly, most trials did not report exclusion criteria in sufficient detail; therefore, the definitions for migraine might be varied among studies. In addition, several studies did not report the confirmation of migraine diagnosis, duration of headache, and prior therapies. As a result, we probably combined studies with varying patient characteristics, making it difficult to determine if our findings are generalizable to other contexts. Finally, this meta-analysis included studies done at different dates (between 1990 and 2020), resulting in the observed heterogeneity.

CONCLUSION

To conclude, metoclopramide was proven to be beneficial to treating migraine in the acute care setting, such as in the ED, compared to placebo. Despite the demonstrated trend of a lower adverse effect, its efficacy compared to other regimens is little comprehended. More studies on this topic should be further conducted to improve migraine treatment in acute care settings effectively.

ARTICLE HIGHLIGHTS

Research background

Metoclopramide may be used to treat people suffering from acute migraine. However, no comprehensive investigation on this issue has been recorded. This review will provide more solid evidence for the use of metoclopramide in treating acute migraine.

Research motivation

Metoclopramide was considered the "probably effective drug", even though several studies showed the efficacy of metoclopramide monotherapy. It has been investigated that the efficacy of metoclopramide was neither inferior to sumatriptan nor opioid. Moreover, apart from the efficacy aspect, metoclopramide showed superiority in other aspects, such as lower adverse severe effects and lower addiction rates.

Research objectives

The objective of this review was to investigate the efficacy of intravenous metoclopramide with other therapies in migraine attack treatment in an emergency department (ED).

Research methods

We conducted a systematic review and meta-analysis of randomized controlled trials.

Research results

The administration of received intravenous metoclopramide was an effective treatment for migraine headache in adults, compared with placebo. However, the benefit of metoclopramide was not superior to other drugs.

Research conclusions

Metoclopramide is more effective than placebo in treating migraine in the ED. Although its effectiveness was not observed on other medications, clinicians may select metoclopramide as one of the first line treatments for acute migraine.

Research perspectives

Despite the observed tendency of decreased side effects, the effectiveness of metoclopramide compared to other regimens is poorly understood. More research on this area is needed to treat migraine in acute care settings effectively.

FOOTNOTES

Author contributions: Ungrungseesopon N and Wongtanasarasin W designed the protocol, contributed to data collection and analysis, and wrote the first draft of the manuscript; Wongtanasarasin W edited and revised the manuscript; both authors read and critically reviewed the final version of the manuscript.

Conflict-of-interest statement: All the authors declare that they have no competing interests.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2021 Checklist, and the manuscript was prepared and revised according to the PRISMA 2021 Checklist.

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S-Editor: Liu JH
L-Editor: Wang TQ
P-Editor: Liu JH

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World Journal of *Methodology*

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The *WJM* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Ji-Hong Liu*.

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Bimonthly

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PUBLICATION DATE
September 20, 2022

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 331-349

DOI: 10.5662/wjm.v12.i5.331

ISSN 2222-0682 (online)

REVIEW

Hemostatic system and COVID-19 crosstalk: A review of the available evidence

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Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Krishnan A, United States; Mukhopadhyay A, India

Received: December 5, 2021
Peer-review started: December 5, 2021
First decision: January 25, 2022
Revised: March 17, 2022
Accepted: July 19, 2022
Article in press: July 19, 2022
Published online: September 20, 2022

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Abstract

Since the discovery of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its resultant coronavirus disease 2019 (COVID-19) pandemic, respiratory manifestations have been the mainstay of clinical diagnosis, laboratory evaluations, and radiological investigations. As time passed, other pathological aspects of SARS-CoV-2 have been revealed. Various hemostatic abnormalities have been reported since the rise of the pandemic, which was sometimes superficial, transient, or fatal. Mild thrombocytopenia, thrombocytosis, venous, arterial thromboembolism, and disseminated intravascular coagulation are among the

many hemostatic events associated with COVID-19. Venous thromboembolism necessitating therapeutic doses of anticoagulants is more frequently seen in severe cases of COVID-19, especially in patients admitted to intensive care units. Hemorrhagic complications rarely arise in COVID-19 patients either due to a hemostatic imbalance resulting from severe disease or as a complication of over anticoagulation. Although the pathogenesis of coagulation disturbance in SARS-CoV-2 infection is not yet understood, professional societies recommend prophylactic antithrombotic therapy in severe cases, especially in the presence of abnormal coagulation indices. The review article discusses the various available evidence on coagulation disorders, management strategies, outcomes, and prognosis associated with COVID-19 coagulopathy, which raises awareness about the importance of anticoagulation therapy for COVID-19 patients to guard against possible thromboembolic events.

Key Words: SARS-CoV-2; COVID-19; Thrombosis; Pulmonary embolism; Disseminated intravascular coagulation

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Core Tip: The pathogenesis of hypercoagulable state and thrombosis related to coronavirus disease 2019 (COVID-19) is unclear. Evidence on endothelial cell injury by direct infection of severe acute respiratory syndrome coronavirus 2 is increasing. Histologic and immunohistochemistry examination of lung autopsies and/or the skin of patients who have died of severe COVID-19 has shown microvascular injury and thrombosis, consistent with intensive and generalized activation of both alternative and lectin-based pathways of complement.

Citation: Wifi MN, Morad MA, El Sheemy R, Abdeen N, Afify S, Abdalgaber M, Abdellatef A, Zaghloul M, Alboraie M, El-Kassas M. Hemostatic system and COVID-19 crosstalk: A review of the available evidence. *World J Methodol* 2022; 12(5): 331-349
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/331.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.331

INTRODUCTION

One of the frequently encountered complications of systemic infections is activation of the coagulation cascade, which can present with a broad spectrum of clinical manifestations varying from subclinical activation, which is expressed by elevated laboratory markers for thrombin and fibrin products, to disseminated intravascular coagulation (DIC) and resultant formation of microvascular thrombi in various body tissues and organs[1]. Inflammation affects all phases of blood coagulation, which in turn, leads to both thrombotic as well as hemorrhagic complications[2]. Various viral infections, such as the human immunodeficiency virus, Dengue virus, and Ebola virus, occur by activation of the coagulation cascade[3-5]. Either direct or indirect activation of endothelial cells by viral infection can affect the balance between the coagulation and fibrinolytic systems[6,7]. The clinical presentation of this altered coagulation appears in hemorrhage, thrombosis, or both. An exaggerated response may even lead to DIC with the formation of microvascular thrombi in various organs[8]. Tissue factor (TF) expression is increased in herpes simplex virus and Dengue virus-infected endothelial cells[9].

The Ebola virus induces TF expression in circulating blood cells, especially macrophages, a condition known as Ebola hemorrhagic fever[4,9]. Stimulation by the poly I:C toll-like receptor 3 (TLR3) agonist induces activation of many proinflammatory cytokines as an antiviral chemokine, which is a selective chemoattractant for both activated type 1 T lymphocytes and natural killer cells. Thus, poly I:C increases TF expression in cultured endothelial cells and activates the coagulation system in mice [4]. On the other hand, inhibition of the TF/factor VIIa (FVIIa) complex was shown to decrease the cytokine storm and mortality in a rhesus monkey model of Ebola hemorrhagic fever[10]. Other hematological disorders that frequently occur with viral infections are hemolytic uremic syndrome, idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura[7]. However, it is not clear why some viruses cause hemorrhage while others are associated with thrombosis as cytomegalovirus or both complications such as varicella-zoster virus[10,11].

Viral respiratory tract infections carry a higher risk for deep venous thrombosis and possibly pulmonary embolism (PE)[12]. Influenza A virus is associated with DIC and 18 pulmonary microembolism[13,14]. In the influenza A virus subtype H1N1, both thrombotic and hemorrhagic complications have been reported such as deep vein thrombosis (DVT), PE, and pulmonary hemorrhage with

hemoptysis, hematemesis, petechial rash, and one case of disseminated petechial brain hemorrhage[15]. Another example of viral infection associated with coagulopathy is H5N1, the highly pathogenic avian influenza that results in DIC, pulmonary hemorrhage, and thrombocytopenia in many cases[16]. The outbreak of severe acute respiratory syndrome (SARS) has been associated with significant morbidity and mortality caused by a broad spectrum of clinical presentation, *e.g.*, DIC, deep venous thrombosis, and pulmonary thromboembolic disasters resulting in pulmonary infarction, due to activated coagulation and vascular endothelial damage in both small and mid-sized pulmonary vessels[17].

Due to the ambiguity of the pathogenesis of the hypercoagulable state related to coronavirus disease 2019 (COVID-19) and the evidence of endothelial cell injury by direct infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, histologic and immunohistochemistry examination of lung autopsies and/or skin of patients who died of severe COVID-19 showed microvascular injury and thrombosis. This review discusses the evidence of coagulation disorders, management strategies, outcome, and prognosis associated with COVID-19 coagulopathy to guard against possible thromboembolic events.

DATA FROM SARS-COV-1 AND MIDDLE EAST RESPIRATORY SYNDROME

SARS-CoV or SARS-CoV-1 emerged in China in 2003 and spread to another 26 countries and is associated with thrombotic complications and hematologic disorders. Histopathological examination of pulmonary vasculature has revealed fibrin thrombi in pulmonary, bronchial, and small lung veins. Many studies of postmortem autopsies identified PE, DVT, and widespread multi-organ infarcts due to thrombi associated with polyangiitis and microcirculation disturbance as ischemic stroke (IS). SARS-CoV-1 causes placental circulation dysfunction through fibrin deposition, avascular and fibrotic villi formation, and prothrombotic tendency resulting in many intrauterine fetal complications such as oligohydramnios, intrauterine growth delay, and small fetal size[18,19]. Laboratory parameters of SARS-CoV-1-infected patients show prolonged prothrombin time (PT), prolonged activated partial thromboplastin time (especially over the first 2 wk), elevated D-dimer, and worsening thrombocytopenia. Increased thrombopoietin level has been reported in SARS-CoV-1 patients in the convalescent phase compared to normal controls with a concomitant increase in platelet count. Anticardiolipin antibodies have been detected in patients with post-SARS osteonecrosis and those with positive lupus anticoagulant tests in children[20,21]. *In vitro* studies have revealed that some genes have procoagulant effects when expressed in SARS-CoV-1-infected mononuclear cells. TLR9 and thromboxane A synthase genes are the targets of the SARS-CoV-1, where the TLR9 receptor is expressed in platelets to increase platelet activation, degranulation, and aggregation while increased thromboxane production promotes vasoconstriction, platelet aggregation, and endothelial dysfunction[22-24]. Upregulation of the five genes is associated with changes in the coagulation pathway in human hepatoma cells. These genes are: (1) The TF pathway inhibitor 2, which usually inactivates the tissue factor-VIIa complex and thrombin generation, and upon upregulation, it counteracts the mechanism that inhibits overt coagulation cascade activation in response to inflammation; (2) Early growth response 1; (3) Plasminogen activator inhibitor 1, which causes inhibition of fibrinolysis and promotes fibrin deposition during inflammatory states; (4) Phospholipid scramblase 1; and (5) Thrombospondin 1[25-27]. Urokinase pathway dysregulation is involved in the pathogenesis of SARS-CoV-1-related coagulation disorders leading to fatal disease in mice. The nucleocapsid protein of SARS-CoV-1 is one of the determinants of the prothrombotic state caused by SARS-CoV-1 as it induces the human fibrinogen-like protein-2 prothrombinase gene with activation of the C/EBP-α transcription factor[28-31]. The Middle East respiratory syndrome (MERS-CoV) that occurred in Saudi Arabia in 2012 is also associated with thrombotic complications and hematologic manifestations. Histopathologic examination of MERS-CoV-infected patients revealed microthrombi on day 4 of infection in the pulmonary vessels associated with parenchymal consolidation, alveolar edema, and cellular infiltrates. Thrombocytopenia was identified in the 1st week of laboratory-confirmed MERS-CoV cases with relatively lower platelet count in MERS-CoV patients than negative controls. DIC was one of the major complications reported in fatal MERS-CoV infections[32-34].

PATHOGENESIS OF COVID-19-RELATED THROMBOSIS

The pathogenesis of hypercoagulable state and thrombosis related to COVID-19 is unclear. Evidence on endothelial cell injury by direct infection of the SARS-CoV-2 virus is increasing. Histologic and immunohistochemistry examination of lung autopsies and/or skin of patients who died of severe COVID-19 showed microvascular injury and thrombosis, consistent with intensive and generalized activation of both alternative and lectin-based pathways of complement[35]. Subsequent activation of the clotting pathway, causing fibrin deposition, might also be implicated[36]. The hypercoagulable state due to profound derangement of hemostasis is another contributor to venous thromboembolism (VTE), PE, and/or DVT of the lower limbs, which has been observed in patients with COVID-19. There is

controversy about the pattern of hypercoagulability associated with COVID-19. Viral, bacterial, or fungal infection elicits a complex systemic inflammatory response as a part of innate immunity. Activation of the host defense mechanism induces subsequent coagulation and thrombin formation as a critical interaction between humoral and cellular mechanisms, a term called thromboinflammation or immunothrombosis[37]. Severe inflammation in patients with COVID-19, proved by elevated levels of interleukin 6 (IL-6), increased erythrocyte sedimentation rate, increased C-reactive protein (CRP), and elevated fibrinogen at presentation[38], results in subsequent activation of coagulation and may cause elevation of D-dimer levels[39]. Some experts have postulated that the predominant hypercoagulability in patients with COVID-19 suggests a unique hypercoagulable multifactorial state termed thromboinflammation or COVID-19-associated coagulopathy (CAC), which seems to be inconsistent with DIC, even though DIC has been reported in severely ill patients[40,41]. Other potential pathogenesis for coagulation abnormalities in patients with COVID-19 includes antiphospholipid antibodies, anticardiolipin and anti–β2-glycoprotein I immunoglobulin G (IgG) and IgA[42]. Another explanation for coagulation abnormalities in the presence of lupus anticoagulant has been observed in a high percentage (88%-91%) of COVID-19 patients[43,44].

Although COVID-19 pathogenesis is associated with pulmonary intravascular coagulopathy (PIC) and thrombosis, it differs from sepsis-associated DIC. The first explanation of the pathogenesis of PIC and thrombosis in COVID-19 was directed to binding of SARS-CoV-2 to angiotensin converting enzyme-2 receptors that are located on type II pneumocytes (and possibly on vascular endothelial cells). This binding results in lysis of the cells immediately causing activation of the endothelium and procoagulant activity with the activation of fibrin deposits and accumulation in pulmonary microcapillary venous vessels, finally ending in PIC and thrombosis[45]. The second opinion is that the immune-mediated mechanism results in marked microvascular thrombosis and hemorrhage linked to extensive alveolar and interstitial inflammation, sharing features with macrophage activation syndrome in terms of lung-restricted vascular immunopathology associated with COVID-19[46].

In this context, infection with COVID-19 presumably induces a process of immune system hyperactivation known as immunothrombosis, in which activated neutrophils and monocytes interact with platelets and the coagulation cascade, leading to intravascular clot formation in small and larger vessels. It is presumed that the exaggerated immunothrombosis that occurs within lung microvessels is the main driver of COVID-19 manifestations[47,48].

Endothelial dysfunction is thought to be the most striking pathophysiological event in COVID-19 that infects vascular endothelial cells leading to cellular damage and apoptosis, decreasing the antithrombotic activity of the normal endothelium[49-51].

Similar to other respiratory infections, leukocyte recruitment to the lungs, a higher percentage of macrophages and neutrophils together with higher levels of proinflammatory cytokines (*e.g.*, IL-6, IL-8, and IL-1β) and chemokines (*e.g.*, CCL2, CCL3, CCL4, and CCL7) found in the bronchoalveolar fluid are major contributors to inflammatory responses in COVID-19 infection[52].

Until recently, the association between COVID-19 and VTE, including PE and DVT, has been published as case reports. The prevalence of VTE in COVID-19 patients appears to be higher than that reported for patients admitted to intensive care units (ICUs) for other disease conditions[53]. Diagnosis of VTE was 12.7% in COVID-19 patients, as shown in a meta-analysis of multiple studies including 1783 ICU patients[54]. Patients with COVID-19 had some laboratory abnormalities, including a marked increase in D-dimer and, in some cases, mild thrombocytopenia, similar to DIC. However, other coagulation parameters in COVID-19, including high fibrinogen and high factor VIII activity, suggest that coagulation factors' consumption is not evident are inconsistent with DIC. Studies based on biochemical markers such as a marked increase of fibrin degradation products (FDP) (*e.g.*, D-dimer), prolonged PT/activated partial thromboplastin time (aPTT), and low platelet counts were compatible with the state of DIC. However, prolonged PT/aPTT is not confirmed in some studies[55]. Other studies using thromboelastography (TEG), a method of testing the efficiency of blood coagulation, together with biochemical parameters, demonstrated that results observed in patients with COVID-19 are not compatible with DIC[55]. In this context, careful monitoring of PT, platelet count, and D-dimer concentrations may help predict the clinical improvement and the expected complications.

EPIDEMIOLOGY AND CLINICAL PRESENTATION OF THROMBOTIC EVENTS IN COVID-19

Despite the plethora of publications regarding SARS-CoV-2, there are no available solid epidemiologic data on the actual prevalence and incidence of hemostatic derangements among patients. Most available data to date are retrospective observational data and can be classified as case series from a single-center experience that cannot be considered a true reflection of the prevalence and incidence of hemostatic derangements associated with SARS-CoV-2. However, there is some light at the end of the tunnel as the World Health Organization registry has several ongoing prospective studies aimed towards accurately determining the incidence. For example, a French study located in Centre Hospitalier Universitaire de Nice, started February 28, 2020[56], aims to screen prospectively any cardiovascular complication in

COVID-19 patients including PE, DVT, and VTE. Another study initiated in Shandong Provincial Hospital[57], where patients are recruited with novel coronavirus pneumonia (NCP), aims to calculate the rates of venous thrombosis among those patients and determine the risk factors. The Centre Hospitalier Universitaire de Nîmes registered in April 2020 is conducting a more dedicated study[58] to analyze coagulopathy. They observed any abnormality resulting from sepsis, including coagulopathy and DIC, and excluded all factors that would alter or influence outcomes such as pregnancy and lactation, anticoagulants, or antiplatelet therapy before recruitment or those with hypercoagulable states. In a study on 81 ICU hospitalized patients with NCP in Wuhan, 25% (20/81) had VTE with a significant increase in their D-dimer levels[59]. Dutch published data from three hospitals (184 patients) found that the cumulative incidence of thrombotic complications was 31%, most commonly PE (in 25 patients), VTE in 27%, and arterial thrombosis in 2.7% of all thrombotic events, despite receiving standard thromboprophylaxis[60]. In Italy, 22.2% of 54 ICU-admitted patients developed VTE despite prophylactic low molecular weight heparin (LMWH)[61].

Thrombocytopenia is one of the earliest observations in COVID-19 patients. A meta-analysis of nine studies suggested that thrombocytopenia was significantly associated with the severity of COVID-19, with more platelets found in non-survivors. Alhazzani *et al*[62] presented the data of 1099 patients from 522 hospitals and found that 36.2% of those patients had thrombocytopenia, which was even more evident in more severe cases (57.7%) *vs* 31.6% in non-severe cases[62]. However, in another case study performed on 150 COVID-19 patients in ICU, PE was reported in 43% of cases, besides extracorporeal circuit clotting, which was detected in 28 of 29 patients on renal dialysis. This research compared a group of patients with COVID-19 related acute respiratory distress syndrome (ARDS) *vs* non-COVID-19 ones and demonstrated a higher incidence of thrombotic events among COVID-19 patients[43]. In another series of 107 ICU-admitted COVID-19 cases, PE was found in 22% of cases despite receiving prophylactic anticoagulation[61]. VTE was noted in 39% of COVID-19 ICU cases in a case series composed of 74 patients, yet it was demonstrated in 25% of severe COVID-19 pneumonia patients in an earlier case series done on a cohort of 81 patients[59,63].

In a screening study done on 26 COVID-19 severely infected patients using Doppler lower limb ultrasound, VTE was detected in around 69% of patients; besides, bilateral DVT was demonstrated in 38% of cases though they were all on prophylactic anticoagulation therapy[64].

One of the earliest alarming laboratory findings observed in COVID-19 patients requiring hospitalization was marked elevation of the D-dimer. Elevated D-dimer levels are correlated with disease intensity and with high levels of proinflammatory cytokines, suggesting a possible relation between hypercoagulability and inflammation[65].

Different arterial thrombotic events have also been described in COVID-19 patients, and at the top of the list are ischemic central nervous system events. In a study performed in New York, 5 COVID-19 patients demonstrated large vessel occlusion and IS, astonishingly all these patients were young (under 50 years)[66]. Moreover, IS was noticed in 3.7% of patients in another case series composed of 184 COVID-19 patients[60]. Acute limb ischemia is the second most common arterial thrombotic event observed in COVID-19 patients. A recent study demonstrated acute lower limb arterial thrombosis in 20 COVID-19 patients; most were men with an average age above 75 years[67]. Another study reported acute lower limb ischemia in 4 patients, but they were young and did not suffer comorbidities[68]. Myocardial infarction was also described in COVID-19 patients and was reported in 2 Chinese studies [69,70]. Figure 1 demonstrates the hemostatic system and COVID-19 interplay, possible complications, organs affected and outcomes.

LABORATORY ABNORMALITIES AND DIAGNOSTIC WORKUP

COVID-19 patients may have many hemostatic abnormalities (which may result in a hypercoagulable state as illustrated in Table 1[71-74]), so appropriate evaluation is mandatory for the correct diagnosis and management of COVID-19-associated thrombosis. Thromboinflammation or CAC is the predominant coagulation abnormality in COVID-19 patients, which will lead to a hypercoagulable state; it seems to be distinct from DIC, although DIC has been reported in severely affected patients[75]. A unique coagulopathy and procoagulant endothelial phenotype associated with a proinflammatory state with COVID-19 infection have a prominent effect on elevation of fibrinogen and D-dimer/fibrin(ogen) degradation products, which in turn results in systemic hypercoagulation and frequent venous thromboembolic events[76].

It is well known that the high level of D-dimer in COVID-19 is triggered by excessive clots and hypoxemia, which is likely reflecting pulmonary vascular bed thrombosis and fibrinolysis and correlates significantly with mortality. In many retrospective studies conducted in COVID-19 pneumonia patients, elevated baseline D-dimer levels are observed with inflammation. However, they cannot be accurately correlated with VTE score, which could help determine whether this is possible anticoagulation is needed or not based on levels of D-dimer[76,77].

The most common hemostatic abnormalities with COVID-19 include mild thrombocytopenia[78]; as reported in the literature, the incidence of thrombocytopenia ranges between 5%–41.7% of COVID-19

Table 1 The various hematological parameters in significant relation to coronavirus disease 2019 and their mechanisms

Hematological parameter	Significant relation to COVID-19	Mechanism
High RDW (greater than 14.5%)	Increase in mortality risk (from 11% to 31%)[86]	Not completely understood however reports suggested elevated RDW was attributed to affection of RBC production kinetics[86]
Leucopenia or lymphopenia (ALC < 1.0 × 10^9/L)	Observed in most of COVID cases especially hospitalized patients and associated with poor prognosis[86]	(1) Defective immune response; and (2) Drug induced as with steroids[87]
Normal or increased platelet count	Found in some cases of COVID-19	May be caused by to the large amounts of platelets produced in response to increased thrombopoietin formation from liver stimulation and megakaryocytes in the lung[88]
Prolonged PT and aPTT, elevations of D dimer, fibrinogen and FDP and decreased levels of antithrombin III	Direct relationship was observed between severity of COVID and affection of coagulation profile, Overt DIC (ISTH score of 5 and higher) is seen more frequently in non-survivors[89]	aPTT prolongation is caused by increased Factor VIII level and Factor XII deficiency secondary to the presence of factor XII inhibitors. Von Willebrand factor is quantitatively increased. LA is positive in 91% of those with prolonged aPTT. The presence of both LA and Factor XII deficiency are not associated with bleeding tendency

ALC: Absolute lymphocyte count; aPTT: Activated partial thromboplastin time; COVID-19: Coronavirus disease 2019; DIC: disseminated intravascular coagulation; ISTH: International Society on Thrombosis and Hemostasis; LA: Lupus anticoagulant; PT: Prothrombin time; RBC: Red blood cell; RDW: Red cell distribution width.

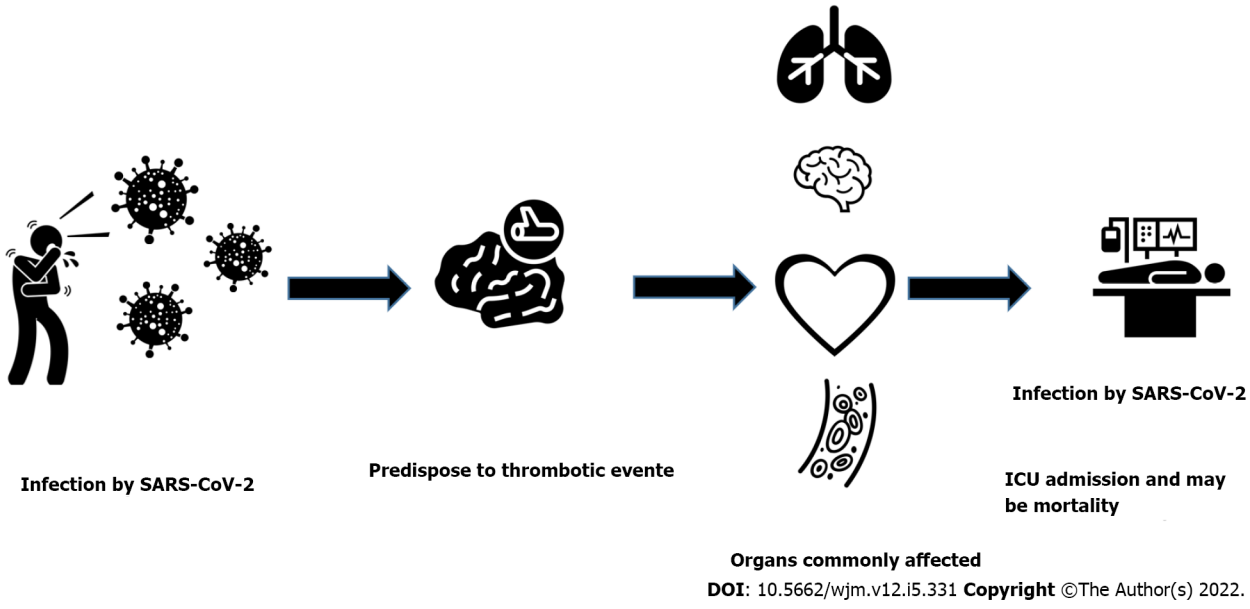

Figure 1 Hemostatic system and coronavirus disease 2019. All icons above are from http://thenounproject.com. ICU: Intensive care unit; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

infected patients, and it varies according to the disease severity. Moreover, rebound thrombocytosis was also reported in some cases[79,80]. Several mechanisms of COVID-19-associated thrombocytopenia have been reported, such as direct viral-platelet interaction activation, platelet autoantibody formation, subsequent platelet clearance, splenic/hepatic sequestration, and/or marrow/megakaryocyte suppression owing to inflammatory response, direct viral infection, or reduced thrombopoietin level [81].

One study suggested that patients with COVID-19 have higher platelet counts than patients with other coronavirus infections[82] and elevation of D-dimer[83], which were related to increased risk of requiring mechanical ventilation, and death[65]. However, high D-dimer levels are common in acutely ill individuals with various infectious and inflammatory diseases. Disease severity is variably related to PT prolongation[84], thrombin time[85] and shortened aPTT[86]. The retrospective analysis of 99 COVID-19 patients conducted by Wuhan Jinyintan Hospital showed that 36% of patients had elevated D-dimer, 16% showed a reduced aPTT, 6% showed an extended aPTT, 30% showed a shortened PT, and 30% showed an extended PT[87]. In a large meta-analysis of 7613 COVID-19 patients, it was found that in severe infection and non-survivors, the platelet count was lower, indicating that platelet counts may be a predictor of COVID-19 mortality[88,89]. COVID-19-associated thrombocytopenia primarily affects clot formation kinetics and clot strength on Quantra viscoelastic analysis; however, the details of *in vivo* fibrinolysis in COVID-19 have not yet been thoroughly investigated[89].

A retrospective analysis of the routine coagulation parameters of 183 patients with COVID-19 revealed that plasma FDP and D-dimer in non-survivors were significantly above those in survivors; PT and aPTT were also significantly prolonged[39]. A retrospective analysis of 138 COVID-19 patients confirmed that D-dimers increased after admission[90]. Previous studies have shown that elevated D-dimer is an independent risk factor for ARDS and mortality in COVID-19 patients[91].

COVID-19 infection has a significantly elevated vWF level together with increased FVIII clotting activity; this likely reflects the combined effect of the more significant release of Weibel-Palade bodies from endothelial cells and the acute-phase reaction. Meanwhile, ADAMTS13 activity was found to be mildly to moderately reduced in COVID-19 patients[75,92,93]. Fibrinogen level is increased to 5.0–7.0 g/dL on average for COVID-19-infected patients, CRP is also increased as an acute-phase reactant associated with elevated IL-6[94,95]. Meanwhile, antithrombin is consumed during coagulation, and the mild antithrombin deficiency occurs in COVID-19 infection whereas protein C has not been decreased in any of the patients assessed[96]. Mildly prolonged aPTT clotting times have been reported in some COVID-19 patients, indicating a prothrombotic state[96].

In a series of 24 intubated patients with severe COVID-19 pneumonia, PT and aPTT were either normal or slightly prolonged, platelet counts were normal or increased (mean, 348000/mL), fibrinogen increased (mean, 680 mg/dL; range 234 to 1344), D-dimer increased (mean, 4877 ng/mL; range, 1197 to 16954), factor VIII activity increased (mean, 297 units/dL), vWF antigen significantly increased (mean, 529; range 210 to 863), *per* endothelial injury. A slight decline in antithrombin and free protein S, with a slight increase in protein C, were also reported. Regarding TEG, there was shortening in reaction time (R) in 50% of patients and in clot formation time (K) in 83% of patients. There was an increase in maximum amplitude in 83% of patients, and also a reduction in clot lysis (LY30) in 100% of patients. Other studies have reported similar hypercoagulable states, including very high D-dimer, vWF antigen and activity, and factor VIII activity[43,97]. Two studies showed a high rate of lupus anticoagulant in patients with prolonged aPTT [50 of 57 tested individuals (88%) and 31 of 34 tested individuals (91%)] [42]. Another study reported 3 cases with severe COVID-19 and cerebral infarction, one with bilateral limb ischemia, within the setting of elevated antiphospholipid antibodies. Whether antiphospholipid antibodies play a significant role in the pathophysiology of thrombosis related to COVID-19 requires further investigation[41]. DIC manifests as coagulation failure and an intermediate phase within the development of multiple organ failure, which is common in many critically ill patients[98]. Tang *et al*[25] recently assessed 183 patients with COVID-19, of whom 21 (11.5%) died. The primary common differences between those who died and survivors were the increased levels of D-dimer and FDPs (an approximate 3.5- and approximately 1.9-fold increase, respectively) and PT prolongation (by 14%, $P < 0.001$), 71% of these patients who died fulfilled the International Society on Thrombosis and Hemostasis (ISTH) criteria for DIC compared with only 0.6% among survivors[40,99]. The COVID-19-related hypercoagulable state has been described as a DIC-like state, especially because many affected individuals are acutely ill and meet the criteria for probable DIC in the ISTH scoring system[99]. However, the main clinical finding in COVID-19 is thrombosis, whereas the main finding in acute decompensated DIC is bleeding. COVID-19 has similar laboratory findings of DIC, including elevated D-dimer and thrombocytopenia in some patients. However, in COVID-19, there is high fibrinogen and high factor VIII activity which are not found in DIC[40,99]. According to the recommendations from ISTH, the American Society of Hematology (ASH), and also the American College of Cardiology, routine testing for inpatients should include complete blood count, coagulation studies (PT and aPTT), fibrinogen, and D-dimer, and it will be repeated according to the clinical situation[100]. According to the American Society of Hematology recommendations regarding the diagnosis of PE, a normal D-dimer is sufficient to exclude the diagnosis of PE. In patients with suspected PE because of unexplained hypotension, tachycardia, worsening respiratory status, or other risk factors for thrombosis, computed tomography with pulmonary angiography (CTPA) is the preferred test. Ventilation/perfusion (V/Q) scan is an alternative if CTPA cannot be performed or is inconclusive, although the V/Q scan is also unhelpful in individuals with significant pulmonary involvement from COVID-19[101]. To date, whether these hemostatic changes are characteristic for SARS-CoV-2 or are an element of cytokine storm, as observed in other viral diseases, is unknown[102,103].

Regarding COVID-19 induced coagulopathy, we conclude that it meets the criteria of sepsis-induced coagulopathy (SIC), defined as a reduced platelet count, increased INR, and higher organ dysfunction score[40,104]. Table 2 shows the various laboratory parameters altered in SARS-CoV-2 and their implications in COVID-19 severity[105,106].

MANAGEMENT STRATEGIES

The cumulative incidence of COVID-19-associated VTE risk has raised concerns. Table 3 shows the frequency of venous thromboembolic complications in COVID-19 patients in different studies[64,107,108].

Many international societies and ministries of health have to publish their interim guidance to overcome this challenging situation[49,65,109-111]

Table 2 Various laboratory parameters that are altered in severe acute respiratory syndrome coronavirus 2 and their implications in coronavirus disease 2019 severity

Clinical index	Alterations with COVID-19 severity	Ref.
Neutrophil-to-lymphocyte ratio	Increased	[84,122-124,131,134-136]
CRP	Increased	[122,124,125,128,129,131,134-136,137-144]
Platelets	Decreased	[78,83,122,126,129,131-133,136,145,146]
Lymphocytes	Decreased	[78,128,129,131,134-136,147,148]
D-dimer	Increased	[55,83,84,127,128,131,137,144-146,149-152]
Ferritin	Increased	[91,94,128,129,131,134,135,137-139,144,153-155]
Procalcitonin	Increased	[83,84,128,144,156-158]
Lactate dehydrogenase	Increased	[106,129-131,152,159-173]
Albumin	Decreased	[111,116,128,129,136,148,174-186]

COVID-19: Coronavirus disease 2019; CRP: C-reactive protein.

Table 3 Frequency of venous thromboembolic complications in coronavirus disease 2019 patients

Ref.	Proportion	Cumulative incidence	Median follow-up	Patients
Cui *et al*[59]	20/81 (25%)	NR	NR	ICU patients
Klok *et al*[60]	68/184 (37%)	57% or 49% adjusted for competing risk of death	14 d	ICU patients only.19 PE were limited to subsegmental arteries.65/68 venous events were PE (95.6%)
Poissy *et al*[61]	VTE 22.2% of 54 ICU admitted			
Helms *et al*[44]	27/150 (18%)	NR	NR	ICU patients with ARDS 25/27 events were PE (92.5%)
Poissy *et al*[61]	PE only 22/107 (20.6%)	20.4% calculated at ICU day 15	6 d	ICU only
Middeldorp *et al*[63]	Venous thromboembolism 39% of COVID-19 ICU cases 74 patients			
Llitjos *et al*[64]	DVT: 18/26 (69%); PE: 6/26 (23%)	NR	NR	ICU patients. Systematic ultrasound screening
Léonard-Lorant *et al*[183]	PE only 32/106 (30%)	NR	NR	24/32 (75%) PE-positive patients were in the ICU
Grillet *et al*[184]	PE only 23/100 (23%)	NR	NR	Ward: 6/61 (9.8%); ICU: 17/39 (43.6%)
Middeldorp *et al*[63]	33/198 (17%)	15% at 7 d; 34% at 14 d	5 d	Ward: 4/123 (3.3%); ICU: 35/75 (47%); 11 (5.4%) clots detected on screening 11/33 events were PE (33%)
Lodigiani *et al*[185]	16/362 (4.4%)	21% (time not reported)	10 d	ICU 4/48(8.3%); Ward 12/314 (3.8%)
Thomas *et al*[186]	6/63 (9%)	27%	8 d	ICU patients
Cattaneo *et al*[108]	DVT only 0/388 (0%)	NR	NR	Non-ICU Ward 64 patients had screening ultrasound. All negative

DVT: Deep vein thrombosis; ICU: Intensive care unit; NR: Not reported; PE: Pulmonary embolism.

Although the general adoption of many societies[112] of the interim guidance of the ISTH[110], some institutions may vary in their management strategy of thromboembolic complications and would encourage enrollment in clinical trials to determine the best approach[113,114]. The ISTH recommends that all inpatients (ICU, medical non-ICU, and perioperative surgical and obstetric patients with COVID-19) receive prophylactic anticoagulation unless contraindicated after careful stratification with a DIC score. The low prophylactic dose molecular weight (LMW) heparin is preferred [*e.g.*, enoxaparin in a dose of 40 mg to 60 mg once daily for patients with creatinine clearance (CrCl) > 30 mL/min, and 30

mg once daily for patients with CrCl 15 to 30 mL/min]. Dalteparin, nadroparin, and tinzaparin are also recommended. In a retrospective study of 449 patients with severe COVID-19, 99 patients who received enoxaparin in prophylactic doses showed a better prognosis concerning mortality, especially those with high SIC score and markedly elevated D-dimer[115]. Moreover, LMWH could have anti-inflammatory properties that would help in COVID-19 patients where proinflammatory cytokines are markedly elevated[116]. The high incidence (43%) of VTE reported in a multicenter prospective study of ICU patients, mainly PE, despite being on a regular prophylactic dose of LMWH[43], prompted many experts to suggest higher doses and call for more aggressive anticoagulation with intermediate-dose or even therapeutic dose anticoagulation for thromboprophylaxis. For patients with CrCl < 15 mL/min or renal replacement therapy, unfractionated heparin can be used. Doses should be modified according to weight and pregnancy conditions. Full-dose anticoagulation is indicated in those with documented VTE like DVT or PE in the same way as those without COVID-19 infection.

Not all patients have access to confirmatory tests for VTE in real life. The empirical initiation on full-dose anticoagulation can be justified by the local consultation of expertise in hemostasis and thrombosis and clinical evaluation of individual patients. Sudden respiratory status deterioration in a previously stable intubated patient not explained by a cardiac cause indicates a high suspicion of PE. Moreover, those with highly elevated fibrinogen and/or D-dimer and otherwise unexplained respiratory failure, superficial thrombophlebitis, retiform purpura, recurrent clotting of arterial lines, or central venous catheters despite prophylactic anticoagulation are highly indicated for full-dose anticoagulation. The dose dilemma for critically ill ICU COVID-19 patients is still not resolved. Whether the regular prophylactic, intermediate, or therapeutic dose would better treat disease morbidity and mortality needs future clinical trials to improve our practice. This strategy is supported by the American Society of Hematology, which recommends against empiric full-dose anticoagulation because of the increased risk of bleeding in the same setting of VTE with this approach[55]. Tissue plasminogen activator (tPA) is suitable for use in its known indications, *e.g.*, massive limb DVT, extensive PE, acute cerebrovascular stroke, and acute myocardial infarction. TPA use was described in a case series of three advanced COVID-19 patients with ARDS that improved their respiratory status and laboratory parameters[117]. Of note, all patients with proven VTE must be maintained on anticoagulation for at least 3 mo after discharge. Immobility, old age, recent surgery, and other risk factors for thrombosis should be considered before deciding thromboprophylaxis in outpatients with COVID-19 with close observation. Patients undergoing clinical trials for COVID-19 new therapeutic options should be closely monitored for possible drug-drug interactions with thromboprophylaxis treatment. The British Thoracic Society recommends therapeutic LMWH for inpatients with COVID-19 disease who are managed on general wards and require supplemental oxygen.

In contrast, the patients with no evidence of VTE or other indication for therapeutic anticoagulation who require high-flow oxygen, CPAP, NIV for severe ventilatory failure, or invasive ventilation should receive less than therapeutic dosing[118]. Meanwhile, The Italian Society of Thrombosis and Hemostasis strongly recommends prophylactic anticoagulation with LMWH, UFH, or fondaparinux for the entire hospital stay for 7–14 d more after hospital discharge[119]. Furthermore, the American College of Chest Physicians and Global COVID-19 Thrombosis Collaborative Group recommends standard dose anticoagulation for inpatients with COVID-19 disease and ICU/Critical Care patients; meanwhile, SIGN and NICE NG-191 exerts intermediate-dose/ standard dose anticoagulation for those patients[120-122].

Much International and National guidance regarding VTE thromboprophylaxis has been published; however, more extensive studies are required to investigate the potential therapeutic approach. Most of the international guidelines and recommendations (ISTH-IG, ACF, CDC, and ASH) adopt stopping anticoagulation in patients who developed bleeding or severely thrombocytopenic; furthermore, they also do not recommend a particular platelet count threshold[123]. Furthermore, the expert panel reports by CHEST/AIPPD/AABIP stated that empiric use of therapeutic anticoagulation regimens in ICU patients with COVID-19 is not beneficial and may be harmful, while its use in hospitalized, noncritically ill patients with COVID-19 remains uncertain[123].

OUTCOME AND PROGNOSIS

The catastrophic event of unopposed coagulopathy and DIC is a strong predictor of mortality in patients with COVID-19. On a laboratory basis, a significant elevation in D-dimer and INR with a decrease in fibrinogen level was also observed in non-survivors at days 10-14, and this was considered a poor prognostic sign[55]. For this reason, continuous and close monitoring of their levels is essential to determine prognosis and outcome, D-dimer level above 1 μg/mL was a strong and independent risk factor for death in this population[124]. In an observational study, a mean D-dimer level of 2.12 mg/L was observed in patients who did not survive compared to a concentration of 0.61 mg/L in survivors [55]. Another study revealed that patients admitted to ICU had significantly higher median D-dimer concentrations than patients who did not receive ICU care[84]. A third study reported that D-dimer on admission greater than 1 mg/L resulted in an 18-times increased risk of death[125]. These data provided strong evidence that D-dimer could be used as an excellent prognostic sign[125]. A retrospective study

that included 449 patients admitted to the hospital with severe COVID-19 infection showed that the use of prophylactic heparin was associated with a lower mortality rate than in patients who did not receive prophylactic heparin[115]. The available data about coagulopathy in COVID-19 patients suggest that regular monitoring of PT, platelet count, and D-dimer concentrations could predict prognosis and expected complications. Accordingly, there is justifying evidence supporting using a prophylactic dose of LMWH to prevent VTE in critically ill COVID-19 patients[126].

COVID-19 AND BLEEDING

Indeed SARS-CoV-2 is not as pathogenic as other RNA viruses (Ebola and hemorrhagic fever viruses) in causing severe hemorrhagic manifestations[127]. Owing to the abnormal coagulation cascade and subsequent high risk of thrombosis necessitating pharmacologic VTE prophylaxis, especially in severe COVID-19, the risk of bleeding with COVID-19 due to over anticoagulation, SIC, or DIC is inevitable. Although there are few reported data about clinically-overt bleeding in the setting of COVID-19, close observation for the occurrence of bleeding or thrombosis is mandatory for all COVID-19 patients who develop SIC or DIC[128]. In the absence of overt bleeding, the correction of coagulopathy is not mandatory in most COVID-19 patients. It is recommended to monitor full blood count, coagulation profile, and/or TEG and Rotational Thromboelastometry are all needed in cases of minor bleeding. However, in cases of significant bleeding as observed with a decrease in systolic blood pressure to less than 90 mmHg and/or increase of heart rate more than 110 beats *per* minute, management should be started immediately with FFP (15-25 mg/kg if PT/INR or aPTT ratios are greater than 1.5), platelet transfusion (for platelet count < 50 × 10^9/L), fibrinogen replacement (when fibrinogen level is < 1.5 g/L).

Additionally, prothrombin complex concentrate will be given if FFP transfusion is not feasible and/or tranexamic acid (in a dose of 1 g over 10 mi) followed by a further dose (of 1 gm) if bleeding persists or restarts in the following 24 h provided that the patient does not have any evidence of DIC and followed by repeated monitoring with coagulation screens[129]. In a unique observation from Thailand on 41 COVID-19 infected patients initially presented with bleeding and petechiae, no specific additional treatment for this hemorrhagic problem was needed, and fortunately, no deaths occurred. This study and other studies may be of great value to raise awareness about the hemorrhagic presentation associated with COVID-19. Therefore, investigation and follow-up for possible hemorrhagic problems induced by COVID-19 are highly recommended[130]. A retrospective study comparing the risk of thrombosis *vs* the risk of bleeding in COVID-19 patients showed that critically ill patients had an increased incidence of bleeding (26.7%). This was a complicated situation in the setting of VTE prophylaxis and could be explained by dysregulated hemostasis in severe COVID-19. However, in noncritically ill COVID-19 patients, the prediction risk of VTE and major bleeding was minor. Based on that, critically ill COVID-19 patients are predisposed to both high risk of thrombosis and bleeding, so prevention strategies should be individualized according to the assessment of thrombosis *vs* bleeding risk[131]. Another study reported two cases of a significant hemorrhagic complication in severe COVID-19 patients presented by spontaneous abdominal, internal bleeding. Patients had bilateral interstitial pneumonia, and there were no other apparent predisposing factors for bleeding. Patients were managed with interventional radiology, with no mortalities recorded. These imbalances (or disruption) in platelet production and disorders of the coagulation system induced by SARS-CoV-2 need to be further clarified in extensive prospective studies[132]. Only a few published data about COVID-19 infection with known bleeding disorder patients are available. A case report of mild COVID-19 in a known hemophilia-A patient reported no evidence of bleeding linked to COVID-19 infection, and the patient recovered completely with only home isolation, antiviral agents, empirical antibiotics, and supportive therapies. Indeed, mild COVID-19 is not known to increase the risk of bleeding, even in patients with known bleeding disorders[133]. Transfusion therapy should be restricted for those with active bleeding, requiring an invasive procedure, or at otherwise high risk for bleeding complications and accordingly to be managed similar to those in ISTH guidelines for DIC[134].

CONCLUSION

In conclusion, and based on all the previously discussed data, we should highlight the importance of using empirical therapeutic anticoagulation for COVID-19 patients to guard against possible thromboembolic events with close observation for the occurrence of bleeding.

ACKNOWLEDGEMENTS

Authors acknowledge the great effort and sacrifice of the medical staff and nurses working in COVID-

19 quarantine hospitals all over the world.

FOOTNOTES

Author contributions: Morad MA, El Sheemy R, Abdeen N, Afify S, Abdalgaber M, Abdellatef A, and Zaghloul M contributed equally to the original writing of the manuscript; Wifi MN and El-Kassas M contributed equally to reading and revising the subsequent versions of the manuscript; Alboraie M contributed to revisions and provided approval of the final manuscript; All authors read and approved the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Fan JR

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 September 20; 12(5): 350-364

DOI: 10.5662/wjm.v12.i5.350

ISSN 2222-0682 (online)

REVIEW

Syndemic aspects between COVID-19 pandemic and social inequalities

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Specialty type: Social science

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Clinton M, Lebanon; Solanki SL, India

Received: March 20, 2022
Peer-review started: March 20, 2022
First decision: June 8, 2022
Revised: June 22, 2022
Accepted: July 25, 2022
Article in press: July 25, 2022
Published online: September 20, 2022

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Abstract

Although the coronavirus disease 2019 (COVID-19) pandemic has reached all over the world population, it has demonstrated a heterogeneous impact on different populations. The most vulnerable communities which coexist daily with the social inequalities like low access to hygiene and personal protection products, crowded residences, and higher levels of chronic diseases have a higher risk of contact and the spread of infection, beyond unfavorable clinical outcomes. The elevation of the risk of infection exposure can be related to gender due to the presence of a larger contingent of women in essential services, as well as frontline and cleaning professionals who regardless of gender have the greatest exposure to the virus. Such exposures can contribute to the development of fear of contaminating themselves or their family members associated also with the work stress, both of which are related to the emergence of mental disturbances in these populations. Furthermore, conditions of unsanitary living and low socioeconomic status, populations at war, pre-existing social barriers, and ethnicity have contributed to more impact of the pandemic both in the exposure to the virus and access to health services, COVID-19 management, and management of other pathologies. At the same time, factors such as the closing of non-essential services, the loss of jobs, and the increase in household spending aggravated the social vulnerabilities and impacted the family economy. Lastly, the COVID-19 pandemic contributed still more to the impact on women's health since it propitiated a favorable environment for increasing domestic violence rates, through the segregation of women from social life, and increasing the time of the victims with their

aggressors.

Key Words: COVID-19; Minority groups; Pandemic; Social inequalities; Socioeconomic factors

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Core Tip: The social inequalities interact continuously with the coronavirus disease 2019 pandemic, influencing the development and heterogeneity of the disease while they are potentiated by the pandemic context. Therefore, understanding the individual features of each group is of fundamental importance to the compression of the illness risk, morbidity, and mortality of the infection, data that can be used to create specific measures for health prevention and recovery of the population.

Citation: Apolonio JS, da Silva Júnior RT, Cuzzuol BR, Araújo GRL, Marques HS, Barcelos IS, Santos LKS, Malheiro LH, Lima de Souza Gonçalves V, Freire de Melo F. Syndemic aspects between COVID-19 pandemic and social inequalities. *World J Methodol* 2022; 12(5): 350-364
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/350.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.350

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had its first case in Wuhan, China in 2019, quickly spreading to other countries[1-3]. This virus colonizes the respiratory tract causing, normally, mild symptoms such as cough, fever, dyspnea, sputum, and sneezing. Severe cases of cardiac damage, shock, kidney, and respiratory failure can occur, mainly in individuals with pre-existing chronic diseases[4,5].

On the other hand, the severity of the disease can also be influenced by the social context in which the person is inserted since unique features of each population can propitiate the elevation of the risk of contagion and increase the morbimortality of the infection[6]. Studies have demonstrated that during the pandemic, the social inequalities have been responsible for aggravating the damages caused by COVID-19[7]. Therefore, in addition to considering the biological characteristics of the virus and individual, it is also essential to evaluate the functional vulnerabilities of the population such as the necessity to exercise the work activity during the lockdown because both are important predictors of the illness risk[8].

Thus, although the COVID-19 pandemic has affected the world, its dissemination and impact on the health of different social groups have been demonstrated not to be homogeneous[9]. Access to health information, different age groups, gender, minority groups, socioeconomic and schooling levels[10], inadequate housing quality, absence of potable water and electricity, overcrowding, and bad sanitary conditions can influence the infection heterogeneity[11]. Such conditions are visualized in populations that daily coexist with armed conflicts, in regions historically more vulnerable with low access to health services, provisions, high rates of infectious and chronic diseases, and factors able to add to the COVID-19 pandemic and potentiate its impact on health[12].

Besides the grievances on population health, the proper pandemic also significantly influences the increase of these inequalities[7], reverberating also on the world and family economy. Data from 2020 demonstrate that during this period, there was increasing poverty and hunger, and jobs were lost with an approximate mark of 400 million, beyond the reduction in the workers' income worldwide[13]. The lockdown, although it has been an effective measure to control the spread of COVID-19, also contributed to negative impacts on family economy through the closing of some non-essential services, an increase in the unemployment rates, and the impossibility of going to work while there was an increase of household expenses with personal entertainment, toiletries, cleaning products, and face masks and gloves[14]. Although such misfortunes can reach all the population, they affect with more intensity the populations more vulnerable and with a low socioeconomic level, contributing to the increase of socioeconomic fragility of these groups[15]. Besides that, both the prioritization of essential or non-essential services and the contagion fear resulted in the reduction of access and seeking for health services such as sexual and mental health and oncology, influencing the increase of the vulnerability of these populations[16].

