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META-ANALYSIS

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ABOUT COVER

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EDITORIAL

Perilipin 2 inhibits replication of hepatitis B virus deoxyribonucleic acid by regulating autophagy under high-fat conditions

M Victoria Delpino, Jorge Quarleri

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Abstract

Hepatitis B virus (HBV) infection poses a global health concern without a definitive cure; however, antiviral medications can effectively suppress viral replication. This study delves into the intricate interplay between lipid metabolism and HBV replication, implicating molecular mechanisms such as the stearoyl coenzyme A desaturase 1 autophagy pathway, SAC1-like phosphatidylinositol phosphatase, and galectin-9 mediated selective autophagy of viral core proteins in regulating HBV replication. Within lipid droplets, perilipin 2 (PLIN2) emerges as a pivotal guardian, with its overexpression protecting against autophagy and downregulation stimulating triglyceride catabolism through the autophagy pathway. This editorial discusses the correlation between hepatic steatosis and HBV replication, emphasizing the role of PLIN2 in this process. The study underscores the multifaceted roles of lipid metabolism, autophagy, and perilipins in HBV replication, shedding light on potential therapeutic avenues.

Key Words: Perilipin 2; Hepatitis B virus; Non-alcoholic fatty liver disease; Liver; Autophagy

Core Tip: Hepatitis B virus (HBV) infection poses a global health concern without a definitive cure. This study delves into the complex interplay between lipid metabolism and HBV replication. It reveals that heightened lipid metabolism may exert an inhibitory effect on HBV replication. Specifically, increased fatty acids lead to the accumulation of lipid droplets and the upregulation of perilipin 2 in hepatocytes. This, in turn, inhibits autophagy and subsequently hinders HBV replication.

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INTRODUCTION

Hepatitis B virus (HBV) infection represents a significant global health concern[1]. The main objective of treatment is to achieve prolonged suppression of HBV replication. Nevertheless, it is important to note that no method can completely eradicate (sterilizing cure) the HBV[2]. Therefore, it is imperative to explore novel mechanisms of HBV DNA replication, investigate biological processes linked to its replication, and discern effective methods for clearing the virus. This exploration aims to establish a foundation and generate renewed ideas for the treatment of HBV infection.

Autophagy is a cellular process responsible for delivering and degrading cytoplasmic components within lysosomes to support intracellular stability. These components include misfolded proteins, damaged organelles, and various intruding pathogens[3].

Many infectious microorganisms either inhibit autophagy, thereby dampening the immune response, or manipulate autophagy to favor pro-microbial activities[4]. Viruses that specifically target the liver, such as HBV, hepatitis C virus, dengue virus, and severe acute respiratory syndrome coronavirus 2, exploit autophagy for their pro-viral objectives. This mechanism assumes a crucial role in the life cycle of HBV, actively participating in viral assembly, envelope formation, and degradation processes[5]. Autophagy has also been shown to be associated with lipid metabolic diseases[6]. The accumulation of excess triglycerides (TG) in the liver is a fundamental factor in the development of fatty liver disease, a highly prevalent condition. The correlation between HBV infection and metabolic dysfunction-associated fatty liver disease (MAFLD), formerly known to as non-alcoholic fatty liver disease[7], has been a persistent focus in research.

The study by Choi *et al*[8] suggests that the combination of MAFLD and chronic hepatitis B can collectively exacerbate liver damage. The coexistence of chronic hepatitis B and MAFLD amplifies the likelihood of liver fibrosis, increases the risk of cancer, and contributes to an overall elevated mortality rate. However, another study has shown that individuals with MAFLD concurrent with chronic HBV infection exhibited lower levels of serum lipid metabolism-related index compared to the uninfected group. This indicates that chronic HBV infection may have a beneficial impact on lipid metabolism and liver impairments related to steatosis, potentially due to improvements in lipidomic profiles[9]. The relationship between HBV infection, MAFLD, and the effects of chronic HBV infection on lipid metabolism is still controversial and deserves further studies to elucidate the precise mechanism.

HBV can induce autophagy and use to increase replication[5]. The removal of fat by autophagy, also known as lipophagy, is currently considered an alternative pathway for lipid metabolism in liver cells[10]. This degradation of autophagy prevents hepatotoxicity and steatosis[11]. However, it is not known whether there is a correlation between HBV-related autophagy and the progression of MAFLD. Consequently, it is imperative to investigate this relationship from various approaches and explore multiple directions to gain clarity on the pathogenesis of HBV infection complicating MAFLD. Hence, we postulate that delving into autophagy represents a crucial avenue for research to understand the mechanism underlying the association of HBV with MAFLD.

The molecular mechanisms involved in the relationship between autophagy, lipids, and HBV replication has been started to be elucidated in the last two years. Among these, the stearoyl coenzyme A desaturase 1 (SCD1) autophagy pathway has been implicated in inhibiting HBV replication by fatty acid stimulation[12], SAC1-like phosphatidylinositol phosphatase (SACM1L/SAC1), a membrane protein integrated into the endoplasmic reticulum, promotes the autophagic degradation of HBV virions[13], and galectin-9 mediates selective autophagy of viral core proteins to restrict HBV replication[14].

In the present issue of *World Journal of Virology*, Wang *et al*[15]'s study aimed to explore mechanisms underlying the association between lipid metabolism and HBV-DNA replication. The study included 1603 HBsAg-seropositive patients, of which 661 were HBeAg-seropositive, and 942 were HBeAg-negative, with no prior antiviral treatment. The aim was to evaluate the effect of the lipid profile on HBV viral replication. The findings revealed a negative correlation between hepatic steatosis, serum triglyceride load, and blood HBV-DNA load in both the HBeAg-positive and HBeAg-negative groups. These results suggest that heightened lipid metabolism in the body may exert an inhibitory effect on HBV replication. A crucial factor to consider is that the patients enrolled in the study were untreated, emphasizing that the observed outcomes stem directly from the virus's unmitigated impact. This significance arises from prior research, which suggests that IFNα-2a treatment could potentially disrupt various intracellular signaling pathways, initiate autophagy, and impede autophagic degradation, potentially leading to a modest increase in HBV replication[16].