Lastly, although masked by the impacts of the pandemic, domestic violence emerges as an important aggravating factor for women's health in the current times[17]. The lockdown made possible a greater psychological and financial domain of abusers on their victims while making it difficult for these women to seek help[18]. This exposure to violence was related to the elevation of the risk of death and suicide in females, as well as the development of mental disturbances such as post-traumatic stress

disorder, anxiety, and depression[17]. Table 1 synthesizes the relation between the individual characteristics of the populations and the impacts of the COVID-19 pandemic.

The present article aims to evaluate the socioeconomic aspects that permeate the COVID-19 pandemic and how these influence the disease.

METHODOLOGY

The present minireview was based on the articles published in the United States National Library of Medicine (PubMed), which were searched using the following descriptors: COVID-19; SARS-CoV-2; gender; pandemic; disparity; chronic diseases; inequalities; socioeconomic; race; ethnicity; home working; social impact; social distancing; essential services; unemployment rate; domestic violence; vaccine; war situation; and indigenous people. Initially, 5908 articles were found. The inclusion criteria encompassed articles that presented the descriptors in their title or abstract, and manuscripts were written in the last 10 years in the English language. Paid manuscripts, articles not available in full text, and texts that do not address the research topic were considered the exclusion criteria. Lastly, the repeated articles were excluded and 136 were used in the construction of this minireview.

MINORITY GROUPS DURING THE PANDEMIC

The concept of ecopandemic injustice, which seeks to explain the interrelations between pandemic and ecological systems, demonstrates how COVID-19 reveals and deepens the structural inequalities that are formed along the lines of environmental health[19]. Some individual features such as gender, socioeconomic conditions, and ethnicity play a major role in COVID-19 susceptibility and progression, leading to a higher risk of infection, mortality, and hospitalization in the most vulnerable groups[20-22]. On the other hand, the global crisis triggered by the pandemic made the links between racism, poverty, and health more visible and exacerbated[19,23].

Gender

Although men and women are equally susceptible to COVID-19, studies demonstrated that the rates of fatality and admission to the intensive care units (ICUs) are higher in males[20,24,25]. Hypotheses such as the differences in the angiotensin-converting enzyme 2 (ACE2) expression between genders seek to explain these sex disparities[24,25]. Many studies have shown that ACE2 expression is higher in males than in females, probably due to differences in sex hormone activity[20,25,26], since estrogen may present a regulatory effect on ACE2, controlling its expression in human bronchial epithelial cells[26,27]. Besides that, the transmembrane protease serine 2 (TMPRSS2), an enzyme necessary for the priming of the viral S protein and for spreading the virus in the body[26], suffers from the influence of androgen receptors (ARs), higher expressed in males due to the presence of dihydrotestosterone and acting in the transcription of TMPRSS2[24,26]. Lastly, behavioral and underlying comorbidity differences, such as alcoholism, smoking, and hypertension, are higher among men than women and contribute to the gender gaps in COVID-19 mortality[20,28].

Nevertheless, the women face secondary effects of the pandemic that place them in a vulnerable condition. The female gender represents most of the essential care employees such as frontline health care professionals[20,29], laundry and cleaning staff, administrative assistants working in hospitals, social workers, cashiers, and food service workers. Their close physical proximity to the population in general and high interaction with others contribute to increasing the risk of exposure and infection[20,30,31], which also can elevate the hospitalization and death risk[32]. In addition, pregnant women are considered one of the most vulnerable groups regarding COVID-19[33], because they present a greater risk of developing severe complications in respiratory infections. On the other hand, these individuals must continue with prenatal care appointments, which may increase the risk of exposure to the virus[33,34]. Studies have reported that the crowded hospitals and staff and supply shortages may affect the quality of care and increase the risk of obstetrics complications[33]. Lastly, life-saving treatments and vaccines may be denied or hampered to pregnant women due to a lack of data or concern for fetal safety [34,35].

Socioeconomic conditions

Lower socioeconomic status has been related to higher SARS-CoV-2 infection rates and worse clinical outcomes[36-38]. Such facts can be explained by delay in seeking help in COVID-19 cases[37] and higher rates of comorbidities, such as cardiovascular diseases, diabetes, and cancer in the most vulnerable populations[21,39]. In addition, the use of public transportation, lack of adequate personal protective equipment, poor general health and nutritional status, housing conditions, living in poverty or deprivation, lack of insurance, household overcrowding, lower level of education, speaking in a language other than the national language in a country, being an immigrant, and unemployment are

Table 1 Relation between individual characteristics and the coronavirus disease 2019 pandemic

Individual characteristic	Risk factors	Repercussion
Gender	Higher expression of ACE2 and ARs in males[20,25,26]	Higher mortality among men[20,28]
	Greater rates of alcoholism, smoking, and hypertension in men[20,28]	
	Women as most of the essential care employees[20,29]	Increased risk of exposure among women[20,31]
Socioeconomic conditions	Delay in seeking help and higher rates of comorbidities[37,39]	Higher infection rates and worse clinical outcomes[36,37]
	Use of public transportation, household overcrowding, lack of personal protective equipment, smoking, alcoholism, poor diet, and being an immigrant[36,37,40,41]	Higher exposure and mortality[36,37,40,41]
Ethnicity	High rates of comorbidities in the minority ethnic groups[51]	Risk of severe forms of COVID-19[22,45]
	Household crowding, language barriers, and difficulties in accessing healthcare systems[22,52]	Increased mortality for COVID-19[50]
	Usually workers in essential industries[51,52]	Higher exposure to the virus[51,52]
Health service accessibility	Resources reallocation to COVID-19 management[76]	Delay in the realization of elective surgeries[78]; reduction of managing chronic disease[84], services of sexual education[82] and family planning[79,82,83]
	High cost of vaccines against COVID-19[92] and discrepancies in the immunization strategies[93]	Reduction of the vaccine access, increase in the infection and death rates[92,93]
	Language Barriers[97]	Low knowledge about the vaccination process[97]
	Mistrust with the health systems[95,96], immigrants with pending documentation, negativism, and having to work during the vaccination process[94]	Reduction of vaccine access by minority groups[94,96,97]
Labor vulnerability	Frontline or essential work[100]	Higher exposition rates, sleep disturbances, suicide anxiety, depression, PTSD[104]
	Marginalized population, low level education[99], and lockdown policies[104]	Unemployment, reduced family income, food insecurity[98]
	Work at home[116]	Sedentary lifestyle, risk of cardiovascular events[115,117]
Domestic violence	Less social interaction and opportunities for denouncing, and socioeconomic problems[119,125]	Physical and psychological consequences (anxiety, depression, and stress)[132]

COVID-19: Coronavirus disease 2019; ACE2: Angiotensin-converting enzyme 2; ARs: Androgen receptors; PTSD: Post-traumatic stress disorder.

factors that may increase the exposure to and mortality of COVID-19[36,37,40,41].

In association with the aforementioned, lower education levels and lack of information may influence lifestyle and behavior, leading to habits such as smoking, drinking, and poor diet, which are risk factors for severe forms of COVID-19[40]. People with lower education levels tend to work in jobs that do not offer the opportunity to work remotely, increasing the exposure risk[39]. Correspondingly, one study conducted in Spain in 2021 reported that workers with low salaries, unemployed, and people on minimum integration income had an increased probability to contract COVID-19 than workers with salaries equal to or higher than €18000 per year[42]. In this context, in certain communities, social distancing is an inaccessible privilege, because it is impossible to depart from work for the period necessary to carry out quarantine[23,43]. Similarly, homeless people, displaced populations, and prisoners cannot choose to be physically distant from each other, which impairs the realization of isolation[43].

On the other hand, past evidence and experience suggest that marginalized and low-income communities suffer the greatest impact from the current pandemic, since they have health systems historically fragile, overloaded, and with few resources. Therefore, it is clear that COVID-19 shows disparities in several areas, particularly the potentially serious healthcare discrepancies[23,43]. Consistent with this, although medical advice is the adoption of safe practices which include hand hygiene and the use of masks in public environments[23], the water insecurity, and lack of access to basic sanitation and hygiene products in many parts of the world[42] create a new barrier for certain marginalized groups[23]. Such facts are corroborated by current data suggesting that 1 in 4 people of the global population do not have access to clean water or soap to wash their hands at home[44].

Ethnicity

The racial/ethnic minority population also face gaps and disparities in the COVID-19 pandemic[22,45]. In a systematic review conducted with 52 studies, 11 reported that racial/ethnic minority groups were at higher risk of exposure to COVID-19 when compared to the White population and 11 studies demonstrated an increased risk of death for these minority groups[40]. Data from National Center for Health Statistics report that Hispanic populations represent approximately 21% of excess deaths[45], which is related to another study that reported a two times higher risk of Hispanics dying from COVID-19 than Whites[45]. This research also demonstrated that American/Black and Hispanic populations present an increased risk of contracting COVID-19 and similar rates of case fatality[46].

The disparity in the consequences of the pandemic among ethnic groups is so evident that The Washington Post revealed in one of its articles that African-American people are contracting SARS-CoV-2 at higher rates and are more likely to die[23]. The Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Report (MMWR) reported that the Black population is disproportionately affected by COVID-19, accounting for 33.1% of hospitalizations for the disease, despite representing only 18% of the population in the area analyzed[47]. Similarly, data from the Johns Hopkins University and American Community Survey revealed that the infection rate is 3 times higher and the mortality rate is 6 times higher in municipalities with a predominance of Black residents when compared to those with White predominance[23]. Such data correspond to the findings of other studies that described that regions where the Black population was present in an above-average proportion also had higher rates of cases, moderated by social segregation[48], and deaths from COVID-19[49].

Some hypotheses such as the greater burden of obesity and other comorbidities in the minority ethnic groups[50], along with ethnic differences in economic status, the density of residence, household crowding[22,51], language, and other structural barriers to accessing healthcare systems would be related to the increased mortality for COVID-19 in this population[50]. Furthermore, minority ethnic groups are usually employed in essential industries, which do not provide opportunities for working from home, leading to closer proximity with other individuals and higher exposure to the virus[51,52].

Similarly, COVID-19 also impacted the health of approximately 900000 indigenous people (IP) in Brazil. Even if part of this population lives in native lands, theoretically more isolated from society in general, the interaction is inevitable. The first reported case in IP occurred on April 9, 2020 in the Kokama tribe after contact with an infected doctor. As of June 5, there were already 70 deaths in patients aged between 0 and 88 years. Among the victims were the so-called Caciques, a title bestowed on the oldest and leader of the tribe. These deaths can mean an irreparable loss for the maintenance of the culture and traditions of these people[53]. The invasion of protected lands by illegal activities such as mining, drug trafficking, and logging, as well as tourists, missionaries, and traders, are other means of contact between IP and infected people[54].

An ecological study, using spatial analysis techniques and government databases, carried out between March 24 and October 26, 2020, revealed the occurrence of 32024 cases and 472 deaths from COVID-19 in IP, with approximately 85% of the fatal cases occurring in individuals over 50 years old, mainly in the north and midwest regions of Brazil. This study also calculated the mortality rate of COVID-19 in IP at 265.37 deaths per 100000 inhabitants, against 41.1 deaths per 100000 inhabitants in the general population[55]. There are some possible explanations for this higher rate in IP, among which are the high prevalence of comorbidities such as obesity, hypertension, diabetes mellitus, and malnutrition, in addition to the low access to health services, potable drinking water, and good sanitary conditions of housing or even soap and alcohol gel[53,54].

To reinforce the vulnerability of IP, another retrospective study identified that the excess of deaths in the general Brazilian population in 2020 showed an increase of 18.1% in relation to the expected value, while in IP this growth was substantially higher, at 34.8%. It is worth noting that this excess of deaths is directly related to the fatal cases of COVID-19[56]. This scenario can be even more serious as studies also raise the possibility of under-reported cases of COVID-19, leading to the belief that these rates may have been higher[57,58], and even leading to the possibility of risk of the decimation of the entire indigenous villages in the southern region of the country[59].

The world perspective on the COVID-19 pandemic and IP is not very different from what happens in Brazil. The difficulties in dealing with COVID-19 are closely related to limited state and federal assistance and this is a reality for IP in different parts of the world[60]. A recent study looked at differences in stress, anxiety, and depression experienced by different ethnic groups during the COVID-19 pandemic. Their results demonstrated that indigenous ethnicity is a specific risk factor for the psychiatric disorders studied and suggested that greater attention to the mental health of this population is needed[61]. In addition, the socioeconomic marginalization and the social inequalities that affect with more severity the indigenous communities and potentiate the pandemic effects in these populations around the world[62] also can hinder these populations' access to vaccination[63].

COVID-19 PANDEMIC AND WAR STRESS

Syria and Lebanon are Middle Eastern countries that have been living with internal armed conflict for

about 11 years since the Arab uprising in 2011. This reality of combat and altercation generated, in addition to an undeniable and expressive number of deaths and injuries, the population displacement and collapse of health systems[64]. With the emergence of the COVID-19pandemic, the situation of poor health in these places has become even more evident, given that if even countries with health systems and more advanced resources struggled with difficulties to fight COVID-19, those affected by conflicts ended up facing even more devastating outbreaks of the disease[65]. Yet, statements from several international public entities, such as the International Crisis Group, warned of concerns about these locations, where the already challenge of global health was met with wars and political conditions that generate an extremely weak health system, mass displacement, and lack of basic infrastructure, resulting in more impacts of the COVID-19 pandemic[65].

There are several factors that put the Syrian population, of about 3.5 million people, at greater risk than the others in the face of the current global health condition. In addition to the presence of more than 2.8 million internally displaced people, this could be a potential route for the spread of the virus and an increase in the number of cases of the disease, as well as overcrowding of urban centers and rural areas, and the existence of more than 500 concentration camps arbitrarily built in the region[65]. Finally, the high rate of extreme poverty – estimated to be that about 83% of Syrians live below the poverty line – added to the inability of the Syrian health system, shaken and weakened by the 9 years of armed conflict, end up contributing to a lack of adequate and sufficient resources and supplies[66].

Thereby, unequal distribution of wealth, sociopolitical instability, and underreporting result in several COVID-19 incidences[67]. The first case of SARS-CoV-2 infection in Lebanon was confirmed in February 2020; since then, the case numbers have increased. However, it remains limited due to national confinement, closed borders, and care measures. Nevertheless, since August 2020, with the explosion of the Beirut Port, there was a decline in socioeconomic status, reaching 534968 positive cases and 7569 deaths by May 2021[68]. Yet, the COVID-19 dissemination coincided with a period of political instability [69].

Focus on public health sectors preparing to meet the infected patients threatens the continuity of some basic services, besides the fear of getting the infection and obligatory reclusion, which has stopped many individuals from visiting the psychological support. Yet, the lockdown measures negatively affect the maintenance of millions of people and about 30% of young people in Lebanon are unemployed in this context[70]. Moreover, one of the most important psychological impacts of the infected or suspected patients was the prejudice and stigma of having the disease, among the front line professionals. Those in quarantine were more likely to be stigmatized and rejected[71].

However, when comparing mortality rates in Middle Eastern countries, including Syria and Lebanon, there are lower figures than, for example, those in Europe and the Americas[72]. Such an estimate could lead to the erroneous conclusion that the pandemic has hit countries in conflict less strongly, as their estimates are less alarming than global ones. However, the conclusion must be precisely inverse: Given an overloaded health system, unequal distribution of wealth, and lack of sociopolitical stability, there is a high number of undetected and unreported cases in this region, making official data not reflect reality [67,72].

Therefore, although politics and health are subjects sometimes seen as unrelated, the index of political stability can and should be used as a predictor of the management capacity of a pandemic[73]. Thus, in countries in a constant war situation, such as Syria and Lebanon, the position in the face of a global health problem is complex, since its inhabitants and political leaders must deal with the pandemic and the ongoing war, two serious obstacles, which add up to the death and invalidity numbers[74].

RESTRICTION TO HEALTH SERVICES ACCESSIBILITY

The primary objective of the health system in Brazil is to provide health services to the population, regardless of gender, age, race, ethnicity, religion, nationality, social class, sexual orientation, or political position, promote treatment, monitor diseases, minimize pain, whether physical or psychological and, when possible, promote the cure[75]. However, COVID-19 generated a growing additional demand in the public health system, mainly in the increase of ICU beds and mechanical ventilation devices, necessary measures for the treatment of contaminated people in moderate and severe states[76]. As elective operations resume, operating room (OR) access has become increasingly challenging because of the large backlog of cases. Before the pandemic, many hospitals were running their ORs at near capacity, leaving little room to accommodate additional surgeries and forcing scheduling delays as long as 20 mo. As a result, patients are facing mounting challenges to the receipt of timely surgical treatment as outpatients and inpatients[77].

The pandemic represents a barrier to access to health services since these are organized for priority care for potentially infected patients and with professionals away from care for various reasons, with an overload of the remaining. In addition, people avoid going to services, due to social distancing recommendations and fear of contamination[78-80]. Thus, Brazil faces some challenges in the battle against the COVID-19 pandemic, including the risks of cross-infection (community infection) increase in densely populated areas, and low access to health services in areas where the number of beds in ICUs is scarce and poorly distributed, mainly in states with a low population density[76,81].

Experience from past outbreaks indicates the need to pay attention to the potential effects of the COVID-19 pandemic on sexual health outcomes, both in the immediate and long term[80]. The greater risk of sexually transmitted infections during the pandemic for women in situations of domestic violence or other conditions of psychosocial risks, such as the use of alcohol or drugs, poverty, among other situations of vulnerability, also needs to be recognized and should be a priority for health services [81]. Attendance at family planning services has also dropped dramatically in different countries. The consequences can involve an increase in the number of unwanted pregnancies and unsafe abortions, as well as maternal and neonatal deaths, and an impact on sexually transmitted infections. The effects could linger during the recovery phase of the pandemic, hitting disadvantaged and neglected groups again and reversing gains made in recent decades[76,82,83].

The treatment and follow-up of chronic diseases also suffer the impact of the pandemic. A study showed that diabetes (38%) was the disease most affected by resource reallocation and prioritization to COVID-19, followed by chronic obstructive pulmonary disease (COPD, 9%), hypertension (8%), heart disease (7%), asthma (7%), cancer (6%), and depression (6%)[84]. Non-infectious chronic respiratory diseases such as obstructive sleep apnea, asthma, and COPD were also negatively impacted. Both diagnosis/treatment and follow-up have been compromised by a reduction of resources, lack of adherence to face-to-face care, and interruption of clinical trials with possible innovative therapies, and these events can have negative consequences in the medium and long term for patient survival[85]. Recently, another study demonstrated a reduction in hospital admission for cardiovascular disease at the beginning of the pandemic and another study reported a lower overall hospital mortality and higher out-of-hospital mortality for patients with cardiovascular disease during rigid periods of isolation compared to other times of the pandemic[86,87]. These studies raised the hypothesis that the changes and interference of the conditions generated by the pandemic in the treatment and monitoring of diseases may negatively affect patients with cardiovascular diseases not infected by SARS-CoV-2[88]. Furthermore, the pandemic has also significantly affected cancer patients. The allocation of resources to deal with patients positive for SARS-CoV-2 has led to a shortage of essential drugs for the care of cancer patients, given that the replacement of therapy is a complex condition and not always possible since the limitation in the treatment of cancer can be fatal[89]. In addition, the diagnosis of some types of cancer such as gastrointestinal cancer was compromised by the risk of infection of patients[90].

In addition to the aforementioned, the inequalities present in the immunization process have contributed to the harm to human health and postponed the pandemic end[91]. Research demonstrated that the cost of vaccines against the COVID-19 impeded the access and the immunization process of some countries which suffered from the economic impact of the pandemic, and the adaptation of their health systems to attend to the population with the disease[92]. Furthermore, the discrepancy between the high stimuli to the creation of vaccination strategies in developed countries like the United States of America (USA) which vaccinated over half of its the population until September 2021 and detriment to countries like India which vaccinated about 13% of the population in the same period, made possible that new infection waves formed in these last countries, increasing the infection and death rates and also propitiating the emergence of new virus variants[93].

Vaccine access also is affected by the way that the communities are structured, since the necessity to work during the vaccination periods, the mistrust of the health system, documents pending related to immigration, religious negativism, and political opposition are individual factors that have contributed to decreasing the vaccine access by Latin and Hispanic people in the USA[94]. Similarly, studies have demonstrated a greater hesitation to vaccination by the people belonging to minority groups, mainly the Black population in the United Kingdoms and the USA, which could be related to possible historic disbelief of these people about the health system due to events like the Tuskegee Experiment[95,96]. Furthermore, immigrant people can present a reduction in the seeking of immunization due to spatial barriers that restrict the mobility to the locals of vaccination and language barriers since not speaking the language of the countries where they live can reduce the access to information about the process of vaccination[97].

LABOR VULNERABILITY AND IMPACT ON THE FAMILY NUCLEUS

Exposure to infection caused by SARS-CoV-2 is directly related to the nature of people's profession. In this context, frontline work can be mentioned, such as the health area and certain essential industries, in which there is greater interaction with other individuals[52]. This scenario becomes more serious in places with a high population density, households with shared sanitation facilities, and ineffective health systems, as is common in poorer regions of developing countries[98]. Furthermore, the COVID-19 pandemic has not only affected infection and mortality rates. With the adoption of restrictive measures to control transmission in several countries in 2020, such as the closing of establishments considered non-essential and rules of social isolation, economic and social aspects were also influenced. Thus, several changes were noticed in work relationships that had consequences on income and family management[98,99].

Social isolation involves exceptions such as essential workers (EWs), which include healthcare professionals (HCP), individuals working in the food production and distribution, emergency and protection services, communications, information technology, logistics, and delivery services. These EWs vary according to regulations and local economy[100] and their contacts, which increase the contagion risk itself and to other people, need to be retained [101]. The recommendations include support work from home, face shield, and individual protection equipment (IPE) for functions where social distancing is not possible, workplace layout changes, and improved cleaning and disinfection. However, working from home is often not feasible[102]. Besides that, achieving a balance between the provision of essential health care and protection of the HCP against infection, mainly due to the deficiency of the IPE, is challenging for the frontline team[103]. Furthermore, the COVID-19 pandemic affects the mental health of work-people. Anxiety, depression, post-traumatic stress disorder (PTSD), and sleep disturbances are more often present in HCP on the frontline, migrant workers, and those in contact with the public, where job insecurity, long-term isolation, and uncertain future worsen the psychological condition[104]. A systematic review showed that a high proportion of the HCP experience elevated levels of anxiety, depression, and insomnia, being more prevalent in the nurse team when compared with physicians [105]. Yet, rates of suicide are reported in this population, due to the psychological pressure, loneliness, financial crisis, and fear of dying[106,107]. Another study that evaluated Spanish health professionals described that about 56.6% of workers presented with PTSD, 59.6% had anxiety, and 41.1% had emotional exhaustion[108]. Among Chinese physicians, 50.4% and 71.5% of the study participants reported depression and anguish, respectively[109].

On the other hand, the economical and productive consequences of the pandemic can also affect labor sectors, while some individuals were forced to stop their work activities due to lockdown policies or effective job loss[104]. A study reported that almost two-thirds of the participants had their family income reduced during the pandemic and approximately half of them had reduced work hours or lost their job due to COVID-19[110]. Yet, the Spanish population estimates an increase in the unemployment rate of 27.88%, mainly in service sectors[111]. A search performed in Hawaii showed that the interviewees reported having difficulties spending for essential items and expected problems to increase in the next 3 mo, such as paying for alimentation, rent, and car expenses, as well as utility bills, and mobile/internet costs[112]. Other data obtained in the USA showed that about 28% of respondents declared that school closures were a factor that affected the finances of low-income families, as children no longer received free or reduced-cost meals in schools[113]. Moreover, domestic work gains importance in pandemic scenarios due to the great demand for care for both children and the elderly, but their employment situation, exposure, and vulnerability affect most of these workers. Therefore, they are at serious risk of losing their jobs, beyond the contagion danger, family estrangement, and violence in the house[114].

Generally, families belonging to marginalized or low-income populations tend to suffer the most severe effects. In this way, existing inequities were further aggravated by COVID-19[99,113]. In Liberia, Africa, it was identified in a study that about 67% of participating families had reduced income due to the pandemic. This situation contributed to the fact that 68% of respondents only had food in stock for a week or less, and 35% reported that they had skipped a meal in the last 7 d[98]. A study in Indiana, USA found that 55% of participants were worried about their family finances because they had lost their jobs. Another factor involved in greater economic precariousness was education, with people who did not have a university degree having twice the risk of food insecurity compared to those who had any college degree, while those without complete high school were 4 times more likely[99].

Another relevant issue is that the COVID-19 pandemic accelerated the process of transitioning from face-to-face work to remote work at home, and this affected the health of individuals[115,116]. A survey carried out in Japan with company workers showed that the average number of days of working from home per week went from 0.2 in 2019 to 1.0 during the pandemic in 2020. In this context, there was an increase in sedentary lifestyle, with more time dedicated to activities such as sitting, watching TV, and using the PC. A sedentary lifestyle is a problem that increases the risk of chronic diseases and fatigue and reduces workers' productivity[115]. In Pittsburgh, USA, a survey was carried out to assess the consequences for desk workers, most of whom had to migrate to remote work. The results show that these people also had an increase in sedentary time on rest days, and worse sleep quality, in addition to a reduction in work-related health, such as loss of productivity, concentration, and personal satisfaction [116]. A study highlighted that in Italy, the number of people working from home rose from 4.6% in 2019 to 19.4% in the second quarter of 2020. The findings point to an increase in physical inactivity and a reduction in outdoor physical exercise, indicating that this increase may have been greater in people who lost their jobs compared to those who could keep them. Such a scenario, which, added to an increase in hours of working and the adoption of less healthy diets, can contribute to an increased risk of cardiovascular events, such as obesity and hypertension[117]. Thus, several studies reported that unemployment also contributes to mental health commitment, especially among young people[118, 119]. It is important to note that even with the end or loosening of restrictions on social isolation, it is very likely that most companies will opt for remote work, either by popularizing available technologies or by saving costs. Thus, the health problems related to a sedentary lifestyle caused by COVID-19 may persist beyond the pandemic, requiring a joint effort among families, companies, and governments to reduce these effects[116,117].

INCREASE IN DOMESTIC VIOLENCE IN THE FACE OF SOCIAL ISOLATION

Domestic violence is defined in The Protection of Women from Domestic Violence Act as "any act of commission or omission or conduct resulting in physical, verbal, emotional, sexual, and economic abuse"[82,120]. Especially during the first 6 mo of the pandemic, support mechanisms for victims of domestic violence such as specialized centers in Spain, Cyprus, Brazil, and the United Kingdom reported an increase of 20%, 30%, 40%-50%, and 25%, respectively, in complaints. Furthermore, Google's search engine detected an elevation of about 75% in searches related to supporting domestic violence [121-123]. It is possible to observe a trend already experienced in other moments of the crisis, in which, as in the current pandemic, there were mainly economic and social problems, linked to the loss of jobs, reduction of family income, food insecurity, stress, reduced interactions and social support, and increase in the consumption of alcoholic beverages and drugs, which corroborate the increase in violence rates [124,125].

The measures of confinement and social isolation restricted contact with external family members, neighbors, and co-workers, which makes it difficult to search for help or the opportunity to talk about the violence faced at home[126,127]. Isolation has made it more complicated for the victim to denounce her aggressor since she is confined with him[128,129], as well as made access to social services and health services and assistance to the population more difficult[129]. Therefore, in a situation of aggression, in addition to the violence suffered, the victim still needs to deal with a series of barriers to defend themselves. This is in agreement with studies that reported how victims of domestic violence felt that social support was weakened during confinement measures, especially in the first 6 wk, associated with lower trust in social and health services[130,131].

The rise in domestic violence and other stressors generated during the pandemic, contribute to aggravating the victims' lack of mental health, bringing physical and psychological consequences to the female population throughout their lives. In this way, the health impact can be translated through higher levels of stress, anxiety, depression, post-traumatic stress symptoms, and chronic environmental stress[132]. A study in the United Kingdom proved this by stating that women and people living with young children experience greater mental distress during the pandemic[133]. In addition, other studies around the world also confirmed that, in relation to men, women were at greater risk of acquiring mental health problems in this period[134-136].

CONCLUSION

In conclusion, the complex interaction between the biological and the social inequalities continually assists the development of the infection. The social inequalities contribute to the illness process, increasing the risk of contamination and morbimortality of the disease. On the other hand, the pandemic context favored the increase of the gaps and structural barriers pre-existing against the more vulnerable groups, leading to distress, social change in daily life, and greater illness of this population. Therefore, understanding the nuances that permeate the infection can assist both in the evaluation of the disease impacts and formulation of targeted measures able to encompass the individual necessities of the population, potentiating the prevention and recovery process of the health.

FOOTNOTES

Author contributions: All authors equally contributed to this paper with the conception and design of the study, literature review and analysis, drafting, critical revision, and editing of the manuscript, and final approval of the final version.

Conflict-of-interest statement: There is no conflict of interest associated with any of the authors or coauthors who contributed their efforts to this manuscript.

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S-Editor: Liu JH
L-Editor: Wang TQ
P-Editor: Liu JH

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 September 20; 12(5): 365-380

DOI: 10.5662/wjm.v12.i5.365

ISSN 2222-0682 (online)

MINIREVIEWS

COVID-19 neuropsychiatric repercussions: Current evidence on the subject

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Specialty type: Medicine, general and internal

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Tiejun W, China

Received: February 28, 2022
Peer-review started: February 28, 2022
First decision: June 16, 2022
Revised: June 30, 2022
Accepted: July 25, 2022
Article in press: July 25, 2022
Published online: September 20, 2022

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has affected the entire world, causing the coronavirus disease 2019 (COVID-19) pandemic since it was first discovered in Wuhan, China in December 2019. Among the clinical presentation of the disease, in addition to fever, fatigue, cough, dyspnea, diarrhea, nausea, vomiting, and abdominal pain, infected patients may also experience neurological and psychiatric repercussions during the course of the disease and as a post-COVID-19 sequelae. Thus, headache, dizziness, olfactory and gustatory dysfunction, cerebrovascular disorders, neuromuscular abnormalities, anxiety, depression, and post-traumatic stress disorder can occur both from the infection itself and from social distancing and quarantine. According to current evidence about this infection, the virus has the ability to infect the central nervous system (CNS) *via* angiotensin-converting enzyme 2 (ACE2) receptors on host cells. Several studies have shown the presence of ACE2 in nerve cells and nasal mucosa, as well as transmembrane serine protease 2, key points for interaction with the viral Spike glycoprotein and entry into the CNS, being olfactory tract and blood-brain barrier, through hematogenous dissemination, potential pathways. Thus, the presence of SARS-CoV-2 in the CNS supports the development of neuropsychiatric symptoms. The management of these manifestations seems more complex, given that the dense parenchyma and impermeability of brain tissue, despite protecting the brain from the infectious process, may

hinder virus elimination. Still, some alternatives used in non-COVID-19 situations may lead to worse prognosis of acute respiratory syndrome, requiring caution. Therefore, the aim of this review is to bring more current points related to this infection in the CNS, as well as the repercussions of the isolation involved by the pandemic and to present perspectives on interventions in this scenario.

Key Words: SARS-CoV-2; COVID-19; Central nervous system; Quarantine; Neurologic disorders; Mental disorders

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Core Tip: Severe acute respiratory syndrome coronavirus 2 infection may also involve neurological and psychiatric manifestations, both by the viral action itself and by social distancing and quarantine. Headache, dizziness, cerebrovascular disorders, olfactory and gustatory dysfunction, neuromuscular abnormalities, anxiety, depression, and post-traumatic stress disorder may occur in this setting. Supporting these repercussions, this virus is able to reach the central nervous system by the interaction between the angiotensin-converting enzyme 2 and the transmembrane protease serine 2 expressed in the host nerve cells, and the viral spike glycoprotein. Finally, the management of these patients is complex and we review current evidence on the subject.

Citation: da Silva Júnior RT, Santos Apolonio J, Cuzzuol BR, da Costa BT, Silva CS, Araújo GRL, Silva Luz M, Marques HS, Santos LKS, Pinheiro SLR, Lima de Souza Gonçalves V, Calmon MS, Freire de Melo F. COVID-19 neuropsychiatric repercussions: Current evidence on the subject. *World J Methodol* 2022; 12(5): 365-380
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/365.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.365

INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak has affected the whole world, causing fear and concern due to its transmissibility and severe life-threatening conditions. This pandemic infectious disease was first discovered in Wuhan, China, in December 2019[1]. Since then, the number of cases has increased, spreading rapidly globally and becoming a major pandemic disease[2]. By February 1, 2022, the World Health Organization confirmed more than 370 million cases worldwide, leading to 5658702 deaths[3].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a β-coronavirus with positive spherical single-stranded RNA and spike proteins that project on the surface of the virion, which is characterized by its crown-shaped morphology[4]. Common symptoms are fever, fatigue, cough, dyspnea, diarrhea, chest tightness, nausea, vomiting, sputum production, anorexia, pharyngalgia, hemoptysis, and abdominal pain[5]. Frequently, SARS-CoV-2 contamination is associated with the nasopharyngeal and pulmonary tracts. However, important findings show that manifestations of this virus can be found in the central nervous system (CNS).

Neurological alterations have been described in patients with COVID-19, which vary from mild to fatal effects and can occur in severe or asymptomatic infection[6]. On the other hand, the global effects of SARS-CoV-2 infection result in various viral-related physical and mental health problems[7]. Thus, physical and social isolation, financial stress, and fear of contagion contribute to this scenario[8]. Therefore, this infection can present neuropsychiatric repercussions, such as headache, dizziness, anosmia, ageusia, neuromuscular dysfunction, anxiety, and depression[9], shown in Figure 1, in addition to other symptoms related to physiological and psychiatric changes, such as post-traumatic stress disorder (PTSD) and neuropsychiatric syndromes[8]. These manifestations seem to be caused as much by the infection itself as by social distancing and quarantine, which means that specific therapy should be used according to each case, seeking the most efficient healing process.

Therefore, this review describes the reported CNS manifestations associated with COVID-19, in order to help professionals who treat these patients, review the manner in which the virus reaches the CNS, and the intervention possibilities available to date in the literature.

METHODOLOGY

For this minireview, the authors surveyed relevant and current articles published in the United States

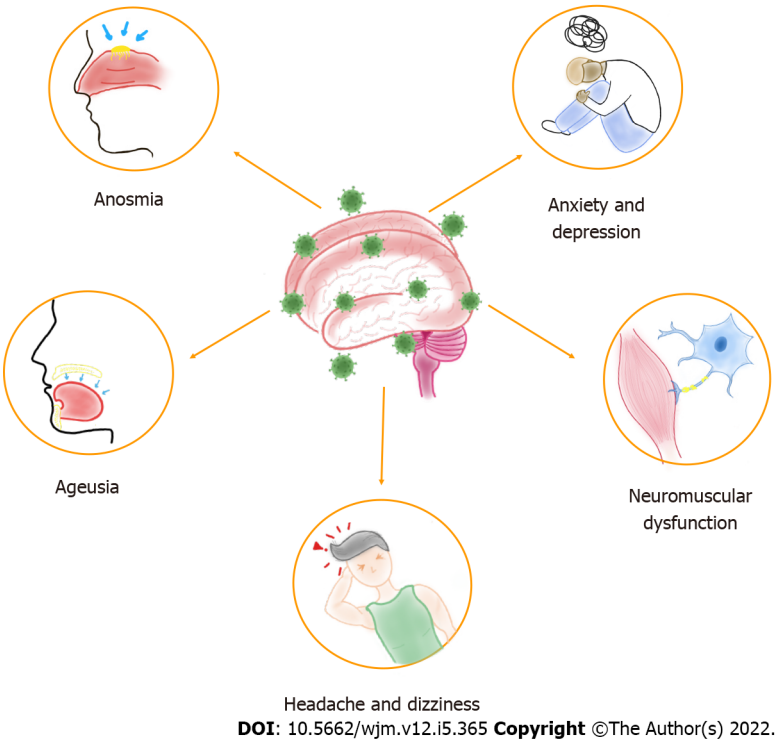

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Figure 1 Main neuropsychiatric repercussions of the severe acute respiratory syndrome coronavirus 2 infection, quarantine, and social distancing.

National Library of Medicine (PubMed). The descriptors used were COVID-19; SARS-CoV-2, coronavirus; angiotensin-converting enzyme 2 (ACE2), central nervous system, neuroimmune, cytokine storm, pathophysiology, neuroinvasion, neurological symptoms, neurological manifestations, olfactory dysfunction, gustatory dysfunction, ischemic stroke, hemorrhagic stroke, Guillain-Barré syndrome, neuropsychiatric symptoms, mental health, mental suffering, psychiatric disorder, and quarantine. The eligibility criteria were based on the discussion of aspects related to the neuropsychiatric repercussions of SARS-CoV-2 infection, dealing with everything from viral neuroinvasive mechanisms to neurological and psychiatric manifestations, due to the infection itself or to the need for full isolation in the read full-text. Thus, 26035 articles were found in the database, of which 109 complied with the inclusion criteria. The exclusion criteria were articles that did not address the topics in the title and/or abstract, or were written in languages other than English. The search was complemented by a manual search of the references of the included articles to identify additional references, 13 of which were added later, totaling 122 articles included in this review.

COVID-19 NEUROPSYCHIATRIC REPERCUSSIONS: CURRENT EVIDENCE

SARS-CoV-2 neuroinvasive mechanisms

One of the main mechanisms of neurological invasion of SARS-CoV-2 is through ACE2 receptors on host cells[6,10]. Several studies have shown the presence of ACE2 protein in human brain vessels, mainly in dopaminergic neurons, astrocytes, oligodendrocytes, and neurons[11-13]. ACE2 has also been observed in the substantia nigra, ventricles, middle temporal gyrus, posterior cingulate cortex, and olfactory bulb[11,13]. One study reported that ACE2 is more highly expressed in neuronal cell bodies than in axons and dendrites[14]. During infection, transmembrane protease serine 2 (TMPRSS2) activates the spike (S) glycoprotein on the SARS-CoV-2 envelope, which allows the virion to bind to ACE2 receptors[12,15]. TMPRSS2 is also found in oligodendrocytes and astrocytes located in the substantia nigra, cortex, and endothelial cells of cerebral capillaries[6,14], and is fundamental in the priming and activation of S proteins, which leads to membrane fusion. This interaction is responsible for SARS-CoV-2 entry on the CNS[14,16]. Later, the virus may affect the nervous system by disturbing the renin angiotensin system[11]. This process is exacerbated by slower circulation in the brain capillaries, which intensifies the interaction between the viral S glycoprotein with the ACE2 on brain cells[9,16]. Thus, CoV interacts with ACE2 expressed in the capillary endothelium, causing neuronal death and neurodegeneration[9,18].

Compared with SARS-CoV, SARS-CoV-2 has higher affinity to ACE2[15]. This enzyme is also known as a cardiocerebral vascular protection factor, and influences blood pressure regulation and anti-atherosclerosis mechanism, in view of its vasoconstrictor function and pro-inflammatory effects[9,19]. When the virus binds to the enzyme, it may cause elevated blood pressure and increase the risk of arterial wall rupture, cerebral hemorrhage, and ischemic stroke[13,17]. On other hand, ACE2 and TMPRSS2 have been detected in the nasal mucosa, one of the main mechanisms of entry into the brain[15,20]. Once the infection of the olfactory system occurs, the virus may be internalized in the nerve by endocytosis *via* the olfactory bulb and be transported retrogradely and disseminated to the brain *via* the cribriform plate[6,12,20].

SARS-CoV-2 may also affect the CNS indirectly, as the virus provokes alveolar and lung tissue damage[17,21]. This inflammation and edema caused by lung invasion disturbs oxygen exchange and results in hypoxemia. Thus, this scenario may lead to increased anaerobic metabolism in brain cells, brain hypoxia with vasodilation, hyperemia, ischemia, and brain edema and injury[17,20,21].

As previously mentioned, the virus can access the CNS through the olfactory nerve and also through the hematoretinal route. However, there is another potential pathway that allows CNS infection, which is *via* the blood-brain barrier (BBB), which occurs through hematogenous dissemination[22]. The BBB is one of the body's protections against disturbances in the nervous system. It is composed of endothelial cells, astrocytes, microglia and neurons, which act together. Under normal circumstances, these cells accurately regulate what enters and leaves the nervous system. However, in pro-inflammatory situations, such as that caused by SARS-CoV-2, this homeostasis is disturbed, which may be the genesis of virus entry into the CNS[23-25].

One factors that has been extensively studied currently is what causes this damage to the BBB, enabling infection in the CNS. The most likely one is the hyperinflammatory situation caused by the cytokine storm[1]. When viral replication occurs, damage-associated molecular patterns, which induce inflammatory states in neighboring cells through Toll-like receptors, are released. These receptors promote several cytokine production pathways. However, in COVID-19, there is hypercytokinemia. The main cytokines involved in this exacerbated process are tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin 2 (IL-2), IL-1RA, IL-2, IL-6, IL-7, IL-8, IL-9, and the granulocyte-macrophage colony-stimulating factor. These cytokines ultimately potentiate the activation of immune system cells, creating a cycle of increasing and perpetuating inflammation[26,27]. These processes are represented in Figure 2. Severe patients can also present with elevated levels of IL-17 compared to non-severe patients [28]. A comparative study showed a particularly strong inflammatory response triggered with the activation of this cytokine[29].

This cytokine storm damages the vascular endothelial cells of the CNS, affecting the integrity of tight junction proteins in the BBB, allowing the virus to enter. In addition to increased cytokines, the virus itself has cytopathic power, which can lead to pathogenic inflammation with cellular damage in the CNS including edema, ischemia, bleeding, and neurodegenerative disorders[1,30]. In pathological situations, the migration of cells from the immune system to the CNS is increased and in severe SARS-CoV-2 infection, it is even greater. This is supplanted in histopathological examinations of the brain parenchyma where large numbers of macrophages and lymphocytes were found[27].

Another factor that corroborates this increase in cell migration is that in the presence of damage to endothelial cells, the number of intercellular adhesion molecules increases. This favors the entry of the virus into the CNS, which is transported by cells of the immune system through a mechanism known as the "trojan horse" in which the virus enters the nervous system inside the host cell[31,32].

NEUROLOGICAL REPERCUSSIONS

SARS-CoV-2 infection can reach the CNS through the olfactory tract and access the cortex, basal ganglia and midbrain, which may be affected during propagation[33], supporting the existence of neurological symptoms as headache, anosmia, dysgeusia, dizziness, and impaired consciousness[34,35].

Headache and dizziness

Although this symptom is very nonspecific, several studies have reported the prevalence of headache in patients infected with SARS-CoV-2. The symptom may be present at the beginning of the disease, or even be the initial presentation of the clinical picture, as it may also be present after resolution of the infection[9]. In addition, this symptom may be related to other diseases present in the patient, and therefore, the prevalence varies greatly according to the work[36]. One study[28] indicated a combined prevalence of headache in about 8% of patients, whereas others reported higher numbers such as 20% [37] and 25%[38], as well as variation from 0.6% to 70.3%[39,40]. Accordingly, among the neurological effects reported in COVID-19 infection, especially headache, may be the result of complications of the viral infection, the host immune response, the presence of severe installed disease or even the drug therapy used[41].

The prevalence of dizziness is estimated to be about 8%[42] to 9%[5], which indicates a combined overall prevalence of 8.77% in a systematic review that analyzed neurological symptoms in patients

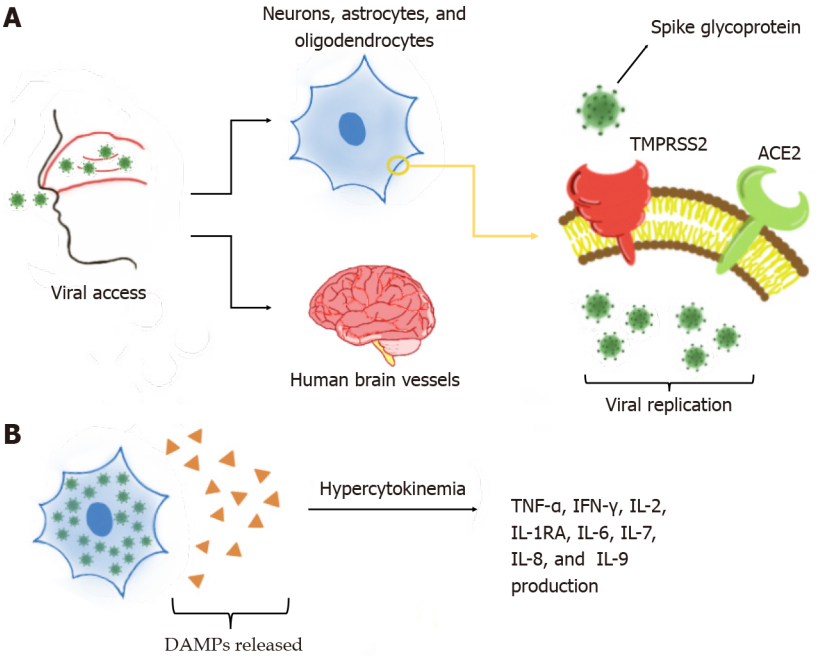

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Figure 2 Main neuroinvasive mechanisms of the severe acute respiratory syndrome coronavirus 2. A: Viral entry by olfactory epithelium and bringing between spike glycoprotein and angiotensin converting enzyme-2/transmembrane protease serine 2 expressed in the nasal mucosa; B: Cytokine storm induced by the damage-associated molecular patterns release. ACE2: Angiotensin converting enzyme-2; TMPRSS2: Transmembrane protease serine 2; DAMPS: Damage-associated molecular patterns; TNF-α: Tumor necrosis factor alpha; IFN-γ: Interferon gamma; IL: Interleukin.

infected with SARS-CoV-2[43]. As with headache, studies vary in determining the period in which dizziness appears in the clinical picture. However, a retrospective and observational case series reports dizziness as the main neurological symptom[44]. This is not a surprise, after all, since dizziness is historically reported in patients with viral infections. This symptom has been proposed to result from the neuroinvasive potential of the virus, such as direct injury by binding to the ACE2, or even by hypoxia and coagulation disorders[45]. Because it is nonspecific, it is important that the healthcare team perform a thorough investigation to determine its cause, given that it can be due to acute labyrinthitis, acute otitis media, vestibular neuritis, or even stroke due to COVID-19[46,47].

Disturbances of consciousness

In retrospective studies that analyzed the incidence of neurological symptoms in patients with COVID-19, a range of 3.3% to 19.6% was reported for disturbances of consciousness/delusion[48,49]. The cause of this involvement is still poorly understood, and may even be related to the post-inflammation inflammatory state, meningoencephalitis and encephalopathy, or may just be a sequela after a traumatic event [48]. Thus, a long-term follow-up is necessary for these patients, so that the real cause of this condition can be investigated with a detailed clinical history and serial imaging examinations.

Acute cerebrovascular disease

Viral neuroinvasion and subsequent central neuronal injury have been proposed to contribute to the pathogenesis of the disease. The interaction of SARS-CoV-2 with ACE2 receptors may be related to the episodes of intracerebral hemorrhage found in some cases, resulting in receptor inactivation and consequent dysfunction in blood pressure regulation[50]. In patients who already have pre-existing vascular risk factors, ischemic stroke is related to late complications in the severity of COVID-19 infection. The elderly with alterations in vascular hemodynamics resulting from age or associated pathology, once again, are groups that deserve special attention for the involvement of these injuries[51-54].

Studies have observed that patients presented neurological symptoms on average 3 to 4 d after the onset of respiratory symptoms, with hemifacial paresis, dysarthria, hemiparesis, loss of level of consciousness, hemiparesthesia and ataxia being symptoms found less frequently[51,52,55,56]. One study reported that the sex-based distribution of patients affected by COVID-19 shows that female patients report more central nervous system-related symptoms than males. This sex-based difference may be attributed to humoral and innate immune responses to viral infections that are more pronounced in women than in men[54-57]. Yet, autopsy results from COVID-19 patients showed that brain tissue was hyperemic and swollen and that some neurons degenerate[55,58].

Olfactory and gustatory dysfunction

Although they are now among the most well-known symptoms during SARS-CoV-2 infection, olfactory dysfunctions (OD), mainly hyposmia and anosmia, were initially seen as less relevant conditions in the pandemic context. Thus, OD throughout the pandemic became part of the symptoms that carry a warning sign of a possible ongoing infection, even when they appear in isolation[59].

Hyposmia, reduced sense of smell, and anosmia, the complete loss of smell, are common in patients with COVID-19. The OD can be evidenced subjectively, when the patient reports any degree of alteration, or objectively, when specific tests are applied in order to evidence the conditions of each individual. These parameters have guided researchers worldwide to carry out studies in which OD was evaluated in patients with COVID-19. In this context, a meta-analysis involving 3563 patients found that 47% of individuals had a self-reported loss of smell. In addition, researchers show that OD is more frequent in women and young patients. However, despite the relationship with the infectious condition, studies suggest that OD is not related to the severity of the condition[59,60].

The mechanisms that can cause OD have not yet been fully defined. In this context, studies point to several possibilities that can lead to olfactory impairment, such as conductive loss due to edema in the olfactory cleft, injury to the respiratory epithelium - due to the local inflammatory response with an increase in pro-inflammatory cytokines and chemokines such as IL-6 and IFN-γ, or lesion in the olfactory bulb, with neuronal damage. Symptoms begin on average within the 1st week of infection, vary in duration, and can last for weeks or months[61,62].

The impairment of smell has a direct impact on the quality of life of the individual, since it can make it difficult to recognize odors in food that indicate its disposal and odors associated with risks, such as flammable or toxic substances, for example. In addition, it can cause impairment in social interactions, and several studies associate olfactory dysfunction with a higher risk of developing depressive disorders[63].

Gustatory dysfunction (GD), mainly hypogeusia and ageusia, are prevalent symptoms in infected individuals. They are mostly associated with OD, but they may manifest in isolation in some cases, as pointed out by a systematic review that revealed a combined prevalence of GD of 43.93% in SARS-CoV-2-positive individuals[64]. Like OD, GD is more prevalent in young and female patients[65]. Furthermore, similarly to OD, the causes of DG are still uncertain. There are several hypotheses mainly involving the ACE2 receptors present on the tongue, which, being fundamental for the virus, may cause local inflammatory reactions, compromising taste functions. In addition, taking into account the potential for damage to the nervous system, there are hypotheses that relate DG to dysfunction of cranial nerves VII, IX, and X[66].

The gustatory function is able to identify sweet, salty, sour, bitter, and umami flavors. Among patients infected by SARS-CoV-2 with DG, sweet and sour tastes had the most altered sensitivity[65]. Among the DG, hypogeusia, mild to moderate, is more common than ageusia. For example, an Italian study with 72 participants found that hypogeusia occurred in 47.1% of cases, while ageusia occurred in only 1.4% of patients[67]. Like OD, GD is a common manifestation mainly in the 1st week of symptoms and has a resolution in a variable period with most patients completely regressing in approximately 10 d[68].