TG are stored in lipid droplets, which serve as the primary organelle for lipid storage in the liver. Within these lipid droplets, a group of proteins known as perilipins (PLINs) reside, with PLIN2 being the most abundant. Encoded by five different genes (Plin1 to Plin5), PLIN2 stands out as the only constitutive and widely expressed lipid droplet protein. Consequently, PLIN2 is commonly utilized as a protein marker for lipid droplets. In the liver, PLIN2 functions as a guardian of its own domain, the lipid droplet[17]. When PLIN2 is overexpressed, it acts as a protective shield against autophagy. Conversely, when PLIN2 is downregulated, it triggers TG catabolism through the autophagy pathway[18].

In the present issue of *WJV*, Wang *et al*[15] revealed, using *in vitro* assays, that the treatment of HepG2.2.15 cells with fatty acids (oleic and palmitic acid) increased lipid droplet deposition but decreased the level of HBsAg, HBeAg, and HBV-DNA load in cell supernatant. This mechanism was found to involve PLIN2 by inhibiting autophagy, as revealed by

knocking down and overexpressing the protein. These results are in contrast with previous findings demonstrating that HBV replication is inhibited when autophagic degradation of HBV virions is promoted[12-14]. The article by Popescu *et al* [19] revealed that the depletion of SAC1 Leads to the accumulation of phosphatidylinositol 4-phosphate, hindering the trafficking of the HBV envelope protein to multivesicular bodies in SAC1-knockout Huh7 cells. Consequently, this disruption inhibits the envelopment and secretion of HBV nucleocapsids, suggesting that SAC1 could play a crucial role as a host cell factor in controlling viral morphogenesis. SAC1 plays a vital role in inducing autophagy, and its deficiency hinders the fusion of autophagosomes with lysosomes[20]. A series of experiments conducted by Zheng *et al*[13] further supports the conclusion that SAC1 actively facilitates the autophagic degradation of HBV virions. Also, Du *et al*[12] demonstrated that under conditions of elevated lipid levels, SCD1 acts to inhibit HBV replication by regulating autophagy. Alternatively, galectin-9 could inhibit HBV replication *via* selective autophagy of viral core proteins in a mechanism that involved type I IFN genes[14].

The study in the present issue of *WJV* by Wang *et al*[15], together with previous findings, offers a theoretical foundation and innovative concepts for the treatment of individuals with chronic HBV infection and disrupted lipid metabolism.

CONCLUSION

In summary, Wang *et al*'s study suggests that the stimulation of fatty acids inhibits the replication of the HBV by increasing the expression of Plin2 and suppressing autophagy in hepatocytes. This process is linked to lipid metabolism, the autophagy pathway, and HBV replication[15]. Further research on the interplay between Plin2 and autophagy is crucial for gaining a better understanding of HBV-host interactions and the pathogenesis of HBV infection. It also proposes a potential avenue for treating individuals with chronic HBV infection coupled with MAFLD. These findings contribute to comprehending the intricate dynamics of HBV infection, lipid metabolism, and autophagy, opening an important potential link in the study of new drug targets in the process. While the role of autophagy in HBV infection has become evident, it remains unclear whether selective autophagy plays a crucial role in restricting HBV.

FOOTNOTES

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REVIEW

Herpes simplex keratitis: A brief clinical overview

Mutali Musa, Ehimare Enaholo, Gladness Aluyi-Osa, George Nnamdi Atuanya, Leopoldo Spadea, Carlo Salati, Marco Zeppieri

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Abstract

The aim of our minireview is to provide a brief overview of the diagnosis, clinical aspects, treatment options, management, and current literature available regarding herpes simplex keratitis (HSK). This type of corneal viral infection is caused by the herpes simplex virus (HSV), which can affect several tissues, including the cornea. One significant aspect of HSK is its potential to cause recurrent episodes of inflammation and damage to the cornea. After the initial infection, the HSV can establish a latent infection in the trigeminal ganglion, a nerve cluster near the eye. The virus may remain dormant for extended periods. Periodic reactivation of the virus can occur, leading to recurrent episodes of HSK. Factors triggering reactivation include stress, illness, immunosuppression, or trauma. Recurrent episodes can manifest in different clinical patterns, ranging from mild epithelial involvement to more severe stromal or endothelial disease. The severity and frequency of recurrences vary among individuals. Severe cases of HSK, especially those involving the stroma and leading to scarring, can result in vision impairment or even blindness in extreme cases. The cornea's clarity is crucial for good vision, and scarring can compromise this, potentially leading to visual impairment. The management of HSK involves not only treating acute episodes but also implementing long-term strategies to prevent recurrences and attempt repairs of corneal nerve endings *via* neurotization. Antiviral medications, such as oral Acyclovir or topical Ganciclovir, may be prescribed for prophylaxis.

The immune response to the virus can contribute to corneal damage. Inflammation, caused by the body's attempt to control the infection, may inadvertently harm the corneal tissues. Clinicians should be informed about triggers and advised on measures to minimize the risk of reactivation. In summary, the recurrent nature of HSK underscores the importance of both acute and long-term management strategies to preserve corneal health and maintain optimal visual function.

Key Words: Herpes simplex virus; Herpes simplex keratitis; Acyclovir; Neurotization; Reactivation

Core Tip: Our minireview is based on herpes simplex keratitis. This type of corneal viral infection is caused by the herpes simplex virus (HSV), which can affect several tissues, including the cornea and deeper uvea. Its ability to remain dormant for extended periods and reactivate with serious morbid ocular presentation makes it an important pathogen to be reviewed. The body's immune response to HSV is another potential cause of herpes simplex keratitis. Clinical management is short-term and long-term to prevent reactivation. Clinicians should be informed about triggers and advised on measures to minimize the risk of reactivation.

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INTRODUCTION

The eye is the organ of sight responsible for collecting and converting visual stimuli for onward transmission to the brain. The cornea, specifically, was believed to have full 'ocular immune privilege'[1] but recent studies challenged this submission[2]. Infectious keratitis ranks fifth among the leading causes of severe visual impairment globally[3]. Microbial keratitis can be bacterial, fungal, or viral/protozoal[4,5]. Viruses are implicated in up to 4/5th of all conjunctivitis in humans[6]. Herpes simplex virus (HSV) is the most common ocular disease-causing viral agent[7-9]. Ocular HSV infection can affect various local tissues[10]. Corneal manifestations of HSV-1 are termed Herpes simplex keratitis (HSK). It often presents with dendritic ulceration and eventual corneal scarring[11].

Systemic conditions like diabetes are not known to exacerbate HSK[12]. Dendritic corneal epithelial lesions found commonly on eyes infected with HSV, may also be seen in diabetes-associated neurotrophic keratopathy[13,14]. HSK may manifest as only mild keratitis at one end of its spectrum but may potentially result in ulcerative keratitis and perforation at its end stage[15,16]. HSV intrastromal keratitis was more common than epithelial keratitis in large-group studies[17, 18]. The herpes virus may infect an eye as a single infective organism or may occur in a polymicrobial form together with other types of microbes[19-21]. HSV causes diseases around the genitalia (herpes labialis) and the brain (herpes encephalitis)[22].

METHODOLOGY

Published articles in English were sought by searching through the PubMed database and with Reference Citation Analysis (https://www.referencecitationanalysis.com) with search net of the past 5 years between 2018 and 2023. Articles without full texts and abstracts were not considered. Also, articles written in other languages, out of the scope of the topic, or deemed unclear in methodology were screened out. The PubMed search query was: "("keratitis, herpetic" [MeSH Terms] OR ("keratitis" [All Fields] AND "herpetic" [All Fields]) OR "herpetic keratitis" [All Fields] OR ("herpes" [All Fields] AND "simplex" [All Fields] AND "keratitis" [All Fields]) OR "herpes simplex keratitis" [All Fields]) AND (2018:2023 [pdat])".

A total of 606 records were returned from the search string and preferred reporting items for systematic reviews and meta-analyses[23] table showing selection criteria is shown below in Figure 1.

UNDERSTANDING THE PATHOGENESIS OF HERPES SIMPLEX KERATITIS

The HSV causes HSK[24], It is among the most ubiquitous human pathogens on earth[25-27]; and a major blinding disease in developed countries[28,29]. It is the second most common cause of corneal scarring according to a large study [30], It has a seroprevalence in the range of 60%-90%[31]. It is also commonly seen in immunocompromised individuals

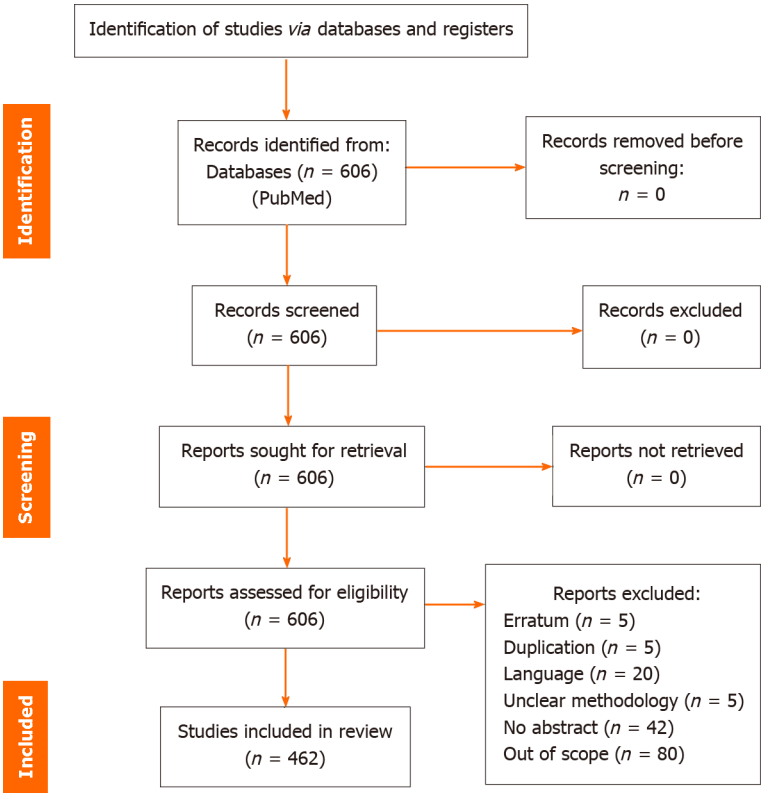

Figure 1 The preferred reporting items for systematic reviews and meta-analyses 2020 table showing review process.

[32]. Multiple strains of the virus present with differing symptomatology according to duration and virulence[33,34]. HSV can deceive toll-like receptors (TLR) which would ordinarily signal the innate immune system to attack the virus[35]. Cui *et al*[36] reported on quantitative proteomics of the infected corneal epithelial cells suggesting that P4HB, ACLY, HSP90AA1, and EIF4A3 proteins are involved in the relationships between hosts and viruses [36]. It is known that HSV-1 infection alters the host metabolism to suit its propagation *in vitro*[37]. Costimulatory molecules like CD80 and CD28 may also reduce the expression of HSV symptoms[38-40].

The virus typically affects humans more than other animals. After initial infection with HSV, hosts carry the infection for life[41-43]. Transmission generally is by direct contact of the skin or mucous membranes with lesions or secretions bearing virions[44,45]. HSV is generally divided into two which include HSV-1 and HSV-2[46,47]. HSV-1 is primarily the cause of infections around the mouth, face, and eyes[48]; while HSV-2 is majorly transmitted sexually and causes genital diseases. HSV-2 could also infect the eyes by contact or spread from lesions present in the genital area. This explains the presence of HSV-2 infection in the eyes of neonates, the route of infection being the mothers' birth canal. This underscores the importance of HSV testing in pregnant women especially those showing signs such as cold sores, and blisters[49]. Biological sex differences do not seem to induce changes in ocular HSV-1 disease expression, as evidenced by BALB/C and C57BL/6 murine models[50,51].