Guillain-Barré syndrome

Guillain-Barré syndrome (GBS) is considered a post-infectious and immune-mediated syndrome characterized mainly by manifestations such as rapid, progressive, and symmetrical limb weakness, impairment of tendon reflexes, which may be reduced or absent and to sensory impairment. Their subtypes are acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy, and acute motor and sensory axonal neuropathy, in addition to Miller Fisher syndrome, a variant. It is usually associated with respiratory or gastrointestinal infections caused by *Campylobacter jejuni*, the most common agent, *Mycoplasma pneumoniae, Haemophilus influenzae,* Epstein-Barr virus, influenza A, and *Zika virus*, for example[69-71].

However, during the COVID-19 pandemic, an increase in the number of GBS cases associated with individuals infected with SARS-CoV-2 was observed. In this context, a study conducted suggested an up to 2.6-fold increase in the baseline GBS case rate, from 0.93/100000/year to 2.43/100000/year. Furthermore, the authors noted that GBS associated with COVID-19 is more severe than those not associated with this virus[69]. In this sense, among patients positive for SARS-CoV-2, GBS is more prevalent in males and those aged over 60 years and, among the GBS subtypes, the most prevalent is AIDP[71-73].

As with other conditions arising from SARS-CoV-2 infection, the onset of GBS-related symptoms is variable, but studies suggest that the onset of symptoms occurs approximately during the 2nd week of infection, reinforcing the hypotheses of a post-infectious etiology of GBS. The causal mechanisms are still uncertain. However, there are hypotheses that relate the development of GBS to the cytokine storm that occurs during the second phase of infection, which usually occurs in the 2nd week of infection, related to the elevation of cytokines such as TNF-α, IL-1β, -6, -17 and IFN-γ, which are capable of causing tissue damage. In addition, there are hypotheses that relate GBS to autoimmune mechanisms by cross-reaction against ganglioside components of peripheral nerves, causing impairment of nerve

structures and, consequently, the development of the syndrome[72,74,75].

A summary of the hypotheses and mechanisms related to development of the neurological manifestations of the SARS-CoV-2 infection, as well as prevalence/incidence of these repercussions, are shown in Table 1.

Manifestations after infection

Neurological complications following COVID-19 infection are still poorly understood due to the recent onset of the pandemic and the question remains whether neurological symptoms are a definite sequelae or just a late effect of the disease. As the virus attacks and grows in lung tissue, alveolar gas exchange is disrupted due to systemic inflammation and edema, which can cause hypoxia and acid accumulation. A study describing the autopsy of 8 confirmed cases with SARS-CoV-2 infection reported brain swelling and severe neuronal damage[78], as did another study, with 18 brain autopsies of patients testing positive for the viral infection, indicated alteration by hypoxia in the cerebellum and brain, with loss of neurons in the cerebral cortex and hippocampus[79]. In addition, recent studies have shown that the infection caused by SARS-CoV-2 affects the central nervous system and the peripheral nervous system, and also directly or indirectly damages neurons, which causes long-term neurological sequelae[80].

PSYCHIATRIC REPERCUSSIONS

Along with the physical damages caused by SARS-CoV-2 infection, quarantine and social distancing significantly impacted the mental health of the population. In this sense, studies have demonstrated that during this period there was an increase in the rates of psychiatric manifestations such as irritability, stress, insomnia and depression[81], which can influence the individual's daily life even after the epidemic has ended.

Depression and anxiety

The subjectivity of life demonstrates an impact in reference to the development of mental disorders which contributes to different mental suffering rates for each group of people[82]. The necessity of staying at home for long periods[83], the vulnerability of the risk groups, increase in unemployment rates and publicity of false information contribute to both the illness of the population and increased number of suicides cases[84]. Other factors such as living alone, having children, being a student or a health worker, poor sleep quality, family support lack, less contact with friends and previous psychiatric history or substance abuse were also associated with the emergence of depression and anxiety[85].

A study conducted with the Chinese population showed that of the 1.210 respondents, about 30% had severe to moderate anxiety symptoms and 17% had severe to moderate depression[83]. Similarly, an online cross-sectional study conducted in China included 1.456 participants and assessed factors that influenced the mental health of adults during the pandemic. Its results showed that loneliness, depression and anxiety were associated with more somatic symptoms and lower self-efficacy. In addition, depression was associated with fear of infection, excessive alcohol consumption, and longer screen time. Loneliness was associated with single, divorced or widowed marital status, low education, medication use and frequent going out[86]. Furthermore, a survey of the Belgian population aged 18 years to 65 years reported that in just 2 wk of isolation the stress of individuals increased by about 25% [87].

PTSD

Literature reports also describe an increase in rates of PTSD in children, parents and even health care professionals after contact with infection, which demonstrates that subjective experience is also related to mental illness[88]. PTSD manifestations were related to higher perceived risk of infection, fear of infection, and self-assessment of higher negative influence due to the epidemic[86]. Corroborating, an electronic records cohort of 69 million individuals, of which 62.354 tested positive for infection, found that patients with COVID-19 had a higher incidence of unprecedented psychiatric diagnoses between 14-90 d after infection compared to other illnesses such as respiratory tract infections, skin infection, large bone fracture, urolithiasis, and cholelithiasis. Interestingly, previous psychiatric diagnoses also appear to be an independent risk factor for COVID-19[89].

Susceptibility to mental distress

The unpredictability of the outcomes of a possible infection can also increase susceptibility to mental distress, especially in people considered to be in the risk groups, which shows that subjective experience is an important predictor of the onset of psychological problems[90]. Studies performed during the pandemic reported a higher prevalence of mental disorders, such as anxiety in people with comorbidities or depression in individuals diagnosed with type 2 diabetes mellitus, when compared to the general population[91]. Similarly, minority groups such as immigrants, individuals with low access to health care and low socioeconomic status are more prone to mental disorders, since not only does the

Table 1 Main hypotheses, mechanisms related to development of the neurological manifestations of coronavirus disease 2019 and prevalence/incidence of these repercussions

Neurological repercussion	Hypotheses/mechanisms related to their development	Prevalence/incidence of manifestation
Headache	Complications of the viral infection, the host immune response, the presence of severe installed disease or even the drug therapy used[41,45]	The headache prevalence varies from 8%[56] to 25%[38] and 70.3%[40], according to the study
Dizziness	The direct injury by binding to the ACE2, or even by hypoxia and coagulation disorders may be related[45]	For dizziness, the prevalence is estimated to be around 8%[42] to 9%[5]
OD	Conductive loss due to edema in the olfactory cleft, injury to the respiratory epithelium or lesion in the olfactory bulb[61,62]	About 47% of individuals had a self-reported loss of smell. More frequent in women and young patients[59,60]
GD	Local inflammatory reactions and the relationship with cranial nerves VII, IX, and X[66]	About 43.93% of the patients[64]. More prevalent in young and female patients[65]
Disturbances of consciousness	Post-inflammatory state, meningoencephalitis, or may just be a sequela after a traumatic event[48]	A range of 3.3% to 19.6% in COVID-19 patients[48,49]
Acute cerebrovascular disease	Intracerebral hemorrhage can be caused by viral interaction with ACE2 receptors and ischemic stroke is related to late complications in the disease severity[50]	The incidence of ischemic stroke in patients with COVID-19 was reported to be between 0.9%[76] and 4.6%[77]
GBS	There are hypotheses that relate the development of GBS to the cytokine storm and autoimmune mechanisms by cross-reaction[74,75]	A study suggested an up to 2.6-fold increase in the baseline GBS case rate, from 0.93/100000/yr to 2.43/100000/yr

ACE2: Angiotensin-converting enzyme 2; COVID-19: Coronavirus disease 2019; GBS: Guillain-Barré syndrome; GD: Gustatory dysfunction; OD: Olfactory dysfunction.

pandemic contribute to increasing marginalization and discrimination of these groups, but they are also considered more susceptible to infection and deaths caused by COVID-19[92]. In addition, women were also more affected than men by major depressive disorders and anxiety[93]. Violation of women's human rights with increasing rates of domestic violence and restrictions on access to prenatal health care services also contribute to greater mental illness in this group[94].

On the other hand, a preexisting mental health condition may be aggravated by the pandemic, as people diagnosed with mental disorders are considered more vulnerable to changes in their health status due to varying risk factors[95]. Interestingly, Pan *et al*[96] noted that people who did not have a psychiatric diagnosis of anxiety, depression, or obsessive-compulsive disorder prior to the pandemic reported an increase in symptoms related to these comorbidities. However, those individuals who already had a diagnosis of one of these disorders did not experience greater worsening of symptoms post-pandemic.

Mental health of the healthcare professionals

Healthcare professionals who worked on the front lines in the pandemic are a particularly affected population at higher risk of developing psychological distress and mental health impairment[97]. In this context, a cross-sectional study of 3852 healthcare professionals assessed the mental health of professionals who worked in the COVID-19 pandemic and SARS outbreak. The authors reported that health care workers achieved moderate and severe scores for symptoms of PTSD, anxiety, and depression[98]. A summary of highlights related to development of the psychiatric disorders in COVID-19 are shown in Figure 3.

INTERVENTIONS

The management of SARS-CoV-2 infection seems more complex when it involves the CNS. The dense parenchyma and impermeability of brain tissue, despite protecting the brain from the infectious process, may also hinder virus elimination. Still, the treatment of patients with neurological complications from this infection requires caution, because some drugs used in non-COVID-19 situations can lead to worsening of the disease-related acute respiratory syndrome, such as corticosteroids and immunosuppressant[99,100]. Moreover, viral damage can affect renal, immunological, hematological, hepatic, pulmonary and cardiac organ systems, as well as lead to pharmacokinetic changes that influence the absorption, distribution, metabolism and/or excretion of medications, such as psychotropic drugs. Susceptibility to side effects may be increased and adjustments in treatment regimens should potentially be considered[101], in addition to the pro-inflammatory, pro-thrombotic and arrhythmogenic implications of this infection[102]. Therefore, the approach to COVID-19-positive patients with neuropsy-

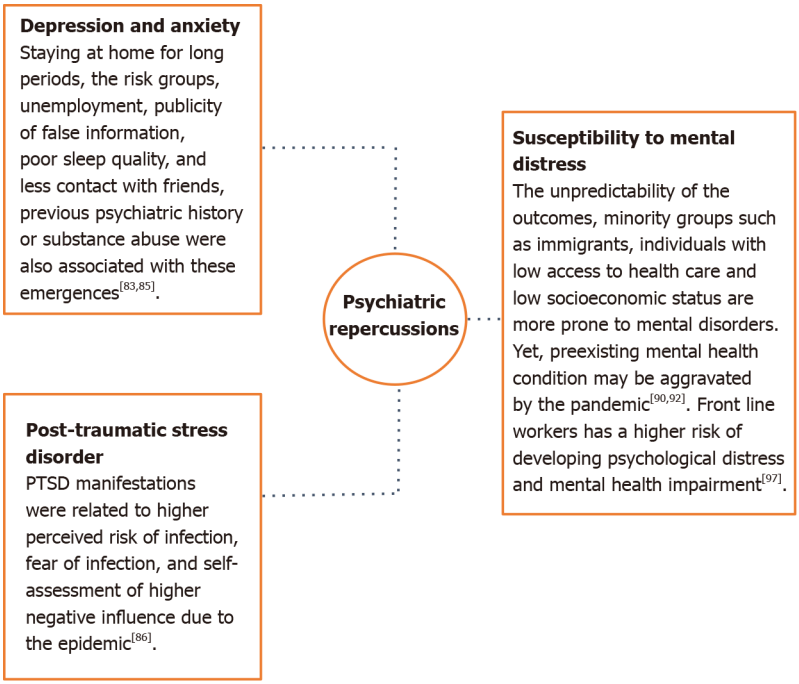

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Figure 3 Highlights related to development of the psychiatric disorders in coronavirus disease 2019. PTSD: Post-traumatic stress disorder.

chiatric repercussions still needs further long-term studies for evaluation and establishment of guidelines.

Neurological repercussions

One study reports the requirement for appropriate neuroimaging protocols in coronavirus infections to detect encephalitis, leptomeningeal and vascular changes such as stroke, microhemorrhages and cerebral infarction[103]. It is important that appropriate therapies are applied at the correct time, and that assessment of adjacent comorbidities, as well as damage to other organs and general condition is done using the sequential organ failure assessment score, which influences the COVID-19 prognosis [104,105]. Severe individuals can present right levels of the inflammatory markers, as C-reactive protein and D-dimer, and administration of tissue plasminogen activator in these patients with ischemic stroke predicted worse prognostic[44,106]. Thrombectomy can be used, evaluating the risks and benefits of therapy, and antiplatelet and anticoagulant agents remain uncertain. Thus, due to the lack of definitive studies, it is recommended to follow the existing guidelines[104].

Patients with demyelinating conditions and mild infection may be acceptable to continue treatment and the interruption may be considered in the use of potent immunosuppressant with risk factors for severe disease, returning after 4 wk or complete remission of symptoms[107]. The remission time of olfactory and gustatory dysfunction is controversial in literature, with studies reporting spontaneous resolution in 1 to 3 wk[108]. On the other hand, the smell performance of SARS-CoV-2-positive patients with mild or no symptoms can also not recover completely after 4 mo or more of acute infection[109]. Therefore, this treatment is still uncertain, but studies point to benefits of practicing olfactory training for those with persistent symptoms. In addition, some studies evaluate the role of local corticosteroids in recovery, but there is no consensus[110]. A study evaluating the efficacy of locally applied steroids in the form of fluticasone nasal sprays for olfaction disorders and triamcinolone paste for taste disorders reported that olfaction and taste function improved significantly in patients with COVID-19 within 1 wk [111]. Treatment for GDs is rarely addressed in the currently published literature, so more studies are needed to understand the best therapeutic options, especially in cases in which symptom regression does not occur as expected. Yet, there are different treatments that can be used against GBS, depending on the health structure and clinical context of each individual. Thus, among treatment possibilities, the use of intravenous immunoglobulins, plasmapheresis, or corticosteroids, alone or in combination, may be necessary[73,112].

The management of headache during SARS-CoV-2 infection can be accomplished by administering previously established therapeutic regimens for the treatment of acute crisis[113]. Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, are already widely used drugs in the treatment of headache crises and, although they are demonstrated during infection, their use is questioned. Reports in the literature note that NSAIDs could be related to increased ACE2 levels[114], which would contribute to ease the entry of the virus into the host cell as more viral receptors would be expressed,

and negatively impact the immune system through cyclooxygenase inhibition, decreasing neutrophil chemotaxis and leading to an inefficient response against the virus, and reducing the expression of lipoxins and resolving that contribute to the resolution of inflammation. However, there are not enough studies to prove or rule out these theories[115]. On other hand, the administration of triptans like sumatriptan has been shown to be quite effective in the treatment of migraine, but despite this, for the drug choice, it is also important to consider both the patient's pre-existing comorbidities and the severity of the infection at the time[116]. Other therapies such as the administration of paracetamol, which is considered a safe and effective drug in these cases[114], neuroleptics such as chlorpromazine and neuromodulation devices can also be considered for treatment[113,117]. Lastly, the use of oral corticosteroids has also been related to improving migraine cases and managing the transition to cluster headache; however, studies have reported that these drugs could also contribute to perpetuating the replication of the virus[117].

Psychiatric repercussions

With regard to psychiatric manifestations secondary to SARS-CoV-2 infection, delirium is mainly related to the hyperactive/mixed variety associated with elevated anxiety, and isolation itself is considered a factor that can both trigger and/or increase delirium symptoms[118,119]. This makes management difficult and lower potency antipsychotics such as olanzapine and quetiapine are preferred[118] and haloperidol is the most considered for agitation control in delusional patients[101]. Immune modulation therapies for depression secondary to infection-initiated hyperinflammation are being investigated, such as IL-6 inhibitors and melatonin[120], but more studies are required. On the other hand, technologies with online psychotherapies can support the pediatric population in this situation[121], cognitive behavioral therapy and mindfulness-based cognitive therapy can also assist in stress reduction[122].

Although low dosages of benzodiazepines are indicated in anxiety, these drugs have the potential for respiratory depression and the risk and benefits in patients with respiratory symptoms should be considered. Thus, according to the situation, gabapentin, hydroxyzine or lower doses of selective serotonin reuptake inhibitors (SSRIs) can be used, as well as non-pharmacological interventions, such as psychotherapy[101]. Treatment of PTSD typically involves SSRIs and serotonin-norepinephrine reuptake inhibitors, and the potential risks should be analyzed on a case-by-case basis. Paroxetine is not recommended due to the short half-life, anticholinergic side effect profile, and increased risk of drug interactions[102].

CONCLUSION

Thus, in this review we described the SARS-CoV-2 ability to infect the CNS and to cause manifestations related to neurology and psychiatry. Some nonspecific symptoms, such as headache, may be part of the initial clinical presentation as also be present after the resolution of the infection. Viral interaction with ACE2 receptors may be related to the onset or worsening of episodes of cerebrovascular disorders and demyelinating conditions, and to the development of olfactory and taste dysfunction by migration through the olfactory tract, one of the virus pathways. Yet, mental illnesses such as depression, anxiety, and PTSD may be caused by the social distancing and quarantine in both patients and health care workers who worked on the front lines, and these disorders may remain even after the pandemic has ended. Our work contributes to the elucidation of the disease pathogenesis, as well as the understanding of clinical presentation, since not all patients will present with a classic respiratory condition. Finally, the pandemic effects still need to be evaluated in the long term and more studies are necessary to clarify guidelines and establish the adequate management of these individuals.

FOOTNOTES

Author contributions: All authors equally contributed to this paper regarding the conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: The authors have no conflicts to declare.

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S-Editor: Zhang H
L-Editor: Filipodia
P-Editor: Zhang H

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 September 20; 12(5): 381-391

DOI: 10.5662/wjm.v12.i5.381

ISSN 2222-0682 (online)

MINIREVIEWS

Diagnosis and management of small bowel neuroendocrine tumors: A state-of-the-art

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Pausawasdi N; Tang D, China

Received: March 6, 2022
Peer-review started: March 6, 2022
First decision: April 12, 2022
Revised: April 22, 2022
Accepted: August 5, 2022
Article in press: August 5, 2022
Published online: September 20, 2022

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Abstract

This review provides an update on the epidemiology, pathophysiology, symptoms, diagnosis and treatment of neuroendocrine neoplasms (NENs) of the small bowel (SB). These NENs are defined as a group of neoplasms deriving from neuroendocrine cells. NENs are currently the most common primary tumors of the SB, mainly involving the ileum, making the SB the most frequently affected part of the gastrointestinal tract. SB NENs by definition are located between the ligament of Treitz and the ileocecal valve. They are characterized by small size and induce an extensive fibrotic reaction in the small intestine including the

mesentery, resulting in narrowing or twisting of the intestine. Clinical manifestations of bowel functionality are related to the precise location of the primary tumor. The majority of them are non-functional NENs and generally asymptomatic; in an advanced stage, NENs present symptoms of mass effect by non-specific abdominal pain or carcinoid syndrome which appears in patients with liver metastasis (around 10%). The main manifestations of the carcinoid syndrome are facial flushing (94%), diarrhea (78%), abdominal cramps (50%), heart valve disease (50%), telangiectasia (25%), wheezing (15%) and edema (19%). Diagnosis is made by imaging or biochemical tests, and the order of request will depend on the initial diagnostic hypothesis, while confirmation will always be histological. All patients with a localized SB NEN with or without near metastasis in the mesentery are recommended for curative resection. Locoregional and distant spread may be susceptible to several therapeutic strategies, such as chemotherapy, somatostatin analogs and palliative resection.

Key Words: Neuroendocrine; Tumor; Small bowel; Small intestine; Gastrointestinal disease; Treatment; Survival

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Core Tip: There are reviews in the literature regarding neuroendocrine tumors in the gastrointestinal tract specifically in the small bowel. Nevertheless, this is a first mini review to synthesize the latest data related to epidemiology, pathophysiology, clinical manifestations, diagnosis and treatment of small bowel neuroendocrine tumors.

Citation: Gonzáles-Yovera JG, Roseboom PJ, Concepción-Zavaleta M, Gutiérrez-Córdova I, Plasencia-Dueñas E, Quispe-Flores M, Ramos-Yataco A, Alcalde-Loyola C, Massucco-Revoredo F, Paz-Ibarra J, Concepción-Urteaga L. Diagnosis and management of small bowel neuroendocrine tumors: A state-of-the-art. *World J Methodol* 2022; 12(5): 381-391

URL: https://www.wjgnet.com/2222-0682/full/v12/i5/381.htm

DOI: https://dx.doi.org/10.5662/wjm.v12.i5.381

INTRODUCTION

Langhans was the first person to describe small bowel (SB) neuroendocrine neoplasms (NENs) in 1867, as a polypoid tumor of the small intestine[1]. Nowadays, NENs are described as a heterogeneous group of neoplasms derived from neuroendocrine cells. The term NENs encompasses well-differentiated NENs and poorly differentiated neuroendocrine carcinomas (NECs)[2]. NENs commonly arise from the gastrointestinal tract[3,4,40].

NENs can progress throughout the gastrointestinal tract, but are specifically seen in the small intestine (45%), rectum (20%), appendix (16%), colon (11%), pancreas (5%-10%) and stomach (7%)[5] (Figure 1).

NENs account for 1.0%-1.5% of all gastroenteropancreatic neoplasms[6]. SB NENs continue to increase in incidence and are today the most frequent primary malignancies of the SB[2]. This growing phenomenon seen since the 1970s is possibly due to the detection of early-stage disease[7,8].

The aim of this manuscript is to carry out not only an updated narrative review on the diagnosis and treatment but also to synthesize the data related to epidemiology, pathophysiology and clinical manifestation of SB neuroendocrine tumors.

MATERIALS AND METHODS

We conducted a bibliographic review using articles indexed in PubMed/Medline, Scopus, Embase and Scielo, published between 2000 and 2022. The Medical Subject Headings used were: "Neuroendocrine Tumors", and "Small Bowel" or "Small Intestine". The research was limited to human-related articles. The type of articles included were: Clinical trials, prospective cohort studies, retrospective and cross-sectional studies, as well as systematic reviews and meta-analyses.

The quality of our narrative review was assessed using the SANRA scale[9], which covered the following topics: Description of the literature search, statement of the review aims, referencing, explanation of the review's importance, presentation of relevant and appropriate endpoint data and scientific reasoning.

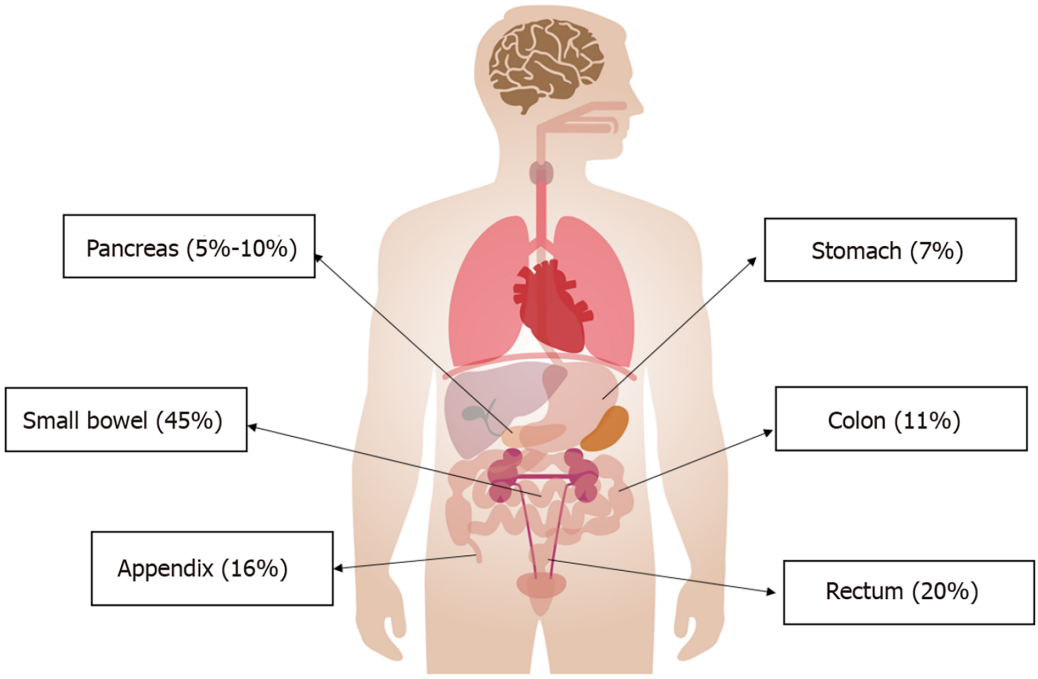

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Figure 1 Incidence of neuroendocrine tumors in the gastrointestinal tract.

EPIDEMIOLOGY

Small bowel cancers represent only 0.6% of all cancers and less than 5% of gastrointestinal (GI) cancers according to figures from the United States[10]. However, its incidence is increasing, reaching a growth of over a 100% in the last 40 years[10].

The two main types of SB neoplasms are adenocarcinoma of the small intestine, and NENs. Although in the 1980s, adenocarcinoma was predominant with 42%, in 2005 it had decreased to 33%, while NEN at the same time increased from 28% to 44%, positioning itself as the most frequent type of primary tumor[11]. Yao *et al*[6] also reported an increase in NENs of 6.4-fold from 1.31/100000 to 6.98/100000 over the timeline 1973 to 2016. In 2020, the incidence of NENs of the small intestine was estimated to be 1.2 cases per 100000 population in the United States[2].

It is thought that the rise in cases is the result of the development of better diagnostic methods, as often they are detected incidentally in endoscopic or imaging studies[11,12]. In addition, the increase in NENs compared to adenocarcinomas may be explained by the increased survival of patients with small intestine cancers, as NENs usually have a better prognosis[11].

Unlike adenocarcinomas, which are more frequent in the duodenum, small intestine NENs are more frequent in the ileum[11]. Some genetic mutations predispose to the development of NENs. The most common predisposing condition is multiple endocrine neoplasia type 1 and represents around 5 to 10% of these tumors[11].

Studies reporting the duration of symptoms preceding diagnosis varies widely, from a median of 4.3 mo up to 9.2 years[1]. Liver metastasis is seen in as many as 61%-91% at the time of diagnosis[11]. Among the risk factors associated with metastatic disease are the location in the jejunum or an unspecified site, the histology of neuroendocrine carcinoma and being a patient from a rural area[13].

The median overall survival (OS) of SB NENs is 14 years, while localized and well differentiated tumors showed a better survival. In multivariate analyses, factors that had a significant correlation were race, age, stage and site. In contrast to pancreas NENs, patients with bowel NENs are 1.5 times more likely to survive[11].

There is no objective way to define the prognosis of these patients; however, tools have been created such as the Modlin Score Nomogram that addresses 15 parameters whose objective is to determine the prognosis and guide treatment[14]. The use of this tool in tertiary referral hospitals made it possible to identify patients accurately with low and high risk of death, although Kelly and co-authors in their 2019 study indicated that it was not applicable to all patients[15].

The Epidemiology and End Results (SEER) database included 73782 patients diagnosed with NENs between 1973 and 2014 in a surveillance analysis. SB NENs were found to be the second tumor with the best prognosis, after rectum NENs[16]. Summing up the localized, regional and metastatic forms of the disease, despite the heterogeneity of these tumors, the decrease in mortality rates of all forms is well-known, regardless of an increasing incidence. In addition, although comparisons between studies is

difficult due to different patient classifications, cohorts and methodology, the observations in diagnostic and therapeutic advances made, are usually similar[13].

PATHOPHYSIOLOGY

Neuroendocrine cells release hormones by stimulation of the nervous system. They are found throughout the body, such as in the skin, lungs, gonads, pancreas, the GI tract, pituitary gland and adrenal glands. NENs are neoplasms that originate from these cells. Depending on their location, their clinical behavior is very heterogeneous[2] (Figure 2).

SB NENs are often small, multifocal, difficult to locate pre-surgically, and may not be found during surgical exploration[17]. They represent 30% of neoplasms found in the SB[18]. By definition, SB NENs are located between the ligament of Treitz and the ileocecal valve. Although duodenal NENs are sometimes included with jejunal and ileal NENs under the umbrella term "SB NEN", these tumors are clinically and biologically different and should not be considered as representatives of the same entity [1].

After the lung, the small intestine is the next most common location for NENs[1]. The risk factors that increase the incidence of SB NENs, have to be considered and include: A habit of smoking[19], a possible family history of cancer and the antecedent of gallbladder disease and cholecystectomy. All of them are associated with a 1.5-fold higher risk of developing SB NEN[20].

Mutation of the MutY human homologue (MYH) gene is associated with SB neuroendocrine tumors and is the main genetic background described in DNA base repair by excision[21-23] which fail in a hereditary form of SB NEN. Clinically, hereditary forms tend to be isolated endocrinopathies; however, further research is necessary.

SB NENs present in many forms, depending on the stage of the disease and the tumor burden at diagnosis. Approximately 30% of patients with SB NENs will have metastasis at the time of diagnosis, and another 40% will have regional lymph node involvement[5]. Primary tumors, in spite of being characteristically small, may cause an extensive fibrotic reaction in the SB and mesentery, resulting in narrowing or twisting of the intestine and potentiate mesenteric ischemia[1].

Metastasis of SB NENs is most commonly from the frequently seen primary site of both the small intestine as well as the pancreas. Some patients with SB NENs have synchronous or metachronous pancreatic NENs (PNENs), and it is frequently unclear whether these are separate primary tumors or metastasis. In a case series, in almost two-third of the evaluated patients, the pancreatic tumor was a metastasis of the SB NEN primary tumor, while in the remaining third of patients it represented a separate primary tumor. Determining the origin of these tumors can guide the choice of systemic therapy and surgical management[24].

CLINICAL MANIFESTATIONS

The clinical manifestations are caused by the location of the primary NEN and its functionality. Most of them are non-functional, which usually have no or very few symptoms in the early stages of the disease; late symptoms are due to its mass effect or liver metastasis[25-28].

In general, the most common symptom of intestinal NENs is nonspecific abdominal pain that leads to Computing Imaging studies. Intestinal NENs can present with GI bleeding and anemia. Occasionally, NENs grow large enough to obstruct the extrahepatic bile duct or GI tract, causing jaundice or intestinal obstruction, respectively. Rarely, an intra-abdominal mass is palpable on physical examination, prompting further diagnostic studies[26]. In addition, around 15%-20% of SB NENs are symptomless and are detected incidentally, which is more frequent in patients with localized disease[1].

In patients with metastatic disease, about 10% develop carcinoid syndrome (CS), with predominance in liver metastasis. Of the wide variety of manifestations, the main manifestations are: Facial flushing (94%), diarrhea (78%), abdominal cramps (50%), heart valve disease (50%), telangiectasia (25%), wheezing (15%) and edema (19%)[29]. Almost all SB NENs produce a wide variety of biologically active peptides, including serotonin, neurokinin A, and histamine, which are responsible for CS. However, for tumors limited to the SB and its regional lymph nodes, these components are inactivated by the liver and hormonal symptoms are rare[1].

With the development of distant metastasis, the hormones secreted by SB NENs are able to bypass the portal circulation, leading to the development of CS. This syndrome was first described by Thorson in 1954. Carcinoid symptoms may be spontaneous or caused by stress, exercise, or ingestion of ethanol and amine-rich food such as chocolate or cheese[5]. The flushing associated with CS is typically transient and affects the face, neck and the upper part of the trunk[1].

The cardiac manifestations of CS, called "carcinoid heart disease", primarily affect the right side of the heart, causing valvular fibrosis. Cardiac involvement is seen in at least 20% of patients. The cause is believed to be related to high levels of serotonin that induce a fibrotic reaction in the right heart. However, the incidence is declining, possibly due to the widespread use of somatostatin analogs (SSAs).

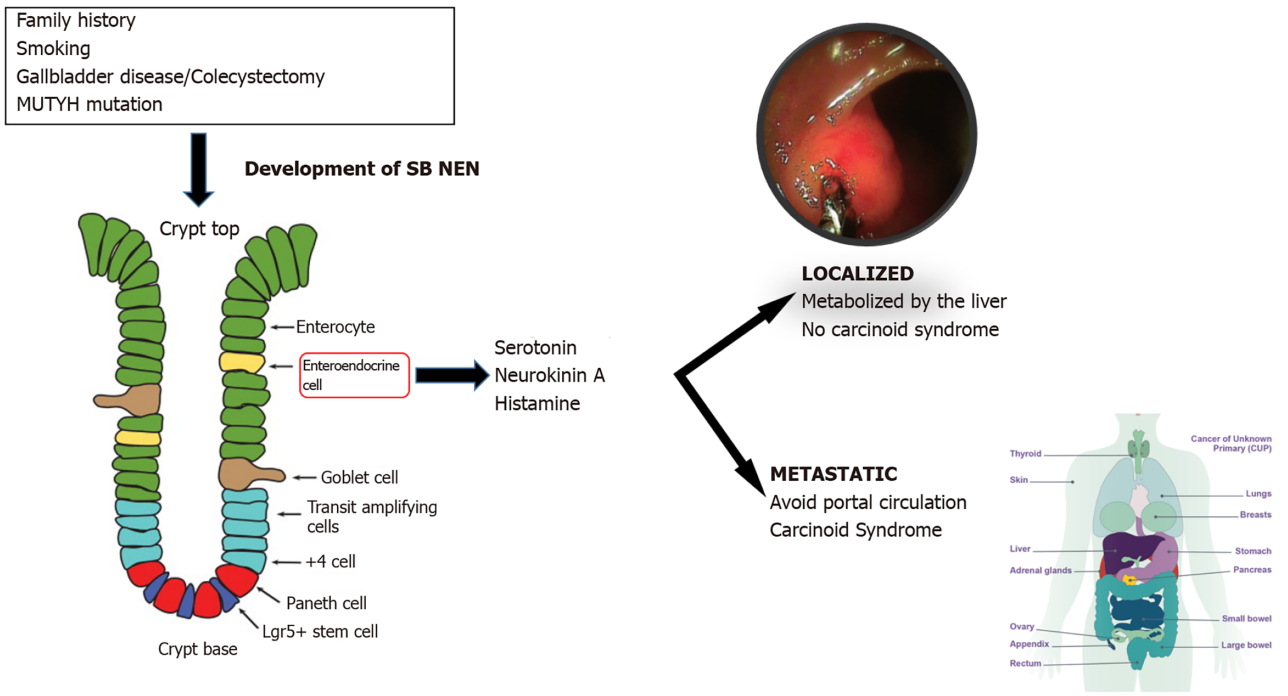

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Figure 2 Pathophysiology of small intestine neuroendocrine tumors. MUTYH: Human mutY homologue; NEN: Neuroendocrine neoplasms; SB: Small bowel.

The presence of carcinoid heart disease predicts a worse prognosis[1]. This variety in presentation, combined with the relative rarity of the tumors and the nonspecific nature of the symptoms, makes diagnosis of these tumors difficult. Although the median duration of symptoms before diagnosis is 4 to 5 mo, misdiagnosis is common and a delay in diagnosis of up to 10 years has been described in the literature[1].

Published series from tertiary referral centers, mention the proportion of patients with distant metastasis of around 60% to 80%. One possible explanation for this is perhaps because early-stage lesions are removed in emergency surgeries for intestinal obstructions in less complex hospitals, while the more advanced stage of the disease is referred to these larger hospitals[30,31].

DIAGNOSIS

For the diagnosis of NENs, there are currently various methods available. The initial methods can be both imaging and laboratory tests; the order in which they are requested will depend on the form of clinical presentation and the initial diagnostic hypothesis. Confirmation will be histological, requiring a biopsy by endoscopy. Octreotide scan, video capsule endoscopy (VCE) and double-balloon enteroscopy (DBE) are the auxiliary exam options for diagnosis. Series reports catalog them as having a diagnostic yield of 85%, 10% and 83%, respectively. In occult SB NENs, capsule endoscopy appears to be superior to enteroscopy but may underestimate tumor burden[17].

Biochemical testing

For most patients, biochemical testing and anatomic or functional imaging will have preceded definitive diagnosis of SB NEN made by an immunohistochemical study of the tumor[6]. In addition to the hormones and neuroamines responsible for CS such as 5-hydroxyindoleacetic acid (5-HIAA) in plasma or urine[32], SB NENs secrete chromogranin A (CgA), pancreastatin, and serotonin which can be used as biomarkers for diagnosis and surveillance[32]. CgA is an acidic glycoprotein secreted by NENs, and has been extensively studied. CgA is sensitive and specific for the diagnosis of NEN, correlates with disease burden, and can predict survival. Nevertheless, renal failure, severe hypertension, vitamin B12 deficiency and proton pump inhibitor therapy can cause false CgA elevations. Serial pancreastatin measurements are useful in predicting and monitoring response to therapy[33]. A 24-h urine sample monitoring 5-HIAA, indicates serotonin breakdown. This test is highly specific for the diagnosis of SB NEN, but patients should be advised to avoid various serotonin-rich foods during collection[2].

Biochemical tests are widely used both for the diagnosis of SB NEN and for monitoring the course of the disease, but there is no agreement on how often they should be measured or how their measurement

should influence treatment decisions[33].

Endoscopic, radiological and molecular investigations

The endoscopic technique of VCE and DBE are the most helpful exams in jejunal and ileal NENs. They allow location of the primary NEN in metastatic disease, where a basic study has been negative, to identify multifocal disease. This might change the management and prognosis. In addition, other studies have reported that multifocality does not seem to have an impact on survival or recurrence[18].

Those patients who present with hot flashes and diarrhea will probably undergo biochemical tests first, while those whose main symptom is abdominal pain or obstructive symptoms will require anatomical imaging such as computed tomography (CT) or may even be diagnosed only after an emergency surgical intervention[33].

For anatomical studies of SB NEN, CT, magnetic resonance imaging, and ultrasound are performed, while for functional studies positron emission tomography (PET) with Gallium and somatostatin receptor-based single photon emission computed tomography are carried out. Functional imaging using PET is essential for detecting small lymph node metastasis, tiny primary tumors in the SB, initial bone and bone marrow metastasis and more accurate assessment of occult liver metastasis[3]. Anatomical images provide the location of the tumors for surgical planning, while functional images have higher sensitivity and indicate the occult presence of metastasis or mistaken evidence of recurrence[2,33].

NENs of the SB are rarely visualized on CT. They are usually just millimeters in size. However, mesenteric lymph node metastasis might well appear as spiculated masses on contrast-enhanced CT, sometimes including calcifications and the regional presence of fibrosis due to its desmoplastic reaction. Additionally, as many as 30% can be multifocal[2]. CT angiography can provide details of valvular involvement. Despite this, morphological images generally significantly understate the disease[2].

Pathology

For tumor classification, the Ki67 index or the number of mitoses per 10 high power fields (HPF) is used. NENs are subclassified into NENs and NECs. Grade 1 NENs have < 2 mitoses per 10 HPF or a Ki67 of < 3%. Grade 2 NENs show a Ki67 index from 3 to 20%, or 2 - 20 mitoses per 10 HPF. Grade 3 NENs give a Ki67 index of > 20%, or > 20 mitoses per 10 HPF. Further classification into G3 NENs and G3 NECs is based on their differentiation. G3 NECs are poorly differentiated but Grade 3 NENs are well differentiated[2].

Figure 3 summarizes the initial approach sequence of the patient with a suspected SB NEN and which tests should be requested depending on the form of clinical presentation.

TREATMENT

Treatment of a SB NEN depends on the staging of the disease, and whether it is locoregional or metastatic (Figure 4).

Management strategies for SB NEN include not only possible treatment of all stage tumors or metastasis, and if present, carcinoid heart disease or tumor-related symptoms and syndromes[2].

The management of these lesions is complex due to the difficulty in diagnosis, hormone secretion and more frequently, its presentation as an advanced disease. Even patients with advanced disease can have a long survival time. There are different aspects that make it difficult to determine the optimal management[34].

All patients with localized SB NEN with or without regional mesentery metastasis should be considered for curative resection. Therefore, multimodal treatment is required[2]. Although surgery is curative in most cases, recurrence rates of 42% in liver NEN have been published[1,35].

In the surgical area, meticulous exploration of the abdomen with palpation of the SB is recommended intraoperatively; this is superior to reference imaging for the detection of SB NENs, as up to 70% of these tumors are overlooked by imaging. Additionally, between 30%-54% of SB NENs are multifocal and just millimeters in size, which are very difficult to see on imaging. Therefore, a laparoscopic study is not recommended[2]. The abdomen should also be carefully examined for evidence of liver and peritoneal metastasis, reported in 20% and 60% of cases, respectively, undergoing SB NENs surgery[1].

In SB NENs, total resection it is not necessary, only the primary tumor and selective resection of the mesenteric nodes is required, taking into account the preservation of bowel function. The length of bowel resected is independent of the number of lymph nodes removed. In up to two-thirds of patients, metastasis outside the "expected" lymph node region is found, and to prevent unresectable locoregional recurrence, an extensive lymphadenectomy is required[2]. A series of reports of surgeries for SB NENs where the resection included 12 or more nodes, was related to better OS outcomes, in patients without distant metastasis[2].

If the primary tumor is located in the terminal ileum, a right hemicolectomy with or without lymphadenectomy is indicated[36]. In cases with stage IV asymptomatic SB NEN, early locoregional surgery as a prophylactic measure is controversial, as there are no convincing data associated with favorable survival outcomes, compared with locoregional surgery later in its development. SB NEN can

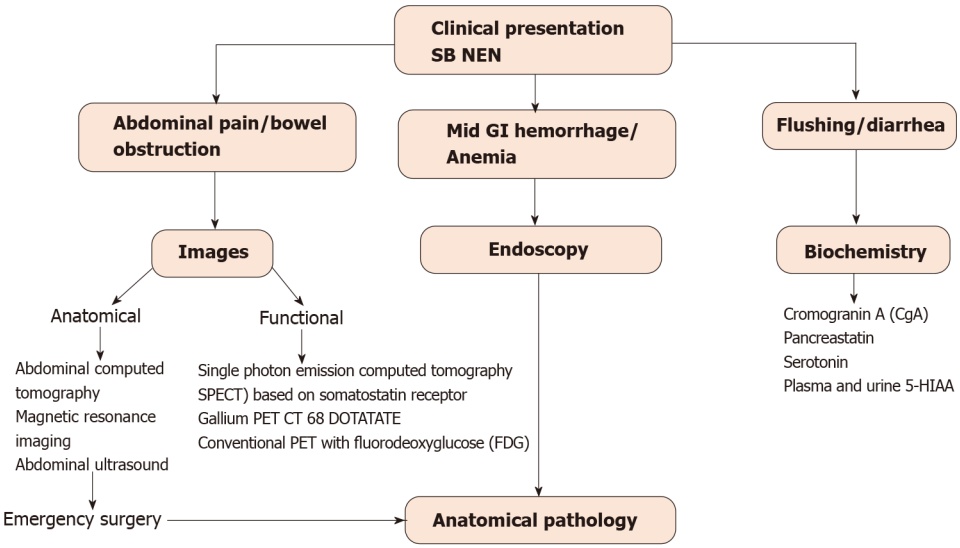

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Figure 3 Diagnostic algorithm for small intestine neuroendocrine tumors. GI: Gastrointestinal; NEN: Neuroendocrine neoplasms; SB: Small bowel; PET: Positron emission tomography.

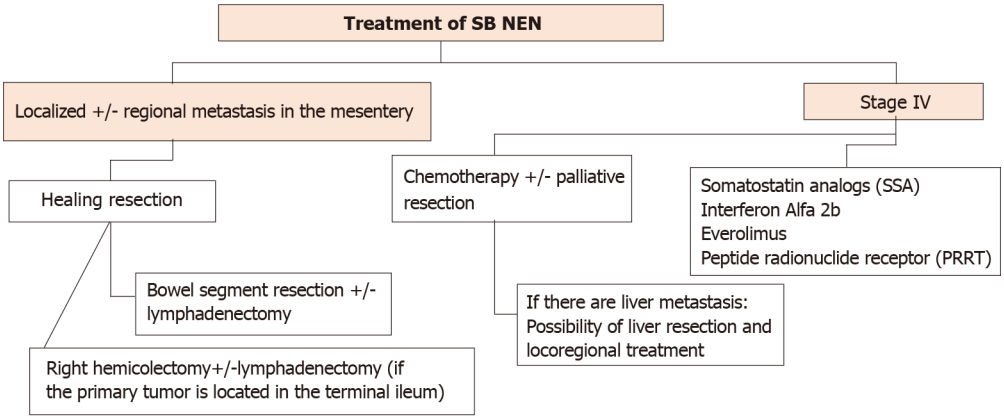

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Figure 4 Management of small intestine neuroendocrine tumors. NENs: Neuroendocrine neoplasms; SB: Small bowel.

be associated with peritoneal carcinomatosis (PC) in up to 30% of cases. As PC can cause fatal intestinal obstruction with a registered mortality of 40%, resection of peritoneal tumors should be part of the locoregional surgery[2].

Resection of the primary tumor in the setting of unresectable SB NEN liver metastasis may prevent ileus, intestinal obstruction, and desmoplastic reactions, and is registered in a retrospective study to prolong survival, independent of the tumor grade. However, such studies are biased toward an aggressive approach in patients with better baseline status, so it is unclear whether this intervention is beneficial versus the underlying characteristics of the cases[2].

Patients with metastatic NENs to the SB have a favorable prognosis, compared with other GI malignancies. An OS of 103 mo for cases with well-differentiated tumors was reported in some series between 2000 and 2012[6].

The first-line treatment of NENs consistes of SSAs, which is also the case in functional and non-functional metastatic NENs of the SB, to control CS symptoms and due to their antiproliferative effects [37,38]. The treatment consists of injections of octreotide LAR or lanreotide, which are long-acting SSAs, every four weeks. Short-acting octreotide injections are given in cases to improve symptomatic control or as a rescue therapy[1]. Octeotride LAR plus interferon alpha have shown beneficial effects by inhibiting hormone secretion and proliferation in NENs in the past decades[39].

Everolimus has been studied in advanced stages of NENs. It is a rapamycin inhibitor, used to treat CS (RADIANT-2 trial) and advanced non-functional NENs (RADIANT-4 trial). In the RADIANT-2 trial, better OS was observed after treatment with everolimus and octreotide LAR versus treatment with octreotide LAR only; however, the difference was not statistically significant[33]. Results from the

RADIANT-4 trial did show a statistically significant improvement in median progression-free survival when everolimus monotherapy was compared with a placebo (11.0 *vs* 3.9 mo). Based on these findings, everolimus is only approved for use in progressive non-functional NENs, but is often used in patients with progressive disease regardless of tumor functionality[1,38].

Since 1992, peptide receptor radionuclide therapy (PRRT) has been used for the treatment of NENs. In PRRT, radionuclides such as Yttrium-90 (90Y) and Lutetium-177 (177Lu) are directly delivered to the tumor by radiolabeled SSA8. In the 229 patient NETTER-1 trial, all patients had well-differentiated, metastatic NENs. It was found that the PRRT treatment group had a significantly better median OS and a better response rate compared with the placebo group (18% *vs* 3%)[1,40].

Cytotoxic chemotherapy is also used in the treatment of PNENs, and has been shown to have an inferior role in well-differentiated SB NENs[1]. Due to easy oral administration, and their low adverse effect profile, capecitabine and temozolomide remain good practice second- or third-line choices in patients with progressive SB NENs[1].

Small intestine NECs are extremely rare. Regardless of the primary site, cisplatin or carboplatin and etoposide are used as first-line treatment, and due to the poor prognosis of NEC, they are generally not recommended for surgical intervention and treatment[41,42]. NECs with an Ki-67 index between 20% and 55% have shown low response rates to platinum-based chemotherapy, and there is no standard treatment regimen for these patients[1].

Patients with metastatic NENs of the SB are not excluded from surgery. Several studies have shown an improvement in OS together with control of symptoms following resection of metastatic lymph nodes and liver metastasis. However, these procedures are seldom curative and the recurrence rates at 5 and 10 years are 95% and 99%, respectively[1].

Finally, at the time of surgery for metastatic NENs of the SB, a cholecystectomy should be included due to the high presence of gallstones in patients receiving SSAs[1]. In addition, minimally invasive resection techniques should be performed in younger patients less prone to obstruction, without metastasis, or with small tumors. However, these techniques have limitations that will require surveillance[17].

CONCLUSIONS

Neuroendocrine tumors are neoplasms that can be found in any part of the body. This review is focused on those with a location or origin in the digestive tract at the level of the small intestine due to its variable form of presentation and difficult diagnosis, as well as the treatment approach, emphasizing a multidisciplinary effort. We observed that reports of current series place them in several cases as one of the most frequent tumors in the small intestine. As their incidence is increasing, the importance of understanding their behavior and how to approach them correctly increases. The presence of small bowel NENs results in variable gastrointestinal symptoms, which are frequently a cause of the delay from symptom onset to diagnosis. In addition, a suspected SB NEN must be confirmed by biochemical tests, anatomical and functional images and an anatomopathological study of tissue, the latter preferably carried out by a pathologist experienced in NENs. Each of these will facilitate clinical decision-making. Finally, treatment depends on the extent of the disease; patients with localized disease are considered for surgery and NENs in metastatic stage will be prescribed SSAs, interferon alpha, everolimus or PRRT together with consideration for resection of the primary tumor and cytoreductive surgery. It is necessary to know and understand the behavior, forms of presentation and therapeutic options for NENs of the small intestine in order to improve current patient management.

ACKNOWLEDGEMENTS

The research team appreciates all the contributions and suggestions received from different colleagues prior to publication of this manuscript.

FOOTNOTES

Author contributions: Gonzáles-Yovera JG, Roseboom PJ, Concepción-Zavaleta M, and Gutiérrez-Córdova I were the main writers and performed the literature review; Alcalde-Loyola C, Massucco-Revoredo F and Roseboom PJ were translators and prepared the manuscript; Plasencia-Dueñas E, Paz-Ibarra J, Quispe-Flores M, and Ramos-Yataco A performed written contribution to the text body; Massucco-Revoredo F, Paz-Ibarra J and Concepción-Urteaga L performed a general literature review; all authors have read and approved the final manuscript.

Conflict-of-interest statement: All the authors declare no conflict of interest.

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S-Editor: Liu JH
L-Editor: Webster JR
P-Editor: Liu JH

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 392-401

DOI: 10.5662/wjm.v12.i5.392

ISSN 2222-0682 (online)

MINIREVIEWS

Pandemic control - do's and don'ts from a control theory perspective

Latchezar Tomov, Dimitrina Miteva, Metodija Sekulovski, Hristiana Batselova, Tsvetelina Velikova

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ait Addi R, Morocco; Khidhir ZK, Iraq

Received: March 16, 2022
Peer-review started: March 16, 2022
First decision: June 16, 2022
Revised: July 6, 2022
Accepted: August 10, 2022
Article in press: August 10, 2022
Published online: September 20, 2022

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Abstract

Managing a pandemic is a difficult task. Pandemics are part of the dynamics of nonlinear systems with multiple different interactive features that co-adapt to each other (such as humans, animals, and pathogens). The target of controlling such a nonlinear system is best achieved using the control system theory developed in engineering and applied in systems biology. But is this theory and its principles actually used in controlling the current coronavirus disease-19 pandemic? We review the evidence for applying principles in different aspects of pandemic control related to different goals such as disease eradication, disease containment, and short- or long-term economic loss minimization. Successful policies implement multiple measures in concordance with control theory to achieve a robust response. In contrast, unsuccessful policies have numerous failures in different measures or focus only on a single measure (only testing, vaccines, *etc.*). Successful approaches rely on predictions instead of reactions to compensate for the costs of time delay, on knowledge-based analysis instead of trial-and-error, to control complex nonlinear systems, and on risk assessment instead of waiting for more evidence. Iran is an example of the effects of delayed response due to waiting for evidence to arrive instead of a proper risk analytical approach. New Zealand, Australia, and China are examples of appropriate application of basic control theoretic principles and focusing on long-term adap-

tive strategies, updating measures with the evolution of the pandemic.