Yadav *et al*[52] documented 10 cases of HSV-2-linked blepharokeratoconjunctivitis over 16 years[52]. HSV-1 typically affects the mucocutaneous distribution of the trigeminal nerve. It usually does not present any symptoms but could sometimes manifest as a nonspecific upper respiratory tract infection. The virus then spreads from the infected epithelial cells to close-by sensory nerve endings and is carried along the nerve axon to the cell body of the trigeminal ganglion. At this level, the virus genome enters the nucleus of a neuron where it stays indefinitely in a dormant state[52,53]. Studies on non-human primates also revealed the persistence of HSV-1 in the ciliary ganglion and cornea during latency[54].

Harrison *et al*[55] hypothesized that stress stimulates viral gene expression and productive infection during reactivation from the latency stage[55]. Yan *et al*[56] also postulated that stress can reactivate a dormant HSV infection [56].

There are typically four subtypes of HSK which include: (1) epithelial keratitis; (2) immune stromal keratitis; (3) stromal necrotic keratitis; and (4) endotheliitis.

This classification depends on the layer of cornea affected; and whether the keratitis is caused by a reactivated or primary infection. The most common subtype is epithelial keratitis which appears as rough granular spots that form punctate lesions on the cornea; they usually coalesce quickly to create the typical dendritic lesions. These dendritic cells typically change in density wildly along the course of herpetic keratitis[57]. Keratic precipitates may also be observed [58]. Shah *et al*[59] reported that stromal keratitis (with no identifiable ulceration) was the most common presentation of HSK[59]. There were reports of both acute & subacute dendritic epithelial keratitis findings following incisional cataract surgeries; and transepithelial photorefractive keratectomy in one patient[60,61]. It is key to differentiate toxic epithelial keratitis following cataract surgery from HSK[62]. HSV geographic corneal epithelial defects increase predisposition to ocular infections *via* fastidious bacteria[63]; and *Stenotrophomonas maltophilia*[64].

An ocular manifestation of HSV specifically in the cornea is termed HSK: Which had a 2016 prevalence of about 1.7 million people worldwide[65]. It is the most prevalent infectious blindness-causing disease in the developed world[66,67], and is also prevalent in developing countries[68]. HSK commonly occurs monocularly, but can also present binocularly in certain conditions such as HIV and rheumatoid arthritis[69]. HSK has been known to affect all layers of the cornea and can be a cause of interstitial keratitis when the stroma is involved[70]. Whether inherent reports of unilateral posterior interstitial keratitis with hypoesthesia can be wholly attributed to HSK remains unproven[71]. Primary infection of any branch of the trigeminal nerve can cause an inactive infection of nerve cells in the trigeminal ganglion. Without prior ocular HSV infection, a person could still develop HSV keratitis due to the interneuronal spread of HSV within the trigeminal ganglion[72]. While a majority of patients initially present with corneal epithelial inflammation, about a third of them either present with or develop stromal keratitis[73].

Neutrophil and CD4+ mediated mechanisms are involved in the pathogenesis of herpetic stromal keratitis following viral spread along the corneal epithelium[74]. In mouse models of recurrent HSK, corneal sensory nerve retraction and replacement with aberrant sympathetic nerves potentiate pathologic processes with CD4+ T cells post-HSV-1 reactivation [75]. Damage at the level of sensory corneal nerves is termed neurotrophic keratopathy[76,77]. Substance-P (SP) production is largely depleted during the early stage of corneal nerve damage in HSK, followed by increased levels, possibly *via* a positive feedback loop during corneal disease manifestation; this surge of SP binds to neurokinin-1-receptor, upregulating the release of pro-inflammatory cytokines on the ocular surface[78]. Bell's facial nerve palsy reportedly occurred with herpetic stromal keratitis in the case of an immunocompetent individual[79].

Recurrence of HSV infection is widely agreed to be the result of virus reactivation in the trigeminal nerve ganglion; which travels along nerve axons to introduce its genome into the eye and initiate replication using the eye's cellular processes to make new copies of the virus. US-11 protein encoded in HSV-1 may play a role in the pathogenesis of worsening keratitis by promoting the translation of viral amino acids following the reactivation of HSV[80]. There is evidence supporting the presence of latent virus in cornea tissue, this could be the cause of recurrent donor-derived HSV, however, this is still very controversial[81].

Evidence shows the reactivation of HSV among patients using latanoprost in managing glaucoma[82,83]. HSV reactivation has also been linked to local, systemic, and topical steroid medications including the use of intravitreal triamcinolone injection[84]. Narang *et al*[85] further reported a case of reactivation of HSK after bilateral botulinum toxin injection to manage epiphora[85]. Ishimaru *et al*[86] reported that the HSV-1 virus replicated in host tissue partly due to proteasomal degradation of the Ras-GRF2 factor, while also demonstrating that this can be reversed by the proteasome inhibitor MG132[86]. Differentials for HSK include Acanthamoeba, Mycobacterium[87], Nocardia[88], Microsporidia[89] and Arthrographis kalrae keratitis[90]. Dong *et al*[91], identified trauma to be the main cause of infectious keratitis in their study[91]. HSK may also complicate the course of acute retinal necrosis in 20% of sufferers[92-93].

Research has shown that endosomal and cytoplasmic Pattern Recognition Receptors and the cell surface recognize HSV and as such start a cascade of immune response which includes Interferons (IFN), Chemokine, and Cytokine production as well as the recruitment of other inflammatory cells to the location of the infection[94]. IFN-1 release in acute HSV keratitis limits dendritic lesion enlargement[95]. Tripartite motif 21 (TRIM21) proteins reportedly inhibit IFN-beta; increasing the release of more proinflammatory cytokines, *e.g.* interleukin (IL)-6, TNF-alpha[96]. Reactive oxygen species (ROS) release from neutrophils in HSK-infected eyes was hypothesized to be catalyzed by nicotinamide adenine dinucleotide phosphate oxidase 2 enzyme[97]. The protein Osteopontin may also mitigate the inflammatory process observed in ocular HSV inflammation at the risk of also upregulating viral replication[98].