Key Words: COVID-19 pandemic; Control; Control theory; COVID zero; Flattening the curve

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Core Tip: Controlling an epidemic is a massive challenge due to the nonlinear systems involved and interactions that are hard to model and predict well. Therefore, any pandemic control policy must apply at least the basic principles of control theory, including having multiple measures simultaneously and having models and predictions to combat the time delay between exposure and symptom onset that could lead to loss of life and controllability of the pandemic. In addition, a control-theoretic-based policy needs to factor in a large set of mutual interactions between people, animals, and pathogens that includes media and social networks and their influence on people's behavior, including fake news and the viral spread of disinformation.

Citation: Tomov L, Miteva D, Sekulovski M, Batselova H, Velikova T. Pandemic control - do's and don'ts from a control theory perspective. *World J Methodol* 2022; 12(5): 392-401
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/392.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.392

INTRODUCTION

Control theory has been developed for the control of dynamic systems in engineering. Its main principle of feedback control is to use the measurement of the output and/or the states of the system and their deviations from the desired levels or trajectories to reduce the deviations to zero and achieve stability [1]. Although mathematical control theory is being developed to control different engineering processes, dynamic feedback systems are often found in biology at different levels, from cellular to whole organisms[2]. For example, the pituitary axis is involved in hormonal level stability control *via* multiple negative feedback loops to maintain homeostasis under different external stressors from the interactions of the system (the organism) with the environment[3]. Termination of the stress response is one prime example. Sustaining constant levels of different hormones and blood sugar are other examples (blood sugar needs to be at constant levels with the variability of demand for it from various physical and intellectual activities, especially in humans)[3]. Therefore, pandemic control is a natural area of application of control theory to achieve a certain level of disease prevalence, being zero or another appropriate level that does not burden healthcare systems. Sadly, many implemented pandemic control policies are not developed in accordance with the main principles of control theory, leaving suboptimal results and increased incidence of deaths from infectious diseases and financial losses in healthcare systems.

Time delay as a factor in pandemic

One of the main characteristics of an ongoing epidemic is that any implemented measure will have an effect with pure time delay. Even full quarantine that stops transmitting in a matter of a single day will leave many infected people in their incubation periods, who will get sick with an unavoidable delay, depending on it. The presence of asymptomatic transmission as in coronavirus disease-19 (COVID-19) means that if they are not found and isolated in time, they will still be able to transmit it in their households during quarantine - and thus, the measure will have a delay in its effects, which will increase the number of infected, sick, and deceased people[4]. The presence of time delay in a dynamical feedback system leads to old and less usable information in the feedback that has to stabilize it, thus decreasing the stability margins and introducing oscillatory behavior of the output. This is known from the beginning of control theory with the work of James Clerk Maxwell on governors[5].

So, how do we deal with a time delay? One mechanism is with modeling and prediction - making decisions about implementing specific policies at a current level and a projected level. For example, suppose we want to keep the number of occupied hospital beds under a certain threshold. In that case, we do not wait until it is reached to implement the policy. Instead, we rely on modeling and forecast to tell us when it is the last possible moment to implement without reaching it. This approach in Proportional-Integral_Derivative (PID) control uses its derivative part to make linear extrapolations and to use projections to compensate for the older information in the feedback loop due to pure time delay. Stability can be either improved or worsened by predictions. However, too much reliance on the future can decrease stability due to model errors and amplification of noise to signal ratio, just as the derivative part of the PID controller increases high-frequency noises. The future may not happen, and we must be

careful not to base the policy solely on unreliable models with long-term predictions.

Controlling unstable systems

Although the pandemics have been perceived as stable processes that eventually lead to endemic situations, that is not so. Every pandemic brings existential risk to the species that it attacks[6]. Stability can be achieved at a population level as small as zero - far from the desired level that we aim to control. Thus, this system is deemed unstable[7]. The risk of loss of life due to pandemics is fat-tailed. It is worse even than the risk of nuclear war[8,9]. As the history of the plague shows, other possible scenarios, such as endemics, may result in periodic oscillatory behavior without a significant decrease in mortality[10]. Unlike stable systems, an unstable system cannot be left unregulated, so the concept of "no policy" cannot be implemented with the expectation of achieving any measurable results in either death rate, hospitalizations, or economic growth targets. Pandemics must be controlled.

Controlling nonlinear systems

The basic and most often used epidemiological models that capture the dynamics of epidemics are nonlinear. Thus, a system that includes the pathogen and the population has to be considered a nonlinear system.

A significant difference between linear and nonlinear systems is that the principle of superposition does not hold for the latter - the result of a linear combination of inputs is not a linear combination of outputs. In other words, if we put inputs U_1 and U_2 to a system separately and archive outputs Y_1 and Y_2, the result of the input $aU_1 + bU_2$ will not be $aY_1 + aY_2$. This decreases the predictability of the behavior of nonlinear systems. Its direct consequence is that the size of inputs defines a nonlinear system's behavior - doubling the input will not double the output.

Studying the influence of a given set of inputs over the system does not provide us with information for other sets of inputs - we cannot construct the combination of outputs due to the variety of inputs. An example, in pandemic control - implementing policy to decrease the prevalence of a disease depends on the prevalence level - the higher the number of active cases, the longer it will take to reach the desired aim due to the diversity.

WHAT ARE THE MEASUREMENT GOALS IN THE COVID PANDEMIC

Aims of the pandemic control system

To assess proper from improper methods for pandemic control, we need to know what our aim is. Do we want to minimize the lost number of lives? Instead, do we want to keep the healthcare system working and minimize only the collateral damage from the pandemic? Do we want to minimize short-term economic loss of value, or do we want to reduce long-term loss? Do we want to preserve the population's overall health for long-term goals related to spending on healthcare or other incentives to do so?

Any viral pandemic poses risks to the long-term health of a population. For example, children born second or third in a family have substantially increased exposure to respiratory disease than the first-born child, which has long-term consequences for their educational level and labor market outcomes [11]. Similar long-term outcomes were seen with 1918 influenza[12]. Minimizing long-term health damage thus coincides with the number of infected, which controls mostly the number of deceased where no specific treatment is available.

Disease eradication

The goal to eradicate a disease globally is the most ambitious and often includes eradication *via* immunization, as with measles or polio[13]. Although local eradication, as seen in China, can be successful for prolonged periods, the pandemic control policy needs to be adapted continuously to respond to the mutating virus from widespread outside of the country or region that implements the policy[14].

Chinese control policy implements multiple different key elements from control theory to achieve this success, such as early response, traffic control, and predictive mechanisms: Early and rapid response that allows the local reaction to a local cluster without disrupting the socio-economic activity. Delay is the critical variable in the economic costs of any pandemic control strategy - costs increase exponentially over time. Fast and complete isolation of exposed individuals includes extensive data analysis to locate all registered infected contacts automatically, so contact tracing is ahead of the transmission chain. This is the *predictive part* of the control mechanism - who is in incubation and will spread the disease next time? Why is the need for prediction?

Due to asymptomatic spreading in the case of COVID-19, all exposed individuals need to be isolated, which needs to happen before some of them display symptoms to rapidly break the chain of transmissions for up to two incubation periods. For this strategy to work, both in government and people, compliance is needed. Excessive community involvement, government funding, motivation

mechanisms, and constraint mechanisms require serious investment and may be at odds with the political system in a given country, such as data privacy protection policies and traffic restrictions. A control system designed to achieve local zero policy also controls people and their motivation and compliance, which can be expensive and hard to achieve or require actions incompatible with specific legal systems. A high technology approach is also beyond the reach of many countries with smaller budgets for pandemic control.

Although the local disease zero policy is possible and is still successful in China, it has been used before, as in the SARS pandemic in 2003, which was eradicated[15]. It is unstable until global eradication is achieved.

Disease containment

Due to various restrictions in implementing pandemic response, sometimes "flattening the curve" is the main goal - to avoid hospital system overburdening, which can cause excessive deaths due to lack of hospital treatment for other diseases and the infected during the pandemic. This also aims to sustain the long-term quality of healthcare due to the hazardous effects of infections among medics, nurses, and staff and the impact of accumulated fatigue for prolonged periods of overburdening.

Measures and effects

Although different measures suit different regional specifics during a pandemic, countries with multiple standards have seen the most robust outcome[16,17].

This is in line with the basics of control theory - having more degrees of freedom in a controller satisfies many constraints while maintaining stability[18]. Thus, redundancy improves robustness - stability in the face of changing parameters of the system, such as the pathogen transmissibility and severity of disease.

In contrast, in countries with poor outcomes of pandemic control, such as Iran, multiple control mechanisms are broken due to socio-economic conditions, including education, economic disparity, and lack of coherent response from the healthcare community due to limited evidence and scientific controversies, which lead to premature actions from the government towards reopening[19].

Still, when implementing multiple different measures, some key measurements need to be highlighted as high-priority ones.

One very effective non-pharmaceutical intervention for that aim is to localize traffic - reducing interstate traffic in the United States substantially affects deaths and intensive care unit admissions[20]. In addition, the estimation of imported cases shows their significant influence in the case of COVID-19[21].

Another cheaper measure is the use of protection such as masks which are highly effective in decreasing case counts and mortality in multiple different ways, including socio-cultural norms and improvement of long-term behavior during pandemics[22]. For countries with limited budgets, universal mask-wearing must control any respiratory disease pandemic.

Another key and high-priority measure that has a high cost that we already described - rapid response to newly found cases and predictive approach to contact tracing - isolates exposed individuals before they become contagious. This is the golden standard that is difficult to achieve for many already described reasons. However, any effort on contact tracing will impact the health prevention of the infected (early discovery and treatment).

EFFICACY OF PANDEMIC CONTROL MEASURES IN COVID-19 PANDEMIC

When the COVID-19 pandemic began in 2019, precautionary measures started to be taken worldwide to limit the increasing number of cases. However, countries demonstrated that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission could be gradually retard or stopped. Effective strategies have been developed because the entire scientific and medical community cooperated in identifying cases and developing strategies for diagnoses, therapies, and vaccines. As a result, quick identification of close contacts, and testing and confirming symptomatic and asymptomatic patients have begun. Thus, all institutions' multidisciplinary approaches and collaborative work have led to containment and case management[23].

The main strategies for mitigating the COVID-19 pandemic are based on measures of social distance and health system reinforcement. Many countries have introduced strict lockdowns due to the widespread virus transmission in the community. However, prolonged strict lockdowns have various unfavorable social, economic, and health effects. Permanent lockdowns are not a long-term solution to limiting the COVID-19 pandemic. Lockdowns have reduced the effective reproduction number (R)[24]. But developing all kinds of resources to test and confirm all cases and using other non-therapeutic prevention will prevent SARS-CoV-2 transmission[17,25,26].

Prolonged strict lockdowns can lead to more deaths forward in time and can do more damage than benefits. Not all models analyzing lockdowns consider the potentially possible effects on other diseases. In England and Wales, cardiovascular deaths at home have increased by 35% compared to 2014-2019 [27]. In Italy, mortality from myocardial infarction increased 3 times in March 2020 during the blockade

period compared to the same period in 2019[28]. However, we must consider that lockdowns are not worse than the epidemic. Plenty of literature on the subject shows the effectiveness of lockdowns. Here, the variable that makes a lockdown good or bad is its duration. That is why China makes theirs within 28 d with the help of the strategy described here. An increase in infarcts can be a confounding variable with the disease because it causes such mortality directly and indirectly.

There is also a reduced hospitalization rate for the acute coronary syndrome. The most likely reason is people's fear of infestation with SARS-CoV-2 in hospitals[29,30]. Most of these deaths reported at the time were not due to COVID-19[27]. Children and adolescents are also not spared from the unfavorable effects of lockdowns. The interruption of the educational process and the lack of socialization caused even more problems[31].

The economic effects are expected to lead to rising unemployment and poverty in the long term[32]. Therefore, careful planning is necessary, but the positive results of any decision must exceed the negative effects on the people. Furthermore, all measures must be based on scientific facts and evidence to be as correct as possible to the situation when they are taken to achieve the long-term goal[33].

Hospital preparedness has been one of the main strategies of governments. Still, even the best health care system cannot cope if the viral transmission and infections continue too long. Therefore, increasing the number of beds, adapting infrastructure and redistributing human resources and equipment, implementing measures to protect healthcare staff and patients, and training staff are essential measures to tackle the COVID-19 crisis[34].

In Europe, governments have mobilized special funds to increase labor capacity and equipment. However, even in regions with high resources, such as the USA and Italy, hospitals and intensive care units came under tremendous pressure from the first wave of the disease. There have been situations with difficult decisions to prioritize cases based on patients' age, comorbidities, and health status[35,36]. The hospital overcrowding and pressure on the health care system led to the first lockdown, which imposed severe restrictions on movement worldwide. Therefore, even if significant financial resources were allocated to hospitals' preparation, that would not be enough to mitigate the effects of the pandemic unless other measures are taken.

Initial mortality data were based on confirmed COVID-19 cases, but actual mortality from COVID-19 was established later[37].

A significant part of infected people is undetected because they are asymptomatic or usually do not seek medical attention. In addition, significant differences in the percent of mortality have been found between different age groups[38]. Therefore, many patients with confirmed COVID-19 will not need to be hospitalized, especially the younger ones.

Primary health care can play a crucial role in unburdening hospitals. Previous data have shown that access to family doctors helps treat patients. They can become the first line of defense in diagnosing and preventing COVID-19[39,40]. Thus, severe patients will be referred to hospitals, while those with mild COVID-19 will be treated at home. A well-coordinated and planned process of primary health care will ensure control of the disease spread and identification of vulnerable groups that need to be protected. Early detection of COVID-19, monitoring during isolation, individual risk assessment, treatment of mild COVID-19 cases, and timely identification of worsening conditions could be priorities for family physicians.

Primary healthcare and home care are taken together with more measures that are selective and are the only realistic long-term strategy to mitigate the COVID-19 pandemic.

Controlling the extended pandemic system, with an account for communications impact on behavior, several countries have successfully reduced COVID-19 cases and deaths by maintaining these results for a long time with long-term maintenance of some of the measures related to masks, social distancing, and control of imported cases. The success depends on the reaction and resolution of the governments and how the information has been presented to the public. Unfortunately, there is no universal communication policy for providing information during a prolonged crisis. But if the right, comprehensive, and scientific information reaches the citizens, it also will help control and mitigate the pandemic. Clear and accurate messages made by medical and scientific professionals delivered through appropriate platforms (media, social networks, and other non-government organizations) will ultimately lead to long-term success. But this is a complicated process and much depends on maintaining public confidence.

An overall policy can be outlined, including a communication strategy to refute the available disinformation with scientific data and evidence and different variants to clarify the importance of vaccination programs during the COVID-19 pandemic.

In December 2020, data showed that New Zealand had 420 reported cases and 5 deaths per million population. In the United States, there were 51655 cases and 937 deaths per million. Australia also reported lower numbers for the second week of December 2020 than other European countries - 1094 cases and 35 deaths per million population[41,42].

One of the main factors contributing to Australia's success was its geographical isolation and consensus among political circles and scientific councils on public health about the measures. A multidisciplinary group was formed, including experts from the country's eight leading universities [43]. The aim was to prepare an independent report to acquaint the entire government with the country's situation and give recommendations and guidelines for managing the crisis[44]. They pro-

posed a strategy in which communication in public health is paramount to tackling the pandemic and involves both politicians and communities

There are various recommendations for communication during a crisis. It is imperative to provide specific information on what to do and avoid for certain periods. Clear rules are essential when some restrictions are stringent. There must be absolute consistency in the messages and maximum reliability of the data provided to the public. The field's specialists and scientists should be used, although the public trust in them is not by presumption[45]. Confidence can be quickly lost if the expert is politically committed.

Politicians should listen to the community's needs and concerns when communicating. People are more likely to follow the pieces of advice if they understand the logic behind them. Therefore, explaining why specific actions are critical, beneficial, or problematic is essential. In addition, information concealment can motivate people to look for information elsewhere, promoting belief in rumors, misinformation, and conspiracy theories[46]. There is always uncertainty in crisis management, so there should be no illusion of false security for people because trust will be undermined[47].

People must also be allowed to get involved in the action. This means that communication must be attended to by appropriate measures to favor changes in the behavior and motivation of the population [48]. People are more likely to comply with quarantine if they have the financial and economic resources to endure a period of unemployment. The role of the government is to call for public solidarity and sustainability[49]. Fear and stress are reduced when people are part of a group and are supported. Then work, responsibilities, home life, and even helping others are often at the forefront of their minds.

The outbreak of the COVID-19 pandemic showed another lousy feature of humanity - its lack of faith in science and the scientific community. As a result, we have witnessed the rise of misinformation and conspiracy theories[50,51]. Transparent providing of factual information prevents susceptibility to emerging misinformation and conspiracy.

In combat against misinformation, specific techniques are used to reduce the spread of fake news significantly[52,53]. But again, public trust in government and health institutions is the most important and protective factor against people looking for opportunities for conspiracies[50].

Another critical factor that plays a crucial role in mitigating and controlling the COVID-19 pandemic is vaccination[13,54-58]. Several vaccines have already been approved and available, but vaccination campaigns in some countries are going slowly. It was assumed that the presence of collective immunity would control the pandemic[56]. However, suppose high morbidity, high mortality and adverse economic effects should be avoided. In that case, the vast part of the population must acquire immunity through vaccination but not through past infection. While the global eradication of COVID-19 may prove very difficult to achieve[13,58], successful vaccination programs can focus on regional elimination in the short term. Vaccinations will therefore have a critical impact on the dynamics and management of the COVID-19 pandemic.

If vaccination programs are modeled and combined with disease dynamics and available virus transmission data, a very effective strategy can be obtained that will lead to the successful long-term management of COVID-19. Monitoring, testing, and isolation will remain important factors in controlling the COVID-19 pandemic. Still, the effectiveness of the vaccination program and the level of vaccination will outweigh these factors in eliminating and stabilizing the COVID-19 pandemic.

The proposed approaches to control in all possible directions are not without challenges. We have seen that many factors affect the management of a pandemic. In addition, SARS-CoV-2 is evolving quite rapidly, and the efficacy of the available vaccines against new strains can be much lower. Despite these challenges, the successful implementation of COVID-19 vaccination programs will lead to pandemic control and a return to everyday life.

CONTROLLING IMPORT OF CASES

One of the most important aspects of nonlinear systems is size-dependent behavior. Large systems are inherently harder to stabilize, while smaller systems are much more easily influenced. This explains the hierarchical nature of multicellular organisms with cells, tissues, organs, and systems with separate feedback control mechanisms for different levels[59]. Pandemics are interactions in nonlinear systems comprised of humans, other life forms, and pathogens. A straightforward way to control a pandemic better is to decrease its size. This can mean lowering prevalence in a given area or localizing the pandemics by breaking the dependencies between different world regions. This necessitates control of imported cases. Imported cases can sustain a pandemic even in the absence of local transmission, and failing to account for them will lead to inappropriate responses with measures that are not focused on targeting the imports and exert unnecessary harsh efforts on the community[60]. Controlling imported cases is much easier and more reliable to be done at the borders of a given region instead of doing it after the patient has arrived, which is essential. The latter requires a high-cost, high-tech approach, while the former does not. This is a standard practice in the Chinese response to both pre- and after case imports[61].

CONCLUSION

Although control theory has been developed for engineering, it has found applications in systems biology and pandemic control. It is an abstract, mathematical theory that can be used to analyze and control any dynamic system as long as the calculated measures have the means to be physically applied. The ongoing COVID-19 has been mainly a challenge for epidemiology and pandemic control. It is a novel type of pandemic with multiple different variants emerging consequentially, resulting from the varied responses in other countries and regions and the abandoning of principles of pandemic control in some of them.

However, we know what to do and what not, thanks to management theory and the real-world applications in countries such as New Zealand, China, Australia in zero COVID and Norway, Denmark, Finland, South Korea, and Japan in disease containment. Mathematical modeling has been crucial in pandemic control as means of prediction that allow rapid response to newly found clusters and proper choice of working measures such as social distancing, masks, control of imported cases, *etc.* In addition, localization of pandemic control has been crucial by dividing the world into smaller regions that are easier to manage.

Communication strategies and transparency have helped with compliance and the overall success of non-pharmaceutical interventions. Focusing on long-term health and economic results helped motivate large parts of the industry and the politicians to join the effort. These are the prosperous regions. In policies that fail, multiple causes exist - weak links at the government level and at the social and community levels, including the scientific community that leads to a slow and reactive approach. Measures that are being implemented and lifted too early, lack of consistency with policy, and lack of adaptivity diverged from the basic control theoretic principles.

FOOTNOTES

Author contributions: Tomov L and Velikova T designed the research; Sekulovski M, Miteva D, and Batselova T performed the research; Tomov T contributed analytic tools; Tomov T, Velikova T, and Batselova H analyzed the data; Tomov T, Sekulovski M, Miteva D, and Batselova H wrote the paper; Velikova T revised, supervised, and edited the paper. All authors revised and approved the final version of the manuscript.

Conflict-of-interest statement: All authors declare no conflict of interest for this article.

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S-Editor: Wang LL
L-Editor: Wang TQ
P-Editor: Wang LL

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World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2022 September 20; 12(5): 402-413

DOI: 10.5662/wjm.v12.i5.402

ISSN 2222-0682 (online)

MINIREVIEWS

Non-medicalization of medical science: Rationalization for future

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Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Kamrul-Hasan ABM, Bangladesh; Ng HY, China

Received: March 20, 2022
Peer-review started: March 20, 2022
First decision: June 8, 2022
Revised: June 13, 2022
Accepted: July 20, 2022
Article in press: July 20, 2022
Published online: September 20, 2022

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Abstract

As we delve into the intricacies of human disease, millions of people continue to be diagnosed as having what are labelled as pre-conditions or sub-clinical entities and may receive treatments designed to prevent further progression to clinical disease, but with debatable impact and consequences. Endocrinology is no different, with almost every organ system and associated diseases having subclinical entities. Although the expansion of these "grey" pre-conditions and their treatments come with a better understanding of pathophysiologic processes, they also entail financial costs and drug adverse-effects, and lack true prevention, thus refuting the very foundation of Medicine laid by Hippocrates "Primum non nocere" (Latin), *i.e.*, do no harm. Subclinical hypothyroidism, prediabetes, osteopenia, and minimal autonomous cortisol excess are some of the endocrine pre-clinical conditions which do not require active pharmacological management in the vast majority. In fact, progression to clinical disease is seen in only a small minority with reversal to normality in most. Giving drugs also does not lead to true prevention by changing the course of future disease. The goal of the medical fraternity thus as a whole should be to bring this large chunk of humanity out of the hospitals towards leading a healthy lifestyle and away from the label of a medical disease condition.

Key Words: Prediabetes; Subclinical hypothyroidism; Osteopenia; Mild autonomous cortisol secretion; Pre-clinical; Medicalization

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Core Tip: In this article we discuss several pre-clinical conditions (subclinical hypothyroidism, pre-diabetes, osteopenia, and minimal autonomous cortisol excess), highlighting the futility of early treatment which may not alter the course of future disease. The medical community needs to exercise restraint with pharmacological measures where changes in lifestyle could play a more decisive role in leading a healthy life. Although the expansion of these "grey" pre-conditions and their treatments come with a better understanding of pathophysiologic processes, they also entail financial costs and drug adverse-effects, and lack true prevention, thus refuting the very foundation of Medicine laid by Hippocrates "Primum non nocere" (Latin), *i.e.,* do no harm.

Citation: Mittal M, Jethwani P, Naik D, Garg MK. Non-medicalization of medical science: Rationalization for future. *World J Methodol* 2022; 12(5): 402-413
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/402.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.402

INTRODUCTION

Background

It was David Humes, who more than 250 years ago had discerningly said that "It is impossible to separate the chance of good from the risk of ill", an adage that holds even more meaning today than ever before. In today's era of rapid progress in medical science, the lines have been blurred between "ease" and "dis-ease" with not only medical terms for pre-clinical medical conditions but also various pharmacological treatments being encouraged to "prevent" the development of clinical diseases. The rationale for such treatments remains debatable and controversial.

The concept of medicalization dates back to 1968 when Zola[1] (1972) defined medicalization as "an effective means of social control". Thereafter, Conrad, one of the pioneers of medicalization, defined it as a "process by which non-medical problems become defined and treated as medical problems, usually in terms of illness and disorders"[2]. As a continually evolving term over decades, medicalization can range from sexuality to garden variety mood disturbances, from childbirth to menopause, from cancer to ageing, blurring the lines between physiological and disease states. While physicians' social movements used to be at the crux of medicalization when it was first introduced as a concept in the 1970s, the major players driving medicalization today have been the pharmaceutical and biotechnology industries, posing ethical concerns. The other main reason is the urge to find something "new" or target newer/higher/lower goals in therapeutics by medical scientists, leading to a flurry of pre-clinical labels to almost every chronic disease.

In this article we discuss a few of these pre-clinical conditions [subclinical hypothyroidism, pre-diabetes, osteopenia, and minimal autonomous cortisol excess (MACS)], highlighting the futility of overtreatment which may be variable early in the course. The medical community needs to exercise restraint with pharmacological measures where changes in lifestyle could play a more decisive role in leading a healthy life.

SEARCH STRATEGY

The following databases were used to identify the relevant studies: PubMed/Medline, Scopus, and Cochrane databases. We also applied RCA (Reference Citation Analysis) to further enhance our search results. All the databases were searched from their inception till March 10, 2022. We did a search again and the search was extended up till June 10, 2022 to look for any additional articles. Keywords used were mainly related to the topics of interest including "prediabetes" or "impaired fasting glucose" or "impaired glucose tolerance", "subclinical hypothyroidism", "osteopenia" or "low bone mass", and "mild autonomous cortisol excess". Reference lists of review articles and guidelines were also scanned for potential articles for inclusion. There was no restriction for study design and language (where English language translation was available). All articles related to non-medicalization were reviewed and those relevant to the topics of interest were considered for inclusion in this scoping review.

MACS

MACS is defined as adrenocorticotropic hormone independent cortisol excess often without clinical signs and symptoms of Cushing's syndrome. Previously referred to with terms including "subclinical Cushing's syndrome", "preclinical Cushing's syndrome", and "subclinical hypercortisolism", currently the European Society of Endocrinology/European Network for the Study of Adrenal Tumors has suggested the use of the term "minimal autonomous cortisol excess" which is universally used[3]. The

term was first introduced as "Pre-clinical Cushing's syndrome" by Charbonnel and coworkers in 1981 [4]. The entity has since made rapid strides and undergone major changes with respect to its understanding, nomenclature, and definitions used for diagnosis.

Approximately 0.3% of adrenal incidentalomas present with Cushing's syndrome[5]. MACS is diagnosed in 5%-48% of patients with incidentally discovered adrenal tumors following evaluation in endocrine clinics[6,7]. The diagnosis of MACS is made in patients with adrenal incidentaloma by an abnormal 1-mg dexamethasone suppression test (DST) with absent stigmata of Cushing's syndrome. The 1-mg DST has been recommended by current recommendations to have the highest sensitivity for diagnosing MACS[3,8]. However, the diagnosis requires exclusion of potential causes of physiological hypercortisolism such as obesity, diabetes, and anxiety/depression. These have also been described as comorbidities associated with MACS and can act as potential confounders in diagnosis.

In a recent systemic review and meta-analysis done by Elhassan *et al*[9] involving more than 4000 patients with benign adrenal incidentalomas, the prevalence of hypertension, obesity, dyslipidemia, and type 2 diabetes in the MACS sub-group was 64%, 41%, 34%, and 28% respectively. This prevalence of dyslipidemia and obesity was almost similar to that of non-functioning adrenal tumors (NFAT) and only hypertension and diabetes were more common in the MACS subgroup. Although hypertension, dyslipidemia, and diabetes were likely to worsen in the MACS sub-group on follow-up, progressive worsening is the *sine qua non* in most chronic conditions. The results have been conflicting with cardiovascular events being prevalent in non-functioning adrenal tumors at baseline (8.7% *vs* 6.3%), and new cardiovascular events developing in MACS than NFAT (15.5% *vs* 6.4%). On top of it, the mortality rates were similar between the two groups despite these differences in metabolic complications. This would mandate a cautionary approach to managing "mild" hypercortisolism based on no difference shown in mortality outcomes, thus putting in doubt the benefit of any intervention at this so-called pre-clinical stage

Patients with MACS have also been found to carry a risk of osteoporosis and mostly asymptomatic vertebral fractures (46%-82%), as compared to 13%-23% of patients with non-functioning adrenal incidentalomas[10]. A study done by Goh *et al*[11] followed 101 patients with benign adrenal adenomas over a 3-year follow-up period. Ninety-two patients had a diagnosis of non-functioning adenomas while nine had a diagnosis of MACS defined as an abnormal 24-h urinary free cortisol and 1mg-DST. After 3 years (range 2.9-4.7 years), four of the nine patients with MACS showed normalization of cortisol parameters (44%), and five of the 92 non-functional AI patients developed MACS (5%). Nearly half of the patients with MACS had normalization of biochemical parameters on follow-up. Whether the initial diagnosis was because of a false-positive test in the first place, or a spontaneous reversal of the cortisol excess, the more important fact is that only 9% of patients had MACS initially and more than half of them normalized on follow-up. Additionally, the risk of progression to overt Cushing's remains very low (< 1%) in patients with MACS, as has been uniformly reported across multiple studies[12-14]. Hence, this should prompt a more vigilant interpretation of any results which imply a higher risk for complications in MACS. This also suggests that caution be exercised with regard to diagnostic interpretation and adopting invasive management decisions.

There remains a need to establish the benefits of adrenalectomy with regard to mortality, quality of life, and potential reversal of these comorbidities in light of the doubtful clinical impact of MACS. A meta-analysis done by Bancos *et al*[15] involving retrospective studies with heterogeneous definitions of MACS showed improvement in hypertension (relative risk [RR] = 11, 95%CI: 4.3–27.8) and diabetes mellitus (RR = 3.9, 95%CI: 1.5–9.9), but not dyslipidemia (RR = 2.6, 95%CI: 0.97–7.2) or obesity (RR = 3.4, 95%CI: 0.95–12) when compared with conservative management. A study done by Salcuni *et al*[16] found a 30% vertebral fracture risk reduction with surgical *vs* conservative management (odds ratio = 0.7, 95%CI: 0.01-0.05, P = 0.008). There have been few studies on the potential use of Mifepristone and Metyrapone in MACS demonstrating beneficial effects on several metabolic parameters[17,18].

All such evidence is largely based on small, heterogeneous studies with inconsistent definitions of MACS and comorbidities as well as degrees of improvement. With the knowledge that half of MACS cases return to normalcy (nonfunctional status), < 1% progress to Cushing's syndrome, and a major surgery involves risks and morbidity, publication bias for positive results certainly calls into question this medicalization.

Moreover, differences in assay factors and the presence of significant comorbidities may lead to possible false-positive results leading to misclassification of patients and adding to bias.

The 2016 European guidelines for the management of adrenal incidentaloma currently suggest an individualized approach. They do recommend surgical management in patients with post-dexamethasone cortisol > 138 nmol/L (> 5 μg/dL) and the presence of at least two comorbidities potentially related to cortisol excess (*e.g.*, type 2 diabetes, hypertension, obesity, and osteoporosis), of which at least one is poorly controlled by medical measures. One needs to weigh the risks and morbidity associated with an adrenal surgery with the perceived benefits. A causal link between MACS and these comorbidities has not been unequivocally established, and these comorbidities can usually be efficaciously managed with medical treatment. Robust randomized trials comparing intensive medical therapy and adrenalectomy, with consistent definitions, appropriately defined endpoints, and a longer duration of follow-up, may bust the myth of operating on MACS.

Subclinical hypothyroidism

As an entity which has been in vogue over the past few decades, subclinical hypothyroidism is essentially a biochemical diagnosis, defined as an elevated thyrotropin (TSH) level with a fT4 level that is within the population specific range.

The prevalence of overt hypothyroidism ranges from 0.2%-5.3%[19] while the prevalence of subclinical hypothyroidism varies from 4.3% to 15% (3× to 150×), with a multitude of factors affecting incidence, including female gender, age, and iodine status[20]. Serum TSH levels in subclinical hypothyroidism have been classified into two categories ranging from the upper limit of normal to 10 and 10 or higher. What is interesting to note is the fact that more than 90% of patients fall in the earlier bracket. Even more striking is the fact that the risk of progression to overt hypothyroidism is only 2% per year in the absence of thyroid peroxidase (TPO) antibodies and 4% per year in the presence of TPO antibodies. As many as two-thirds (approximately 60%) of these individuals see their TSH levels return to the normal range without treatment[21]. Simply put, treating 100 patients for hypothyroidism with TSH between 4-10 mU/mL will potentially avoid progression to overt hypothyroidism in 2-4 patients while what they add on to are unnecessary multiple clinic visits, treatment costs, polypharmacy, and modification of daily habits including taking the tablet 30-60 min before a meal in the rest. The question that matters "is it really worth it?" and are we wasting 96% of our efforts? Would it not be prudent to wait for identifying and then treating only those who actually progress to overt hypothyroidism unless there are significant immediate medical concerns which may benefit from treatment as in infertility or in pregnant females?

There are a number of factors affecting TSH levels. TSH levels tend to follow a circadian fluctuation with nadir levels in the afternoon and only 30% having higher levels in the evening and night. This TSH peak may also be altered in night-shift workers and those with irregular sleep patterns, following vigorous exercise, and mood disorders. Moreover, although population specific reference ranges have been defined for fT4 and TSH concentrations, the intra-individual hypothalamic-pituitary axis set point is largely genetically determined and is minutely sensitive to changes in thyroxine concentrations, which despite being within the population specific range, may be enough to result in an increased TSH concentration. Furthermore, due to alterations in the hypothalamic-pituitary TSH set point, there is a trend towards elevated TSH concentrations with advancing age which is a physiological change. Besides this, there are several conditions which should be ruled out before making a diagnosis of subclinical hypothyroidism (SCH)[22] (Table 1).

Taking these factors into account, the guidelines clearly mention that a diagnosis of subclinical hypothyroidism should be made following confirmation with a repeat TSH and T4 measurement[23] and even when reconfirmed on second testing, only a subset may need treatment. However, one-third of the people are offered treatment after a single TSH measurement making them "patients" and making levothyroxine one of the leading prescriptions worldwide[24].

Symptoms and quality of life: With regard to symptomatology, around one in three patients with subclinical hypothyroidism are asymptomatic. Symptoms when present, tend to predominantly be fatigue, muscle weakness, and cold intolerance. However, around 20%-25% of patients with normal TSH levels report these symptoms. A meta-analysis done by Feller *et al*[25] in 2018 on quality of life showed no difference in hypothyroid symptoms (16.7 *vs* 16.5) or fatigue (28.6 *vs* 29) as assessed using the ThyPRO self-reported instrument (scale 0–100, lower better), or in health related quality of life, depression, cognitive function, or muscle strength. About treatment on the various aspects of hypothyroidism, the largest trial done to date, the TRUST trial, did not demonstrate any effect of levothyroxine on the coprimary outcomes of hypothyroid symptoms and fatigue scores after 12 mo of therapy nor on the secondary outcomes of quality of life, handgrip strength, cognitive function, blood pressure, weight, body mass index (BMI), waist circumference, or carotid plaque thickness[26].

Cardiovascular risk: Amongst the various organ systems that thyroid hormone does play a role in, its effects on the cardiovascular system have been evaluated most extensively. Multiple studies have found that surrogate markers of cardiovascular function (such as left ventricular diastolic function) and lipid profile deteriorate with subclinical hypothyroidism[27,28]. However, while these observations seem to suggest that raised TSH levels may be associated with an increased risk of adverse cardiovascular outcomes, such has not been the case in multiple randomized trials. Subclinical hypothyroidism was not associated with an increased risk of atrial fibrillation, heart failure, stroke, coronary heart disease events, mortality from coronary heart disease, or overall mortality compared with euthyroid individuals in an individual patient meta-analysis performed by the Thyroid Studies Collaboration[29-32]. There are limited randomized clinical trials with sufficient power to examine the impact of thyroid hormone therapy on cardiovascular events, and the TRUST study found that treatment of SCH did not impact the incidence of cardiovascular events within 1 year after initiation of therapy[26].

Treatment: Current guidelines recommend that all individuals with subclinical hypothyroidism would not benefit from treatment and clinicians need to weigh other factors when deciding on treatment[33-35]. Treatment guidelines for subclinical hypothyroidism have been summarized[36-38]. To conclude, there is significant evidence to suggest that subclinical hypothyroidism remains an entity which is

Table 1 Causes of elevated thyrotropin levels

Transient increase in TSH	Permanent increase in TSH
Non-thyroidal illness	Assay interference
Thyroiditis	TSH hormone resistance
Medications: Amiodarone and Lithium	Adrenal insufficiency
Lack of adherence to treatment	Obesity

TSH: Thyrotropin.

misdiagnosed and largely over-treated, and there is a need to improve adherence to the guidelines (Table 2) with periodic reassessment of symptoms, with discontinuation of treatment if and when no benefit becomes evident.

Prediabetes

Pre-diabetes, a term developed to pre-empt the progression to diabetes, represents an intermediate state of hyperglycemia. Varying definitions have been used by the World Health Organization (WHO) and the American Diabetes Association (ADA) to define prediabetes, classifying them into impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) depending on fasting plasma glucose levels and 2-h plasma glucose levels after a 75 g oral glucose tolerance test. ADA, in addition, uses hemoglobin A1c (HbA1c) too to classify diabetes[39,40].

The worldwide prevalence of diabetes ranges from 6% to 10.5% in adults[41,42] while the prevalence of pre-diabetes ranges from 5.5% to around 50%[43] depending on the ethnicity and definitions used to define pre-diabetes (Table 3). The yearly conversion rate from pre-diabetes to diabetes is 5%-20%, with rates ranging from 4%-6% for isolated IGT, 6%-9% for isolated IFG, and 15-19% for those with both IFG and IGT[44]. The reversal rates vary from 45% for individuals with IFG, 37% for individuals with IGT, and 17% for individuals with impaired HbA1c levels[45].

Complications of pre-diabetes: The strongest evidence for pre-diabetes comes with cardiovascular complications[46]. In a meta-analysis done by Huang *et al*[47], IFG, IGT, and HbA1c were independently associated with an increased risk of composite cardiovascular outcomes, coronary heart disease, stroke, and all-cause mortality. In a meta-analysis done by Echouffo-Tcheugui *et al*[48], pre-diabetes was associated with a moderately increased risk of CKD. Several studies have shown similar results with increased rates of micro-albuminuria and progression to chronic kidney disease in prediabetes[49-51]. Pre-diabetes also has been associated with an increased prevalence of diabetic neuropathy, especially autonomic involvement, and increased risk of diabetic retinopathy[52,53].

Treatment: Multiple randomized control trials including the diabetes prevention program (DPP)[54], the Finnish diabetes prevention study[55], and the Da Qing diabetes prevention study[56] demonstrated that lifestyle/behavioral therapy is highly effective in preventing progression to type 2 diabetes. In a recent meta-analysis, lifestyle interventions for obese or overweight individuals with pre-diabetes led to a reduced incidence of diabetes[57]. The DPP for instance demonstrated that an intensive lifestyle intervention could reduce the risk of incident type 2 diabetes by 58%. To prevent one case of diabetes during a period of 3 years, 6.9 persons would have to participate in the lifestyle-intervention program, and 13.9 would have to receive metformin. The ADA currently recommends lifestyle modification for pre-diabetes with a target of 7% weight loss and 150 min/wk of moderate intensity physical activity[58].

While weight loss does lead to a definite reduction in incident type 2 diabetes, it often comes with the challenge of sustaining it long-term[59]. Multiple pharmacological agents have been evaluated with the strongest evidence and long-term safety favoring metformin. In the Indian Diabetes Prevention Programme (IDPP-1) study, metformin and lifestyle intervention reduced diabetes risk similarly at 30 mo although the lifestyle intervention in the Indian DPP-1 was less effective than the DPP[60]. The ADA currently recommends that metformin should be considered in those with BMI ≥ 35 kg/m^2, those aged < 60 years, and women with prior gestational diabetes mellitus[58].

While current evidence does suggest the effectiveness of treatment modalities for the prevention of pre-diabetes to diabetes, the long-term effects on microvascular and macrovascular complications remain debatable. Moreover, although pharmacotherapy has been found to be beneficial in preventing type 2 diabetes, questions regarding the starting and endpoint of therapy, long-term safety of other potential drugs, and economic considerations regarding its cost-effectiveness and health benefits remain unanswered.

Shahraz *et al*[61] using NHANES data showed that a widely promoted web-based risk test by ADA and AMA would label more than 73 million Americans, including more than 80% of those older than 60 years, as being at high risk for "prediabetes", thus elegantly demonstrating how common conditions can

Table 2 Guideline recommendations for treatment of subclinical hypothyroidism

Degree of subclinical hypothyroidism	ATA 2012[36]	ETA 2013[37]	NICE 2019[38]
TSH > 10 mIU/L	Levothyroxine should be considered. (Grade B)	Younger patients (< 65 to 70 yr): Treatment with levothyroxine is recommended, even in the absence of symptoms. (Grade 2); Older patients (> 70 yr): Treatment with levothyroxine should be considered if clear symptoms of hypothyroidism are present or if the risk of vascular events is high. (Not a graded recommendation, but part of the treatment algorithm)	All adults (on 2 occasions, 3 mo apart) consider treatment.
TSH: ULN to 10 mIU/L	Treatment should be considered on the basis of individual factors (*i.e.*, symptoms suggestive of hypothyroidism, a positive test for antibodies to thyroid peroxidase, or evidence of atherosclerotic cardiovascular disease, heart failure, or associated risk factors for these diseases). (Grade B, because of a lack of randomized, controlled trials)	Younger patients (< 65 to 70 yr): A trial period of treatment with levothyroxine should be considered when symptoms suggestive of hypothyroidism are present. (Grade 2); Older patients (especially > 80 to 85 yr): Careful follow-up with a wait-and-see strategy, generally avoiding hormonal treatment, is recommended. (Grade 3)	Age < 65 years (on 2 occasions, 3 mo apart): Consider a 6-mo trial of levothyroxine if symptoms are present.

ATA: American Thyroid Association; ETA: European Thyroid Association; NICE: National Institute for Health and Care Excellence; TSH: Thyrotropin.

Table 3 Criteria for prediabetes

	WHO[39]	ADA[40]
FPG (mg/dL)	110-125	100-125
2-h plasma glucose (mg/dL)	140-199	140-199
HbA1c (%)		5.7-6.4

ADA: American Diabetes Association; FPG: Fasting plasma glucose; HbA1c: Hemoglobin A1c; WHO: World Health Organization.

be "medicalized".

Osteopenia

Osteopenia, a term which came into being by the WHO in 1992, was initially "meant to indicate the emergence of a problem without having any diagnostic or therapeutic significance". The WHO currently defines osteopenia as a bone mineral density (BMD) T score that is higher than −2.5 but less than −1.0[62,63].

The prevalence of osteoporosis ranges from 2% to 26.3%[64] while that of osteopenia is two to three times higher, varying from 54% to 80%[65]. Because osteopenia is so much more prevalent than osteoporosis, the majority of fractures occur in women with osteopenia. In the National Osteoporosis risk assessment study which involved 149542 postmenopausal women followed for 1 year, the gross disparity in the proportion of women with osteopenia and osteoporosis (39% *vs* 6%) meant that more fractures were observed among women with osteopenia despite that they had a lower risk for the same.

When we look at the temporal transition of osteopenia to osteoporosis in a study done by Gourlay *et al*[66] which included 3702 women with osteopenia, the investigators estimated the time for 10% of the women to transition to osteoporosis before having a hip or clinical vertebral fracture. Women were stratified into three subgroups: Mild osteopenia (T score from −1.01 to −1.49), moderate osteopenia (T score from −1.50 to −1.99), and severe osteopenia (T score from −2.00 to −2.49). For 10% of the women to transition to osteoporosis, it took 17 years for those with mild osteopenia, 5 years for those with moderate osteopenia, and 1 year for those with severe osteopenia. Moreover, there was no difference in time needed to transition to osteoporosis among women with normal bone density and mild osteopenia.

Hillier *et al*[67] performed a study including a large cohort of women aged 65 years and older with osteopenia at baseline; 4.7% and 15.7% of these women developed hip and a major osteoporotic fracture, respectively, within 10 years. The corresponding values were 1.2% and 6.3% for women with normal BMD and 14.3% and 30% for women with osteoporosis.

While lifestyle measures along with appropriate intake of calcium and vitamin D can be uniformly recommended to all women with osteopenia, pharmacological treatment remains largely debatable[68,69]. A variety of algorithms and clinical tools such as the fracture risk assessment tool have been developed to enable physicians in stratifying women with osteopenia and decide on potential indications of treatment. Most trials evaluating the efficacy of these agents involve women with

Table 4 Criteria for defining osteopenia

Category	Definition	Treatment recommendation
"Moderate risk", Endocrine Society guidelines 2019[74]	Clinical: No prior hip or spine fractures, BMD T-score at the hip and spine both above -2.5, 10-yr hip fracture risk < 3% or risk of major osteoporotic fractures < 20%	Reassess fracture risk in 2-4 yr. Country-specific guidelines for treatment
ISBMR guidelines 2021 [75]	BMD T-score between -1.0 and -2.5 at the femoral neck or lumbar spine, 10-yr probability of a hip fracture ≥ 3.5%, or a 10-yr probability of a major osteoporosis-related fracture ≥ 10.5% based on the FRAX tool (based on limited data in Indians)	Advisable to initiate treatment

BMD: Bone mineral density.

osteoporosis or prevalent vertebral fractures with fewer trials in osteopenic women. In the fracture intervention trial, alendronate was not associated with a reduced risk of sustaining a vertebral fracture among women with a T score between −1.6 and −2.5 (hazard ratio = 0.8; 95%CI: 0.3–2.1)[70]. In a RCT done by McCloskey *et al*[71], the number needed to treat (NNT) to prevent the occurrence of one clinical fracture was three and a half times higher among women with T>-2.5 than women with osteoporosis (NNT = 66 *vs* 19) despite a similar RR reduction (22% *vs* 30%). Hence, while RR reduction might appear greater in terms of numbers, it does not quite translate into significant numbers in terms of absolute risk reduction. Cost-effectiveness is another factor that needs to be taken into consideration. Studies done by Schousboe *et al*[72] and Meadows *et al*[73] have assessed the cost-effectiveness of prescribing alendronate among post-menopausal women with osteopenia and found that the drug is not cost-effective. The long duration of treatment, lack of defined endpoints, and the adverse effects associated with long-term use are other factors that need to be considered prior to initiating pharmacological therapy in osteopenia (Table 4)[74,75]. Hence, in the absence of unequivocal clinically and epidemiologically relevant benefits of pharmacotherapy, osteopenia essentially remains a radiological diagnosis in the absence of risk factors for fracture and is probably best managed by periodic monitoring and fracture risk assessment.

Reasons for progressive medicalization

There are many reasons why clinicians may provide more care than is needed. A primary reason is "technology creep". After a new drug or device is approved for use in a condition in which there is a proven benefit, its use often expands to lower-risk groups in which the benefit does not outweigh the risk. Others include payment systems that reward procedures disproportionately compared with talking to patients, expectations of patients who equate testing and interventions with better care, the glamour of technology, the fact that it may be quicker to order a test or write a prescription than explain to a patient why they are not being treated, and defensive medicine[76]. Even if a medical intervention has been shown to provide a clear benefit in selected groups, using it in other groups, especially in those with milder disease or at-risk group for disease, can result in harm.

It is worthwhile to note that providing excessive health care service is most likely to occur in situations in which there is less strong evidence to document the benefit and harms of the service. In fact, editors of *JAMA Internal Medicine* took note of "medicalization" of common conditions, as an area of increasing concern[77]. "Less is More" was a series used to highlight situations in which the overuse of medical care could result in harm and in which less care is likely to result in better health[78]. A comprehensive look at the four pre-clinical conditions is summarized in Table 5.

CONCLUSION

A fine balance between indiscriminate acceptance of medicalization of areas of human existence and blind criticism of new medicalization cases needs to be struck. A reasonable way to look at any chronic non-communicable disease should be to avoid unnecessary medicalization by medical labeling of the "grey zone" preceding a disease. Rather, it should seek to identify people at the highest risk for targeted allocation of limited health care resources and address the lifestyle changes which can improve the overall health of the community. Having a proven therapeutic intervention for a disease does not pre-validate its use in the pre-clinical stage of the same disease and can lead to more harm than good.

The time is more than ripe for paying heed to hard facts and sane logic both for the patient as well as the medical community for treading carefully with regard to early interventions for "preclinical and subclinical" conditions in medical science.

Table 5 Clinical spectrum of preclinical conditions: Looking at hard facts

	Prediabetes	Subclinical hypothyroidism	Osteopenia	MACS
Clinical disease	Diabetes	Overt primary hypothyroidism	Osteoporosis	Cushing's syndrome
Prevalence of preclinical condition	5.5%-53.1%[43], IFG - 6.2%[41], IGT -10.6% [41]	4.3%-15%[20]	54%-80%[65]	5%-48%[6]
Prevalence of clinical condition	10.5%[41]	0.2%-5.3%[19]	2%-26.3%[64]	0.3% of patients with adrenal incidentalomas[5]
Dx criteria	FPG: 100-125, 2-h PPG: 140-199, HbA1C: 5.7-6.4	Elevated TSH level with a fT4 level that is within the population specific range	T-score between -1 to -2.5	Abnormal 1-mg dexamethasone suppression test with absent stigmata of Cushing's disease.
Progression	5%-18.3%[54,55,60]	2%-6%[22]	16% risk of major osteoporotic fracture in 10 years [67]	< 1%[13]
Regression/reversal	19%[54]	60%[21]	Stays static or progresses	2%-44%[6,11]
Long-term sequelae	Microvascular and macrovascular complications of diabetes, Cardiovascular risk	Markers of cardiovascular function (such as left ventricular diastolic function) and lipid profile deteriorate with subclinical hypothyroidism	Fractures	Hypertension, Diabetes, Dyslipidemia, Osteoporosis
Short-term consequences		Fatigue, muscle weakness, cold intolerance		
Preventive options	Lifestyle and behavioural therapy, drugs	Lifestyle and behavioural therapy, drugs	Lifestyle and behavioural therapy, drugs	Lifestyle and behavioural therapy, drugs, surgery
Pharmacotherapy	Metformin	L-thyroxine	Calcium and vitamin D	Mifepristone, metyrapone
Surgery	-	-	-	Adrenalectomy
True prevention	x	x	x	x
Adverse effects of treatments available	B12 deficiency	Bone loss, cardiac arrhythmias in elderly	Overtreatment can predispose to hypervitaminosis D	Hypocortisolism
Recommendations/Guidelines	Metformin should be considered in those with BMI ≥ 35 kg/m^2, those aged < 60 yr, and women with prior gestational diabetes mellitus with IGT	TSH > 10 mIU/L, consider treatment; TSH < 10 mIU/L, consider treatment if symptoms suggestive of hypothyroidism, positive antibodies to thyroid peroxidase, or evidence of atherosclerotic cardiovascular disease, heart failure, or risk factors for these diseases	Country-specific guidelines for treatment	Individualized approach to consider patients with 'autonomous cortisol secretion' due to a benign adrenal adenoma and comorbidities potentially related to cortisol excess for adrenal surgery
Grade of recommendation	Level of evidence A[58]	Grade B, BEL 1 (Best evidence rating level)[36]	-	(⊕OOO) Very low level of evidence/recommendation[3]

MACS: Minimal autonomous cortisol excess; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; FPG: Fasting plasma glucose; HbA1C: Hemoglobin A1c; PPG: Photoplethysmography; TSH: Thyrotropin; BMI: Body mass index.