HSV-1-encoded ICP-5 proteins are crucial for capsid reassembly processes during viral replication[99]. Novel studies suggest that the upregulation of tryptophan hydroxylase during viral replication implicates the derangement of serotonin neurotransmitter pathways[99]. Ubiquitination is a process of viral protein modification; its first step is the bonding of ubiquitin to ubiquitin-activating enzyme1; HSV-1 induces bonding of ubiquitin-activating-enzyme 1a isoform with Lys604: Ubiquitination at Lys604 functions as a rate-limiting step of HSV-1 replication[100].

DIAGNOSTIC APPROACHES FOR HERPES SIMPLEX KERATITIS: CHALLENGES AND ADVANCEMENTS

HSK may be diagnosed by its clinical presentation on the slit-lamp biomicroscope[101]. Slit-lamp biomicroscopy provides better sensitivity than other low-resource alternatives[102]. It should be noted that slit-lamp findings in HSV infection are similar to endotheliitis secondary to other Herpesviridae: Cytomegalovirus and varicella zoster[103]. Visually, this diagnosis usually includes dendritic/geographical ulceration[104]. The common presenting symptoms: Photophobia, redness, itching, tearing, irritation, pain, discharge, and watery eyes usually subside after about 2 wk. Pain experienced by HSK sufferers has been likened to post-refractive surgery pain[105]. Deep Learning Artificial Intelligence has been identified as a positive aid in diagnosing HSK, especially in areas with less eye care-related manpower[106-108].

Ancillary Deep Learning methods integrated into clinical practice aided earlier diagnosis of HSK from its differentials [109]. Machine learning-based multinomial regression reduced the frequency of misdiagnosing HSV anterior uveitis from other uveitis etiologies[110]. Optical coherence tomography (OCT) is useful in monitoring patients' reactions to medication therapy[111,112]. Soliman *et al*[113] described the anterior segment-OCT findings of HSK as having sub-epithelial infiltration and specific stromal hyper-reflective patterns. Although these features are not unique to HSK they could help in diagnosing and monitoring HSK[113].

Spectral domain OCT has been used *in vivo* to provide a better understanding of the inflammatory and repair processes involved in HSK. Active HSK shows significant epithelial and stromal thickening while the inactive disease process shows a change in the structure at the site of stromal thinning due to the scarring[114]. Acanthamoeba keratitis (AK) and

HSV-keratitis are mimics[115,116]. Subjective determination of corneal lesion depth *via* anterior segment OCT may distinguish between these pathogens at earlier stages[115]. AK tends to invade the corneal stroma aggressively, causing radial keratoneuritis, in contrast to herpetic keratitis[115].

Diagnosing HSV typically involves identifying the virus or its proteins, HSV-specific antibodies, or HSV genetic materials in the blood[117]. Culture staining is limited as most diagnostic dyes have poor sensitivity to non-bacterial pathogens[118]. As early as 1996, immunofluorescence and polymerase chain reaction were already being used to detect HSV in corneal tissue[119]. Hirota *et al*[120] have reported successful monitoring of HSV levels in tears by using polymerase chain reaction analyses[120].

The conventional strategies for the diagnosis of HSV include serological tests, viral culture, and molecular techniques [121]. Viral culture is done by needle aspiration or the use of a swab and then cultured for a few days before microscopic analysis is carried out to determine HSV cytopathic effects. Viral culturing requires great-quality specimen collection, proper handling, and transportation of the specimen.

Compared to Herpes Zoster ophthalmicus, older age, diabetes mellitus and history of surgery are poor prognostic correlates for HSK[122]. Neonatal herpetic stromal keratitis can be confirmed by polymerase chain reaction (PCR)[123]. Multiplex real-time PCR (RT-PCR) has been found to identify HSV DNA reliably and is ideal in the diagnosis of HSV keratitis in the microbiology laboratory[124]. HSV superinfection can be diagnosed using multiplex PCR[125].

Diagnosing HSK is paramount as other conditions could mimic the typical appearance pattern in the cornea, Chang *et al*[126], showed that antiglaucoma medications could cause pseudo dendritic keratitis which is typically in the center-lower cornea as horizontal linear lesions. Benzalkonium chloride has been implicated in most cases[126]. Haidar et. al published a case report on the subject of the misdiagnosis of a foreign body as HSK[127].

Research has found that Nocardia keratitis, AK, and intraepithelial neoplasia can be misdiagnosed clinically for HSV infection, correct diagnosis of HSV is paramount as treatment modalities are different for these conditions[128-132]. Atypical microsporidial and fungal keratitis may mimic expected HSV findings from the clinicians' perspective also[133, 134]. Another problem occurs when other microbes superimpose on HSV to cause or exacerbate keratitis[135].

The tear HSV-sIgA test has been identified as a technique to identify HSV infection[136]. Diagnosing HSV prenatally has proved difficult as ultrasound results are usually not specific to congenital HSV infection[137].

Quantitative RT-PCR was shown by Mohammadpour *et al*[138], to be an excellent technique for detecting HSK[138] while Tóth *et al*[139] showed that PCR could identify HSV in about every 2.8th patients with a clinical history of HSK[139].

Parekh *et al*[140] have advocated for the use of Shotgun sequencing to analyze samples for the presence of HSK[140]. PCR is not sufficiently reliable in the diagnosis of HSV-induced stromal keratitis and endotheliitis due to limitations associated with assessing diseased corneal tissue specimens; hence, viral etiologies in these cases are often inferred or presumed clinically[141]. Real-time PCR was useful in making differential diagnoses of diffuse HSV endotheliitiswith feathery infiltration from fungal keratitis[142].

Tear film analysis *via* quantitative microfluidic PCR yielded good sensitivity for the detection of Herpesviridae in instances of epithelial HSK[143]. Tear film protective analysis identified high concentrations of IL-1A, IL-12B, DEFB4A, and CAMP for eyes infected with HSV epithelial keratitis[144,145]. Polymerase chain reaction and viral culture sensitivity in the diagnosis of HSV is limited, with the sensitivity of viral culturing being 50% and PCR being between 55%-88% while the sensitivity even reduces further in the identification of recurrent HSV disease[146].

HSV-1 DNA, antigens, and Latency-associated Transcript (LAT) in the cornea can prove crucial to the diagnosis of atypical clinical presentations or post-infectious stages where it might be difficult to identify the cause of an innocuous cornea scar[147].

Detection of the HSV LAT gene by reverse transcriptase quantitative PCR is superior to conventional PCR and Immunohistochemistry[148].

Louise and Sotiria reported the use of cornea pachymetry and epithelial thickness maps to provide an objective assessment of stromal inflammation. They reported that pachymetry and corneal thickness maps helped to identify HSV stromal keratitis; differentiating it from less debilitating HSV keratitis, and even neurotrophic keratopathy. It also offered an objective measurement for stromal inflammation resolution[149]. Studies have shown[149] Amplivue to be a rapid, potentially office-based diagnostic test for detecting HSV-1 and 2 as compared to more expensive and time-consuming PCR testing. *In-vivo* confocal microscopy was successfully used to study microscopic changes in cornea structures of feline and canine models with HSK[150].

Metagenomic deep sequencing can help to identify specific nucleic acids in complex ocular samples and assign them to specific organisms, thereby aiding diagnosis[151].

Ferreira *et al*[152] examined records of 235 keratitis patients presenting to a tertiary center between 2007 and 2015. As part of their comparisons and conclusions, HSK negatively correlated with poor outcomes after management[152].

Clinical characteristics of HSV-induced anterior uveitis can mimic other viral and non-infectious uveitis most particularly at the onset of disease. PCR and Goldman-Witmer coefficient should be carried out on aqueous humor samples in suspected viral cases[153].

Danileviciene *et al*[154], identified the role of the *C21orf91* gene in the development of HSK where they described the condition to be 2.9 times more likely in patients with the rs10446073 genotype being more common[154]. According to Borivoje *et al*[155], the CC IL28B gene has been identified to be present in individuals with recurrent HSK[155].

Cornea sensitivity of patients with HSK especially stromal keratitis or those who had suffered before is usually lower; they typically have lower Cornea hysteresis, and lower Corneal resistance factor (CRF). Even the contralateral eyes of patients with previous HSK infection have less CRF and cornea hysteresis[156].

Cornea esthesiometry and Laser scanning confocal microscopy have been shown to reveal a significant decrease in cornea sensitivity and sub-basal nerve fibers: Which recover after around 6 months but never return to normal anatomy [157].

Studies have shown that obese and overweight individuals are more likely to develop recurrent simplex keratitis[158]. The use of corneal impression membranes led to higher detection of HSK compared to swab techniques[159]. Storing HSV-1 inoculated polytetrafluorethylene impression membranes at +35 °C for three months led to a reduction of DNA recovery; storage at +4 °C, -20 °C and -70 °C for 10 d were optimal for HSV-1[160].

Computational bio-sequencing methodology identified HSK and other corneal virulent organisms *in vitro*[161]. Bioinformatics analyses suggest that UL24.5 is a possible determinant of pathogenesis[162] Miyazaki *et al*[163] in their study showed that RAGEs (receptors for Advanced glycation end products) is a sensor of HSV-1 infection, this is a route to possible diagnosis for HSV[163].

ANTIVIRAL THERAPY FOR HERPES SIMPLEX KERATITIS: EFFICACY AND LIMITATIONS

There are lots of anti-viral prescribing patterns currently, Cabrera-Aguas *et al*[164,165], described the need to standardize the indication and dosage of antiviral therapy in the management of HSK[164,165]. Ultimately, the decision to treat and treatment regimen selection is largely dependent on the individual clinician[166]. Lázaro-Rodríguez *et al*[167] reported isolated primary herpes-simplex virus neuroretinitis in an immunocompetent adult; thus, underpinning the need for starting antiviral therapy for individuals with macular stars who are not immunocompromised but seropositive for HSV IgM after ruling out other infectious causes, ionizing radiation, and arterial hypertension[167].

Antiviral resistance of HSK is generating concern, but the exact mechanisms of resistance have not been fully articulated[168]. Acyclovir is the most common drug used in the management of HSK[169], although eyedrops are now being proposed[170]. Resistance to acyclovir occurs due to extended use[171], and mutation in the viral thymidylate kinase and DNA polymerase which decreases enzyme affinity for its substrate[172-174]. Topical cyclosporine drops and prednisolone acetate drops are statistically similar in potency for stemming inflammation and preventing scar development[175].

HSK may predispose the eye to opportunistic infections. Vigilance must be applied when managing HSK patients with steroid-antibiotic eye drops as this is contraindicated[176]; and can result in epithelial defects and vascularization[177]. On the other hand, steroid-antiviral therapy performs better than fixed antiviral therapy[178].

In scenarios where the clinician is considering treatment-related side effects or conventional antiviral therapies not giving the required results, oral Valganciclovir could be used as an alternative for treatment and prophylaxis against HSK [179]. Watson *et al*[180] described a novel way of attacking the dormant LAT responsible for reactivating HSK after periods of latency using adeno-associated virus vectors to prevent reactivation[180]. A novel antiviral agent, SC93305, reportedly showed effectiveness against acyclovir-resistant strains of HSV-1 & HSV-2; SC93305 also reportedly did not interfere with host humoral immune responses[181]. Amentoflavone was found to inhibit resistant strains of HSV-1 including HSV-1/106, HSV-1/153, and HSV-1/blue by interfering with early-stage transcription of viral genes[182].