FOOTNOTES

Author contributions: Mittal M was involved in conceptualization and design of the manuscript; Mittal M and Jethwani P performed the search and wrote the manuscript; Mittal M, Naik DB, and Garg MK edited and reviewed the manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest to disclose.

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S-Editor: Liu JH
L-Editor: Wang TQ
P-Editor: Liu JH

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 414-427

DOI: 10.5662/wjm.v12.i5.414

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Observational Study

Migraine in physicians and final year medical students: A cross-sectional insight into prevalence, self-awareness, and knowledge from Pakistan

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Specialty type: Neurosciences

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Hasabo EA, Sudan; Lu H, China

Received: April 28, 2022
Peer-review started: April 28, 2022
First decision: June 8, 2022
Revised: June 22, 2022
Accepted: August 21, 2022
Article in press: August 21, 2022
Published online: September 20, 2022

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Abstract

BACKGROUND

Despite its high prevalence, migraine remains underdiagnosed worldwide. A significant reason is the knowledge gap in physicians regarding diagnostic criteria, clinical features, and other clinical aspects of migraine.

AIM

To measure the knowledge deficit in physicians and medical students and to assess the prevalence of migraine in the same population.

METHODS

An online questionnaire was developed and distributed among physicians and final year medical students on duty in various medical and surgical specialties of Allied and DHQ Hospitals, Faisalabad, between October 2018 and October 2019. Inclusion criteria were public practicing physicians who experience headaches, while those who never experienced headaches were excluded. Different questions assessed respondents on their knowledge of triggers, diagnosis, management, and prophylaxis of the migraine headache. They were asked to diagnose themselves using embedded ICHD-3 diagnostic criteria for different types of migraine.

Graphs, tables, and figures were made using Microsoft Office 2016 and Microsoft Visio, and data analysis was done in R Studio 1.4.

RESULTS

We had 213 respondents and 175 fulfilled inclusion criteria, with 99 (52%), 58 (30%) and 12 (6.3%) belonging to specialties of medicine, surgery, and others, respectively. Both genders were symmetrically represented (88 male and 87 female). Fifty-two (24.4%) of our 213 respondents were diagnosed with migraine, with 26 (50%) being aware of it. Females had higher prevalence among study participants (n = 28, 32.2%) compared to males (n = 20, 22.7%, P = 0.19). A majority (62%) of subjects never consulted any doctor for their headache. Similarly, a majority (62%) either never heard or did not remember the diagnostic criteria of migraine. Around 38% falsely believed that having any type of aura is essential for diagnosing migraine. The consultation rate was 37% (n = 65), and migraineurs were significantly more likely to have consulted a doctor, and a neurologist in particular (P < 0.001). Consulters and migraineurs fared better in the knowledge of diagnostic aspects of the disease than their counterparts. There was no significant difference in other knowledge aspects between consulters *versus* non-consulters and migraineurs *versus* non-migraineurs.

CONCLUSION

Critical knowledge gaps exist between physicians and medical students, potentially contributing to misdiagnosis and mismanagement of migraine.

Key Words: Migraine; Headache disorders; Knowledge study; Prevalence; Knowledge; Epidemiology; Public health

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Core Tip: Despite its high prevalence, migraine remains underdiagnosed worldwide. A significant reason is the knowledge gap in physicians regarding diagnostic criteria, clinical features, and other clinical aspects of migraine. The primary objectives of this study were to measure the knowledge deficit in physicians and medical students and to assess the prevalence of migraine in the same population.

Citation: Choudry H, Ata F, Naveed Alam MN, Ruqaiya R, Suheb MK, Ikram MQ, Chouhdry MM, Muaz M. Migraine in physicians and final year medical students: A cross-sectional insight into prevalence, self-awareness, and knowledge from Pakistan. *World J Methodol* 2022; 12(5): 414-427

URL: https://www.wjgnet.com/2222-0682/full/v12/i5/414.htm

DOI: https://dx.doi.org/10.5662/wjm.v12.i5.414

INTRODUCTION

Headache is the top neurological complaint of patients presenting to general practitioners and neurologists. Migraine, one of the commonest headaches, is the sixth most prevalent disease globally and the second largest cause of disability, affecting more than 1 billion people[1,2]. Although not directly fatal, migraine results in considerable loss of work hours, productivity, and quality of life, culminating in a health burden and significant cost. In the United States alone, the annual expenditure on migraine exceeds 78 billion USD[3]. Despite being one of the top causes of morbidity, millions of migraine cases remain undiagnosed worldwide, leading to a preventable burden on the system[4]. This underdiagnosis has been attributed to lapses in physicians' knowledge and lack of patient consultation, besides various other factors[5,6]. A recent study published in 2021 revealed several knowledge gaps in primary care providers concerning migraine diagnosis, with only 6.3% of physicians aware of migraine prevention guidelines[7]. Studies have elucidated a significantly higher prevalence of migraine in physicians, attributed to the better knowledge of diagnostic criteria and a variety of presentations of this headache[8]. Studies have also shown a specifically higher prevalence of migraine in headache specialists (53% compared to 19.3% in general practitioners), relating it to a better knowledge of the diagnostic criteria of migraine[9].

Prevention is the key management strategy for a significant subset of the population experiencing migraines, particularly those who cannot take abortive treatment. Preventive strategies, including drugs, indications of prophylaxis, and avoidance of triggers, constitute an essential piece of knowledge for managing physicians in this regard. Some of the triggers of migraines may not be commonly known

by physicians, leading to incomplete medical advice and counseling. Studies have shown a significant difference in the discussion of migraine triggers (with the patients) among neurologists and other physicians (82% *vs* 51%)[10]. Only a physician adequately equipped with proper knowledge of prevention and triggers can manage migraine patients properly with a comprehensive education of prevention strategies. Lack of awareness of triggering factors among patients increases the frequency of otherwise avoidable exacerbations of migraine[11].

Pakistan's estimated 1-year prevalence of migraine (22.5%) is considerably higher than the global 1-year prevalence of 15%[12,13]. Headache patients present in the outpatient settings of multiple specialties of our hospitals, including surgery. Junior doctors (including sub-interns, *i.e.*, final year medical students) in Pakistan's public hospitals serve as the first contact with health care for most patients with headaches. Therefore, the knowledge, attitudes and practices related to headache serve a pivotal role in the accurate and timely diagnosis and management of patients with headache syndromes, including migraine. Knowing the types of migraines and diagnostic criteria, and screening tools for some common types are essential for correct diagnosis. Transient neurological disturbances, usually in the form of visual or auditory sensory issues that precede migraine headaches, are known as auras. Aura is not experienced by 60%–80% of migraine patients, leading to a diagnosis of migraine without aura[14]. Worldwide, some studies recently have highlighted the gaps in physicians' knowledge regarding the diagnosis of migraine[5,15,16]. We hypothesize similar gaps exist in our clinical settings in Pakistan; viewing aura as an integral part of the diagnosis of migraine being one such gap in knowledge. It is imperative for physicians to be aware of migraine without aura as it constitutes > 70% of migraine cases in Pakistan[17].

The aim of the study was to provide the first insight in the region into physicians' knowledge regarding the diagnosis and management of migraine. The primary objective was to gauge the knowledge of physicians and final-year medical students regarding the triggers, diagnosis, management and prevention of migraine in Pakistan. Secondary objectives included determining the awareness of their own migraine among migraineurs, as well as estimating the point-prevalence of migraine among the physician population in Pakistan. Moreover, we also sought an assessment of the attitudes of our respondents towards medical consult-seeking for their headaches and self-medication (without a medical consult).

MATERIALS AND METHODS

Study design

A web-based 30-question anonymous questionnaire was developed consisting of simple multiple choice as well as multiple choice–multiple response questions.

Participants

The questionnaire was distributed among physicians and final year medical students on duty in various medical and surgical specialties of Allied and DHQ Hospitals, the affiliated hospitals of Faisalabad Medical University. Participants were required to fill in the questionnaire in the presence of a team member to avoid misinterpretation of any question.

Inclusion criteria and data collection

Inclusion criteria were physicians and final year medical students who experienced headaches. Private practitioners and non-practicing physicians were excluded. The data were collected between October 15, 2018 and October 15, 2019.

Tools and variables

Respondents were asked if they thought they had a migraine and were then assessed on their knowledge of the definition, triggers and prophylaxis of the migraine, utilizing various subjective and objective questions. The triggers of migraine and the list of indications of prophylaxis of migraine were adopted from Kelman *et al*[19] and American Headache Society Consensus statement, respectively[18,19]. They were questioned about their knowledge of diagnostic criteria, prophylactic therapy and migraine triggers. They were also asked to choose appropriate answers from a list of available triggers of migraine and indications and duration for prophylaxis. Distractors were introduced in the triggers and indications of prophylaxis checklists to assess better for recognition (Figures 1 and 2). Migraineurs were further asked questions about their triggers, abortive and prophylactic therapy use and efficacy, medical consultation seeking, and over-the-counter (OTC) drug use. For diagnosis, ICHD-3 diagnostic criteria of migraine with aura, migraine without aura, and chronic migraine were embedded in the questionnaire. Respondents were asked to self-diagnose by matching their symptoms to these criteria within the questionnaire. Migraine cases (migraineurs) were the respondents who chose any type of migraine after going through all the diagnostic criteria. Self-awareness of migraine was defined as migraineurs who thought they had a migraine, while cases who answered "no" or "not sure" when

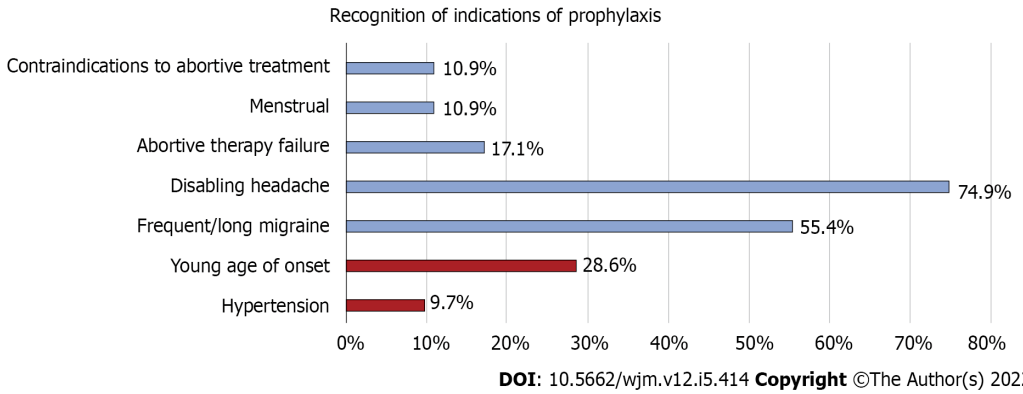

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Figure 1 Knowledge of indications of migraine prophylaxis in physicians. The answers highlighted in red indicates distractors which were not true indications of prophylaxis and added as distractor for more accuracy and to lower bias.

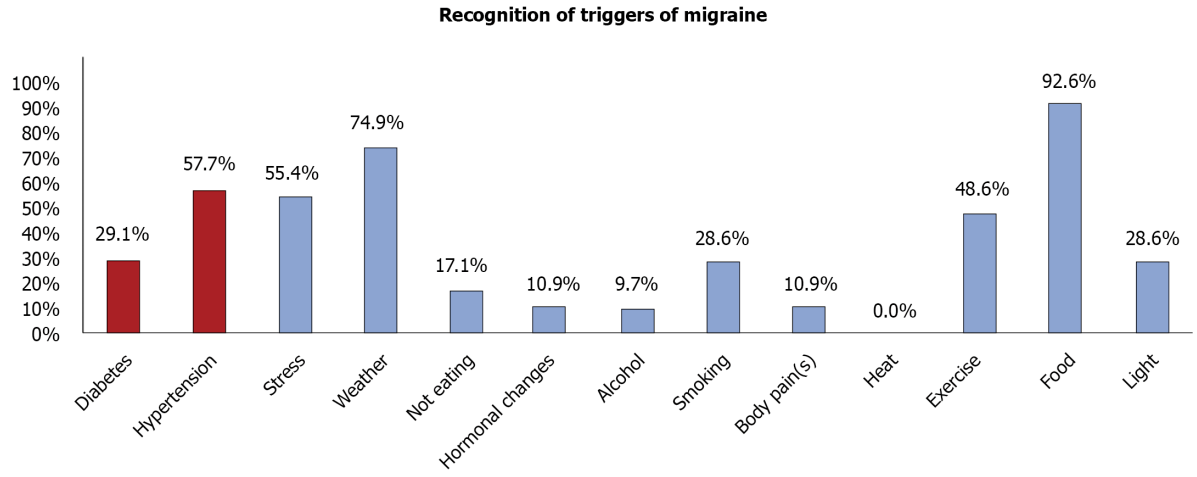

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Figure 2 Knowledge of triggers of migraine in physicians. The answers highlighted in red indicate distractors which were not true triggers of migraine and were added as distractor for more accuracy and to lower bias.

asked if they had migraine were termed unaware. Sample size and sampling: a migraine prevalence of 30% in physicians was assumed (greater than the general population migraine prevalence of 22.5% in Pakistan) and sample size for a prevalence study was calculated for an estimated physician population of 100000 with a confidence interval level and precision of 95% and 6%, respectively. Source Forge's free online sample size calculator was used (sampsize.sourceforge.net). The sample size determined was 186 for the prevalence study.

Statistical analysis and reporting

Reliability testing of migraine awareness was performed with the correlation coefficient (κ) to assess agreement between those who thought they had a migraine (self-aware) and confirmed cases of migraine. The sensitivity and specificity of the self-awareness of migraine were calculated by comparing it with the final diagnosis. The prevalence of migraine was calculated among all the respondents, including the excluded ones, to assess the actual prevalence of the disease in the physician and medical student population. Data analysis was run between groups using R version 1.4.1106, with an additional package of epitools. Chi-square, Mann–Whitney and Fisher's exact tests were applied wherever applicable after tests of the normalcy of distribution (Shapiro–Wilk). Graphs, tables and figures were made using Microsoft Office 2016 and Microsoft Visio. This study was reported in accordance with The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[20].

RESULTS

Participant characteristics

We distributed the questionnaire to a total of 275 subjects and managed to get a response from 213 of them, setting our response rate at 77.5%. One hundred and ninety participants fulfilled the inclusion criteria. After the application of exclusion criteria, we were left with 175 participants. Among them, 39 were medical students, and the rest of them were physicians. Males and females were symmetrically represented, with 88 males and 87 females. The average age was 25.7 ± 4.1 years. Most of our subjects (56%, n = 98) belonged to the specialty of medicine and allied, 30% (n = 51) to surgery and allied, and 5% (n = 9) to others (including pathology, radiology, *etc.*). A majority (62.3%, n = 109) of our respondents never consulted anyone for the headaches. The basic characteristics and demographic data of participants are summarized in Table 1.

Prevalence of migraine

Forty-eight (27.4%) of our 175 respondents were diagnosed with migraine using the questionnaire-embedded, self-diagnosis algorithm. This made the total prevalence of migraine in our sample of physicians and medical students 24.4%. Females, as expected, had higher prevalence (n = 28, 32.2%) compared to males (n = 20, 22.7%) among the final sample.

Awareness of migraine

Only 21 (43.8%) of our 48 migraineurs were fully aware of their disease before the application of the embedded criteria, while eight (16.7%) and 19 (39.6%) were completely unaware and not sure, respectively. Similarly, out of 127 non-migraineurs, 126 correctly thought they did not have a migraine. This made the sensitivity and specificity of this self-awareness 43.8% and 99.2%, respectively. The correlation coefficient Cohen's between self-awareness and migraine diagnosis was 0.52, showing a moderate level of association. The physicians' diagnosis of migraine had similar sensitivity (37.5%), specificity (97.6%), and correlation coefficient values (0.43).

Knowledge of migraine

A major proportion of participants (36.7%) erroneously believed that having any type of aura is essential for diagnosing migraine (Figure 3). Almost half of the respondents (n = 85, 48.6%) said they did not remember the diagnostic criteria of migraine, while 13% had never heard about the criteria at all. 11.4% of respondents did not know that prophylactic therapy even existed, while only 33.7% said they remember prophylaxis indications. Only 13.7% (n = 24) could correctly identify the correct duration of prophylaxis while 57.1% (n = 100) of subjects did not know, 21.1% (n = 37) underestimated, and 8% (n = 14) overestimated the duration of prophylactic therapy of migraine. Respondents were able to recognize, on average, just 1.3 of 5 real indications and 3.5 of 11 real triggers. Stress was by far the most commonly recognized trigger, recognized by 92.6% of respondents.

Consulters versus non-consulters

Only 60.6% (n = 66) respondents had sought consult. In general, consultation seekers had better knowledge of diagnostic criteria and prophylactic therapy in the subjective questions. The difference almost reached significance in the knowledge about prophylactic therapy (Table 2), where 57.8% of consulters said they remembered the indications of prophylaxis compared with just 28% of non-consulters (OR: 1.84, P = 0.05). Similarly, only 40.4% of those who chose not to consult correctly believed that aura is essential for a migraine diagnosis compared with a majority (59.1%) of consultation seekers (OR: 0.47, P = 0.02). Paradoxically, non-consulters were more likely to correctly identify the monthly headache rate cut-off for initiation of prophylaxis (30.3%) *versus* consulters (15.1%), and the difference was significant (P = 0.02). However, upon further analysis, it was revealed that consulters were more likely than non-consulters to underestimate the monthly headache rate threshold for starting the prophylaxis, with a mean monthly rate chosen by this group to be 2.85 *versus* 3.18 by the non-consulters (P = 0.03). There was no significant difference in recognizing triggers and indications of prophylaxis between both groups (Table 2). Additionally, headache frequency in both groups was also similar.

Migraineurs versus non-migraineurs

Migraineurs were much more likely to have visited a doctor than non-migraineurs, and the difference was significant (OR: 3.7, P < 0.001). A neurologist consultation was even more significantly associated with a diagnosis of migraine (OR: 17.4, P < 0.001). Females were more likely (31.8%) to have migraines than were males (22.9%), although the difference was not significant. Similarly, there was no significant difference between knowledge of duration or prophylaxis indications and triggers between migraineur and non-migraineur populations.

Migraineurs were more likely than non-migraineurs to remember the diagnostic criteria (52% *vs* 32%, OR: 2.26, P = 0.016). They were also more likely to know prophylaxis indications (43% *vs* 29.9%), but the difference here was just above significance (P = 0.08). Headache attacks per month were significantly higher in migraineurs (median 2 ± 4) than non-migraineurs (0.5 ± 2 IQR), and the difference was

Table 1 General characteristics and responses of all subjects

Characteristics	Results (*N* = 175), *n* (%)
Gender	Females: 88 (50.3)
	Males: 87 (49.7)
Age (yr)	Mean: 25.7 ± 4.1
Grade	Medical student: 39 (22.3)
	House officers: 74 (42.3)
	Non-trainee medical officers: 17 (9.7)
	Trainee medical officer: 38 (21.7)
	Senior registrar: 4 (2.3)
	Assist professor: 2 (1.1)
	Professor: 1 (0.6)
Specialty of doctors	Medicine & allied: 98 (56)
	Surgery & allied: 51 (29.1)
	Others: 9 (5.1)
	Not answered: 17 (9.7)
Do you have migraine?	Yes: 22 (12.6)
	No: 109 (62.3)
	Maybe: 44 (25.1)
Confirmed migraine after reading the ICHD-3 criteria of all 3 types of migraine	Migraine without aura: 36 (20.6)
	Migraine with aura: 9 (5.1)
	Chronic migraine: 3 (1.7)
	No migraine: 127 (72.6)
Consulted any physician	GP: 19 (10.8)
	Medical specialist: 22 (12.6)
	Neurologist: 10 (5.7)
	Ophthalmologist: 19 (10.8)
	Other: 9 (5.1)
	No consultation: 110 (62.8)
Physician able to diagnose migraine	Yes: 45 (25.7)
	No: 9 (5.1)
	Maybe: 9 (5.1)
	Never consulted: 112 (64)
Knowledge of diagnostic criteria of migraine	Heard and remember it: 66 (37.7)
	Heard about it but don't remember: 85 (48.6)
	Never heard about it: 24 (13.7)
Knowledge of prophylaxis of migraine	I know its indications: 59 (33.7)
	I knew its indications but don't remember: 70 (40)
	Know only that it exists: 26 (14.8)
	Don't know about it at all: 20 (11.4)
Aura is essential for migraine?	Yes: 66 (37.7)
	No: 83 (46.8)
	Not sure: 26 (14.7)

Duration of standard prophylactic therapy of migraine?	Do not know: 100 (57.1)
	1 mo: 10 (5.7)
	3 mo: 27 (15.4)
	6 mo[1]: 24 (13.7)
	12 mo: 14 (8)
Monthly headache rate for prophylaxis	≥ 2 per mo: 64 (36.6)
	≥ 3 per mo: 52 (29.7)
	≥ 4 per mo[1]: 43 (24.6)
	≥ 5 per mo: 15 (8.6)
	Not available: 1 (0.6)
Used abortive (migraine patients only)	Yes: 12 (25)
	No: 34 (70.8)
	Maybe: 2 (4.2)
Used prophylactic therapy (migraine patients only)	Yes: 9 (18.7)
	No: 38 (79.2)
	Not sure: 1 (2.1)

[1]Chosen as the standard in accordance with American Headache Society (AHS) guidelines.

significant ($P < 0.01$).

Aware versus unaware migraineurs

Awareness of one's own disease was more common in females (53%) than in males (40%), but the difference was not significant. Migraine-aware respondents were significantly more likely to have visited a physician (81%) than were unaware migraineurs (44%, OR: 5.0, $P = 0.01$). Neurologist consultation, in particular, was more common in migraine aware (6/21) *versus* unaware (2/27, OR: 9.6, $P = 0.024$) participants. All knowledge questions related to diagnosis and prophylaxis had similar results in both groups (Table 3).

DISCUSSION

Our study presents the first extensive data on awareness and knowledge of migraine among physicians in Pakistan, with a point-prevalence of migraine at 24.4%. A similar prevalence has been reported in neighboring countries in the region[21,22]. Herekar *et al*[12] have previously reported a 1-year prevalence of migraine in the general population of Pakistan to be 22.5%. The differences in prevalence have been attributed to methodological variations and changes in cultural attitudes towards disease that lead to underdiagnosis in certain subsets of the population[23,24].

Lack of awareness and knowledge of migraine and its management among physicians causes a striking yet avoidable burden on its demographics. One of the critical reasons for underdiagnoses of migraine is unfamiliarity with the diagnostic criteria and the reluctance to use diagnostic tools among physicians[5,25]. Kristoffersen *et al*[16], who surveyed knowledge of Neurology residents in Norway regarding migraine, reported lapses in the knowledge of neurology residents below the bare minimum. Only half of the neurology residents had used the diagnostic criteria regularly, undoubtedly leading to inadequate familiarity with migraine presentations and subsequent underdiagnosis. Gültekin *et al*[5] reported that only 10% of primary care physicians in Turkey could give the complete diagnostic criteria of migraine. We report similar findings in our population, as 62% of participants in our study admitted not remembering the diagnostic criteria. When tested objectively, 38% believed in the myth that migraine could not be diagnosed without aura. This further indicates a fundamental unfamiliarity with types of migraine, migraine without aura in particular. The inadequate familiarity with not only the diagnostic criteria but the types of migraine as well can undeniably lead to an underdiagnosis and mismanagement of a plethora of cases.

The migraine triggers originated from self-reports by patients, but some have been experimentally verified[26]. Advice regarding triggers has varied through the years. Historically, it was argued that the best way to avoid headache was to avoid the triggers. Still, recent evidence suggests that the association of triggers with the headache is a learned process of the brain that subsequently attaches it to the

Table 2 Results of analysis between groups based on consult-seeking behaviors and migraine diagnoses

	Consultation seeking			Migraine		
	Non-consulters (*n* = 109)	**Consulters (*n* = 66)**	***P***	**Migraineurs (*n* = 48)**	**Non-migraineurs (*n* = 127)**	***P***
Gender	Females: 54 (49.5%); Males: 55 (50.5%)	Females: 34 (51.5%); Males: 32 (48.5%)	0.8	Females: 28 (58.3%); Males: 20 (41.7%)	Females: 60 (47.2%); Males: 67 (52.7%)	0.19
Knowledge of diagnostic criteria	Remember: 37 (33.9%); Don't remember:72 (66.1%)	Remember: 29 (43.9%); Don't remember: 37 (56.1%)	0.24	Remember: 25 (52.1%); Don't remember: 23 (47.9%)	Remember: 41 (32.3%); Don't remember: 86 (67.7%)	**0.016;** OR: 2.26 (95% CI: 1.1-4.5)
Know prophylaxis indications	Yes: 31 (28.4%); No:78 (71.6%)	Yes: 38 (34.9%); No: 28 (42.4%)	0.05	Yes: 21 (43.7%); No: 27 (56.2%)	Yes: 38 (29.9%); No: 89 (70.1%)	0.08
Aura essential for diagnosis?	Yes: 65 (59.6%); No or not sure: 44(40.4%)[1]	Yes: 27 (40.9%); No or not sure: 39 (51.1%)[1]	**0.016;** OR: 0.47 (95%CI: 0.25-0.87)	Yes: 22 (45.8%); No or not sure: 26 (54.2%)	Yes: 70 (55.1%); No or not Sure: 57 (44.9%)	0.27
Consulted		Neurologist: 10 (15.2%); Other doctor(s): 56 (84.8%); None: 0 (0%)		Neurologist:8 (16.7%); Other doctors:21 (43.7%); None: 19 (39.6%)	Neurologist: 2 (1.6%); Other doctors: 35 (27.6%); None: 90 (70.9%)	**< 0.001**
Monthly attack cutoff for prophylaxis[2]	Correctly Identified: 33 (30.3%); Could not Identify: 75 (68.8%)	Correctly Identified: 10 (15.1%); Could not Identify: 56 (84.9%)	0.02	Correctly Identified: 16; Could not Identify: 32	Correctly Identified: 27 (21.4%); Could not Identify: 99 (78.6%)	0.11
Know correct duration of prophylaxis?[3]	Yes: 28 (25.7%); No: 81 (74.3%)	Yes :20 (30.3%); No: 46 (69.7%)	0.5	Yes: 13 (27%); No: 35 (73%)	Yes: 35 (27.5%); No: 92 (72.5%)	0.94
Frequency of headache attacks (per month)	None: 31 (28.4%); ≤ 1: 39(35.8%); 2: 17 (15.6%); 3: 6 (5.5%); ≥ 4: 16 (14.7%)	None: 13 (19.7%); ≤ 1: 22 (33.3%); 2: 13 (19.7%); 3: 8 (12.1%); ≥ 4: 10 (15.1%)	0.4	None: 4 (8.3%); ≤ 1: 16 (33.3%); 2: 9 (18.7%); 3: 6 (12.5%); ≥ 4: 13 (27.1%)	None: 40 (31.5%); ≤ 1: 45 (35.4%); 2: 21 (15.7%); 3: 8 (6.3%); ≥ 4: 13 (2.4%)[1]	**< 0.01**
Total triggers recognized	Median: 4, IQR: 5	Median: 3, IQR: 4	0.29	Median: 3.5, IQR: 3	Median: 4, IQR: 5	0.297
Total indications recognized	Median: 2, IQR: 1	Median: 1, IQR: 1	0.21	Median: 1, IQR: 1	Median: 1, IQR: 1	0.22
Distractor(s) recognized as triggers	Yes: 47 (43.1%); No: 62 (56.9%)	Yes: 29 (43.9%); No: 37 (56.1%)	0.91	Yes: 22 (45.8%); No: 26 (54.2%)	Yes: 54 (42.5%); No: 73 (57.5%)	0.69
Distractor(s) recognized as Indications	Yes: 37 (33.9%); No: 71 (65.1%)	Yes: 17 (25.7%); No: 49 (74.2%)	0.23	Yes: 16 (33.3%); No: 32 (66.7%)	Yes: 38 (22.1%); No: 88 (87.9%)	0.68
Migraineurs	Migraineurs: 19 (17.4%); Non-migraineurs: 90 (82.5%)[1]	Migraineurs: 29 (43.9%); Non-migraineurs: 37 (56.1%)[1]	< 0.001; OR: 3.7 (95%CI: 1.8-7.5)			

[1]Indicates statistically significant results.

[2]Monthly headache rate ≥ 4 was chosen as standard for initiation of prophylaxis according to American Headache Society (AHS) guidelines.

[3]A 6-mo duration of prophylaxis was chosen as standard according to AHS guidelines.

IQR: Inter-quartile range; OR: Odd's ratio; CI: Confidence interval.

headache. According to this theory, slow desensitization techniques rather than avoidance strategy is the way forward[27]. Nonetheless, knowledge of the trigger itself is vital for physicians if they counsel the patient appropriately for either strategy. The fact that an average physician in our study could not recognize even half of the triggers from the list points to an apparent deficiency in this knowledge.

Table 3 Subgroup analysis among migraineurs based on awareness of their disease

	Migraine awareness		
	Migraine aware (*n* = 21)	**Not aware (*n* = 27)**	***P***
Gender	Females: 13 (61.9%); Males: 8 (38.1%)	Females: 15 (55.5%); Males: 12 (45.5%)	0.65
Diagnostic criteria	Remember: 12 (57.1%); don't remember: 9 (42.9%)	Remember: 13 (48%); don't remember: 14 (52%)	0.53
Know prophylaxis indications	Know indications: 11 (52.4%); do not know about either prophylaxis or its indications: 10 (47.6%)	Know indications: 10 (37%); do not know about either prophylaxis or its indications: 17 (63%)	0.44
Aura essential for diagnosis?	No: 13 (61.9%); yes or not sure: 8	No: 13 (48.1%); yes or not sure: 14 (51.9%)	0.34
Consulted	Neurologist: 6 (28.6%); Any other physician: 11 (52.4%); Didn't consult: 4 (19%)[1]	Neurologist: 2 (7.4%); Any other physician: 10 (37%); Didn't consult: 15[1] (55.6%)	**0.02;** OR: 9.6 (95%CI: 1.5-96)
Monthly attack cutoff for prophylaxis	Correctly identified: 5 (23.8%); Couldn't identify: 16 (76.2%)	Correctly identified: 11 (40.7%); Couldn't identify: 16 (59.2%)	0.22
Know duration of prophylaxis	Correctly identified: 6 (28.5%); Couldn't identify:15 (71.4%)	Correctly identified: =7 (33.3%); Couldn't identify: 20 (66.7%)	0.83
Respondent's frequency of attacks (per month)	None: 1 (4.8%); ≤ 1: 8 (38.1%); 2: 2 (9.5%); 3: 3 (14.3%); ≥ 4: 7 (33.3%)	None: 3 (11.1%); ≤ 1: 8 (29.6%); 2: 7 (25.9%); 3: 3 (11.1%); ≥ 4: 6 (22.2%)	0.5
Average no of triggers recognized	Median: 3, IQR: 3	Median: 4, IQR: 3	0.32
Average no of indications recognized	Median: 1, IQR: 1	Median: 2, IQR: 1	0.17

[1]Indicates statistically significant results.
IQR: Inter-quartile range; OR: Odd's ratio; CI: Confidence interval.

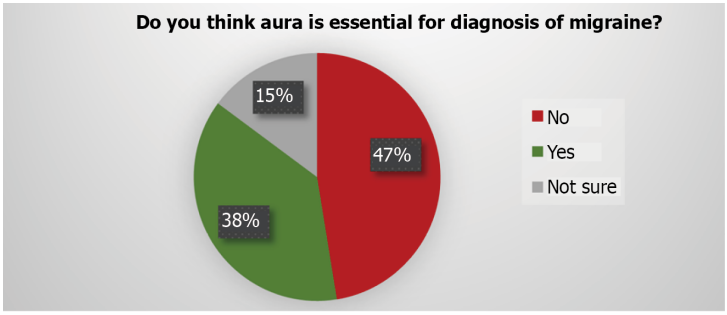

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Figure 3 Belief in the myth that aura is an integral part of migraine diagnosis.

Moreover, perhaps even more worryingly, almost half of the participants chose the distractors (hypertension and diabetes) as triggers.

Menstrual migraine is associated with particularly significant morbidity due to the longer duration, increased severity, and periodicity, and also because of its refractoriness to abortive treatment[28]. The disability associated with it deserves a special mention because it, arguably, is the most common migraine trigger, with 70% of female migraineurs reporting this trigger[28]. It is also one of the most common disabling conditions presented in gynecological practices[29] but 42% of our respondents did not recognize it as a trigger. In comparison, an overwhelming majority (90%) did not know that menstrual migraine can itself be an indication for initiation of prophylaxis, which reveals a vital missing piece in physicians' knowledge on the subject.

Studies on migraine have consistently demonstrated the role of preventive therapy in reducing disease burden[30]. Preventive therapy is central in managing migraineurs with severe and frequent attacks as the overutilization of abortive therapy may frequently lead to medication-overuse headaches or resistant migraine[31]. Preventive therapy is also required in some cases to augment responsiveness to abortive therapy as it reduces the frequency and duration of the migraine attacks and the severity [13]. Silberstein and colleagues demonstrated that preventive therapy (when indicated) combined with abortive was much more effective in reducing the migraine load than abortive therapy alone[31]. Moreover, management of chronic migraine requires an approach involving a combination of abortive, preventive, and behavioral therapy. In our sample, chronic migraine (frequent attacks) as an indication

of prophylaxis was recognized by 50% of respondents, which fares relatively better than recognition of other indications but is still inadequate. On the contrary, only 33% of physicians and medical students thought they remembered the indications of prophylaxis. When tested from a list of indications, a physician could identify only about one indication out of five. These results reveal another vital knowledge gap that needs priority focus.

Physicians, in general, underestimate the role of preventive therapy[31-34]. The American Migraine Communication study highlighted physicians under-rating the disability caused by migraine and thus the need for prophylactic therapy[35]. In contrast, there is some evidence that neurologists, compared with other physicians, tend to emphasize the role of prophylaxis[36]. Physicians' unfamiliarity with preventive therapy means an inability to manage chronic migraine cases properly. Preventive therapy use among physicians in our sample (18%) was similar to the prevalence reported elsewhere in the literature[31,37]. The fact that more than two thirds of physicians in our sample did not remember the indications of prophylaxis when asked subjectively is particularly troubling for a large subset of special cases. These comprise but are not limited to chronic migraine, menstrual-related, resistant migraine, and other more severe forms of migraine, which are contingent upon preventive therapy and are perhaps responsible for several mishandled cases. Additionally, we found no meaningful differences between consulters and non-consulters for recognition of prophylaxis indications. Our inference is that the under-emphasis on preventive therapy in the form of avoidance of triggers or drug therapy is so pervasive that even after consulting a physician for the headache, respondents did not gain any meaningful knowledge of these aspects of the migraine.

Weber *et al*[38] reported that primary care physicians suffering from migraine described receiving more migraine patients in their practice than their healthy colleagues. Their patients were more likely to have a better quality of life. This is perhaps related to the sensitivity of such physicians towards migraineurs. A similar inference can be made from our results, as migraineurs were more likely to state that they knew the diagnostic criteria. However, the difference was not significant when asked the question regarding diagnosis objectively, *i.e.*, the question related to the aura. The improved knowledge of the disease's diagnosis and management in physicians with migraine (Table 2) puts them in a better position to understand and help the patients. Migraineurs in our study were also more likely to have visited a doctor (61.4% *vs* 29.1%). The association was strongest with a neurologist's consultation (OR: 17.4, $P < 0.001$). This potentially represents the role of a consultation, especially with a neurologist, in diagnosing migraine[39]. The subjective feeling of knowledge related to diagnostic criteria as well as prophylactic therapy was also significantly better in consultation seekers (Table 2). We think this is a result of discussion about the disease with their consulting physicians or more intrigue and reflection about the disease resulting from the consultation. The consulting process, the resultant introspection, and perhaps reading about their condition helped physicians improve their knowledge. This is also reflective of the power and efficacy of a medical consultation[39]. The contradictory results on the monthly headache rate threshold of preventive therapy can be logically explained with a further breakdown of data, as consulters favored the prophylaxis more and underestimated the threshold for initiation of prophylaxis, constituting a better trend overall.

Radtke *et al*[25] reported 70% awareness of migraine in their sample in 2012, with a coefficient of agreement value of 0.46 between ICHD-II criteria and awareness. At 44%, migraine in our sample was lower (= 0.52). The sensitivity of physicians' diagnosis among the sample collected by Radtke *et al*[25] was also better at 63%. We did not specify the temporal order of events in our question, *i.e.*, whether they knew about their migraine before consulting a doctor or suspected one after their visit. Hence, this self-awareness of migraine is not mutually exclusive to the consulting physician's diagnosis of migraine in our cases. However, our data hint toward the role of physicians' consultation in producing this self-awareness. Although consulting with physicians could not contribute to better knowledge of the subjects, it still helped diagnose the disease in many cases. The precision of diagnosis was better when consultations came from neurologists (Table 3). However, the number in our sample was too small, and more studies are needed for a more generalizable inference. Our study thus reinforces the earlier findings that the advice to seek consultation for a headache instead of OTC medication use is essential and needs to be practiced by our physicians[39].

The ever-increasing global burden of non-communicable diseases concerning morbidity and the fact that migraine is jumping the ladder of the most prevalent diseases exponentially is alarming[40]. Every effort has to be made for an accurate and timely diagnosis of migraine. Fortunately, with the advent of new data on drug therapy in migraine, this era is also witnessing a remarkable change in its management[41]. Recent studies have shown mechanism-based therapies like anti-calcitonin gene-related peptide monoclonal antibodies (erenumab, tinezumab, fremanezumab and galcanezumabas) as promising drugs in the preventive management of migraine[13,42]. In addition to rapid onset, these drugs also carry fewer adverse effects (mainly injection site reactions). Erenumab specifically has shown effectiveness in a 50% reduction in migraine days, with a favorable safety profile[43]. However, currently, high costs and limited availability of these monoclonal antibodies are a challenge in migraine management on a global level. Various methods can be applied to improve awareness and knowledge of migraine among the physicians who serve as the first encounter with health care for patients with headaches. We suggest that the interventions for improvement have to be incorporated early in the course of a physician's clinical life. One such strategy can be dedicated lectures on migraine in medical

schools, focusing on it as a high-yield topic of examination, including standardized patients with migraine in clinical exams. New graduates should be educated by headache specialists on migraine diagnosis and management before starting internships. For practicing physicians, the interventions can include yearly workshops, continuing medical education activities, and the provision of migraine diagnostic and management posters to be placed in the clinics. Virtual education, which saw its role vastly inflated during the current coronavirus disease-2019 pandemic, can be utilized to maximize education on migraine among physicians. It is also imperative to prospectively study the effects of increased awareness of migraine among physicians to establish the amplitude of change it may carry in decreasing the global burden of disability with regard to migraine. Together with the introduction of more effective preventive and possibly curative treatments, this may also play a key role in reducing the global prevalence of migraine.

Our study had some limitations grounded in the study design used. Firstly, the subjective questions on diagnostic criteria and prophylactic treatment were subject to social desirability bias as most respondents were not open to accepting their knowledge deficit. Secondly, we did not use any migraine diagnosis registry due to lack of the aforementioned, but our data collection team ensured that respondents understood the diagnostic criteria during collection. Thirdly, excluding the cases that did not experience headaches may have potentially excluded a specific subset of doctors whose knowledge was not tested. This, albeit small, was a potential source of sampling bias in our study. Fourthly, although almost all the questionnaires were filled in the presence of one of the study team members and we tried to keep the questionnaire as short as possible, there was still a possibility that some of the participants might not have read the questions and criteria thoroughly; an inherent possibility with the questionnaire-based studies. Despite these limitations, we believe the study was largely free from any systematic biases.

CONCLUSION

Despite its high prevalence and high associated morbidity, migraine diagnosis and management knowledge remain below the minimum functionally required among physicians in Pakistan. Steps need to be taken to bridge the knowledge gap among doctors to address underdiagnosis and mismanagement of the disease.

ARTICLE HIGHLIGHTS

Research background

Despite its high prevalence, migraine remains underdiagnosed worldwide. A significant reason is the knowledge gap in physicians regarding diagnostic criteria, clinical features, and other clinical aspects of migraine.

Research motivation

This research was conducted to see whether migraine follows the same trends of underdiagnosis in Physicians of Pakistan as globally.

Research objectives

We aimed to measure the knowledge deficit in physicians and medical students and to assess the prevalence of migraine in the same population.

Research methods

An online questionnaire was developed and distributed among physicians and final-year medical students on duty in various medical and surgical specialties of Allied and DHQ Hospitals, Faisalabad, between October 2018 and October 2019. Inclusion criteria were public practicing physicians who experience headaches, while those who never experienced headaches were excluded. Different questions assessed respondents on their knowledge of triggers, diagnosis, management, and prophylaxis of the migraine headache. They were asked to diagnose themselves using embedded ICHD-3 diagnostic criteria for different types of migraine. Graphs, tables and figures were made using Microsoft Office 2016 and Microsoft Visio, and data analysis was done in R Studio 1.4.

Research results

We had 213 respondents and 175 fulfilled inclusion criteria, with 99 (52%), 58 (30%) and 12 (6.3%) belonging to specialties of medicine, surgery, and others, respectively. Both genders were symmetrically represented (88 male and 87 female). Fifty-two (24.4%) of our 213 respondents were diagnosed with migraine, with 26 (50%) being aware of it. Females had higher prevalence among study participants (n =

28, 32.2%) compared to males (n = 20, 22.7%, P = 0.19). A majority (62%) of subjects never consulted any physician for their headache. Similarly, a majority (62%) either never heard or did not remember the diagnostic criteria of migraine, and 38% falsely believed that having any type of aura was essential for diagnosing migraine. The consultation rate was 37% (n = 65), and migraineurs were significantly more likely to have consulted a physician, a neurologist in particular (P < 0.001). Consulters and migraineurs fared better in the knowledge of diagnostic aspects of the disease than their counterparts. There was no significant difference in other knowledge aspects between consulters and non-consulters and migraineurs and non-migraineurs.

Research conclusions

Critical knowledge gaps exist between physicians and medical students, potentially contributing to the misdiagnosis and mismanagement of migraine cases.

Research perspectives

Migraine remains an underdiagnosed disease in the general population as well as among healthcare providers. Education, timely diagnosis, and management will help reduce its global burden.

ACKNOWLEDGEMENTS

We are grateful to all the participating medical students, residents and senior doctors for their time and input, without which this project would not have been accomplished. We also want to thank the Department of Neurology, Punjab Medical College Faisalabad, for providing constructive feedback on the project.

FOOTNOTES

Author contributions: Choudry H, Ata F and Naveed Alam M were responsible for study design; Choudry H, Ata F, Naveed Alam M, Ruqaiya R and Qaiser Ikram M did the questionnaire design; Choudry H and Naveed Alam M analyzed the data; Choudry H and Ata F were responsible for the manuscript revision; all authors participated in data collection and manuscript writing.

Institutional review board statement: The study was conducted following the guidelines of the Declaration of Helsinki and approved by the institutional ethical review committee of the Faisalabad Medical University (No. 000319). The ethics committee waived informed consent.

Informed consent statement: Informed consent from patients was waived by the ethics committee.

Conflict-of-interest statement: All authors report no relevant conflict of interest for this article.

Data sharing statement: Data can be made available from the first or corresponding author at reasonable request

STROBE statement: The authors have read the STROBE checklist of items, and the manuscript was prepared and revised according to the STROBE checklist of items.

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S-Editor: Wu YXJ
L-Editor: Kerr C
P-Editor: Wu YXJ

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 428-437

DOI: 10.5662/wjm.v12.i5.428

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Role of the circulatory interleukin-6 in the pathogenesis of gliomas: A systematic review

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Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Tantau AI, Romania; Zhang GL, China

Received: December 13, 2021
Peer-review started: December 13, 2021
First decision: March 24, 2022
Revised: April 1, 2022
Accepted: July 24, 2022
Article in press: July 24, 2022
Published online: September 20, 2022

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Abstract

BACKGROUND

Glioma is the most common primary tumor in the brain originating from glial cells. In spite of extensive research, the overall survival rate is not enhanced. A number of published articles observed differentially circulating levels of cytokines in glioma. Interleukin-6 (IL-6) protein coded by IL-6 gene is regulated by the immune system and it has been found to have a significant role in progression and apoptosis resistance of glioma.

AIM

To review the role of circulatory IL-6 in the development and progression of glioma and its utility as a biomarker.

METHODS

Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines were applied to filter the relevant studies based on inclusion and exclusion criteria. We used a combination of keywords and the *Reference Citation Analysis* (*RCA*) tool to search the potential studies and performed data extraction from selected studies.

RESULTS

The published results were inconsistent; however, most studies showed a significantly higher IL-6 level in glioma cases as compared to controls. Comparative IL-6 level among the different grades of glioma showed a higher level with low-grade gliomas and lower level with high-grade gliomas.

CONCLUSION

IL-6 level significantly differed between cases and controls, and among different cancer stages, which shows its potential as a diagnostic and prognostic marker.

Key Words: Gliomas; Interleukin-6; Circulatory markers; Diagnostic marker; Prognostic marker

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Core Tip: In spite of extensive research in the field of brain oncology, the overall survival is not much improved. There is an urgent need to explore the circulatory markers for diagnosis and prognosis. This systematic review focused on the role of interleukin-6 in brain cancer development and progression and its utility as a diagnostic or prognostic biomarker.

Citation: Singh M, Raghav A, Gautam KA. Role of the circulatory interleukin-6 in the pathogenesis of gliomas: A systematic review. *World J Methodol* 2022; 12(5): 428-437
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/428.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.428

INTRODUCTION

Gliomas are the most common primary brain tumors in adults, accounting for 80% of malignant brain tumors originating from glial cells[1]. Globally, gliomas show a wide variation in incidence, and it is 0.01–12.7 in males and 0.01–10.7 in females per 100 000 people[2]. The lowest incidence is in Africa and highest in Northern Europe[2]. Gliomas are an increasing cause of death in children and the third most common in adolescents and adults[2]. According to the World Health Organization (WHO) classification, the most common occurring histological grade of gliomas is astrocytic tumors (grades I–III) and oligodendroglial tumors (grades II–III), ependymoma (grades I–III) and glioblastoma (grade IV)[3,4]. Glioblastoma is aggressive in nature and the survival rate is low, with death within 2 years of diagnosis despite receiving maximal surgical removal of the tumor and medical therapies including chemotherapy and radiotherapy. Therefore, there is an urgent need to find comprehensive treatment strategies to enhance the survival rate[5].

Adapting the Virchow theory, various studies concluded that inflammation is one of the major hallmarks of cancer formation[6,7]. Within the cancerous microenvironment, inflammatory cells and cytokines have pleomorphic roles. On the one hand, these aid in tumor suppression, while on the other hand, they support malignant cell transformation, tumor growth, inhibition of apoptosis, invasion, angiogenesis, cell migration, tumor cells differentiation and immuno-suppression[8-11]. A number of studies showed varied circulating levels of cytokines in glioma. On the basis of The Cancer Genome Atlas database, interleukin (IL)-6 has a significant role in progression and apoptosis resistance of glioma [12-15].

IL-6 is a pleiotropic proinflammatory cytokine with a 21–28-kDa four-helix bundled glycoprotein with 184 amino acids[16,17]. Under normal conditions, IL-6 secretion is initiated in response to stimuli such as viruses, UV and secretion of other cytokines, and it is released by a variety of cells including macrophages, monocytes, hematopoietic cells, stromal cells, muscles cells and epithelial cells. IL-6 has a significant role in the process of immunity, inflammation, angiogenesis, neural development, reproduction, metabolism hematopoiesis, and bone remodeling[18,19]. In tumor vasculature, IL-6 is released by tumor cells, tumor-infiltrating immune cells and fibroblast stromal cells, and induced by several factors such as prostaglandin E2, IL-1β, hypoxia, nuclear factor (NF)-κB, miRNAs and lack of signal transducer and activator of transcription (STAT)3 inhibitors[16,18,20-23]. IL-6 exerts its function by binding to its receptor either by membrane bound receptor (mIL-6R), the classical pathway or by soluble receptor (sIL-6R), the trans-signaling pathway. Binding of IL-6 to its receptor causes the activation of gp130, which subsequently activates cytoplasmic tyrosine kinases (Janus kinase, JAK) *via* its phosphorylation that is responsible for intracellular signaling by phosphorylation of STATs (especially the STAT3 pathway). Phosphorylated STAT3 dimer translocates to the nucleus, which leads to the transcription of targeted genes (*Bcl-2, Bcl-xL, Cyclin D1, VEGF, etc.*) and production of other proinflammatory cytokines and exerts an acute-phase response[16,18,24]. These activated genes may code for the proteins involved in cell survival (cyclin D1, survivin and MYC)[18], antiapoptotic condition (Bcl-x and MYC)[16,25], angiogenesis (vascular endothelial growth factor; VEGF)[16], invasion (MMP)[16], tumor growth and immunosuppressive factor secretion [transforming growth factor (TGF)-β, IL-10 and VEGF][26,27]. A systematic diagram showing the physiology of IL-6 is shown in Figure 1. The STAT3 signaling pathway is downregulated in different ways, such as suppressor of cytokine signaling (SOCS)3 inhibits phosphorylation of JAK proteins and protein inhibitor of activated STAT3 (PIAS3) inhibits dimerization of STAT3 monomers.