Novel delivery approaches such as prodrugs, nanocarriers, and peptides do cover against the systemic toxicity of oral antiviral prescription, as well as the rapid nasolacrimal clearance of topical antiviral therapy. The use of gel formulations and novel delivery approaches function tremendously to achieve desired outcomes[183].

The prophylactic use of antiviral agents such as acyclovir and valacyclovir is successful in treating HSK[184]. Cacicol® which is a topical eye biopolymer that contains poly-carboxymethyl glucose sulfate solution has been identified to have antiviral action on HSV and Varicella Zoster Virus (VZV)[185]. Due to emerging resistance to antiviral medication, there is a need to use other medications that target other viral proteins. This prompted Guan *et al*[186] to study the effect of stapled peptides on HSV-1 DNA synthesis and HSV-1 infection[186].

In the application of antiviral therapy for recalcitrant HSK, epithelial debridement, high-frequency dosing, and reduction of immunosuppression could help in achieving a better outcome[187].

IMMUNE RESPONSES IN HERPES SIMPLEX KERATITIS: IMPLICATIONS FOR DISEASE MANAGEMENT

The infection of the cornea by HSV secondary to an immune-inflammatory reaction by proinflammatory T cells is a significant cause of vision impairment[188].

There is a greater incidence of HSV infection in patients with atopy and the course of HSV keratitis in patients with severe atopic disease is usually more difficult to manage[189]. Patients with immune deficiencies or atopy usually present with bilateral HSK and it has been proven that long use of antiviral therapy can reduce the recurrence rate[190].

Presenting as an unwanted side effect, HSK has been found to spontaneously occur in those managed for MDA5-DM with rapidly progressive interstitial lung disease with tofacitinib at a dose of 20 mg per day[191].

Lappin *et al*[192] trialed immunotherapeutic management of HSV *via* topical administration of an ocular activating nanoparticle, feline models exhibiting re-occurrence of HSK were managed with this nanoparticle therapy and showed marked improvement as demonstrated by reduced viral shedding and ocular morbidity markers[192]. Davido *et al*[193] derived the KOS-NA mutant HSV mutant as a vaccine for the prevention of HSV-1[193,194]. Their data suggests that the mutated agent performed considerably well by preventing keratitis eruption in a mouse model.

Matundan *et al*[195], suggested that the ICP22 gene of HSV protected a murine model against corneal scarring[195]. Plasmid DNA administered with Interleukin 4, 10, 12, and 18 is reported to reduce inflammation and subsequent scarring in HSK[196]. Naidu *et al*[197] reported that the human HSV1 VC2 vaccine administered intramuscularly to mice mitigated the expression of HSK after subsequent infection with HSV-1 (McKrae) virus[197]. Similar results using the HSV1 VC2 vaccine in mice were shown to protect against HSV virus-linked immunopathogenesis[198].

Optineurin, a host protein, has been suggested as a possible inhibitor of the spread of HSV-1 while also mitigating neural damage[199]. This may be due to its selective autophagy regulatory properties. Hirose *et al*[200] investigated the role of TH17 responses in an HSV-1-infected murine model and concluded that interleukin-17 protects against ocular morbidity secondary to HSV-1[200].

Periocular corticosteroid injection resulted in hypopyon formation in a small sample of patients with HSV stromal keratitis and endotheliitis; the subsequent resolution was reportedly gained with topical antivirals, steroids, and systemic antivirals[201]. Upon infection with HSV-1, innate immune TLR-2 forms dimers with TLR-1, TLR-2, and TLR-6, cytokines, and IFN. TLR-2/2 Ligand activates the expression of specific antiviral genes[202]. Multiple microRNAs (miRNA) suspected to play significant roles concerning host immunity are upregulated in tear film samples of patients with HSV-induced epithelial keratitis[203]. Tenascin-C, an extracellular matrix glycoprotein increased in expression following injury, was discovered on the corneal epithelium of eyes with HSK keratitis[204].

Understanding the crucial role immune response plays in the development of herpes simplex stromal keratitis is necessary to control Stromal keratitis, especially macrophages, T cells, proinflammatory cells such as Th1 and Th17 CD4 T cells, and in some cases CD8 T cells in addition to memory CD19+ and CD27+ cells[205-209]. The transmission properties of HSV keratitis would be better managed if the role of the CD4+ TRM (Tissue Resident Memory T cells) and their induction by vaccines is well understood[210]. CXCR4-expressing cells may be key in the migration of neutrophils and the progression of lymphatics onto HSV-infected corneas[211]. During latent stages, viral proteins maintain low-level sporadic expression without full virion production, and ganglionic HSV-1 specific CD8+ T cell retention during latency serves protective functions[212]. Priming CD8+ T lymphocytes formed a basis for the hypothesis of future vaccination against HSV-1 reactivation[212]. Novel epitope peptide/CXCL-10 based prime/pull HSV vaccine elicited increased migration of HSV-specific CD8+ T-cell lymphocytes to the cornea and trigeminal ganglia of human leukocyte antigen (HLA) transgenic rabbits, thus protecting against ocular herpes virus infection[213].

Plasmacytoid dendritic cells are the main source of IFN-alpha within corneal stroma; higher density of plasmacytoid dendritic cells was associated with better *in-vivo* immunity against HSV-1 inoculation[214]. Higher peripheral blood levels of interleukin-1beta in patients with inactive/Latent HSK were correlated with increased levels of STAT1 and IRF3: Essential proteins for antiviral immune responses[215].

Interleukin-27 production by macrophages limited HSV-1 corneal shedding and consequent disease progression in mice[216]. Enhancement of P13K-Akt pathway signaling was hypothesized to cause increased susceptibility to HSV infection among test mice[217].

TRIM21 has been hypothesized to regulate type-1 IFNs' response to viral pathogens. The absence of TRIM21 proteins in knockout mice reportedly correlated with greater HSV-1 titers within the trigeminal ganglion during acute infection [218]. IFNalpha/beta could represent promising immune-mediated targets in HSV-1.