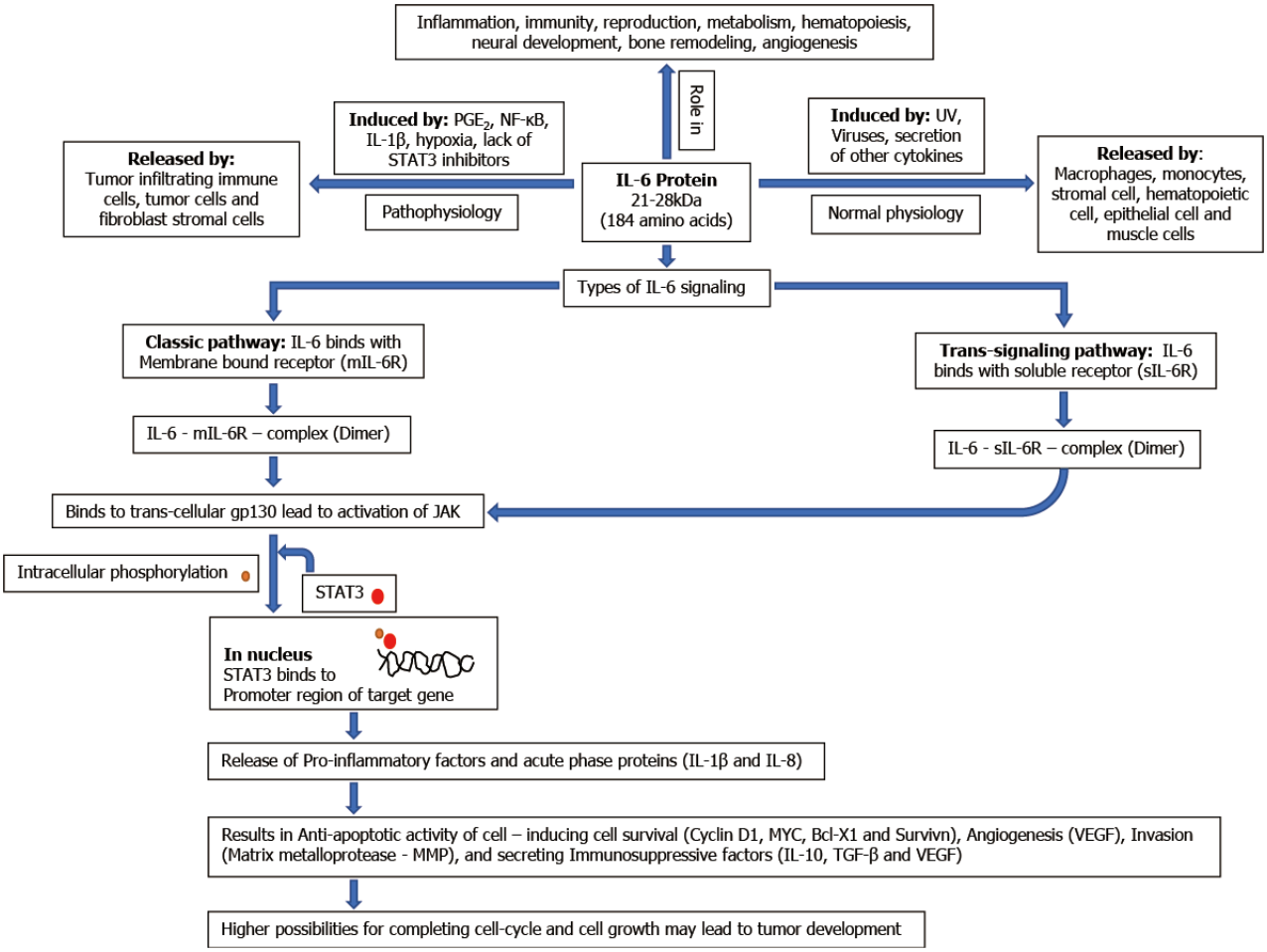

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Figure 1 Physiology of interleukin (IL)-6. IL-6 has regulatory role in various physiological processes such as inflammation, immunity and reproduction. IL-6 is induced by viruses and UV, *etc.*, and is released by macrophages, monocytes and stromal cells under normal physiological conditions, and under pathophysiological conditions, it is induced by nuclear factor-κB, prostaglandin E2, hypoxia and released by tumor-infiltrating immune cells and tumor cells, *etc.* IL-6 can activate the STAT3 signaling pathway either by the classical pathway or trans-signaling pathway. Activation of STAT3 can upregulate a variety of genes and may have an important role in tumor formation.

Besides these key roles, IL-6 also plays key roles in inflammation, proliferation and differentiation of B and T lymphocytes and natural killer cells[28]. IL-6 blocks MHC class II expression of Th1 cells and halts the secretion of IL-2 and interferon-γ and hence reduces cytotoxic T-lymphocyte activity[29]. Inhibition of the activity of T lymphocytes helps cancer cells to inhibit the immune response. Several miRNAs are involved in the production of IL-6 in a paracrine manner[30]

In various studies, a higher level of IL-6 was found to be associated with tumor progression and poor survival rate in several cancers including glioma. In glioma, IL-6 affects tumor formation and progression by triggering the JAK/STAT3 signaling pathway, which may further lead to continuous cell growth[31], tumor development, cell invasion and migration[32,33], angiogenesis[34] and inhibition of apoptosis[35,36]. The mRNA expression of IL-6 gene has been found to correlate with higher grade of glioma (glioblastoma)[37], in addition IL-6 gene amplification in tissues samples was 54% (15 of 36) on glioblastoma and none of 17 in lower grade of glioma[38]. Immunohistochemistry revealed that IL-6 receptors were totally absent in normal brain tissue and all the tissues of glioblastoma samples[39]. STAT3 promotes tumor growth by inhibiting apoptosis in glioma and increased level of phosphorylated STAT3 is found in recurrent glioblastoma as compared to primary glioma[40].

In this systematic review, we reviewed all the published case–control studies investigating the role of circulatory IL-6 in the development and progression of glioma and its utility as a diagnostic or prognostic biomarker.

MATERIAL AND METHODS

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) guidelines[41] were adapted to perform this systematic review.

Literature search strategies

An exhausted literature search on March 1, 2021 was done by two research scientists independently using various combination of keywords "glioma", "glioblastoma", "interleukin-6", "IL-6", "case-control study", "ELISA", "enzyme linked immunosorbent assay" "circulatory levels of IL-6" using the Reference Citation Analysis™ (RCA) tool, which is an artificial intelligence technology-based open multidisciplinary citation analysis database. On RCA window, keywords were entered in the designated area and after selecting the "Find an Article", we obtained a list of the latest highlighted articles that was further filtered by selecting Impact Index Per Article. The systematic search was limited to articles published in English language. The relevant full-text articles were obtained. References were also evaluated to retrieve additional studies. The researchers thoroughly evaluated full-length original articles based on the inclusion and exclusion criteria for the inclusion in this systematic review [41].

Inclusion criteria

All the retrieved studies were screened and filtered on the basis of PICO (patient/population, intervention, comparison and outcomes) strategy as follows: (1) Participants: histopathological confirmed cases of glioma; (2) intervention: conditions including progression and invasion of glioma; (3) comparison: controls free from any malignancy; (4) observation: IL-6 expression level by ELISA or multiplex assay; and (5) case–control studies. A flow chart (PRISMA) showing the search strategy is shown in Figure 2.

Exclusion criteria

The studies were excluded based on the following criteria: (1) Studies with insufficient information regarding the level of IL-6; (2) review articles, meta-analyses, editorials, letters, and duplicate articles; (3) conference proceedings; and (4) not in English language.

Data extraction and study characteristics

Gathering of information from the relevant articles was carefully done on the basis of inclusion criteria. From each relevant study, the following information was collected and organized in Table 1: First author's last name, year of publication, ethnicity of the study population, sample size, sample collected (serum or plasma), method of analysis (ELISA), IL-6 expression and glioma outcome (increased or decreased) in comparison to controls.

RESULTS

A total of 953 studies were identified in the literature search and five studies have been included for full evaluation in this systematic review (Figure 2). The critically evaluated studies are summarized in Table 1.

The study of Doroudchi *et al*[42] comprising 38 cases and 26 controls found a significantly decreased level of IL-6 in the serum of glioma cases (2.34 ± 4.35 pg/mL) as compared to controls (4.67 ± 4.35 pg/mL), while some other studies observed a significantly increased level of IL-6 in cases as compared to controls[8,42,43]. A study including 55 cases of glioblastoma and 20 healthy controls found fourfold upregulation of IL-6 in the cases of glioblastoma as compared to controls[8]. In contrast, Schwartzbaum *et al*[44], with a large number of cases of glioma (n = 487) and healthy controls (n = 487), did not find any significant (OR = 0.77) association of case–control correlation in differentially expressed level of IL-6. Level of IL-6 in glioma patients aged > 30 years showed a lower value as compared to young patients; however, the investigators did not find a significant correlation[42].

Comparative level of IL-6 among the different grade of glioma cancer observed a higher level (4.02 ± 7.80 pg/mL) with low grade of cancer and lower levels (1.74 ± 1.55 pg/mL) with high grade of cancer [42]. In contrast, in a few studies, the serum levels of IL-6 increased with the progression of glioma grading[43]. Univariate analysis indicated that the increased level of IL-6 declined after surgical removal of the glioma[43]. This indicates that, along with immune cells including inflammatory cells, tumor cells can also release the IL-6. Zhenjiang *et al*[45] has compared the circulating level of IL-6 along with other cytokines between glioblastoma multiforme (GBM) and non-GBM malignant glioma. They observed a detectable concentration of IL-6 in 45%–50% of cases, along with IL-4 and IL-5 in GBM patients, while 55%–60% cases with non-GBM glioma expressed IL-6 along with IL-4 and IL-5[45]. The investigators also analyzed the combination effects of selected cytokines (IL-4/IL-5/IL-6) on patients' survival and found that if all were present or all absent, it was associated with better survival rate.

DISCUSSION

Many biomarkers are differentially expressed in cases versus controls using tissue samples; however, the current need is based and focused on circulatory biomarkers. Recently, liquid biopsy has been used

Table 1 Characteristics of the selected studies included in this review literature

No.	Author name	Year	Region	Sample	Case (glioma)	Control	IL-6 level (pg/mL)
1	Doroudchi *et al* [42]	2013	Iran	serum	38	26	Decreased as compared to controls
2	Shan *et al*[43]	2015	China	Serum	86	18	IL-6 level increased with the elevation of grade
3	Albulescu *et al*[8]	2013	Romania	Serum	55	20	3-fold upregulated than control
4	Schwartzbaum *et al*[44]	2017	Norway	Serum	487	487	insignificant association with the disease
5	Zhenjiang *et al*[45]	2018	Sweden	Serum	GBM = 145	Non-GBM = 60	45%–50% cases of GBM observed with detectable level of IL-6 & 55%–60% cases of Non-GBM malignant glioma observed with detected level of IL-6

IL-6: Interleukin-6; GBM: Glioblastoma.

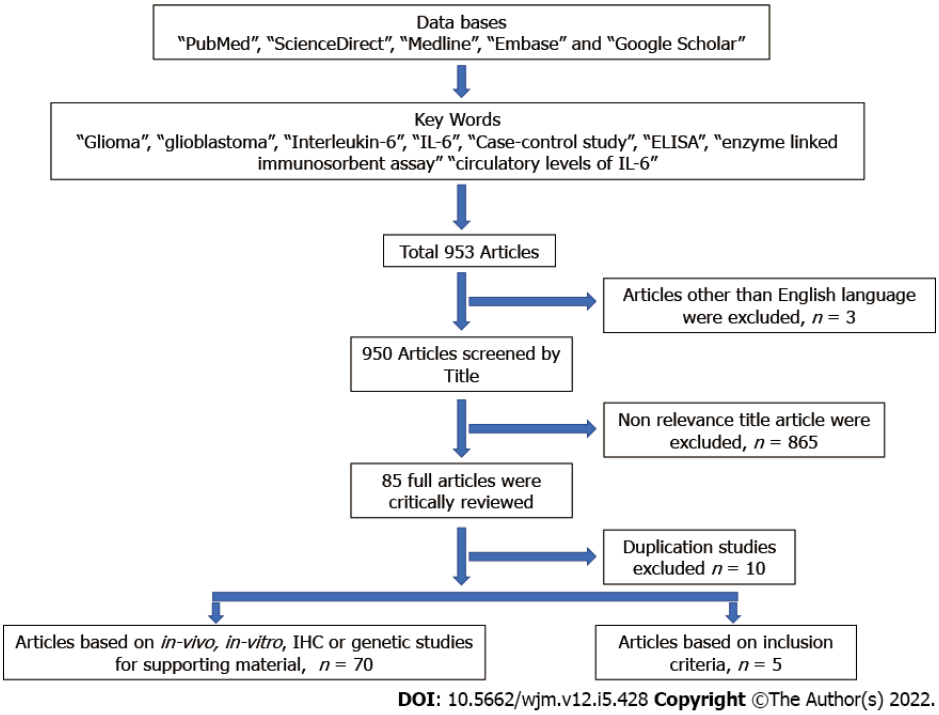

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Figure 2 Electronic search. This review was based on a search of electronic databases using the keywords shown. The resulting 953 articles were screened and assessed by language and three were excluded. The remaining 950 articles were again screened by title and 865 were excluded. The 85 studies were critically reviewed and 10 were duplicates, systematic reviews or meta-analyses and these were excluded. The resultant 75 studies were further divided into two: five studies were included in this mini literature review on the basis of inclusion criteria, and 70 studies based on *in vivo* and/or *in vitro* and/or genetic and/or immunohistochemistry methods were chosen as supporting articles.

to investigate disease development and progression using easily accessible samples like blood or urine or saliva. The published literature shows that there has been a scarcity of studies on the association between human brain cancer and IL-6, and published results are contradictory. However, *in vivo* studies have shown a strong relationship between IL-6 and disease initiation and progression. This indicates an urgent need to design studies to establish how IL-6 can be exploited as diagnostic or prognostic marker.

Glioma is a fatal disease with a reported survival rate of 5% despite surgical resection along with radiotherapy and/or chemotherapy. In spite of extensive research, the overall survival has not much improved[46]. Several experimental studies have shown that IL-6 can be produced by tumor cells, and glioma is characterized by systemic immunosuppression that hinders the response to immunotherapy and helps with tumor progression. Immunotherapy is currently the most explored area of cancer biology and has been shown to increase survival rate in patients with malignancies; however, for glioma its efficacy is currently still being revealed[47]. In glioblastoma, programmed death-ligand 1 (PD-L1) is the critical mediator of immunosuppression and myeloid cells (noncancerous cells) in the tumor microenvironment and circulation express an elevated level of PD-L1[48,49]. Experimental studies have

shown that glioblastoma-derived IL-6 is mandatory and sufficient for the induction of PD-L1, and the correlation between IL-6 and immunosuppression has been recognized *in vitro* and *in vivo*[50,51].

In this systematic review, the overall result was inconclusive. However, we found that most studies observed an elevated level of IL-6 in serum of glioma patients as compared to controls, which indicate the immunosuppressive role of IL-6 in tumor development[3,43]. IL-6, IL-8 and IL-1β are the proinflammatory cytokines and their circulatory expression is upregulated along with downregulated level of anti-inflammatory cytokine IL-4 in glioma, and higher secretion of proinflammatory cytokines is related to the progression of glioblastoma and poor survival rate[8,52,53]. In addition, studies based on expression analysis have shown that expression of IL-6 in glioma cases is significantly different from that in controls. Among grading of glioma, the intensity of IL-6 staining increases with increasing grading, which shows that patients with poorly differentiated tumor have a higher level of IL-6[43]. Therefore, measuring the circulatory levels of IL-6 before and after surgery can be standardized for the prediction of clinical prognosis of glioma.

The uptake and role of IL-6 in glioma invasion has been demonstrated by trans well invasion assay using glioma cell lines (U251 cells, U87 cells T98G cells and A172 cells) incubated with exogenous IL-6 [43]. These studies observed IL-6 in the supernatant of the glioma cell lines[43]. *STAT3* gene is considered to have a conserved sequence and mutation is rare; therefore, it is believed that its constitutive expression is regulated by upstream regulators and IL-6 is one of them[54]. This relationship has been observed in an *in vivo* study that concluded that STAT3 expression is dependent on IL-6 and it is increased in tumor progression[55]; hence, IL-6 has an important role in the development and progression of glioma. Our review found a significant association of IL-6 with disease progression[43, 45] except one study with a lower level of IL-6 in high-grade glioma[42].

The exact regulatory network of IL-6 in the tumor microenvironment is complex; therefore, targeting the underlying mechanism of IL-6 regulation should be undertaken to understand how its upregulation or over-active signaling pathways (especially IL-6/JAK/STAT3 signaling pathway) can help in tumor development, progression or recurrence[56]. Tumor formation is not a consequence of an adverse effect of a single risk factor or cytokine, but rather a group of cytokines, including chemokines, angiogenesis factors and growth factors. Therefore, combinational effects of cytokines can be used to assess their role in glioma and the results may be applied for future tailored immunotherapy and immune-monitoring procedures. Targeting and reducing the molecules hindering the activity of specific therapy may lead to re-sensitization to delivered therapy. Few clinical trials are investigating this idea[57].

CONCLUSION

This systematic review found five published research articles investigating the role of IL-6 as a potential biomarker of glioma in case–controls studies. The overall results are inconsistent; however, most studies found an elevated level of IL-6 in cases of glioma as compared to controls. The level of IL-6 was more than twofold in cases, which means that IL-6 can be considered as potential diagnostic biomarker. In tumors with progressive growth (advanced grade), the circulating level of IL-6 is also increased and hence can be used as a prognostic marker for glioma. Immunotherapy that can produce a durable and tumor-specific immune response can be implemented by disrupting IL-6 signaling and re-sensitizing the immune response to halt or reduce tumor growth and enhance survival rate based on REMARK (reporting recommendation for tumor biomarker prognostics studies) guidelines[58,59].

ARTICLE HIGHLIGHTS

Research background

Interleukin (IL)-6 is a proinflammatory cytokine that is involved in immunity, inflammation, angiogenesis, neural development and reproduction. The tumor microenvironment containing tumor cells, tumor-infiltrating immune cells and fibroblast stromal cells releases IL-6. IL-6 acts on the Janus kinase and signal transducer and activator of transcription factor pathway. These pathways release or associate with proteins that are responsible for major cellular functions.

Research motivation

This systematic review was motivated by a number of research studies that investigated the association between IL-6 and glioma.

Research objectives

In this systematic-review, case-control studies investigating the role of IL-6 with glioma development and progression have been discussed to review the utility of IL-6 as a biomarker.

Research methods

Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines were applied to filter the relevant studies based on inclusion and exclusion criteria. We used a combination of keywords and *Reference Citation Analysis* (*RCA*) tool to search for potential studies and performed data extraction from selected studies.

Research results

Five case–control studies were included for full evaluation. Most studies found a significantly higher level of IL-6 in cases as compared to controls although a study with contradictory results and a study with no difference in IL-6 level was also observed. Il-6 level varies with glioma stage, and some studies have reported lower levels in high-stage of cancer, whereas others have reported higher levels of IL-6 in early-stage glioma. Age at the time of diagnosis of glioma and IL-6 level could also have a significant relationship with glioma.

Research conclusions

IL-6 could be a potential biomarker for the diagnosis and prognosis of glioma as it was increased twofold in cases of glioma as compared to controls.

Research perspectives

Immunotherapy based treatment can be implemented by trigging IL-6 protein associated pathways and re-sensitizing the immune response to inhibit tumor growth and enhance survival rate.

FOOTNOTES

Author contributions: Singh M conceptualized this manuscript; Gautam KG and Raghav A performed the literature search and scrutiny of eligible studies; Gautam KG and Raghav A wrote the manuscript; all authors have read and approved the final draft of the manuscript.

Conflict-of-interest statement: All authors report no relevant conflict of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Wu YXJ
L-Editor: Kerr C
P-Editor: Wu YXJ

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 438-447

DOI: 10.5662/wjm.v12.i5.438

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Growth differentiation factor 15 as an emerging novel biomarker in SARS-CoV-2 infection

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Specialty type: Biochemistry and molecular biology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Hashemi S, Iran; Munteanu C, Romania; Papadopoulos K, Thailand

Received: March 5, 2022
Peer-review started: March 5, 2022
First decision: June 16, 2022
Revised: June 29, 2022
Accepted: August 30, 2022
Article in press: August 30, 2022
Published online: September 20, 2022

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Abstract

BACKGROUND

Growth differentiation factor (GDF)-15 is a member of a transforming growth factor-β cytokine superfamily that regulates metabolism and is released in response to inflammation, hypoxia and tissue injury. It has evolved as one of the most potent cytokines for predicting the severity of infections and inflammatory conditions, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

AIM

To investigate the utility of GDF-15 in predicting the severity of SARS-CoV-2 infection.

METHODS

PubMed, Reference Citation Analysis, CNKI, and Goggle Scholar were explored by using related MeSH keywords and data such as the first author's name, study duration, type and place of study, sample size and subgroups of participants if any, serum/plasma GDF- 15 level in pg/mL, area under the curve and cut-off value in receiver operating characteristic analysis, method of measurement of GDF-15, and the main conclusion were extracted.

RESULTS

In all studies, the baseline GDF-15 level was elevated in SARS-CoV-2-infected patients, and it was significantly associated with severity, hypoxemia, viral load, and worse clinical consequences. In addition, GDF-15 levels were correlated with C-reactive protein, D-dimer, ferritin and procalcitonin, and it had superior discriminatory ability to detect severity and in-hospital mortality of SARS-CoV-2 infection. Hence, GDF-15 might be used to predict the severity and prognosis of hospitalized patients with SARS-CoV-2.

CONCLUSION
Serial estimation of GDF-15 levels in hospitalized patients with SARS-CoV-2 infection appeared to have useful prognostic value and GDF-15 can be considered a clinically prominent sepsis biomarker for SARS-CoV-2 infection.

Key Words: SARS-CoV-2; Growth differentiation factor 15; Biomarker; Risk-stratification; Prognosis

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Core Tip: Growth differentiation factor (GDF)-15 levels are higher in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and higher levels are associated with disease severity, viremia and hypoxemia. The consistent increase in the concentration of GDF-15 during a hospital stay is associated with worse outcomes. Hence, serial monitoring of GDF-15 concentrations may provide useful prognostic value for hospitalized patients with SARS-CoV-2. GDF-15 appears to be involved in the underlying pathophysiology, laying the foundation for a novel therapeutic approach for SARS-CoV-2.

Citation: Parchwani D, Dholariya S, Katoch C, Singh R. Growth differentiation factor 15 as an emerging novel biomarker in SARS-CoV-2 infection. *World J Methodol* 2022; 12(5): 438-447
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/438.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.438

INTRODUCTION

Coronavirus disease-2019 (COVID-19), an extremely contagious disease, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global public health problem. The index case of this viral infection was confirmed in Wuhan, the capital city of Hubei Province, China in December 2019[1]. Then, SARS-CoV-2 quickly disseminated across the globe, infecting around 430257564 individuals with a global mortality of 5922047 people as of February 25, 2021[2]. Considering the massive spikes in cases of COVID-19 across countries within a short period of time, the World Health Organization declared COVID-19 as a public health emergency of international concern, giving it a global risk assessment of extremely high[3]. SARS-CoV-2 primarily infects respiratory tract cells and manifests as mild to fatal pneumonitis[4], especially in older men with comorbidities of hypertension, diabetes mellitus, or vascular disease[5].

SARS-CoV-2 is an enveloped virion with positive sense, single-stranded RNA with a genome size of 29.99 kb encoding for multiple nonstructural and structural proteins. The viral envelope contains four anchored structural proteins, spike protein (S), enveloped protein (E), nucleocapsid protein (N) and membrane protein (M)[6]. S glycoprotein (type 1 transmembrane protein) protrudes from the virus surface and embraces two functional components, S1 and S2. S1 helps the virus to binds with host cell through its receptor-binding domain (RBD) and S2 possesses an element essential for SARS-CoV-2 fusion with the host cell membrane[7].

SARS-CoV-2 enters type II pneumocytes in the lungs by binding with membrane-bound angiotensin-converting enzyme (ACE) 2 receptor through its RBD[6], primed by host cell surface transmembrane serine protease-2[8]. SARS-CoV-2 then starts replicating and migrating down to the airways and enters alveolar epithelial cells in the lungs, resulting in pre-eminent early viral loads and soluble ACE2 (sACE2) protein release into the bloodstream[9]. Cumulative viral load destroys type II alveolar epithelial cells and decreases the synthesis of pulmonary surfactants[10]. Simultaneously, infiltration of macrophages causes secretion of various cytokines namely tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6, instigating migration of lymphocytes and neutrophils and vasodilatation. This dysregulated host immune response plays a crucial role in the pathogenesis of the cytokine storm in SARS-CoV-2 infection[11].

Common clinical manifestations of SARS-CoV-2 infection are pyrexia, tussis, dyspnea, pharyngitis, myalgia, headache, and olfactory and taste dysfunction (hyposmia/anosmia or ageusia)[12]. However, severe consequences such as viral sepsis have been observed in approximately 20% of SARS-CoV-2-infected patients. Sepsis is a life-threatening systemic condition that graduates to cytokine storm followed by immune dysregulation, leading to systemic hyperinflammatory state, acute respiratory distress syndrome (ARDS), multiorgan failure, and development of sepsis-related complications with increased mortality[13].

Apart from inflammation and virulence, tissue tolerance and host response are also important factors for the pathogenesis and resultant consequences of SARS-CoV-2 infection[14]. A member of the transforming growth factor-β superfamily, growth and differentiation factor (GDF)-15, is a multifunctional anti-inflammatory cytokine that increases immunotolerance physiologically. It is an evolving modulator of immune responses and facilitates inflammation-induced tissue tolerance through metabolic adaptation[15,16]. Various pathways such as inflammation, hypoxia and oxidative stress tightly regulate expression of GDF-15[17]. In an animal model infected by human rhinovirus, GDF-15 promotes viral replication and virus-induced inflammation in the lungs[18]. Thus, GDF-15 may attenuate the antiviral immune response and affect the consequences of SARS-CoV-2 infection. Conversely, GDF-15 might increase in SARS-CoV-2 infection due to the altered balance of proinflammatory and anti-inflammatory cytokines[14].

Some biomarkers, such as C-reactive protein (CRP), D-dimer, ferritin[19] and presepsin[20], have been identified as biomarkers to assess the inflammation and consequences of SARS-CoV-2 infection. However, more than a year into the pandemic with little evidence of specific therapeutic regimens, front-line clinicians are still reliant on clinical presentation and basic imaging facilities for assessing risk stratification of SARS-CoV-2[21]. Since there are limited data on the accuracy of laboratory investigations for evaluating the severity of SARS-CoV-2 infection[22], identifying a novel biomarker such as GDF-15 offers the opportunity to triage patients for disease severity, allowing better care and timely management of critical patients. As GDF-15 predicts tissue tolerance in SARS-CoV-2-induced inflammation[14], it is worth reviewing the importance of GDF-15 for diagnosis and risk stratification of SARS-CoV-2. This systematic review emphasizes the importance of GDF-15 in SARS-CoV-2 infection by providing the most current evidence from studies that have examined GDF-15 in SARS-CoV-2 patients.

MATERIALS AND METHODS

Literature search strategy

The highly sensitive systematic literature search was carried out in multiple electronic databases: PubMed, Reference Citation Analysis (RCA), China National Knowledge Infrastructure (CNKI), Web of Science and Google Scholar. The following MeSH keywords were used to search the literature: GDF-15 AND SARS-CoV-2 OR GDF-15 AND COVID-19 OR GDF-15 AND 2019-nCoV OR GDF-15 AND Coronavirus Disease 2019. The inclusion criteria were English language articles published between December 1, 2020 and February 15, 2022. The original research articles, case series, brief reports, and letters were accepted for review. All selected articles' reference list was further screened to identify additional possible research literature. There was no exclusion based on the study outcome and stage or severity of SARS-CoV-2 infection. Finally, seven out of 24 articles were selected for the review after removing the duplicate research literature.

Data extraction

Using the above key terms, the first two authors independently searched for research literature following the inclusion and exclusion criteria, and both authors selected the final articles. The data were extracted in duplicate by standardized data extraction tables by two researchers. The following data were extracted: first author, place of study, sample size, disease severity/stage, intensive care unit (ICU) admission, survivors and nonsurvivors/death, GDF-15 level, and correlation with other inflammatory or sepsis biomarkers.

RESULTS

A total of 14 studies were retrieved after removing the duplicate or repeated publications; 13 of which were evaluated in full text. Among the included studies, seven were considered suitable for the qualitative synthesis. The process flow for the extraction of research literature (Figure 1) was conducted according to the guidelines defined in the PRISMA statement 2020 and was performed in accordance with a predetermined published protocol (PROSPERO ID: CRD42022311838).

DISCUSSION

The primary analysis of this systematic review revealed a high level of GDF-15 in SARS-CoV-2-infected patients and found a significant interaction with the severity of COVID-19. GDF-15 was also found to be positively correlated to predict the disease severity and to some degree is worthier than other inflammatory biomarkers as CRP, D-dimer, procalcitonin and ferritin. This conclusion came firstly from the study by Myhre and colleagues in 2020, which evaluated the utility of serum GDF-15 as a prognostic biomarker in hospitalized patients with SARS-CoV-2, and compared it with other known inflammatory

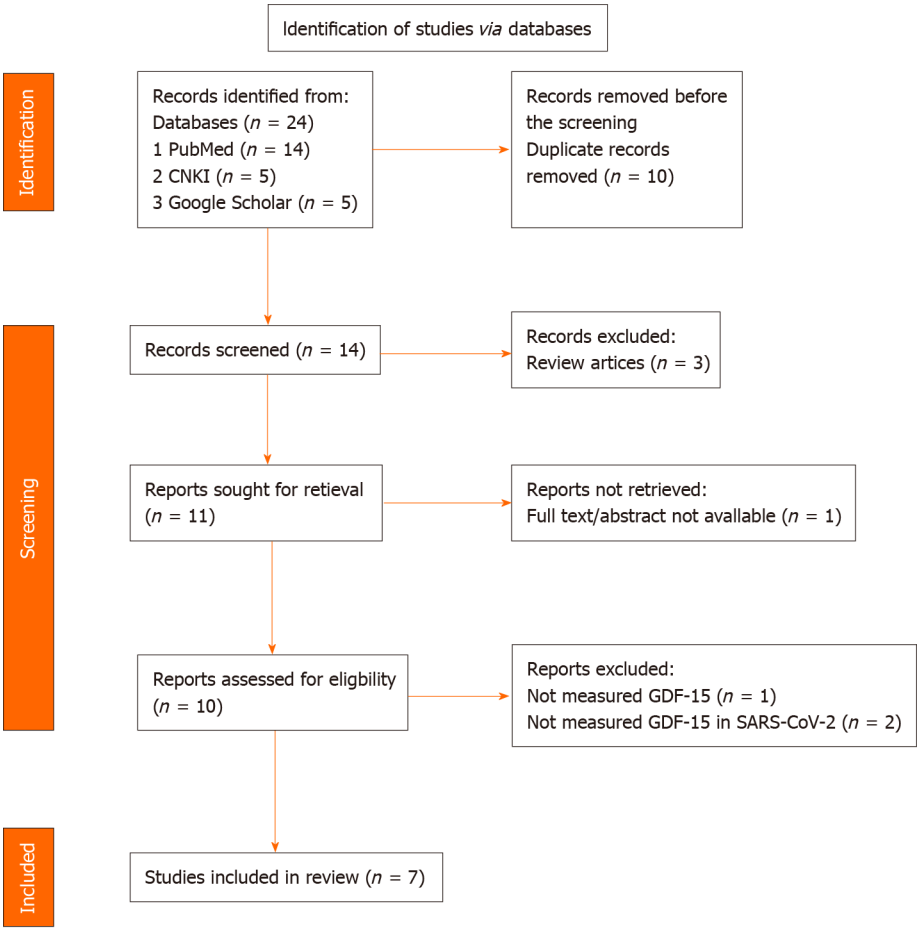

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Figure 1 Flow diagram for selection of research studies from various databases according to PRISMA 2020 guidelines. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

biomarkers (CRP, D-dimer, IL-6, procalcitonin and ferritin) in the Norwegian population from March 18, 2020 to May 4, 2020. The baseline GDF-15 level was elevated in 78% of cases of SARS-CoV-2 infection and it was found to be associated with viral load and hypoxemia. The GDF-15 concentrations were higher in patients who met the primary endpoint of ICU admission or death [4225.0 (3197.0–5972.0) pg/mL *vs* 2187.0 (1344–3620.0) pg/mL, $P < 0.001$]. Patients who reached the primary endpoint had a significant rise in GDF-15 from baseline to day 3 [86.0 (322.0–491.0) *vs* 1208.0 (0–4305.0) pg/mL, $P < 0.001$]. The area under the receiver operating characteristic curve (ROC) was 0.78 (95% confidence interval = 0.70–0.86), indicating a better prognostic significance of GDF-15 than for recognized inflammatory biomarkers such as CRP, ferritin, procalcitonin and IL-6. They derived a cut-off value of 2252.0 pg/mL that differentiated non-ICU survivors from nonsurvivors or ICU admission with good accuracy [23].

Secondly, Notz *et al*[24] measured blood GDF-15 in patients with SARS-CoV-2-induced ARDS in the German population from March 14 to May 28, 2020 and reported an increased level of GDF-15 in patients during their ICU stay. In addition, they testified that comorbidities were unlikely to influence the blood GDF-15 levels, and GDF-15 was not correlated with age, BMI or other anthropometric variables of patients[24]. Subsequently, Luis García de Guadiana Romualdo *et al*[25] evaluated the effect of circulating GDF-15 levels to predict the mortality of hospitalized SARS-CoV-2-infected patients in the Swedish population from March 14 to April 12, 2020. They found a significantly elevated level of GDF-15 in nonsurvivors compared to survivors of SARS-CoV-2 infection [9448.0 (6462.0–11707.0) *vs* 2590.0 (1886.0–4811.0) pg/mL]; a superior discriminatory ability of GDF-15 to predict in-hospital mortality at the cut-off value ≥ 7789.0 pg/mL [AUC = 0.892 (0.792–0.955), $P < 0.001$]. GDF-15 levels were also positively correlated with CRP ($r = 0.527$; $P < 0.001$), ferritin ($r = 0.334$; $P = 0.006$) and D-dimer ($r = 0.260$; $P = 0.035$). They concluded that GDF-15 might be used to predict the prognosis of in-hospitalized patients with SARS-CoV-2[25].

Likewise, Teng *et al*[26] retrospectively evaluated the profile of inflammatory factors in SARS-CoV-2-infected patients and healthy controls in China from January 22 to May 13, 2020. They assessed GDF-15 by categorizing SARS-CoV-2 patients into asymptomatic, mild, moderate, severe and convalescent; GDF-15 at admission, remission and discharge to find the association between dynamic alteration in

GDF-15 with the progression of SARS-CoV-2 infection, and found that GDF-15 concentration escalated consistently with disease severity. GDF-15 expression returned to normal in the convalescent group, as it did in the healthy participants. In continuance, study data revealed GDF-15 levels acutely upsurged with the worsening of symptoms before death, inferring that GDF-15 aptly monitors progression of SARS-CoV-2 infection. They reported an AUC value of 0.89 for GDF-15, which implied that the serum GDF-15 is an effective diagnostic biomarker to assess the severity of SARS-CoV-2 infection[26].

A prospective study conducted in the Swedish population[27] to evaluate the GDF-15 in SARS-CoV-2-infected patients and healthy controls reported a significantly ($P < 0.001$) higher level of GDF-15 in the severe [3562.0 pg/mL (2458.0–5880.0)] and moderate [3450.0 pg/mL (2337.0–4105.0)] type of SARS-CoV-2 infection compared to mild infection [748.0 pg/mL (586.0–1087.0)] and healthy participants [703.0 pg/mL (501.0–949.0)] throughout the acute phase. In the follow-up visit at 6 mo, severe and moderate SARS-CoV-2 infection was recorded with a high GDF-15 level compared to mild type and healthy controls ($P < 0.05$). Like the findings of Myhre *et al*[23], these authors also reported a significant association of GDF-15 with hypoxemia, viral load, and worse clinical consequences in SARS-CoV-2 infection[27].

Ebihara *et al*[28] conducted a prospective, multicenter observational study in the Japanese population to evaluate the role of cytokines in the pathogenesis of SARS-CoV-2 infection, through proteomics analysis. They found: an increased level of GDF-15 in patients with SARS-CoV-2 infection during ICU stay; an AUC of 0.764 and 0.740 for SARS-CoV-2 infection severity and prognosis, respectively; plasma level of GDF-15 was significantly associated with the time to wean off mechanical ventilation and delay recovery in ICU. Based on these results, the authors concluded that GDF-15 was positively related to the severity of SARS-CoV-2 infection and its concentration was significantly higher in patients with sepsis compared with SARS-CoV-2 infection[28].

Alserawan *et al*[29] evaluated serum GDF-15 level and correlated it with SARS-CoV-2 infection severity in the Spanish population. They reported a significantly ($P < 0.0001$) higher level of GDF-15 in SARS-CoV-2-infected patients [2051.0 (1474.0–2925.0) pg/mL] compared to healthy controls [582.0 (370.0–807.0) pg/mL] and in patients who were admitted to hospital for > 9 d. They categorized SARS-CoV-2 patients into $SpO_2/FiO_2 \leq 400$ and > 400 to find an association of GDF-15 with lung involvement. They found high GDF-15 levels in SARS-CoV-2-infected patients with $SpO_2/FiO_2 \leq 400$ or lung impairment. GDF-15 concentrations ≥ 1675.0 pg/mL were found to be a good predictor for impaired pulmonary function or $SpO_2/FiO_2 \leq 400$ compared with CRP and D-dimer, according to ROC analysis (AUC = 0.729, $P < 0.002$)[29]. Wallentin *et al*[30] observed that GDF-15 was associated with sACE2 levels, increased risk of mortality, and cardiovascular disease, which could help identify those at risk for severe COVID-19 infection.

Table 1 abridges the findings of included studies in this systematic review and Table 2 gives an overview of the data pertaining to GDF-15 in the included studies. Gleaned from the included studies, we conclude that GDF-15 has both diagnostic and prognostic importance in SARS-CoV-2 infection. As SARS-CoV-2 invades the lungs, it causes leukocyte migration, endothelialitis, hypoxia and tissue destruction by enhanced innate immunity[31]. All these factors promote secretion of GDF-15 from infected alveolar epithelial cells. Migration of leukocytes releases proinflammatory cytokines such as TNF-α, IL-8, IL-6, IL-1β, interferon- and granulocyte–macrophage colony-stimulating factor, which in turn stimulates the Notch pathway. The Notch pathway may well activate the Wnt and Hippo pathways, which, in succession cause differentiation of IL-17- and GDF-15-mediated inhibition of the T regulatory suppressor cell activity, respectively, which individually and in conjunction with one another results into extreme activation of the immune system. Concurrently, syncytical development further hyperactivates the immune system and results in a cytokine storm. Thus, GDF-15 plays a pivotal role in the immunological context and may influence the pathogenesis of SARS-CoV-2[14,32].

Impaired iron metabolism has also been hypothesized in the development of hyperinflammation and oxidative stress in patients with SARS-CoV-2 infection. GDF-15 has also been found to interact with iron metabolism, hepcidin and erythropoiesis during inflammation. More specifically, elevated GDF-15 during hypoxia and anemia has been found to suppress hepcidin expression, which boosts the iron level for hemoglobin production. As a result, GDF-15 has been considered as an immune modifier to regulate altered erythropoiesis and ferroptosis in patients with SARS-CoV-2 infection with anemia. During inflammation, GDF-15 overexpression has been associated with iron overload, which could increases ferritin, another key biomarker to assess severity of SARS-CoV-2 infection[33]. Hence, this hypothesis supports the association between high GDF-15 and anemia in inflammatory conditions such as SARS-CoV-2 infection, chronic kidney disease[34], diabetes, cardiovascular diseases[35], and cancer[36].

The iron chelation therapy improves innate immunity and endothelialitis in SARS-CoV-2 infection through its antifibrotic and antiviral properties[37] and is substantiated by the fact that the US FDA approved iron chelation therapy as an adjuvant treatment for the management of critical patients with SARS-CoV-2 infection[38]. Consequently, GDF-15 could be considered a crucial biomarker to indicate the prompt use of iron-chelating therapy in SARS-CoV-2 infection.

Metformin has recently been shown to elevate blood GDF15 levels, resulting in decreased satiety and body weight in clinical investigations. In animal studies, metformin was also associated with increased GDF-15 levels, along with increased GDF-15 expression in kidneys and intestines. In addition, metformin supplementation decreased weight in high-fat-fed mice, but not in GDF15-deficient mice and

Table 1 Studies included in the systematic review, with duration, type, region/place, and main findings

Refs	Study duration	Type of study	Region/place	Main findings
Myhre *et al*[23], 2020	March 18, 2020 to May 4, 2020	Prospective, observational study	Norway	GDF-15 has a better prognostic significance than recognized inflammatory biomarkers like CRP, ferritin, procalcitonin, and IL-6
Notz *et al*[24], 2020	March 14 to May 28, 2020	A single-center retrospective study	Germany	There was no evident imbalance of pro-and anti-inflammatory pathways, with higher GDF-15 levels in patients with SARS-CoV-2 infection during ICU stay, implying elevated tissue resilience
Luis García de Guadiana Romualdo *et al*[25], 2021	March 14 to April 12, 2020	Case-series	Spain	The GDF level was significantly high in nonsurvivors compared to survivors of SARS-CoV-2 infection, and it may be useful to predict prognosis
Teng *et al*[26], 2021	January 22, 2020, to May 13, 2020	Retrospective study	China	GDF-15 could be used as a biomarker to predict the severity of SARS-CoV-2 infection. GDF-15 level increased consistently with increased severity of SARS-CoV-2 infection, and GDF-15 expression returned to normal level similarly in a convalescent group compared to the healthy control participants. Hence, it implies that the GDF-15 precisely monitors the progression of SARS-CoV-2 infection
Kanberg *et al*[27], 2021	February 21 to November 5, 2020	Prospective study	Sweden	Patients with severe and moderate SARS-CoV-2 infection exhibited significantly increased GDF-15 levels compared with participants with mild infection and controls throughout the acute phase. Even after 6 mo of infection, GDF-15 concentrations persisted considerably higher in the severe and moderate infections compared to patients with mild infection and controls
Ebihara *et al*[28], 2022	August 2020 to December 2020	Prospective multicenter observational study	Japan	GDF-15 may be beneficial to predict delayed recovery or mortality of SARS-CoV-2-infected patients during ICU treatment
Alserawan *et al*[29], 2021	Not mentioned	Prospective study	Spain	GDF-15 may play a role in categorizing SARS-CoV-2-infected patients based on severityGDF-15 is an excellent biomarker to detect impaired respiratory function compared to CRP and D-dimer

CRP: C-reactive protein; GDF-15: Growth differentiation factor 15; IL-6: Interleukin 6; ICU: Intensive care unit; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

mice deficient for GFRAL (GDNF family receptor α-like, receptor for GDF-15)[39-42]. Thus, metformin supplementation has been associated with reduction in mortality in patients with SARS-CoV-2 infection with diabetes.

A few limitations of this analysis should be taken into consideration when interpreting the results for any potential clinical implications. Firstly, the sample size was small. Secondly, heterogeneity was a major issue in the included studies, especially in terms of methodology, type of ongoing treatment, time of sample collection after hospital admission, non-consideration of the disease-onset time and divergence in adjusting study variables (age, gender, and various comorbidities). Thirdly, variance in the quantification of GDF-15 and subclassification of patient populations in the included studies. Lastly, the literature search and coverage were limited to articles published in English; languages other than English were not considered for analysis, which is susceptible to a local literature bias. Nevertheless, the goal of this study was not to create a predictive model but to investigate the potential importance of GDF-15 as a novel biomarker[39-42]. Hence, despite these limitations, this systematic review offers vital information on the risk stratification of SARS-CoV-2, which could in the future become an important part of the clinical process.

CONCLUSION

GDF-15 appeared to be an important determinant in the etiopathogenesis of disease and might serve as a predictor for onset and severity of SARS-CoV-2 infection. Hence, GDF-15 can be considered a clinically prominent sepsis biomarker for screening, risk stratification, and monitoring SARS-CoV-2.

Table 2 Review of data extracted for growth differentiation factor 15 in SARS-CoV-2 infection from included studies

Refs		Myhre *et al*[23], 2020	Notz *et al*[24], 2020	Luis García de Guadiana Romualdo *et al*[25], 2021	Teng *et al*[26], 2021	Kanberg *et al*[27], 2021	Ebihara *et al*[28], 2022	Alserawan *et al*[29], 2021
Sample size and subgroup of participants, if any		123 confirmed cases of SARS-CoV-2 infection (non-ICU survivor = 88, ICU admission/ death = 28)	13 cases of SARS-CoV-2 infection with ARDS	66 confirmed cases of SARS-CoV-2 infection (non-survival = 58, survival = 6)	111 confirmed cases of SARS-CoV-2 infection and 20 healthy controls (asymptomatic = 14, mild = 12, moderate = 34, severe = 18, and convalescent = 33)	100 confirmed cases of SARS-CoV-2 infection (mild = 24, moderate = 28, severe = 48] and 51 healthy controls	306 confirmed cases of SARS-CoV-2 infection	84 confirmed cases of SARS-CoV-2 infection and 20 healthy controls
GDF-15 level in pg/mL	Healthy controls				13.5 (8.0–79.0)	703.0 (501.0–949.0)	-	582.0 (370.0-807.0)
	Mild				136.4 (44.7–321.4)	748.0 (586.0–1087.0)	-	2051.0 (1474.0-2925.0)
	Moderate		12400.0		256.2 (76.1–341.0)	3450.0 (2337.0–4105.0)	-	
	Severe			-	524.8 (405.1–831.1)	3562.0 (2458.0–5880.0)	Increased during ICU stay	
	Critical				621.0	-	-	-
	Non-ICU survivor	2187.0 (1344.0-3620.0)	-	2590.0 (1886.0-4811.0)	-	-	-	
	ICU admission or death	4225.0 (3197.0-5972.0)	-	9448.0 (6462.0-11707.0)	-	-	-	-
AUC and 95% CI of GDF-15 in ROC analysis		0.78 (0.70–0.86) $P < 0.001$	Not mentioned	0.89 (0.792–0.955) $P < 0.001$	0.89	Not mentioned	For severity: 0.764; For prognosis: 0.740	0.729 (0.602-0.857) $P = 0.002$
The optimal cut-off value of GDF-15		2252.0 pg/mL, to differentiate non-ICU survivors and ICU admission or death	Not mentioned	7789.00 pg/mL, to differentiate non-ICU survivors and ICU admission or death	Not mentioned	Not mentioned	Not mentioned	1675.0 pg/mL, to recognize deprived respiratory function ($SpO_2/FiO_2 \leq 400$)
Method of GDF-15 measurement		ELISA	ELISA	Electro-chemiluminescent	ELISA	Electro-chemiluminescent	ELISA	ELISA
Additional findings related to GDF-15		It was associated with viral load and hypoxemia. Better prognostic significance compared to CRP, ferritin, IL-6, and procalcitonin	It was not correlated with age and BMI	Positively correlated with CRP, ferritin, and D-dimer	GDF-15 indicates the severity and closely monitor the progression of SARS-CoV-2	Elevated GDF-15 was significantly related to hypoxemia, viral load, and worse clinical consequences	The plasma level of GDF-15 was significantly associated with the time to wean-off mechanical ventilation	Positively correlated with CRP, D-dimer, and neutrophil count and negatively correlated with lymphocyte count

ARDS: Acute respiratory distress syndrome; BMI: Body mass index; CRP: C-reactive protein; ELISA: Enzyme-linked immunosorbent assays; GDF-15: Growth differentiation factor 15; IL-6: Interleukin 6; ICU: Intensive care unit; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

ARTICLE HIGHLIGHTS

Research background

Growth differentiation factor (GDF)-15 is a modulator of immune responses and facilitates inflammation-induced tissue tolerance through metabolic adaptation. Experimental studies reveled that GDF-15 promotes virus replication and virus-induced inflammation in the lungs. Thus, GDF-15 may attenuate the antiviral immune response and affect the consequences of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Research motivation

To identify a novel biomarker for the guidance of severity of disease, so as to provide better care and timely management of critical patients.

Research objectives

To investigate the utility of GDF-15 in predicting the risk stratification of SARS-CoV-2.

Research methods

A systematic literature search was carried out in multiple electronic databases: PubMed, Reference Citation Analysis, China National Knowledge Infrastructure (CNKI), Web of Science and Google Scholar using MeSH keywords. The inclusion criteria were research articles of any type written in the English language and published between December 1, 2020 and February 15, 2022. There was no exclusion based on the study outcome and stage or severity of SARS-CoV-2 infection. Finally, seven of 24 articles were selected for the review after removing the duplicate research literature.

Research results

The primary analysis of this systematic review revealed a high level of GDF-15 in SARS-CoV-2-infected patients and found a significant interaction with the severity of COVID-19. GDF-15 was also found to be positively correlated to predict the disease severity and is superior to other inflammatory biomarkers such as C-reactive protein, D-dimer, procalcitonin and ferritin.

Research conclusions

Serial estimation of GDF-15 levels in hospitalized patients with SARS-CoV-2 infection may have useful prognostic value and GDF-15 can be considered a clinically prominent sepsis biomarker for screening, risk stratification, and monitoring SARS-CoV-2.

Research perspectives

Additional prospective studies are warranted in this regard to justify GDF-15 as an ideal biomarker which should provide optimization of disease status.

FOOTNOTES

Author contributions: Parchwani D and Dholariya S contributed equally to this work from the design of study, search and scrutiny of articles, analysis and manuscript writing to proof reading; Katoch CDS designed the research study, scrutiny of articles and manuscript writing; Singh R literature search and analysis; all authors have read and approve the final manuscript.

Conflict-of-interest statement: Authors hereby declare that there in no conflict of intrest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2020 Checklist, and the manuscript was prepared and revised according to the PRISMA 2020 guidelines.

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S-Editor: Liu JH
L-Editor: Kerr C

P-Editor: Liu JH

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 448-458

DOI: 10.5662/wjm.v12.i5.448

ISSN 2222-0682 (online)

META-ANALYSIS

Microvessel density in differentiated thyroid carcinoma: A systematic review and meta-analysis

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

P-Reviewer: Fang L, China; Lee KS, South Korea

Received: October 28, 2021
Peer-review started: October 28, 2021
First decision: December 27, 2021
Revised: December 30, 2021
Accepted: July 18, 2022
Article in press: July 18, 2022
Published online: September 20, 2022

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Abstract

BACKGROUND

Microvessel density (MVD) has been proposed as a direct quantification method of tumor neovascularization. However, the current literature regarding the role of MVD in differentiated thyroid carcinoma (DTC) remains inconclusive.

AIM

To appraise the effect of tumoral MVD on the survival of patients with DTC.

METHODS

This meta-analysis was based on the PRISMA guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. The electronic databases Medline, Web of Science, and Scopus were systematically screened. A fixed-effects or random-effects model was used, according to the Cochran *Q* test. The data were then extracted and assessed on the basis of the *Reference Citation Analysis* (https://www.referencecitationanalysis.com/).

RESULTS

A total of nine studies were included in the present study. Superiority of low MVD tumors in terms of 10-year disease free survival (OR: 0.21, 95%CI: 0.08–0.53) was recorded. Lowly vascularized thyroid cancers had a lower recurrence rate (OR: 13.66, 95%CI: 3.03–61.48). Moreover, relapsing tumors [weighed mean difference (WMD): 11.92, 95%CI: 6.32–17.52] or malignancies with regional lymph

node involvement (WMD: 8.53, 95%CI: 0.04–17.02) presented with higher tumoral MVD values.

CONCLUSION
MVD significantly correlates with the survival outcomes of thyroid cancer patients. However, considering several study limitations, further prospective studies of higher methodological and quality level are required.

Key Words: Cancer; Density; Microvessel; Thyroid; Vascularization

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Core Tip: This systematic review is the first meta-analysis investigating the effect of tumoral vascularity, through microvessel density (MVD) assessment, on the survival of patients with differentiated thyroid carcinoma. Higher intratumoral MVD values were associated with inferior disease-free survival outcomes.

Citation: Perivoliotis K, Samara AA, Koutoukoglou P, Ntellas P, Dadouli K, Sotiriou S, Ioannou M, Tepetes K. Microvessel density in differentiated thyroid carcinoma: A systematic review and meta-analysis. *World J Methodol* 2022; 12(5): 448-458
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/448.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.448

INTRODUCTION

Thyroid cancer is the most common endocrine tumor and includes several subtypes with different histologic, epidemiologic, and prognostic characteristics. Although they display a stable mortality rate, thyroid carcinomas are characterized by a rising trend of overall incidence of nearly 5.5% annually[1-3]. The above-mentioned increase is primarily attributed to a steady increment of new papillary thyroid cancer cases[1-3]. This is translated to an average of 56000 new cases and 2000 deaths per year in the United States alone[4]. Therefore, an attempt to identify survival-prognostic indicators for thyroid cancer has been implemented[5,6]. More specifically, extrathyroidal infiltration, aggressive histological pattern, vascular invasion, lymph node involvement, distant metastases, and BRAF mutations have been linked to a poorer survival outcome[7,8]. Various other serological, genetic, molecular, and immunohistochemical markers have also been considered[5,6,9-11].

Angiogenesis represents a pivotal part of tumor expansion and metastasis development. It includes a cascade of processes such as degradation of the basal membrane, remodeling of the extracellular matrix, migration of the endothelial cells, and maturation of the newly formed capillaries, which are regulated by several angiogenic and angioinhibitory factors[12-14]. Activation of the "angiogenic switch" due to alterations in the concentration of agents, such as vascular endothelial growth factor (VEGF) and thrombospondin-1 (TSP-1), as well as the subsequent upregulation of the *de novo* formation of blood vessels, has been associated with survival outcomes in thyroid cancer[15,16]. Microvessel density (MVD) as described by Weidner *et al*[17] has been proposed as a direct quantification method of tumor neovascularization. The methodology of MVD assessment involves the immunohistochemical staining for endothelium specific markers, such as von Willebrand factor (vWF), cluster of differentiation (CD)31, and CD34, for the labeling of microvessels[18]. The correlation between survival outcome and vascularity of a solid tumor has been extensively validated[19-22].

The current literature regarding the role of MVD in differentiated thyroid carcinoma (DTC) remains inconclusive. Initial studies reported that MVD displayed negative prognostic value in terms of survival, and was reversibly associated with the differentiation status of thyroid carcinomas[9,23,24]. However, subsequent trials did not confirm the prognostic role of MVD value or even document a positive correlation with survival endpoints[25,26]. Taking into consideration the above-mentioned evidence, a systematic literature review and meta-analysis was designed and conducted to clarify the effect of tumoral vascularity - through MVD assessment - on the survival of patients with DTC.

MATERIALS AND METHODS

Study protocol

This review was performed by applying the guidelines proposed in the PRISMA Statement and the Cochrane Handbook for Systematic Reviews of Interventions[27].

Endpoints

The primary endpoint of the present meta-analysis was the pooled odds ratio (OR) of disease-free survival (DFS) between high and low MVD measurements in patients with DTC[28,29]. Secondary endpoints included the hazard ratio (HR) of DFS and the OR of overall survival (OS) and DFS at specific time endpoints (5 and 10 years). Moreover, the effect of MVD on certain disease outcomes was examined, such as lymph node involvement, extrathyroidal infiltration, and recurrence rates.

Eligibility criteria

All prospective or retrospective studies that included a trial population diagnosed with DTC, reported outcomes of interest in English, and could be retrieved were considered as eligible. The MVD assessment of the primary tumor should have been introduced in the study design. The exclusion criteria for this meta-analysis were studies: (1) Written in a language other than English; (2) With no outcome of interest; (3) With insufficient data; (4) With no human subjects; (5) Including a pediatric study population; (6) Including undifferentiated or medullary thyroid cancer; or (7) In the form of editorials, letters, conference abstracts, or expert opinions.

Literature search

A systematic literature search was performed in the electronic scholar databases Medline, Scopus, and Web of Science. The last search date was August 31, 2021. The following keywords were used: "Thyroid", "thyroid cancer", "thyroid carcinoma", "papillary", "follicular", "Hurthle cell", "well differentiated", "MVD", "microvessel density", "microvascular density", and "vessel density".

Study selection and data collection

The first step of our review was removal of duplicate entries, followed by screening of titles and abstracts for consistency with the eligibility criteria. The remaining articles were submitted to a full text review. Searching of electronic databases, study selection, data extraction, and methodological assessment of the studies were performed blindly and in duplicate by two independent investigators (Perivoliotis K, Koutoukoglou P). If disagreement arose between the two investigators, a mutual revision and discussion process followed. If consensus was not achieved, the opinion of a third researcher was considered (Ntellas P). The methodological and quality evaluation was performed on the basis of the Newcastle-Ottawa Scale (NOS)[30]. This evaluation tool ranks non-RCT trials based on different domains, such as selection and comparability of the study groups and confirmation of the exposure. All eligible studies were rated with a score ranging from 0 to 9. Interrater agreement was estimated based on Cohens *k* statistic.

Statistical analysis

The statistical software used for the analyses included the Cochrane Collaboration RevMan version 5.3 and IBM SPSS version 23. All results are presented with the corresponding 95%CI. If the trials included did not directly provide data concerning the HR and OR endpoints, they were then estimated through the implementation of the algorithm proposed by Parmar *et al*[31] and Tierney *et al*[32]. By utilizing digitizing software, an accurate reconstruction of the primary data from the Kaplan-Meier (KM) curves was performed[33,34]. Furthermore, if the mean and standard deviation (SD) of the continuous variables were not reported, they were estimated from the respective median, range, or interquartile range (IQR)[35,36].

The statistical methods applied were the Maentel-Haenszel (MH) and inverse variance (IV), for OR and HR, respectively. If a statistically significant heterogeneity was present (Cochran Q test $P < 0.1$), a random-effects (RE) model was used. Otherwise, the pooled result estimation was based on a fixed-effects (FE) model. Overall heterogeneity was also quantified through the calculation of I^2. Statistical significance was considered at the level of $P < 0.05$.

Risk of bias across studies

Visual inspection of the primary outcome funnel plot was applied, to identify possible outliers. Moreover, Egger's statistical test was calculated.

RESULTS

Study selection

Application of the search algorithm resulted in the retrieval of 2208 citations (Figure 1). More specifically, the number of studies identified through Medline, Scopus, and Web of Science were 992, 517, and 699, respectively. After removal of 507 duplicate records, 1701 titles and abstracts were reviewed. In this phase of literature screening, 1626 studies (125 non-human studies, 130 reviews or meta-analyses, and 1371 irrelevant trials) were excluded. Full text review was applied to 75 articles to assess consistency with the inclusion criteria. After the exclusion of 50 irrelevant records and 16 studies

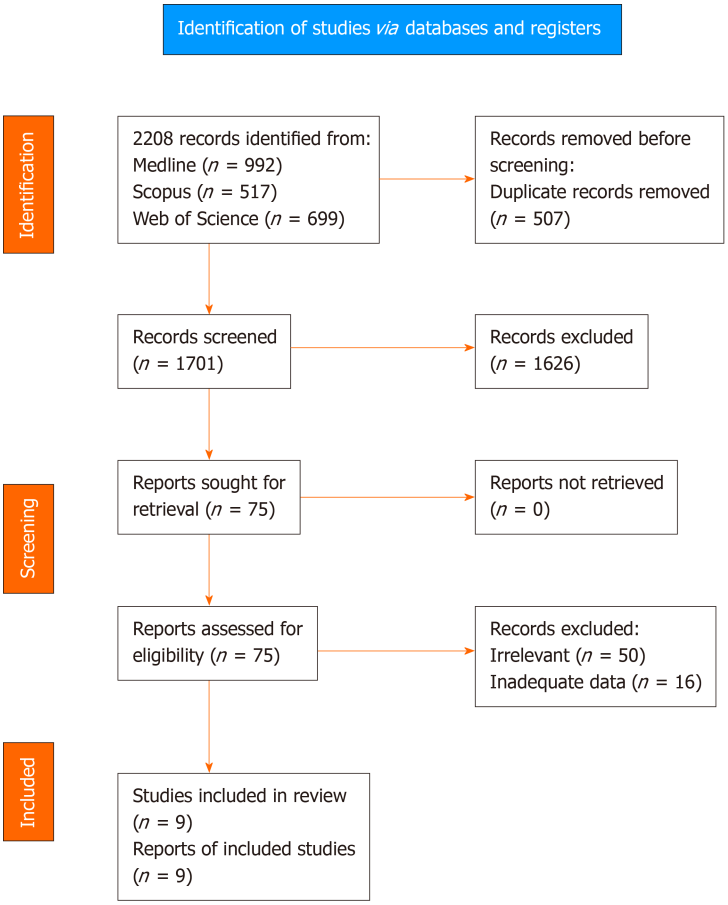

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Figure 1 Study flow diagram.

with inadequate survival data, a total of nine trials[23,25,37-43] were introduced in the qualitative and quantitative synthesis of the present systematic review.

Study characteristics

Table 1 summarizes the characteristics of studies included in the systematic review. Concerning the study design, all trials were retrospective and single centered, with publication years ranging from 1998 to 2017. In total, 738 patients were included in this meta-analysis. Mean age and gender allocation are also presented in Table 1. Mean follow up extended from 61.7 mo to 180 mo.

Supplementary Table 1 provides information regarding the tumor characteristics. The most frequent malignancy was papillary thyroid carcinoma (PTC) (708 cases), followed by follicular thyroid carcinoma (FTC) (27 cases). Although data regarding the tumor stage and the TNM classification were scarce and inconsistent, the respective allocations are also displayed.

Regarding MVD assessment method (Supplementary Table 2), in the majority of the articles[23,25,37,39,40], the technique proposed by Weidner *et al*[17] was implemented. In the remaining studies[38,41-43], variations of the hot spot method, such as the methodology described by Bono *et al*[44], were applied. The antibodies used for the immunochemical staining of the microvessels included the anti-CD34[35-39], anti-CD31[42,43], and anti-VIII antibodies[23,25]. The initial magnification applied spanned from 4 × to 40 ×, whereas the final magnification included values ranging from 200 × to 400 ×. The number of pathologists and hot spots examined varied among studies, thereby increasing the methodological heterogeneity. Blinding of the MVD estimator was applied in four trials[23,37,40,43]. Assessment of both intra- and peri-tumoral vessels was performed in only two studies[25,40]. Furthermore, the MVD cut off values are included in Supplementary Table 2. Overall, 324 total or subtotal thyroidectomies and 71 lobectomies were performed Supplementary Table 3). Lymph node dissection was reported in 574 cases. Data regarding the adjuvant chemotherapy or radiotherapy mode were not systematically provided.

Risk of bias within studies

Supplementary Table 4 provides a detailed report on the quality and methodological evaluation of the included trials. Although the number of stars awarded ranged from 3[39] to 7[42], the majority of trials

Table 1 Study characteristics

Ref.	Type of study	Year	Country	Center	Sample (patients)	Age	Gender (male/female)	Follow-up
Lee *et al*[36]	Retrospective	2017	Korea	Single center	202	43.4 (13.6)	43/159	NA
Liu *et al*[37]	Retrospective	2017	China	Single center	42	49.1 (13.5)	9/33	NA
Hakala *et al*[40]	Retrospective	2014	Finland	Single center	51	52	19/32	NA
Lee *et al*[41]	Retrospective	2012	Korea	Single center	47	> 45: 24	11/36	NA
Yasuoka *et al*[38]	Retrospective	2005	Japan	Single center	49	48.8 (15)	7/42	NA
Kilicarslan *et al*[39]	Retrospective	2003	Turkey	Single center	48	39.8	21/27	61.7 (29.7)
Akslen *et al*[25]	Retrospective	2000	Norway	Single center	128	45.1	36/89	145 (35.8)
Dhar *et al*[35]	Retrospective	1998	Japan	Single center	71	50 (9.8)	11/60	180 mo
Ishiwata *et al*[23]	Retrospective	1998	Japan	Single center	100	48 (9.6)	5/95	101 mo

NA: Not available.

received a 5 star grade. A satisfying rate of interrater agreement was identified (Cohen's k: 72.1%, P < 0.001).

Primary endpoint

Data regarding the primary outcome were extracted from three studies (Figure 2). Pooled analysis of these data showed a statistically significant OR (P < 0.001) for DFS between high and low MVD groups (OR: 0.21, 95%CI: 0.08–0.53). Heterogeneity levels were not significant (Q test P = 0.12, I^2=53%) and as a result, a FE model was applied. Due to the small number of studies reporting on the primary outcome and the moderate heterogeneity, further sub-analyses included only sensitivity analysis Supplementary Figure 1).

Secondary endpoints

In accordance with the primary outcome, a statistically significant OR for DFS at 5 years (Figure 1) was identified (P = 0.004). Therefore, overall HR (P < 0.001) for DFS was in favor of the low vascularity group (HR: 6.31, 95%CI: 2.81–14.17). However, meta-analysis of the raw data at 10 years postoperatively did not show a significant difference in survival terms (10-year OS: OR: 0.78, 95%CI: 0.01–61.19, P = 0.91). In total, five studies (Figure 3) provided data regarding mean MVD measurements between tumors with positive and negative lymph nodes. Although heterogeneity was high (Q test P < 0.001, I^2 = 93%), tumors that involved lymph nodes had higher mean MVD measurements (weighed mean difference [WMD]: 8.53, 95%CI: 0.04–17.02, P = 0.05) when compared to DTCs with negative nodes. Despite this, extrathyroidal infiltration was not associated with tumoral vascularity (OR: 1.86, 95%CI: 0.56–6.15, P = 0.31). Finally, recurrence rates in DTCs were significantly higher in the highly vascularized tumors (OR: 13.66, 95%CI: 3.03–61.48, P = 0.0007). Besides this, the thyroid malignancies that relapsed had significantly higher mean vascularization values (WMD: 11.92, 95%CI: 6.32–17.52, P < 0.001) than those that did not recur.

Risk of bias across studies

Concerning the funnel plot of the primary outcome Supplementary Figure 2), eligible trials were symmetrically distributed on both sides of the combined effect size line. Moreover, Egger's test did not confirm the presence of a significant publication bias (P = 0.585).

DISCUSSION

Our study validated a negative linkage between the intratumoral vascularity and the survival outcomes in DTC. Specifically, higher MVD values translated to a lower HR of DFS. In a similar manner, the DFS probabilities at 5 and 10 years after diagnosis increased when the DTC was hypovascularized. Furthermore, lymph node metastases were associated with a denser microvessel plexus in the primary tumor. In terms of recurrence, higher MVD measurements were correlated to superior relapse rates and *vice versa*. The rate of extrathyroidal invasion, however, did not appear to be affected by the tumor vascularization pattern. The effect of microvessel quantity in thyroid carcinoma is still a matter of controversy[16]. In 1994, Herrmann and his colleagues reported that reduced vascularization was found in less differentiated tumors[9]. Similarly, according to Kavantzas *et al*[45], FTCs and medullary thyroid

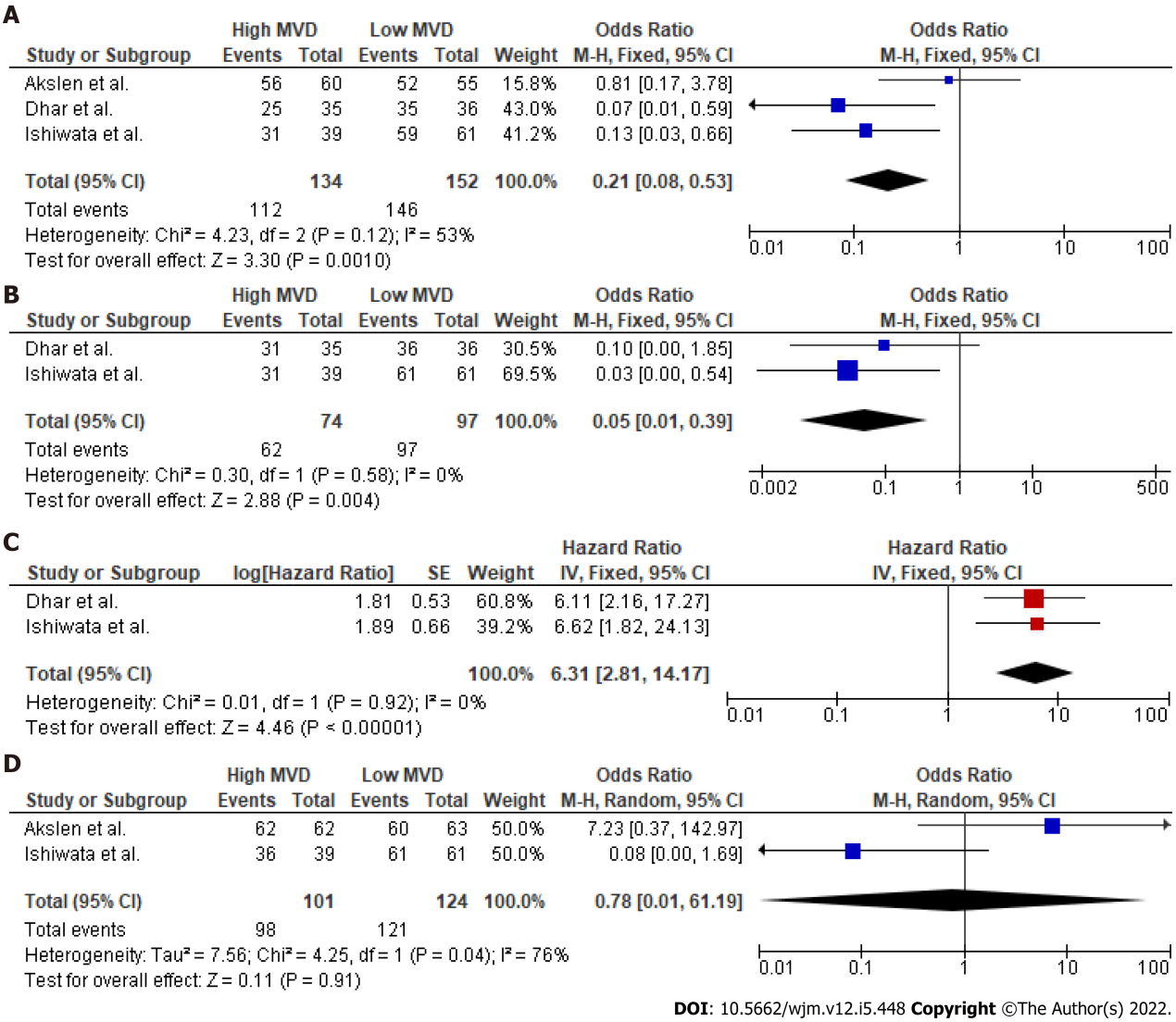

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Figure 2 Survival endpoints. A: 10 year disease free survival (DFS) odds ratio (OR); B: 5 year DFS OR; C: DFS hazard ratio; D: 10 year overall survival OR.

cancers (MTCs) were characterized by different mean MVD values. Diversity in the neovascularization pattern was also found among the differentiated carcinomas. Giatromanolaki *et al*[46] showed that FTCs displayed a higher vascular density, whereas subsequent research by Gulubova *et al*[26] suggested higher CD31 MVD in PTCs. However, several successive studies which applied either a CD34 or CD31 immunohistochemical marker for staining of the endothelium, could not identify a correlation between MVD and histology[10,11].

The fact that most of our quantitative comparisons were statistically significant suggests a possibly strong overall correlation between MVD and prognosis in DTCs. In a retrospective analysis of 71 DTCs, Dhar *et al*[37] correlated a lower recurrence free survival rate with a hypervascularized tumor. Correspondingly, using VIII-related immunohistochemical stain, Ishiwata *et al*[23] identified mean microvessel count as an independent prognostic factor for DFS. A denser angiogenetic pattern was also reported in tumors with a higher metastatic potential[47]. Additionally, MVD has been found significantly higher in malignancies with high risk characteristics, such as extrathyroidal and vascular invasion[48].

In contrast to the above-mentioned statements, a considerable number of studies do not recognize the prognostic character of MVD in thyroid carcinomas. Goldenberg *et al*[11] showed that although mean vessel density in the tumor was higher when compared to the healthy surrounding tissues, MVD lacked a significant correlation with histology or recurrence rates. Furthermore, in the study by Gulubova *et al*[26], postoperative survival rates in PTC patients were not associated with MVD values. Lee *et al*[43] also suggested that lymph node status was not linked to the MVD value of the primary malignancy. Moreover, in a study by Akslen *et al*[25], higher MVD was associated with improved OS rates in PTC patients.

The process of angiogenesis and the corresponding modulators have been extensively studied and related to MVD in thyroid carcinoma. VEGF was directly linked to the number of microvessels, and was characterized as a negative prognostic index for lymph node metastasis as well as local and distant

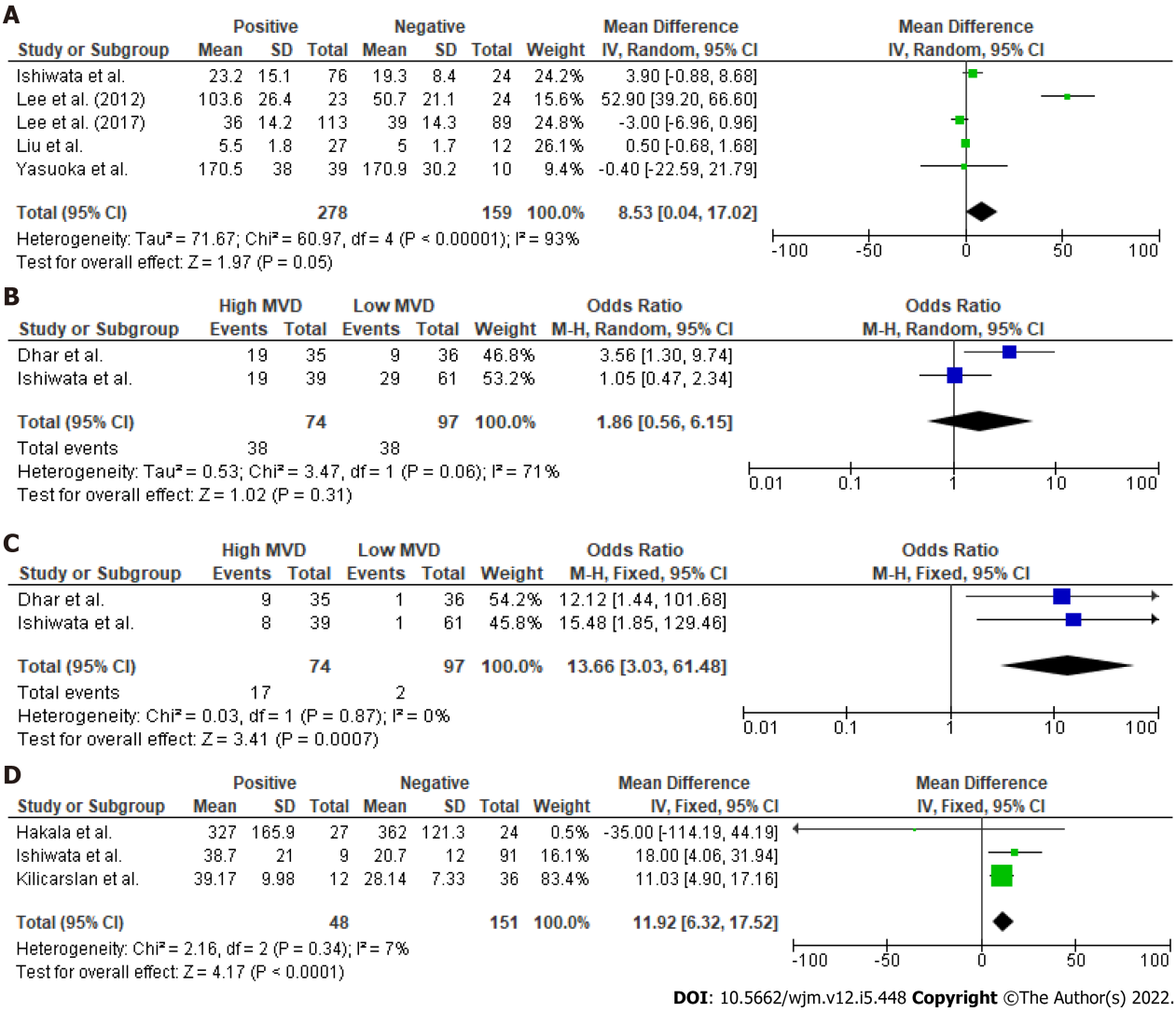

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Figure 3 Secondary endpoints. A: Lymph node involvement; B: Extrathyroidal involvement; C: Recurrence rate; D: Recurrence microvessel density value.

recurrence[26,40,41,43]. A higher rate of immunoreactive cells for metalloproteinase-9, an enzyme necessary for collagen degradation and subsequent angiogenesis, were present in advanced stages of FTCs[14]. Increased values of circulating and tumoral angiopoietins (Ang) have also been linked to poorer outcomes in thyroid cancer[49-51]. Based on the work of Tanaka *et al*, the levels of TSP-1 have been inversely correlated with the infiltration status of the tumor and MVD[52]. As a result, ratios representing the balance of angiogenic and inhibitory factors VEGF/TSP-1, VEGF-C/TSP-1, and Ang-2/TSP-1 have been significantly associated with the number of microvessels[52].

In addition to prognosis, tumor vascularization has also been proposed as a diagnostic tool in thyroid carcinomas. Using color flow Doppler sonographic analysis with a cut off value at 70% of microvessels, differential diagnosis between PTCs and adenomas or adenomatous nodules demonstrated a sensitivity of 92% and specificity of 89%[53]. The administration of contrast agents further validated the correlation of tumoral MVD and ultrasonographic assessment of vascularity, and increased the accuracy of PTC detection at the level of 95.9%[54,55]. In addition, the application of a shear elastography model by Gu *et al*[56] linked tumor stiffness with MVD values. Therefore, subsequent studies examined the role of the relationship between ultrasound estimation of vascularity and MVD, as a potential prognostic and risk assessment factor[38,39].

Before assessing the results of our meta-analysis, several limitations should be appraised. First, only a limited number of studies with a small sample size were introduced in each comparison, thus compromising the validity of our estimations. Moreover, all eligible studies had a retrospective study design, with a moderate-to-low methodological evaluation. Although significant heterogeneity was identified in only two endpoints, bias could be introduced from the non-homogeneous stratification of factors such as the histopathological subtype, the stage, and the TNM status. Another source of potential bias could be the heterogeneous allocation in operative and adjuvant treatment modules. Inconsistency was further identified in technical characteristics of the MVD assessment process. Finally, the estimation of survival endpoints required the reconstruction of raw information from the KM curves; therefore, a

small amount of bias was inherent in our data extraction methodology, although this process has been reported and applied in several studies[31,32,57].

The present systematic review and meta-analysis is the first study that attempts to provide a pooled correlation between MVD and survival endpoints in DTC. Higher intratumoral MVD values were associated with inferior DFS outcomes. Moreover, the thyroid malignancies presenting with lymph node infiltration displayed a higher vascularization pattern. Similarly, relapsing thyroid cancers when compared to non-recurring tumors were characterized by a denser microvascular plexus. Our study concludes that there are significant primary indications of a negative relationship between intratumoral MVD and survival outcomes. However, to clarify the exact effect of MVD on thyroid cancer and due to several study limitations, further prospective studies with a larger sample size as well as a higher methodological and quality level are required.

CONCLUSION

MVD significantly correlates with the survival outcomes of thyroid cancer patients. However, considering several study limitations, further prospective studies of higher methodological and quality level are required.

ARTICLE HIGHLIGHTS

Research background

An attempt to identify survival-prognostic indicators for thyroid cancer has been implemented

Research motivation

Microvessel density (MVD) has been used as a direct quantification method of tumor neovascularization

Research objectives

This meta-analysis attempted to clarify the effect of tumoral vascularity - through MVD assessment - on the survival of patients with differentiated thyroid carcinoma (DTC).

Research methods

The present meta-analysis was based on the PRISMA guidelines and the Cochrane Handbook for Systematic Reviews of Interventions.

Research results

Lowly vascularized thyroid cancers had a lower recurrence rate. Moreover, relapsing tumors or malignancies with regional lymph node involvement presented with higher tumoral MVD values.

Research conclusions

MVD significantly correlates with the survival outcomes of DTC patients

Research perspectives

Further prospective studies and randomized controlled trials have to be conducted in order to elucidate the correlation between MVD and prognosis in DTC.

ACKNOWLEDGEMENTS

Special thanks to Anagnostopoulos L for language editing.

FOOTNOTES

Author contributions: Perivoliotis K, Ntellas P, and Samara AA performed study conception and design; Dadouli K and Koutoukoglou P acquired the data; Perivoliotis K and Ntellas P analyzed and interpreted the data; Perivoliotis K, Ntellas P, and Samara AA drafted the manuscript; Ioannou M, Tepetes K, and Sotiriou S critically revised the manuscript; all authors have approved the final version of the present manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Xing YX
L-Editor: Wang TQ
P-Editor: Xing YX

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 459-460

DOI: 10.5662/wjm.v12.i5.459

ISSN 2222-0682 (online)

LETTER TO THE EDITOR

Radiological evaluation of patellofemoral instability and possible causes of assessment errors: Letter to the editor

Mohamed Kamal Mesregah

Specialty type: Orthopedics

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Mingli F, China; Shariati MBH, Iran

Received: May 6, 2022
Peer-review started: May 6, 2022
First decision: July 12, 2022
Revised: July 12, 2022
Accepted: August 17, 2022
Article in press: August 17, 2022
Published online: September 20, 2022

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Abstract

This letter to the editor is a commentary on the study titled "Radiological evaluation of patellofemoral instability and possible causes of assessment errors". There are some pertinent structural changes and radiological findings that should be considered in the setting of traumatic knee injuries, as their recognition is of paramount importance.

Key Words: Patellofemoral instability; Radiological evaluation; Sliver sign; Avulsion fractures; Osteochondral lesions; Bone oedema

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Core Tip: The radiological diagnosis of patellofemoral instability is pivotal in management as some radiological findings may necessitate surgical intervention. Therefore, image interpretation should be meticulous. Some crucial radiological findings should be considered in the setting of traumatic knee injuries.

Citation: Mesregah MK. Radiological evaluation of patellofemoral instability and possible causes of assessment errors: Letter to the editor. *World J Methodol* 2022; 12(5): 459-460
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/459.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.459

TO THE EDITOR

I read with interest the review article titled "Radiological evaluation of patellofemoral instability and possible causes of assessment errors" by Ormeci *et al*[1], published in the March 2022 issue of *World Journal of Methodology*. The review article focused on the

potential causes of errors that can occur when measuring some radiographic instability factors, including trochlear dysplasia, patella alta, tibial tuberosity-trochlear groove distance, and patellar tilt[1].

I would like to further discuss some pertinent structural changes and radiological findings that should be considered in the setting of traumatic knee injuries, as their recognition is of paramount importance.

On knee radiographs, a small osseous avulsion fracture on the peripheral margin of the medial patellar facet, known as the "sliver sign", may indicate avulsion of the attachment of the medial patellofemoral ligament (MPFL) and potential patellar dislocation[2].

Studies have shown that 30% of these avulsion fractures are only likely to be recognized on the dedicated patellar view; therefore, including a sunrise view in cases of traumatic knee injuries is essential[3]. Moreover, in the case of radiographic avulsion fracture, further evaluation of additional stigmata of previous patellar dislocation by magnetic resonance imaging (MRI) is recommended[4].

Generally, bone edema of the inferomedial aspect of the patella and the lateral femoral condyle and MPFL disruption indicate a recent patellar dislocation[5].

Even after reduction, the patella typically does not fully return to its normal position. MRI usually reveals patella subluxation or tilt in the majority of patients, and medial patellar chondral lesions are seen in more than two-thirds of patients[5,6]. A concave impaction of the inferomedial patella is highly specific for prior dislocation of the patella[7].

Osteochondral lesions of the lateral condyle are present in approximately 40% of patients. The presence of completely separated bone fragments that may appear as intraarticular bodies is an indication of surgery[8].

The radiological diagnosis of patellofemoral instability is pivotal in management as some radiological findings may necessitate surgical intervention. Therefore, image interpretation should be meticulous.

FOOTNOTES

Author contributions: Mesregah MK revised the literature and wrote the letter.

Conflict-of-interest statement: The author declares that they have no conflict of interest to disclose.

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S-Editor: Liu JH
L-Editor: Wang TQ
P-Editor: Liu JH

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World Journal of Methodology

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World J Methodol 2022 September 20; 12(5): 461-464

DOI: 10.5662/wjm.v12.i5.461

ISSN 2222-0682 (online)

LETTER TO THE EDITOR

Mouth shield to minimize airborne transmission risk of COVID-19 and other infectious diseases in the dental office

Mohiddin R Dimashkieh, Mohammad Zakaria Nassani, Yousef Fouad Talic, Ali Alqerban, Amir M Demachkia

Specialty type: Medical laboratory technology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kalani M, Iran; Nalunkuma R, Uganda

Received: June 1, 2022
Peer-review started: June 1, 2022
First decision: June 27, 2022
Revised: July 4, 2022
Accepted: August 7, 2022
Article in press: August 7, 2022
Published online: September 20, 2022

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Abstract

Transmission of coronavirus disease (COVID-19) and other infectious diseases is a significant risk during dental procedures because most dental interventions involve aerosols or droplets that could contaminate the surrounding environment. Current protection guidelines to address the high risk of droplets, aerosols, and airborne particle transmission of COVID-19 in the dental office recommend minimizing aerosol-generating procedures. In this paper, an innovative mouth shield is presented that should minimize water backsplash from the air-water syringe during dental treatment. The mouth shield can be added to the personal protective equipment to provide the dental team with extra protection. It can be made of different materials, is straightforward, inexpensive, and safe to fabricate, and is easy to use.

Key Words: Mouth shield; Transmission; Dentistry; COVID-19; Airborne; Droplets; Aerosols; Infectious diseases

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Core Tip: This letter to the editor presents an innovative mouth shield to increase the protection of the dental team against the water backsplash of aerosols, droplets, and airborne particles during dental procedures.

Citation: Dimashkieh MR, Nassani MZ, Talic YF, Alqerban A, Demachkia AM. Mouth shield to minimize airborne transmission risk of COVID-19 and other infectious diseases in the dental office. *World J Methodol* 2022; 12(5): 461-464
URL: https://www.wjgnet.com/2222-0682/full/v12/i5/461.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i5.461

TO THE EDITOR

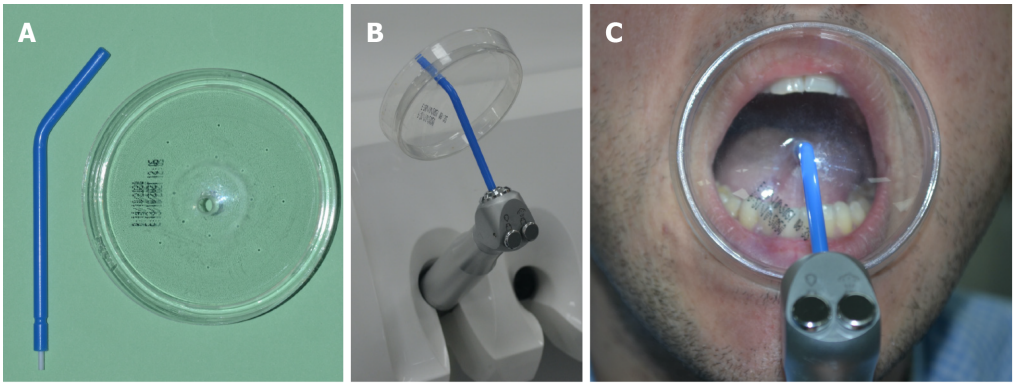
The coronavirus disease (COVID-19) pandemic has spread fear and anxiety across the globe because of its high death toll[1]. Various strategies have been introduced to combat the transmission of COVID-19 and reduce its severity, including the expedited development and approval of vaccines[2]. The risk of transmission of COVID-19 in the dental office has led to specific treatment guidelines and protocols, including the minimal use of aerosol- or droplet-generating procedures[3-6]. However, most dental interventions produce aerosols and droplets, contaminating the surrounding environment and leaving dental personnel at risk of acquiring COVID-19 from infected patients. Although non-emergency dental services were halted at the outset of the pandemic, the long duration of the pandemic has required dental practices to resume their services, but with additional precautions and careful triage of patients [7]. Strict adherence to preventive and protective measures became the mantra for oral care services to maintain an active dental practice at the era of COVID-19[8,9]. The aim of this paper is to introduce an innovative, straightforward, and inexpensive personal protection device that minimizes water backsplash from air-water syringes during cavity washing and drying. The goal was to develop a special mouth shield that should minimize the transmission risk of COVID-19 and other infectious diseases *via* airborne droplets or aerosols in the dental office.

MOUTH SHIELD

The mouth shield attaches to the air-water syringe tip and consists of a transparent shield made from the plastic lid of a conventional, disposable, crystal clear plastic cup. The center of the lid is perforated with a 3.5-mm-diameter twist drill to produce a frictional fit with the tip of an air-water syringe and form a disposable mouth shield (Figures 1A and B). The mouth shield can be positioned to maintain light contact with the patient's lips (Figure 1C). It can be used with most air-water syringes during various dental procedures. Different size lids made from disposable, crystal clear polyethylene terephthalate plastic or polystyrene can be selected to accommodate patients with varying degrees of mouth opening. The front surface of the shield can be relined with a water absorbent liner to capture scattered droplets. The mouth shield can also be easily adjusted forward and backward along the tip (nozzle) of the air-water syringe for convenience (video).

DISCUSSION

The COVID-19 pandemic and the increased risk of infection prompted the authors to develop a cost-effective disposable mouth shield to provide protection against back splashes of aerosols, droplets, and airborne particles during dental treatment. An air-water syringe is essential for dental procedures such as etching, bonding, cavity cleansing, and impression making. Contamination from the aerosol could be a major source of infection[10]. The association between aerosols, droplets, and splatter and the transmission of COVID-19 has been emphasized, and recommendations have been made to reduce their generation during the coronavirus pandemic[4,11-13]. Furthermore, emphasis has been placed on the role of personal protective equipment such as medical masks, protective face shields, and goggles in preventing and minimizing airborne transmission of COVID-19[14,15]. Despite the use of personal protective equipment, transmission of the viral infection is still possible, and additional preventive precautions are advised. For example, while wearing magnifying loops, it is not feasible to wear a face shield, leaving the face of the operator exposed to contamination. The described mouth shield provides additional protection at minimal cost. It is designed to prevent water backsplash out of the oral cavity during mouth/tooth washing and drying, minimizing contamination of the surrounding environment and dental personnel. Being transparent, the shield will allow light to reach the field of operation and



DOI: 10.5662/wjm.v12.i5.461 **Copyright** ©The Author(s) 2022.

Figure 1 Crystal clear plastic cup lid mouth shield. A: Traditional, disposable, crystal clear plastic cup lid perforated in the center using a 3.5-mm-diameter twist drill and a disposable air-water syringe tip; B: The air water syringe tip is inserted with a friction fit through the central hole of the plastic cover to form a mouth shield; C: The mouth shield rests lightly on the patient's lips, sealing the mouth during water/air spray.

allow the operator to easily see into the patient's mouth. The described mouth shield has been successfully implemented and evaluated in our dental practice. Nevertheless, the effectiveness of the mouth shield in minimizing the airborne aerosols and droplets spread during dental treatment should be investigated, and its role in protecting against infectious diseases, with a comparison of the load of produced aerosols, droplets and airborne particles with and without this shield, should be examined before this shield can be adopted for global use.

ACKNOWLEDGEMENTS

The authors would like to thank the Deanship of Graduate Studies and Scientific Research at Dar Al Uloom University for supporting the publication of this paper.

FOOTNOTES

Author contributions: Dimashkieh MR proposed the topic of the paper; Dimashkieh MR and Nassani MZ prepared the original draft; Talic YF, Alqerban A and Demachkia AM reviewed and revised the original draft; all authors discussed and agreed the final draft.

Conflict-of-interest statement: All authors declare no conflict of interest.

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S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

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ISSN 2222-0682 (online)

World Journal of *Methodology*

World J Methodol 2022 November 20; 12(6): 465-487

Published by Baishideng Publishing Group Inc

World Journal of Methodology

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Bimonthly Volume 12 Number 6 November 20, 2022

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World Journal of Methodology

Bimonthly Volume 12 Number 6 November 20, 2022

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ABOUT COVER

Peer Reviewer of *World Journal of Methodology*, Chun-Fu Zheng, Adjunct Professor, Department of Microbiology, Immunology & Infection Diseases, University of Calgary, Health Research Innovation Centre, 3330 Hospital Drive NW, Calgary, AB T2N 4N1, Canada. zheng.alan@hotmail.com

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INDEXING/ABSTRACTING

The *WJM* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Ji-Hong Liu*.

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Bimonthly

EDITORS-IN-CHIEF
Bruno Megarbane

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2222-0682/editorialboard.htm

PUBLICATION DATE
November 20, 2022

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World J Methodol 2022 November 20; 12(6): 465-475

DOI: 10.5662/wjm.v12.i6.465

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Impact of gender-affirming hormone therapy on the development of COVID-19 infections and associated complications: A systematic review

Jennifer J Ferraro, Allie Reynolds, Sylvia Edoigiawerie, Michelle Y Seu, Sydney R Horen, Amir Aminzada, Alireza Hamidian Jahromi

Specialty type: Surgery

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Chavan RP, India; El Sayed S, Egypt

Received: April 11, 2022
Peer-review started: April 11, 2022
First decision: June 27, 2022
Revised: July 14, 2022
Accepted: October 5, 2022
Article in press: October 5, 2022
Published online: November 20, 2022

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Abstract

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can produce a wide range of clinical manifestations from asymptomatic to life-threatening. Various researchers have worked to elucidate the pathogenic mechanisms underlying these variable presentations. Differences in individual responses to systemic inflammation and coagulopathy appear to be modulated by several factors, including sex steroid hormones. Transgender men or non-binary individuals who undergo gender-affirming hormone therapy (GAHT) are a unique population of interest for exploring the androgen-mediated coronavirus disease 2019 (COVID-19) hypothesis. As the search for reliable and effective COVID-19 treatments continues, understanding the risks and benefits of GAHT may mitigate COVID-19 related morbidity and mortality in this patient population.

AIM

To investigate the potential role of GAHT in the development of COVID-19 infections and complications.

METHODS

This systematic review implemented an algorithmic approach using PRISMA guidelines. PubMed, Scopus, Google Scholar top 100 results, and archives of *Plastic and Reconstructive Surgery* was on January 12, 2022 using the key words "gender" AND "hormone" AND "therapy" AND "COVID-19" as well as associated terms. Non-English articles, articles published prior to 2019 (prior to COVID-19), and manuscripts in the form of reviews, commentaries, or letters were excluded. References of the selected publications were screened as well.

RESULTS

The database search resulted in the final inclusion of 14 studies related to GAHT COVID-19. Of the included studies, only two studies directly involved and reported on COVID-19 in transgender patients. Several clinical trials looked at the relationship between testosterone, estrogen, and progesterone in COVID-19 infected cis-gender men and women. It has been proposed that androgens may facilitate initial COVID-19 infection, however, once this occurs, testosterone may have a protective effect. Multiple clinical studies have shown that low baseline testosterone levels in men with COVID-19 are associated with worsening outcomes. The role of female sex hormones, including estrogen and progesterone have also been proposed as potential protective factors in COVID-19 infection. This was exemplified in multiple studies investigating different outcomes in pre- and post-menopausal women as well as those taking hormone replacement therapy. Two studies related specifically to transgender patients and GAHT found that estrogen and progesterone could help protect men against COVID-19, and that testosterone hormone therapy may increase the risk of contracting COVID-19.

CONCLUSION

Few studies were found related to the role of GAHT in COVID-19 infections. Additional research is necessary to enhance our understanding of this relationship and provide better care for transgender patients.

Key Words: COVID-19; Transgender; Gender-affirming hormone therapy; Gender affirmation; Testosterone; Estrogen

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Core Tip: Severe acute respiratory syndrome coronavirus 2 can produce a wide range of clinical manifestations from asymptomatic to life-threatening. Differences in individual responses to systemic inflammation and coagulopathy appear to be modulated by several factors, including sex steroid hormones. Androgens may facilitate initial coronavirus disease 2019 (COVID-19) infection, however, once this occurs, testosterone may have a protective effect. The role of estrogen and progesterone has also been proposed as potential protective factors in COVID-19 infection. Few studies have investigated the role of gender-affirming hormone therapy in COVID-19 infections. Additional research is necessary to enhance our understanding of this relationship and provide better care for transgender patients.

Citation: Ferraro JJ, Reynolds A, Edoigiawerie S, Seu MY, Horen SR, Aminzada A, Hamidian Jahromi A. Impact of gender-affirming hormone therapy on the development of COVID-19 infections and associated complications: A systematic review. *World J Methodol* 2022; 12(6): 465-475
URL: https://www.wjgnet.com/2222-0682/full/v12/i6/465.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i6.465

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent behind the coronavirus disease 2019 (COVID-19) global pandemic, has a wide array of clinical manifestations ranging from asymptomatic to life-threatening disease[1]. Various researchers have worked to elucidate the pathogenic mechanisms underlying these highly variable presentations, with many agreeing that the critical role of the immunological hyper-response (characterized by widespread endothelial damage, complement-induced blood clotting, and systemic microangiopathy) facilitates inflammation and disease progression[2]. Differences in individual responses to systemic inflammation and coagulopathy appear to be modulated by several factors, including sex steroid hormones[2].

Older age and male sex are known risk factors for more severe manifestations of the COVID-19 disease[3-5]. Even after controlling for other risk factors commonly found among men, such as a hypertension, smoking, and cardiovascular disease, the mortality rate of COVID-19 has been shown to be higher in cis-gender males compared with cis-gender females[6]. The molecular basis of the observation can be attributed to transcription of transmembrane protease serine 2 (TMPRSS2), a protease that processes SARS-CoV-2 spike proteins that bind angiotensin converting enzyme 2 (ACE2) receptors and mediate entry of the virus into host cells[7]. The expression of both TMPRSS2 and ACE2 appears to be androgen-mediated[8,9]. For this reason, androgens like testosterone, and other important sex hormones like estrogen and progesterone, have been investigated for their potential role in the age and sex-specific severity of COVID-19[10-12].

Given the risks associated with male sex hormones, patients with gender dysphoria (transgender men or non-binary individuals) who undergo gender-affirming hormone therapy (GAHT) have become another population of interest for exploring the androgen-mediated COVID-19 hypothesis[13]. Through GAHT, transgender women are prescribed natural or synthetic estrogens[14], while transgender men take exogenous testosterone titrated to physiological female range estradiol levels and male-range serum testosterone levels, respectively[15]. For the latter, there is a paucity of data on the safety and health risks associated with long-term testosterone administration in transgender men[16]. As the search for reliable and effective COVID-19 treatments continues, understanding the risks and benefits of GAHT (especially masculinizing treatments) may mitigate COVID-19 related morbidity and mortality in a unique and vulnerable patient population.

The purpose of this study was to perform a systematic review and meta-analysis of the literature pertaining to potential role of GAHT in the development of COVID-19 infections and associated complications.

MATERIALS AND METHODS

The current systematic review implemented an algorithmic approach to review all of the available English medical literature on the impact of GAHT on the development of COVID-19 infections using the preferred reporting items for systematic reviews and metanalysis (PRISMA) principles (Figure 1). A comprehensive search of the medical literature in the "PubMed," "Scopus," "Reference Citation Analysis (RCA)," "Google Scholar" top 100 results, and previous issues of *Plastic and Reconstructive Surgery* was performed by two authors (A.R. and S.E.) on January 12, 2022 using the key words "gender" AND "hormone" AND "therapy" AND "COVID-19" as well as associated terms.

The search string was generated, and records that were not specific about GAHT or COVID-19 were excluded. Foreign language (non-English) articles were not eligible for inclusion. Articles published prior to 2019 were excluded as being prior to the COVID-19 pandemic and therefore not relevant to complications associated with COVID-19 infection. Titles and abstracts were screened by two authors (A.R. and S.E.) after which full-text articles were assessed for eligibility and inclusion. On initial and secondary searches, papers in review, commentaries, letters, or those without accessible full-text articles were excluded. References of the selected publications were additionally screened with the aforementioned inclusion criteria.

RESULTS

In total, 14 studies were included in this review per the inclusion/exclusion criteria (Figure 1). Two studies were laboratory-based research (Table 1), while the remaining were clinical studies, including one randomized-control trial (Table 2). Only two studies directly involved and reported on COVID-19 in transgender patients (Table 3).

DISCUSSION

The sexual dimorphism seen in COVID-19 morbidity and mortality outcomes has contributed to the hypothesis that the male sex hormone, testosterone, may be an independent risk factor associated with COVID-19 infection and severity, while female sex hormones, estrogen and/or progesterone may endow a protective effect[17,18].

TESTOSTERONE AND COVID-19

It has been proposed that androgens are needed for initial entry of SARS-CoV-2 into the cell *via* the

Table 1 Laboratory studies

Ref.	Study type	Focus	Results
Youn *et al*[37]	*In vitro* cell line treatment	Investigated potential protective effects of estrogen on endothelial cells against oxidative stress induced by IL-6 and by SARS-COV-2 spike protein (S protein)	17B-Estradiol reversed S protein induced activation of NADPH oxidase isoform 2 (NOX2) and ACE-2 dependent ROS production, as well as ACE2 upregulation and induction of pro-inflammatory gene monocyte chemoattractant protein-1 (MCP-1) in endothelial cells, effectively attenuating endothelial dysfunction completely Implications: Estrogen inhibits initial viral response and attenuation of cytokine storm induced endothelial dysfunction, especially in men and post-menopausal women. Data supports hypothesis that estrogen may be used to alleviate viral infection and cytokine storm-induced endothelial dysfunction, a critical mediator of ARDS/multi-organ failure. Thus, attenuating disease progression, severity and mortality
Samuel *et al*[42]	*In vitro* stem cell lines and high throughput drug screens	Established a screening strategy to identify drugs that reduce ACE2 levels in human embryonic stem cell (hESC)-derived cardiac cells and lung organoids. Target analysis of hit compounds revealed androgen signaling as a key modulator of ACE2 levels	Inhibitors of 5-alpha reductase, which dampen androgen signaling reduced ACE2 levels in target cells; Treatment with antiandrogenic drugs reduced ACE2 expression and protected hESC-derived lung organoids against COVID-19 infection; Study also found that clinical data on COVID-19 patients with prostate cancer, which is associated with elevated androgen levels, are significant risk factors and that genetic variants that are associated with higher androgen levels are associated with higher diseases severity

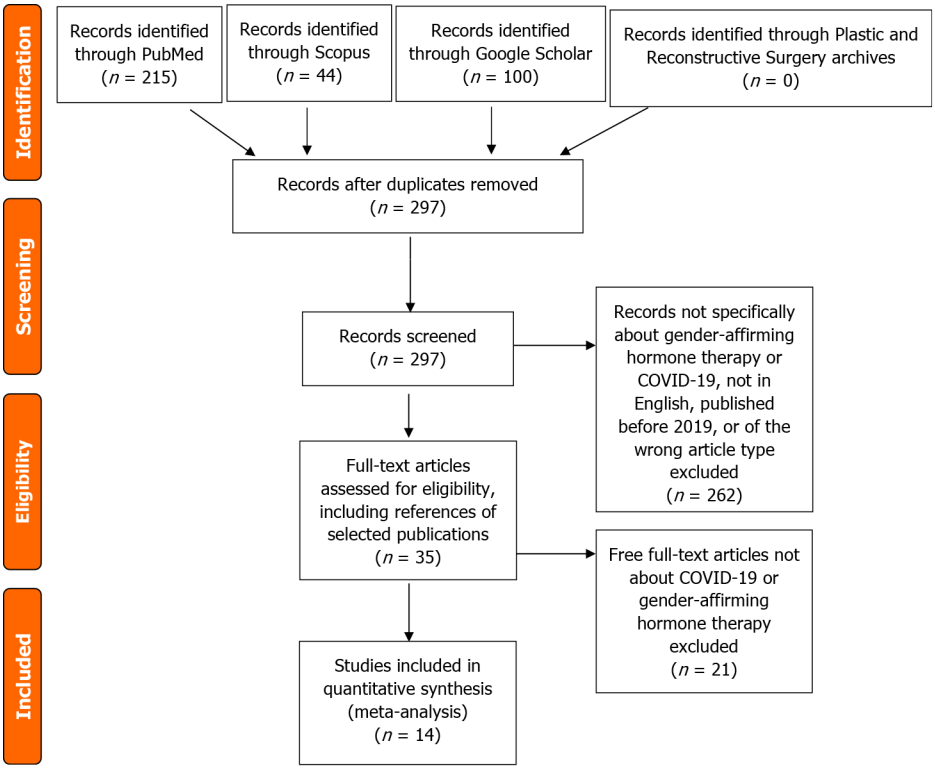

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Figure 1 Search strategy for our systematic review to find the currently published medical literature describing the impact of COVID-19 infections on gender-affirming hormone therapy.

activation of TMPRSS2, whose expression is increased by testosterone[8,9]. This gave rise to the theory that higher androgen levels in cis-gender men may account for the higher rates of infection and worse outcomes compared with their cis-gender women counterparts. Likewise, based on this logic, clinical trials have begun to look at the use of anti-androgens and TMPRSS2 inhibitors as prophylactic agents in the setting of SARS-CoV-2 infections[19]. However, once the initial infection occurs, testosterone is hypothesized to have a protective effect by limiting the collection of free radicals in cells and reducing the risk of a cytokine storm and subsequent development of acute respiratory distress syndrome (ARDS)[20]. Taken together, these two androgen dependent theories suggest that while low testosterone may reduce the risk of initial infection, testosterone later protects against more severe forms of disease and may prevent detrimental outcomes in individuals with COVID-19 infections. Further complicating the role of male sex hormones in gender outcome differences, testosterone levels are highly variable among men, with lower testosterone levels seen in men of older age, as well as men with other

Table 2 Current literature on hormone therapy and coronavirus disease-2019 clinical trials

Ref.	Focus	Predictors or conditions	Sample Population	Outcomes/Findings
Ghandehari *et al*[36]	Los Angeles, California; Effect of progesterone therapy in men with moderate to severe COVID-19	Randomized control trial	42 hospitalized men with confirmed moderate to severe COVID-19	There was a 1.5 point overall improvement in median clinical status score on a seven-point ordinal scale from baseline to day 7 in the progesterone group (n = 18) compared with the control group (n = 22)
			Experimental Cohort: re received 100 mg of progesterone subcutaneously twice a day for 5 d while hospitalized Control Cohort: Standard of care	This study shows that the use of progesterone may help to lower the length of hospital stay, use of supplemental O2 and need for mechanical ventilation
Dhindsa *et al*[12]	Association of concentration of serum sex hormones with COVID-19 Severity	Prospective cohort study	152 consecutive patients (59% men and 40.8% women) presenting with COVID-19 to the hospital were recruited. Of the participants, 143 (94.1%) were hospitalized. The mean age of participants was 63 yr	Lower testosterone concentrations and increased estradiol to testosterone ratios during hospitalization are associated with disease severity, inflammation, and mortality in men with COVID-19. Men with severe COVID-19 had 65%-85% lower testosterone concentrations compared with men with milder disease course, and was independent of other known risk factors associated with COVID-19 severity
van Zeggeren *et al*[25]	Assess the association between androgen levels and mortality in patients with severe COVID-19	Observational Case-control study	16 postmenopausal women (age > 55), and 24 age matched men	Total and free testosterone were lower in deceased men than in survivors. Significantly lower SHBG levels were associated with in both deceased men and women compared with survivors
				Low SHBG levels were associated with mortality rate in patients with COVID-19 and low total and free testosterone levels were associated with mortality in men. However, whether these hormone levels influence the disease severity, or are a marker of disease severity needs elucidation
Seeland *et al* [31]	Evidence for treatment with estradiol for women with SARS-COV2 infection	Retrospective cohort study	Electronic health record for a large, 68,466 case international COVID-19 cohort	Incidence of SARS-CoV-2 infection is ≥ 15% higher in women than men, but fatality rate is higher is 50% higher in men. Age stratification showed, that while preadolescent men and women had same risk of infection and fatality rate, compared with men of the same age, premenopausal women had a higher risk of infection, but peri and post-menopausal infection rates were similar to men of the same age; -fatality risk for women > 50 yr receiving hormone therapy with estradiol was reduced by > 50% (OR 0.33, HR 0.29) compared with women not receiving HRT. For younger women, (15–49 yr of age) risk of COVID-19 fatality was the same irrespective of estradiol treatment
Infante *et al* [24]	Asses testosterone levels at time of admission with inflammatory state and in-hospital mortality rate	Retrospective cohort study	40 symptomatic men with confirmed COVID-19 infections admitted to hospital. Patients were divided into two groups, survivors (n = 20), and non-survivors (n = 39)	Low total testosterone levels and elevated E2/T ratios (a marker of aromatase activity) were associated with a hyperinflammatory state. Low testosterone was an independent risk factor for in-hospital mortality
Rambhatla *et al*[27]	Assessed the outcomes of COVID-19 infection in men on testosterone replacement therapy	Retrospective case control study	32 men diagnosed with COVID-19 on testosterone replacement therapy (TRT) were matched to 63 men with COVID-19 diagnosis but not on TRT	No statistically significant difference in outcome endpoints (hospitalization, ICU admission, ventilator utilizations, thrombotic event, death) between two groups. Results suggest that no statistically significant difference in outcomes for men treated with TRT than men not on TRT.
Salonia *et al* [26]	Evaluated testosterone levels in men with COVID-19 compared with healthy men	Retrospective case control study	286 symptomatic men with COVID-19 requiring hospital admission Control group: 281 healthy men	Men with COVID-19 had significantly lower serum testosterone levels than healthy men. Lower testosterone levels were independently associated with COVID-19 infection status, and lower levels of testosterone predicted more severe clinical outcomes
Ding *et al* [30]	Examined how menstrual status and sex hormones affect the progression and outcomes of	Retrospective cohort study	All confirmed hospitalized COVID-19 patients from three hospitals (n = 1902). Cohort 1: Sex differences and disease severity (n = 1902); Cohort 2:Women with menstrual status	Non-menopausal (NM) women had milder severity and better outcomes compared with age match males. Menopausal(M) patients had longer hospitalization times compared with NM patients. -Anti Mullerian hormone (AMH) and estradiol (E2) negatively correlated with infection severity. Menopause is an independent risk factor for female COVID-19 patients, AMH and

	COVID-19		(*n* = 509)Cohort 3:Serum hormone levels (*n* = 78), Cytokines levels (*n* = 263)	E2 inversely corelated with COVID-19 severity. Thought to offer protective benefits, E2 specifically through regulation of cytokines related to immune inflammatory response
Lee *et al*[43]	Assessed the effects of female sex hormones on clinical outcomes of COVID-19 using national claims data	Retrospective cohort study	Adult patients with COVID-19 infection (*n* = 5061). Subgroup analyses using aged matched case-control data	There was no significant difference in mortality rate between males and females, and HRT was not associated with improved clinical outcomes

Table 3 Transgender care and coronavirus disease 2019

Ref.	Study type	Findings
Masterson *et al*[38]	Prospective Case study	TW patients treated with E+P as part of feminizing GAHT showed reduced testicular ACE-2 R expression in testicular tissue. In comparison to control group (cis-gender males with no hormone therapy) and the TW cohort treated with E only, O+E cohort also had higher degree of tissue fibrosis. Significance: Support the possibility that short course of E+P or P alone could help protect men against COVID-19 infection through downregulation of ACE-2 Receptor
Durcan *et al* [39]	Single center, cross-sectional web-based survey	Of 238 participants (179 FTM, 59 FTM) with GD receiving hormone therapy, the risk of contracting COVID-19 was 3.46x higher in FTM receiving testosterone therapy, compared with FTM patients receiving estrogen and anti-androgen therapies. Furthermore, among the FTM cohort, longer treatment periods with testosterone was associated with increased risk of contracting COVID-19; Significance: TM receiving Testosterone as part of GAHT are at an increased risk for contracting COVID-19

comorbidities that concurrently increase the risk of COVID-19 severity and morbidity, *i.e.* type 2 diabetes, chronic lung disease, obesity, and renal insufficiency[20-22]. While the above-mentioned arguments consider endogenous testosterone as a potential factor impacting the risk of SARS-CoV-2 infection, severity, and morbidity associated with COVID-19, whether exogenous hormone consumption in the setting of GAHT in transgender individuals would confer the same risks is mostly a matter of speculation.

Multiple clinical studies have shown that low baseline testosterone levels in men with COVID-19 at the time of admission are associated with worsening outcomes. A recent prospective study by Dhidsa *et al*[12] found that lower testosterone concentrations and increased estradiol to testosterone (E2/T) ratios (a marker of aromatase inhibitor activity) during hospitalization are associated with disease severity, inflammation, and mortality in cis-gender men with COVID-19. The authors did not specify if there were transgender individuals in their studied population. Men with severe COVID-19 had 65%-85% lower testosterone concentrations compared with men with a milder disease course. Similarly, a retrospective cohort study by Infante *et al*[24] evaluated men who were admitted with COVID-19 and found that compared with the survivor cohort, non-survivors had a significantly lower testosterone level at time of admission, which was inversely correlated with E2/T ratios and inflammatory marker levels. The study found that low testosterone levels at time of admission were an independent risk factor for in-hospital mortality and may serve as a surrogate marker for disease severity in male patients [24]. In addition, an observational cohort study in the Netherlands found that lower sex hormone binding globulin (SHBG) levels were associated with a higher mortality rate in both men and women, but low testosterone levels were only associated with mortality in men and not women.

The association of low testosterone and worse outcomes in male patients in these studies supports the theory that low testosterone levels may lead to an increase in proinflammatory cytokine markers, facilitating the development of a cytokine storm and subsequent disease severity and morbidity in men with COVID-19. The findings are consistent with a larger case-control study that found lower serum testosterone in men infected with SARS-CoV-2 at time of admission compared with the unaffected controls, and that the level of testosterone on admission was associated with worse outcomes. Interestingly, this study found that in as many as 85% of cases, sex hormone levels were suggestive of secondary hypogonadism[26].

Despite the repeated observed association between low testosterone levels and COVID-19 disease severity in males, it is not clear if low testosterone in males predisposes individuals to COVID-19 infection and increases the chance of higher severity of the disease, or if low testosterone is simply a marker of illness severity. Further studies looking at testosterone levels prior to infection are required to clarify this relationship. Adding to the possible immune role of testosterone levels in COVID-19 infection and disease course, a retrospective case-control study examining the outcomes of COVID-19 infection in men on testosterone replacement therapy (TRT) (*n* = 32), found no statistically significant difference in outcomes compared with men not on TRT[27]. However, considering the limited number of cases evaluated and the considerable number of potential confounding factors, the study was not powered enough to draw strong and valid conclusions.

Another possible explanation for the dimorphism in outcomes between cis-gender males and females may be that regardless of testosterone levels in men, female sex hormones provide a much greater protection. Thus the higher levels of female sex hormones in cis-gender women may account for the disparity in outcomes between the two sexes.

ESTROGEN AND PROGESTERONE AND COVID-19

The role of female sex hormones, including estrogen and progesterone, have also been proposed as potential protective factors contributing to the dimorphism in COVID-19 infection between cis-gender men and cis-gender women. Earlier studies have shown that estrogen plays an important modulatory role in both cellular and humoral immune responses, including causing a reduction in T-cell exhaustion and suppression of inflammatory cytokines[29,30].

In line with the hypothesis of female sex hormones playing a significant protective role, a retrospective study from China by Ding *et al*[30], indicated that non-menopausal (NM) females presented with milder disease severity and had better outcomes compared with age-matched males, but these differences disappeared between menopausal (M) females and age-matched men. This supports the idea that female hormones of NM women (pre-menopausal cis-gender) may provide protection, and the authors further noted that estradiol (E2) and anti-Mullerian hormone (AMH), which serves as a marker for ovarian reserve and function, showed a negative correlation with severity of infection in women. The study also found that E2 Levels specifically were negatively correlated with cytokines related to immunity and inflammation[30]. Further illustrating the potential protective role of E2, a retrospective cohort study from Germany found that compared with PM women not taking hormone replacement therapy (HRT), PM women receiving HRT, containing E2, had a 50% lower risk of mortality following SARS-CoV-2 infections (Odds Ratio 0.33; Hazard Ratio 0.29)[31]. In addition to the immunoprotective role of E2, progesterone is also thought to play a significant immunomodulatory role, including the prevention of free radical formation and suppression of proinflammatory cytokines[32,33]. This inflammatory dampening facilitated by high endogenous progesterone levels is thought to be protective against cytokine storms and subsequent development of ARDS in COVID-19 patients[34,35]. Results from a recent randomized control trial found that subcutaneous progesterone administration was associated with significant clinical improvement in hypoxemic men hospitalized with COVID-19[37].

Researchers investigating the potential protective effects of estrogen on endothelial cells against oxidative stress induced by interleukin (IL)-6 and by SARS-COV-2 spike protein (S protein) demonstrated that in response to S protein or IL-6 exposure of endothelial cells, estrogen inhibits initial viral response and alleviates cytokine storm-induced endothelial dysfunction, a critical mediator in ARDS/multi-organ failure, ultimately attenuating disease progression, severity, and mortality. This lab based research supports the notion that estrogen provides significant protection against COVID-19 in cis-gender females and underlines the potential utility of estrogen administration as a treatment option for COVID-19 to reduce disease severity and improve survival. While not reviewed in this paper, several clinical studies are currently taking place to study the utility of estrogen treatment in infected cis-gender males and females[38].

GENDER-AFFIRMING HORMONE THERAPY AND TRANSGENDER CARE

While several studies have looked at the interplay of hormone and innate hormone levels on cis-gender male and females, less is known about the impact of COVID-19 on individuals undergoing GAHT. Similar to studies that have looked at the protective effects of progesterone and estrogen in cis-gender females, the mechanism of estrogen and progesterone in relation to COVID-19 infection and susceptibility can also be readily studied in the transgender population. A recent study by Masterson and colleagues has been one of the first to examine the impact of feminizing GAHT in transgender individuals being treated for gender dysphoria GD[39]. Transgender women (TW) are routinely treated with estrogen (E) or estrogen plus progesterone (E+P) as part of feminization GAHT. Compared with orchiectomy samples of cis-gender men and TW on E alone, the TW cohort receiving E+P therapy prior to gender-affirming orchiectomy surgery had fewer Leydig cells and less ACE-2 expression when examined with immunohistochemistry. Their findings suggest that P appears to significantly diminish ACE-2 expression in the testes. This reduction in ACE-2 expression helps to support the hypothesis that a short course of exogenous P or E+P therapy may downregulate ACE-2 expression and help offer protection against COVID-19 infection and limit disease severity in cis-gender men and TW undergoing GAHT. While this study demonstrated the differences in ACE-2 expression in gonadal tissues when exposed to P+E therapy, it is unclear if the lower rate of expression in the studied group confers a lower risk of COVID-19 infection and severity, nor is it broadly applicable to the cis-gender population at large. The findings support prior work published by Montopoliet al[39], which showed that men undergoing prostate cancer treatment who received androgen deprivation therapy (ADT) were four times less likely to be diagnosed with COVID-19 compared with those who did not receive ADT. In

contrast, a more recent prospective cohort study consisting of 1779 men with prostate cancer found a higher rate of COVID infection in the ADT group (17.1% ADT group *vs* 5.7% no ADT group), but upon further multivariable analysis did not indicate a difference in infection rate for men treated with ADT compared with no ADT once confounding variables were accounted for (OR 0.93 95%CI: 0.54–1.61, P = 0.8)[40].

In addition to histochemical and laboratory studies on the mechanism of sex hormones, clinical studies looking at the infection rates and outcomes among transgender individuals treated with hormone therapy help to further deepen our understanding of the role of sex hormones. A recent web-based survey evaluation by Durcan *et al*[13] found that the risk of COVID-19 infection was 3.46 times higher in transgender men (TM), who were receiving testosterone therapy compared with TW, who received estrogen and anti-androgen therapy. In addition, the TM cohort who contracted COVID-19 had a longer androgen therapy treatment history compared with TM patients who did not contract the virus. While these findings suggest that TM individuals who receive androgen therapy as part of GAHT are at greater risk of COVID-19 infection, the study was limited by the small cohort size and retrospective design. Additionally, while the study stated that most patients who contracted COVID-19 did not require hospital admission, further studies looking at the severity of COVID-19 infection and need for ICU admission among transgender individuals undergoing GAHT would further help to demonstrate the risk of COVID-19 and its relationship with the COVID severity and morbidities in patients receiving supplemental androgen therapy (testosterone).

Despite the interest in the use of exogenous hormone therapies to help reduce COVID-19 infection and severity, still, very little research on the impact of COVID-19 on the transgender community and transgender individuals underdoing GAHT is available, as that cohort has been largely overlooked in demographic data, research studies and public health surveillance data collection[41]. While the currently available literature suggests that GAHT has a role in COVID-19 infected individuals, the current small sample sizes and limited understanding make generalizing to the overall transgender community (TM, TW, and non-binary individuals) or cis-gender individuals receiving sex hormone supplement difficult. As of now, the current hypothesis and available data on transgender-identifying individuals suggests that those undergoing MTF HRT (transgender women) may be more protected from becoming infected or suffering from severe COVID-19. In contrast, those who undergo FTM GAHT (transgender men), including androgens, may carry a higher risk. While the above current literature broadly supports this hypothesis, the impact of other biological and behavioral factors, including genetic differences in biological men and women, and higher rates of comorbidities, including smoking and other chronic illnesses in transgender individuals.

LIMITATIONS

Despite the unique opportunity to study hormone therapy and its impact on COVID-19 in this population, only two studies to date have reported on this subject. The studies are generally retrospective in nature and on a small number of individuals, thus are not powered adequately to draw valid and strong conclusions.

CONCLUSION

Transgender care and use of GAHT within this population represents a unique opportunity to study the implications of these treatments, as currently used, in relation to COVID-19 and biological sex. While several clinical trials looking at the use of E+P in COVID-19 infected cis-gender men, the understanding of their role in transgender care is limited. While clinical trials investigating the utility of hormone therapy in COVID-19 may prove useful, studying the effects of GAHT in transgender individuals already taking these medications may prove a more efficient route to understanding the role of hormone therapy in the treatment of COVID-19. Not only would studying transgender individuals in COVID-19 studies help to further broaden our understanding of the role of biologic sex and hormone treatment in disease susceptibility and course, but it would also serve to benefit those in the transgender community, who are often a vulnerable and underserved population within healthcare.

ARTICLE HIGHLIGHTS

Research background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can produce a wide range of clinical manifestations from asymptomatic to life-threatening. Differences in individual responses to systemic inflammation and coagulopathy appear to be modulated by several factors including sex steroid

hormones. Androgens may facilitate initial coronavirus disease 2019 (COVID-19) infection. however, once that occurs, testosterone may have a protective effect. Few studies have investigated the role of GAHT in COVID-19 infections. Additional research is necessary to enhance our understanding of this relationship and provide better care for transgender patients.

Research motivation

The role of estrogen and progesterone has also been proposed as potential protective factors in COVID-19 infection.

Research objectives

To investigate the potential role of GAHT in the development of COVID-19 infections and complications.

Research methods

The current systematic review implemented an algorithmic approach using PRISMA guidelines. PubMed, Scopus, Google Scholar top 100 results, and archives of *Plastic and Reconstructive Surgery* was on January 12, 2022 using the key words of "gender" AND "hormone" AND "therapy" AND "COVID-19" as well as associated terms.

Research results

The database search resulted in the final inclusion of 14 studies related to GAHT COVID-19. Of the included studies, only two studies directly involved and reported on COVID-19 in transgender patients. Several clinical trials looked at the relationship between testosterone, estrogen, and progesterone in COVID-19 infected cis-gender men and women. It has been proposed that androgens facilitate initial COVID-19 infection, however, once that occurs, testosterone may have a protective effect. A number of clinical studies have shown that low baseline testosterone levels in men with COVID-19 are associated with worsening outcomes. The role of female sex hormones, including estrogen and progesterone have also been proposed as potential protective factors in COVID-19 infection. This is exemplified in multiple studies investigating different outcomes in pre- and post-menopausal women as well as those taking hormone replacement therapy. Two studies related specifically to transgender patients and GAHT found that estrogen and progesterone could help protect men against COVID-19, and that testosterone hormone therapy may increase the risk of contracting COVID-19.

Research conclusions

Few studies were found related to the role of GAHT in COVID-19 infections. Additional research is necessary to enhance our understanding of this relationship and provide better care for transgender patients.

Research perspectives

SARS-CoV-2 can produce a wide range of clinical manifestations from asymptomatic to life-threatening. Differences in individual responses to systemic inflammation and coagulopathy appear to be modulated by several factors, including sex steroid hormones. Androgens may facilitate initial COVID-19 infection, however, once that occurs, testosterone may have a protective effect. The role of estrogen and progesterone has also been proposed as potential protective factors in COVID-19 infection. Few studies have investigated the role of GAHT in COVID-19 infections. Additional research is necessary to enhance our understanding of this relationship and provide better care for transgender patients.

FOOTNOTES

Author contributions: Hamidian Jahromi A contributed to conceptualization and manuscript editing; Ferraro JJ, Reynolds A, Edoigiawerie S, Seu MY, Horen SR, Aminzada A contributed to writing, statistical analysis, and manuscript editing; All authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors declare that they have no conflicting interests.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Ma YJ

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World Journal of Methodology

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World J Methodol 2022 November 20; 12(6): 476-487

DOI: 10.5662/wjm.v12.i6.476

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Associations between SARS-CoV-2 infections and thrombotic complications necessitating surgical intervention: A systematic review

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Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Al-Ani RM, Iraq; Du BB, China

Received: April 15, 2022
Peer-review started: April 15, 2022
First decision: August 1, 2022
Revised: September 1, 2022
Accepted: November 4, 2022
Article in press: November 4, 2022
Published online: November 20, 2022

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Abstract

BACKGROUND

Several unique clinical features of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19) infection, have been identified and characterized. One such feature, mostly among patients with severe COVID-19 infection, has become known as COVID-19-induced coagulopathy. Surgical patients with a history of or active COVID-19 infection bear a significantly higher risk for postoperative thrombotic complications. These patients may require surgical intervention to treat severe thrombotic complications. Few studies have been carried out to better characterize this association. The purpose of this study was to perform a systematic review and meta-analysis of the literature on COVID-19 infections that led to thrombotic complications necessitating surgical intervention. We hypothesized that patients with recent or active COVID-19 infection would have high rates of thromboembolic complications both arterial and venous in origin.

AIM

To perform a systematic review and meta-analysis of the literature on COVID-19 infections that led to thrombotic complications necessitating surgical intervention.

METHODS

The current systematic review implemented an algorithmic approach to review all

the currently available English medical literature on surgical interventions necessitated by COVID-19 thrombotic complications using the preferred reporting items for systematic reviews and meta-analysis principles. A comprehensive search of the medical literature in the "PubMed", "Scopus", "Google Scholar" top 100 results, and archives of *Plastic and Reconstructive Surgery* was performed using the key words "COVID-19" AND "surgery" AND "thromboembolism" AND "complication". The search string was generated and the records which were not specific about surgical interventions or thrombotic complications due to COVID-19 infection were excluded. Titles and abstracts were screened by two authors and full-text articles were assessed for eligibility and inclusion. Finally, results were further refined to focus on articles that focused on surgical interventions that were necessitated by COVID-19 thrombotic complications.

RESULTS

The database search resulted in the final inclusion of 22 retrospective studies, after application of the inclusion/exclusion criteria. Of the included studies, 17 were single case reports, 3 were case series and 2 were cross sectional cohort studies. All studies were retrospective in nature. Twelve of the reported studies were conducted in the United States of America, with the remaining studies originating from Italy, Turkey, Pakistan, France, Serbia, and Germany. All cases reported in our study were laboratory confirmed SARS-CoV-2 positive. A total of 70 cases involving surgical intervention were isolated from the 22 studies included in this review.

CONCLUSION

There is paucity of data describing the relationship between COVID-19 infection and thrombotic complications necessitating the need for surgical intervention. Intestinal ischemia and acute limb ischemia are amongst the most common thrombotic events due to COVID-19 that required operative management. An overall postoperative mortality of 30% was found in those who underwent operative procedures for thrombotic complications, with most deaths occurring in those with bowel ischemia. Physicians should be aware that despite thromboprophylaxis, severe thrombotic complications can still occur in this patient population, however, surgical intervention results in relatively low mortality apart from cases of ischemic bowel resection.

Key Words: Thromboembolic; COVID-19; SARS-CoV-2; Surgical intervention; Complications; Surgery

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Core Tip: Surgical patient with a history of or current active infection with severe acute respiratory syndrome coronavirus 2 bear a significantly high risk for postoperative thrombotic complications. These patients may require surgical intervention to treat severe thrombotic complications. In total, 70 cases of thromboembolic complications necessitating surgical intervention have been documented. These patients have an overall mortality rate of 30%. Intestinal ischemia and acute limb ischemia are the most common thrombotic complications that required operative management. Physicians should be aware that severe thrombotic complications can occur in this patient population, however, surgical intervention results in relatively low mortality apart from cases of ischemic bowel resection.

Citation: Ferraro JJ, Reynolds A, Edoigiawerie S, Seu MY, Horen SR, Aminzada A, Hamidian Jahromi A. Associations between SARS-CoV-2 infections and thrombotic complications necessitating surgical intervention: A systematic review. *World J Methodol* 2022; 12(6): 476-487
URL: https://www.wjgnet.com/2222-0682/full/v12/i6/476.htm
DOI: https://dx.doi.org/10.5662/wjm.v12.i6.476

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a pandemic infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. Since December 2019, COVID-19 has spread throughout the world and changed the landscape of biomedical research and healthcare in a myriad of ways. Several unique clinical features of the virus have been identified and extensively characterized. One such feature, mostly among patients with severe COVID-19 infection and to some extent in less severe cases, is known as COVID-19-induced coagulopathy (CIC), which manifests as considerable elevation in D-dimer and fibrin split products, with little to no associated change in activated partial thromboplastin time and prothrombin time[2].

A large proportion of patients with CIC have been reported to develop venous and arterial thromboembolic complications[3]. Critically ill patients and patients undergoing surgeries are generally predisposed to thromboembolism due to a combined immobility, systemic inflammation, endothelial dysfunction, and circulatory stasis[4,5]. The progression of CIC can be insidious, with some cases of pulmonary embolism (PE) identified as the first sign of SARS-CoV-2 infection in patients with no early evidence of virus upon testing with nasopharyngeal swab[6]. Thrombotic risks posed by CIC cannot be underestimated, as it is not limited to patients within intensive care or other high-dependency settings [7].

Postoperative thrombotic complications such as venous thromboembolism (VTE) and PE are responsible for significant morbidity and mortality among patients undergoing invasive procedures and surgeries[8,9]. Approximately 50% of all reported VTEs are provoked by prolonged immobilization, trauma, surgery, or hospitalization within the last 3 mo[10,11]. Therefore, a surgical patient with a history of or active COVID-19 infection would be at a significantly higher risk for postoperative thrombotic complications than the general population[12,13]. Traditionally cases of VTE are treated with systemic anticoagulation (*i.e.*, heparin, low molecular weight heparins, direct oral anticoagulants, and vitamin-K antagonists) following a careful evaluation of the risks and benefits. The thrombolysis is reserved for clinically serious and massive PE conditions in an attempt to dissolve the clot more rapidly than with anticoagulation options and reduce the mortality[14]. Severe cases of thromboembolic complications may require surgical intervention (*i.e.*, mechanical thrombectomy, catheter direct thrombolysis) to reduce the risk of post thrombotic syndrome and venous insufficiencies [in case of deep venous thrombosis (DVT)][15] or the risk of pulmonary insufficiencies, hemodynamic instability and or death (in cases of PE)[14]. Therefore, a patient with previous or active COVID-19 infection may require surgical intervention to treat severe thrombotic complications. Few studies have characterized this association. The purpose of this study was to perform a systematic review and meta-analysis of the literature on COVID-19 infections that led to thrombotic complications necessitating surgical intervention.

MATERIALS AND METHODS

The current systematic review implemented an algorithmic approach to review all the currently available English medical literature on surgical interventions necessitated by COVID-19 thrombotic complications using the preferred reporting items for systematic reviews and meta-analysis principles (Figure 1). A comprehensive search of the medical literature in the "PubMed", "Scopus", "Google Scholar" top 100 results, and archives of *Plastic and Reconstructive Surgery* was performed by two authors (Reynolds A and Edoigiawerie S) on January 4, 2022, using the key words "COVID-19" AND "surgery" AND "thromboembolism" AND "complication" as well as associated terms.

The search string was generated and the records which were not specific about surgical interventions or thrombotic complications due to COVID-19 infection were excluded. Foreign language articles were not eligible for inclusion. Articles published prior to 2019 were excluded as being prior to the COVID-19 pandemic and therefore not relevant to complications associated with COVID-19 infection. Titles and abstracts were screened by two authors (Reynolds A and Edoigiawerie S) after which full-text articles were assessed for eligibility and inclusion. On initial and secondary search, papers in review, commentary, or letter format or those without accessible full-text articles were excluded.

Finally, results were further refined to focus on articles that featured surgical interventions that were necessitated by COVID-19 thrombotic complications. For completion of the search, the references of the selected publications were additionally screened with the priorly mentioned inclusion criteria. We also cited high-quality articles in *Reference Citation Analysis* (https://www.referencecitationanalysis.com).

RESULTS

The database search resulted in the final inclusion of 22 retrospective studies, after application of the inclusion/exclusion criteria. Of the included studies, 17 were single case reports, 3 were case series and 2 were cross sectional cohort studies. All studies were retrospective in nature. Twelve of the reported studies were conducted in the United States of America, with the remaining studies originating from Italy, Turkey, Pakistan, France, Serbia, and Germany. All cases reported in our study were laboratory confirmed SARS-CoV-2 positive. A total of 70 cases involving surgical intervention were isolated from the 22 studies included in this review. The 22 studies which were included in the review are listed in detail in (Table 1).

Table 1 Synopsis of reviewed studies on coronavirus disease 2019 thromboembolisms necessitating surgical intervention

Ref.	Location	Study design	No. of patients	Age (yr)	Sex: Males, females (%)	Comorbidities	Thromboprophylaxis	Thromboembolic complication(s)	Surgical intervention(s)	Outcome
Adekiigbe *et al*[43], 2020	NY, United States	Case report	1	47	Male	DM	Yes	Cutaneous vasculitic lesions and gangrene of all toes, bilateral DVT	Bilateral transmetatarsal amputations of all 10 toes	Discharged home
Ali Nasir *et al* [20], 2021	Pakistan	Case report	1	64	Male	T2DM, HTN	No	Acute LLI	Above knee amputation	Discharged home
Balanescu *et al*[33], 2021	MI, United States	Case series	4	20-77 (median 52)	Male (50)	Obesity (50%)	Unknown	PE	Mechanical thrombectomy (100%)	Discharged home (100%)
Bilge *et al* [21], 2021	Turkey	Case report	1	73	Male	HTN	No	Upper extremity arterial thromboembolism	Left upper extremity arteriotomy and arterial thrombectomy. Repeat thrombectomy 12 h later. Amputation at the level of the forearm 13 d later. Stump revision with amputation 22 d later	Discharged home
Bozzani *et al* [22], 2020	Italy	Case series	6	71 (49-83)	4 males (66)	3 PAD, other unknown	Unknown	Acute LLI	Urgent revascularization procedures (embolectomy in 3 cases, and hybrid open/endo procedures in other 3)	1 rethrombosed day 5, died 30 d later of MOF. 1 rethrombosed day 5, repeat embolectomy, above knee amputation. 4 discharged home. 23 discharged home in good condition
Cheung *et al* [55], 2020	NY, United States	Case report	1	55	Male	HTN	No	SMA thrombosis, bowel ischemia	Emergency exploratory laparotomy and SMA thrombectomy, necrotic small bowel resection	Discharged home
Dao *et al*[56], 2021	CA, United States	Case report	1	61	Male	HTN	Yes	Free floating descending aortic thrombus	Percutaneous vacuum assisted aortic thrombectomy	Discharged home
Dinoto *et al* [23], 2021	Italy	Case report	1	78	Male	DM, obesity, prior remote endovascular surgery for large popliteal aneurysm	No	Acute LLI. Thrombosis of left femoral-popliteal stent	Mechanical thrombectomy	Discharged home
Galastri *et al* [34], 2020	Brazil	Case report	1	57	Male	DM, obesity, HTN	Yes	Massive PE	Catheter directed thrombolysis	Discharged home
Gutierrez *et al*[39], 2022	NY, United States	Case report	1	53	Male	HTN, remote smoking, DM	Yes	Due to phlegmasia cerulean dolens	Fasciotomy and mechanical thrombectomy	Discharged PAD 70
Hwabejire *et*	MA,	Case series	20	58 ± 7	13 males	Obesity (60%)	85% (17) received	Acute bowel ischemia	Laparotomy with resection of bowel	50% overall mortality rate: (1)

al[19], 2021	United States				(65)		preoperative anticoagulation			100% mortality in patients ≥ 65 yr; (2) 33% mortality < 65 yr; and (3) 40% (8) developed
Jamshidi *et al* [40], 2021	CA, United States	Case report	1	51	Male	Tricuspid atresia status post Fontan and extracardiac Shunt	Yes	Bilateral lower extremity DVT, phlegmasia cerulea dolens of the left lower extremity	Catheter directed mechanical thrombectomy (PAD 13), left below knee amputation (PAD 41)	Discharged to rehabilitation facility PAD 50
Khanna *et al* [32], 2021	PA, United States	Case report	1	67	Female	HTN	No	Acute stroke from bilateral anterior circulation large vessel occlusion	Bilateral simultaneous mechanical thrombectomy	Full neurologic recovery
Nascimbene *et al*[35], 2021	TX, United States	Case report	1	44	Male	Patent foramen ovale, T2DM, HTN, dyslipidemia, obesity	No	Massive PE with a large right atrial thrombus	Percutaneous right and left atrium embolectomy	Discharged home
Naudin *et al* [24], 2021	France	Case report	1	56	Male	T2DM, HTN, obesity	No	Acute aortoiliac thrombus and LLI	Aortoiliac and lower limb artery mechanical thrombectomy and left lower limb fasciotomies, subsequent left below knee amputation	Extubated but still in ICU 6 wk post operatively
Szeles *et al* [25], 2021	NY, United States	Case report	1	67	Male	DM, hyperlipidemia, HTN	No	Acute LLI and aortic mural thrombosis	Emergency bilateral aortoliliac and distal embelectomies, followed by transmetatarsal amputation of the right foot and below knee amputation of the left limb	
Topcu *et al* [26], 2021	Turkey	Single center cross sectional study	3	62 (58-70)	3 (100)	1 ex-smoker	Yes (100%)	Acute LLI	3 emergency surgical thrombectomy	1 minor amputation (33.3%); 1 death (33.3%); 1 bilateral major amputation (33.3%)
Traina *et al* [18], 2021	Italy	Case report	1	80	Male	CVD, prior endovascular aortic repair in 2019 for abdominal aneurysm repair, and dyslipidemia	No	Bowel ischemia with aorto-enteric fistula formation	Laparotomy with resection of necrotic small bowel (occult COVID-19, diagnosed on histologic examination of resected small bowel)	Discharged home
Vyas *et al* [36], 2020	NY, United States	Case report	1	32	Male	None	No	Large saddle pulmonary embolus	Bilateral percutaneous pulmonary artery mechanical thrombectomy	Discharged home 3 d post procedure
Yang *et al* [17], 2021	Germany	Cohort study	20	69 (62-72)	15 males (75)	65% (13) obese	25% (5)	Colonic ischemia	12 (60%) underwent (sub)total colectomy, 7 (35%) right hemicolectomy, 1 (5%) ileocecal resection	9 (45%) surgical complications, 10 (50%) required revision surgery, 9 (45%) mortality
Zivkovic *et al* [57], 2021	Serbia	Case report	1	44	Female	None	No	Ascending aorta floating thrombus with acute right arm ischemia	Surgical thrombus extraction through open sternotomy and bypass surgery	Discharged POD 6

Madani *et al* [27], 2021	CA, United States	Cases report	1	40	Male	HTN, T2DM	Yes	Acute LLI	Right lower extremity above knee amputation	Discharged 41 d after admission

DM: Diabetes mellitus; DVT: Deep venous thrombosis; HTN: Hypertension; LLI: Lower limb ischemia; PE: Pulmonary emboli; MOF: Multi organ failure; SMA: Superior mesenteric artery; PAD: Peripheral artery disease; ICU: Intensive care unit; CVD: Cardiovascular disease; COVID-19: Coronavirus disease 2019; POD: Post op day.

DISCUSSION

Thromboembolic complications are a well-known sequela of COVID-19 infection, and their incidence has been the subject of many recent studies. The predisposition to the development of both venous and arterial thromboembolic complications by COVID-19 has also been well established, with incidences of thromboembolic complications in COVID-19 patients ranging from 7.2% to 40.8%[16]. The high complication rate poses a public health concern due to the increased morbidity, mortality and high costs associated with their development[16]. Such complications also pose a significant challenge to physicians treating them, as the need for a surgical intervention must be weighed against the risk of operation in an unstable and high-risk individual while the patient is in an already prothrombotic state. There is a significant gap in the literature describing the relationship between COVID-19 infections and thrombotic events requiring surgical intervention.

Of the 70 COVID-19 patients with thromboembolic complications necessitating surgical intervention found in our study, 85% (n = 60) had thrombotic complications considered to be arterial in origin. The most common complication reported was intestinal ischemia at 60% (n = 42)[17-19]. The second most common complication was acute limb ischemia (ALI) at 23% (n = 16), which included 14 cases of lower limb ischemia and 2 cases of upper limb ischemia[20-27]. ALI is defined as a sudden decrease in arterial perfusion of an extremity that compromises the viability of a limb[28]. Prior to the COVID-19 pandemic, the incidence of ALI in the general population was found to be 10 to 15 per 100000 cases each year (0.0001%-0.00015%)[20]. However, one study performed in a New York City hospital found the rate of ALI in the COVID-19 population to be as high as 0.38%[29]. Treatment of ALI includes endovascular or open surgical revascularization, however 10% to 15% of patients end up undergoing amputation during their hospitalization[30,31]. Of the 16 cases of ALI in the population being investigated, 8 (50%) of these patients eventually underwent some form of amputation of the affected extremity. In addition to the above cases, our cohort had 1 case of stroke due to bilateral arterial thrombosis of the anterior circulating vessels, which was treated with bilateral simultaneous mechanical thrombectomy[32].

In contrast to arterial thrombotic events, venous thrombotic events are a more common sequela of COVID-19, with the PE and DVT at an estimated incidence of 13.5% and 11.8% respectively[16].In our study, PE accounted for 10% (n = 7) of thrombotic events necessitating surgical intervention[33-36], notably higher than both the reported incidence of PE amongst non-intensive care unit (ICU) hospitalized patients and ICU patients with COVID-19 (1.3% and 6.2%, respectively)[37,38]. Interestingly, our study included two cases of phlegmasia cerulean dolens (PCD)[39,40], a rare and life-threatening form of DVT that results in arterial occlusion secondary to compartment syndrome caused by total venous occlusion[41]. PCD has been reported to have an amputation rate close to 50%, as well as a mortality of up to 40%[42]. Of the two patients in our study with PCD, only one required amputation. Also included

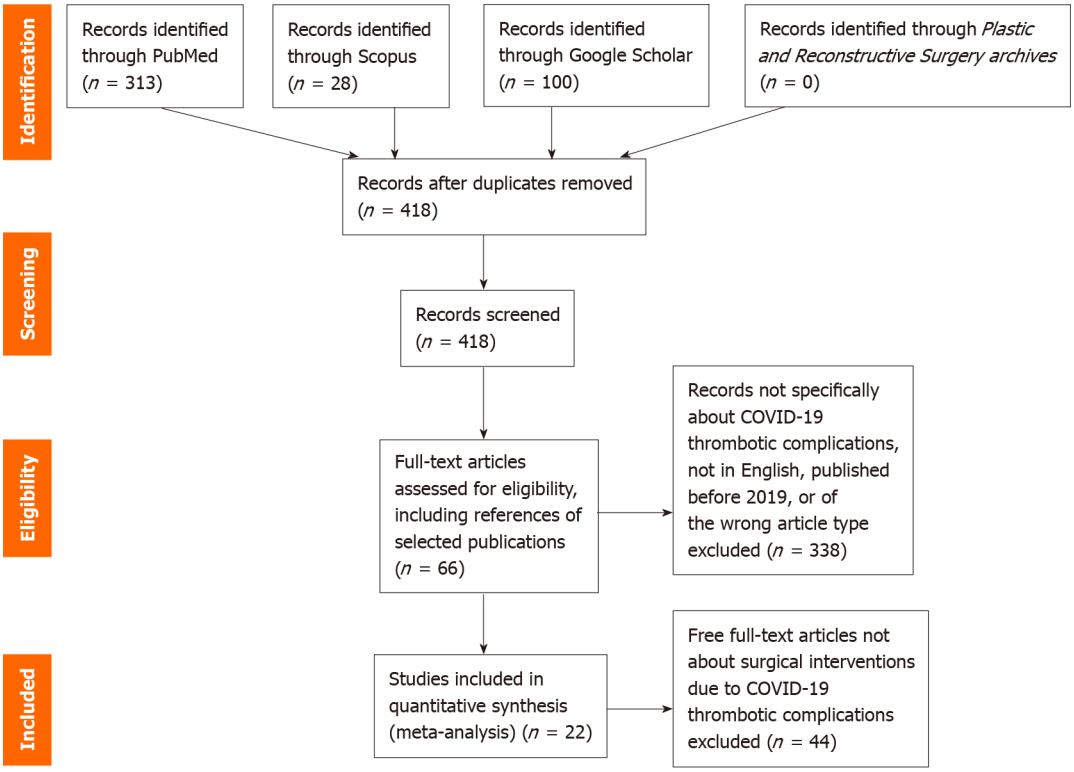

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Figure 1 Search strategy for our systematic review to find the currently published medical literature describing surgical interventions necessitated by coronavirus disease 2019 thrombotic complications. COVID-19: Coronavirus disease 2019.

in our cohort was one patient who required bilateral transmetatarsal amputations due to the development of "COVID toes", thought to be due to either microvascular thrombosis or related to a rare complication of venous thrombosis manifesting as venous gangrene[43].

Mortality

Several studies have attempted to quantify mortality related to thromboembolic events in COVID-19 patients. One study found that COVID-19 patients with a thromboembolic event had a 40% mortality rate, over twice that of COVID patients without a thrombotic event[44]. Another study, a meta-analysis of 8271 patients, found that patients with COVID-19 who had thromboembolic events had a pooled mortality rate of 23%, with thromboembolism significantly increasing the odds of mortality by as high as 74%[45]. Similarly, Gonzalez-Fajardo *et al*[46] found a mortality rate of 23.58% in their retrospective review of COVID-19 patients with thrombotic events, with a higher mortality seen in patients with peripheral arterial thrombosis and ischemic stroke compared to those with DVT and PE. Our study of COVID-19 patients with thrombotic events needing surgical intervention produced an overall mortality rate of 30% (n = 21). Notably, the highest mortality rate was seen in patients with acute intestinal ischemia who underwent bowel resection (45%, n = 19 of 42), followed by patients treated for ALI (13%, n = 2 of 15). It is unclear at this time if COVID-19 infection significantly complicated the cases of bowel resection, as acute intestinal ischemia has been noted to have a mortality rate as high as 80%, even without the added complexity of COVID-19 infection[47]. This is partly due to difficulty in diagnosis, importance of early diagnosis, and the rapid deteriorating nature of ischemic intestinal tissue and the patient's condition. It is possible that severe COVID-19 infection delayed the diagnosis of intestinal ischemia in several of the patients included in this study, leading to higher mortality rates. Difficulties in accessing medical and surgical care due the widespread impact of the current COVID-19 pandemic in every aspect of the health care could also be influential although the true nature and depth of such an impact is a matter of speculation.

Comorbidities

In total, 17 of the 70 patients in this review had specific comorbidity data readily available. The most common comorbidities amongst our cohort of patients were hypertension (64%, n = 11), diabetes mellitus (53%, n = 9), and obesity (35%, n = 6), all of which have previously been associated with a prothrombotic state[48,49]. Hypertension has been noted to be an independent risk factor for the development of deep vein thrombosis in a large study of over 18000 patients[50], so it is not surprising that patients with hypertension and COVID-19 infection were at an increased risk of thrombotic

complications necessitating surgical intervention. However, a recent study by Xiong *et al*[51] demonstrated no increase in thrombotic events among COVID-19 patients who were obese or had hypertension. Interestingly, their meta-analysis also found a previous diagnosis of diabetes mellitus to have a protective rather than potentiating effect on thrombotic events in this population. These results have been attributed to the use of medications such as statins and metformin, which have some degree of anti-inflammatory effects. While concomitant medication use was not a variable under investigation in our study, future studies may look at the relationship between medications with anti-inflammatory effects and reduced thrombotic complications of COVID-19.

Thrombotic prophylaxis

Since it became apparent that COVID-19 produces a prothrombotic state, much of the focus on thrombotic complication management has been shifted towards prevention. In May of 2020, the International Society on Thrombosis and Hemostasis published a statement regarding hospitalized COVID-19 patients in the ICU, recommending routine thromboprophylaxis with standard-dose low molecular-weight heparin or unfractionated heparin, unless contraindicated[52]. Yet our study found that 44%(n = 31) of patients who developed thrombotic complications requiring surgical intervention received some type of prophylactic anticoagulant therapy. This finding is consistent with the current literature, as studies have shown a high rate of thromboembolic complications in COVID-19 patients despite the use of prophylactic anticoagulation[53], with one study estimating this phenomenon to occur in almost one-third of all critically ill COVID-19 patients[54]. As previously stated, CIC has been reported to be the presenting symptom of some severe COVID-19 infections, making it possible for some patients in our study to have had thrombotic events prior to their presentation or COVID-19 diagnosis. Additionally, in several of the studies analyzed by this systematic review, dosage information and duration of thromboprophylaxis was not described, therefore it is unclear if some patients were subtherapeutic with their thromboprophylaxis regimen. Further studies to look at the dose and choice of anticoagulant in relation to severe thromboembolic events in the setting of COVID-19 infection is warranted.

Limitations

Our study is one of the first to analyze the relationship between COVID-19 infection and thrombotic complications that required surgical intervention, but there were several limitations. As all the included studies in this review were retrospective in nature, bias cannot be eliminated. Additionally, differences between the studies included in this review may lead to an additional bias, including the reporting of and variation of type and dosage of thromboprophylaxis. The reporting of outcomes and mortality, location of thrombotic events, and the method of surgical management also varied between many of the studies. Finally, our review drew a relatively small sample size, and our search criteria included only those studies in which patients were reported to have surgical intervention for their thrombotic events, and therefore incidence data could not be calculated.

CONCLUSION

There is paucity of data describing the relationship between COVID-19 infection and thrombotic complications necessitating the need for surgical intervention. Intestinal ischemia and ALI are amongst the most common thrombotic events due to COVID-19 that required operative management. An overall postoperative mortality of 30% was found in those who underwent operative procedures for thrombotic complications, with most deaths occurring in those with bowel ischemia. Physicians should be aware that despite thromboprophylaxis, severe thrombotic complications can still occur in this patient population, however, surgical intervention results in relatively low mortality apart from cases of ischemic bowel resection.

ARTICLE HIGHLIGHTS

Research background

It is well-known that coronavirus disease 2019 (COVID-19) infection is associated with hypercoagulability among affected patients. This has become known as COVID-19 induced coagulopathy (CIC). This study investigated CIC-related thrombotic complications through a systematic review and meta-analysis of the existing literature.

Research motivation

There is paucity of data describing the relationship between COVID-19 infection and thrombotic complications necessitating the need for surgical intervention. Intestinal ischemia and acute limb ischemia (ALI) are amongst the most common thrombotic events due to COVID-19 that required

operative management. An overall postoperative mortality of 30% was found in those who underwent operative procedures for thrombotic complications, with most deaths occurring in those with bowel ischemia. Physicians should be aware that despite thromboprophylaxis, severe thrombotic complications can still occur in this patient population, however, surgical intervention results in relatively low mortality apart from cases of ischemic bowel resection.

Research objectives

Main, overarching objective was to conduct a systematic review to find the currently published medical literature describing surgical interventions necessitated by COVID-19 thrombotic complications. We achieved this objective and identified intestinal ischemia and ALI as the most common thrombotic events necessitating surgical intervention.

Research methods

The current systematic review was performed using an algorithmic approach to review all the currently available articles in the English medical literature on surgical interventions necessitated by COVID-19 thrombotic complications using the preferred reporting items for systematic reviews and meta-analysis principles. A comprehensive literature search in the "PubMed", "Scopus", "Google Scholar" top 100 results, and archives of *Plastic and Reconstructive Surgery* was performed by two authors (Reynolds A and Edoigiawerie S) on January 4, 2022, using the key words "COVID-19" AND "surgery" AND "thromboembolism" AND "complication" as well as associated terms. The search string was generated and the records which were not relevant were excluded. Articles published prior to 2019 were excluded as being prior to the COVID-19 pandemic and therefore not relevant to complications associated with COVID-19 infection. Titles, abstracts, and full-text articles were assessed for eligibility and inclusion. On initial and secondary search, papers in review, commentary, or letter format or those without accessible full-text articles were excluded. Finally, results were further reviewed and refined to focus on articles that featured surgical interventions that were necessitated by COVID-19 thrombotic complications. For completion of the search, the references of the selected publications were additionally screened with the previously mentioned inclusion criteria.

Research results

The database search resulted in the final inclusion of 22 retrospective studies, after application of the inclusion/exclusion criteria. Of the included studies, 17 were single case reports, 3 were case series and 2 were cross sectional cohort studies. All studies were retrospective in nature. Twelve of the reported studies were conducted in the United States of America, with the remaining studies originating from Italy, Turkey, Pakistan, France, Serbia, and Germany. All cases reported in our study were laboratory confirmed severe acute respiratory syndrome coronavirus 2 positive. A total of 70 cases involving surgical intervention were isolated from the 22 studies included in this review.

Research conclusions

Physicians should be aware that despite thromboprophylaxis, severe thrombotic complications can still occur in this patient population, however, surgical intervention results in relatively low mortality apart from cases of ischemic bowel resection.

Research perspectives

Future directions could focus on how to prevent thrombotic complications and mitigate mortality among patients at risk for ALI and bowel ischemia in particular.

FOOTNOTES

Author contributions: Hamidian Jahromi A contributed to conceptualization and manuscript editing; Ferraro JJ, Reynolds A, Edoigiawerie S, Seu MY, Horen SR, and Aminzada A contributed to writing, statistical analysis, and manuscript editing.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

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