Antibody-dependent cellular cytotoxicity immune mechanisms provide protection from both cutaneous and ocular manifestations of HSV-1 and-2 infection[219]. Hence, novel vaccines eliciting the production of polyfunctional antibodies can offer protection to the immune-privileged eye[219].

The absence of Lymphotoxin-α was reported by Wang *et al*[220] to affect the expression of HSK in mice[220]. Dhanushkodi *et al*[221] also discovered IFN-γ-producing PLZFloRORγtlo as the most prevalent Invariant Natural Killer 1 cells in HSK-infected corneas[221]. At the level of the infected endothelial layer, IFN regulatory factor-7 has been shown to upregulate acquired immunity and mediate the major histocompatibility complex resulting from HSV infection[222].

RECURRENT HERPES SIMPLEX KERATITIS: CLINICAL FEATURES AND PREVENTION STRATEGIES

HSK is known to lie latent for extended periods before being reactivated by any number of factors[223]. These factors may be as simple as ultraviolet radiation[35,126,129] to major ocular surgeries.

Tear fluid exosomes may be a site of HSV-1 Latency; involved in the spread of HSV-1 infection to human corneal epithelial cells (HCECs). Corneal grafts can also be a source of HSK infection if not properly screened[224]. With a global shortage of available viable cornea graft tissue, Li *et al*[225] demonstrated that porcine corneal tissue is a good option for human corneal tissue[225], Recurrent HSK has been implicated in Deep Anterior Lamellar Keratoplasty failure[226]. It is therefore important to differentiate between HSV-linked endotheliitis and actual graft rejection[227]. The use of tectonic grafts for non-healing keratitis was reported by Tourkmani *et al*[228], with around 50% postoperative recurrence of HSK lesions by Suzuki *et al*[229]. Being elderly, male, and having a large graft > 9 mm are all risk factors for developing epithelial defects after penetrating keratoplasty[230].

Researchers have reported cases of HSV relapse with ocular presentations after Botulinum Toxin injection[231-233]. Recurrence of HSK dendritic epithelial keratitis should be considered for following CXL in keratoconus patients with suspicious history[234,235]. It has been postulated that the ultraviolet radiation A light, damage to the epithelial/stroma trauma, or damage to the nerves of the cornea during the cornea cross-linking could result in recurrent HSV[236,237]. However, other authors have advocated for corneal cross-linking as a therapy for HSK[238].

Mohanty *et al*[239] recommended a two-week steroid regimen for the management of HSV-induced interstitial keratitis [239]. Topical steroids have been reported to reduce the risk of stromal progression of HSK by up to 60%[240]. Stromal keratitis commonly occurs following Varicella-Zoster viral infection in children[241]. Instituting steroids empirically in all clinically suspected HSK cases without prior culture may result in aggravation of microbial differentials and long-term topical agent reliance[242].

Research has shown that HSV keratitis could recur after ocular surgery such as strabismus[243], penetrating keratoplasty, cataract, corneal crosslinking, lamellar keratoplasty, photorefractive, and phototherapy[244-247]. Intraocular

surgery, it seems, is also a risk factor for HSV reactivation with uveitic presentation[248]. Latent HSV in morphologically normal donor corneal grafts reactivated following keratoplasty[249].

There is substantive evidence for the prophylactic use of oral medications in penetrating keratoplasty who have had a previous history of HSK[250]. Herpetic keratitis lesions were found in a section of keratoconus eyes after collagen cross-linking procedures[251]. Small incision lenticule extraction has also been reported to precede a case of HSK manifestations[252]. Laser-assisted in-situ keratomileusis and photorefractive keratectomy triggered HSV reactivation which presented as endotheliitis in some patients post-corneal refractive surgery[253]. In another case reported by Basak and Basak[254] and Samak, oral Valacyclovir was used to resolve acyclovir-resistant endotheliitis resulting from deep membrane epithelial keratoplasty[254].

Nutritional deficiencies with subsequent hematologic abnormalities may trigger a recurrence of HSK[255]. Long-term use of systemic immunosuppressants for treating autoimmune disorders like systemic lupus erythematosus (SLE), lymphoproliferative diseases; and prophylaxis of organ transplant rejection, increases the likelihood of HSV recurrence/reactivation[256-259]. In certain individuals, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination triggers a cascade of autoimmune responses that can reactivate herpetic keratitis[260,261]. There were some reported incidences of reactivated herpetic keratitis following the reception of SARS-CoV-2 vaccines; patients' immune status before vaccination was not specified[262-266]; one patient had prior PKP surgery owing to HSK scarring[263]. Palpebral demodex infestation was associated with refractory and recurrent herpetic keratitis[267].

A retrospective study reviewing a large sample size from Canadian health databases reported no causal recurrence of HSK due to topical antiglaucoma medications[268]. Ozturk *et al*[269] reported a case of HSK in an adult male patient one week after receiving Ranibizumab. Behera *et al*[270] reported another case of reactivation of HSK in eyes and they investigated aster instilling bevacizumab[270]. A similar occurrence was also detailed by Al-Kaabi and Choremis[271]. Eyes with previous herpetic keratitis or uveitis were reported to manifest more recurrent episodes following phacoemulsification without longer periods of disease quiescence[272].

COMPLICATIONS OF HERPES SIMPLEX KERATITIS: CORNEAL SCARRING AND VISION LOSS

Kim *et al*[273] described the clinical features of HSK showing the most common subtype as epithelial keratitis (49.7%) followed by stromal keratitis (23.5%). They also show that epithelial keratitis had the highest likelihood of recurrence. The most common complication was cornea opacity. There was a 32.2% recurrence of HSK. The recurrence rate was seen to be less in the group that used prophylactic antiviral agents and the ascorbic acid treatment group[273]. There are bi-directional relationships between HSK and Atopic dermatitis[274].

Stromal involvement is usually a precursor for corneal scarring due to the infiltration of inflammation regulatory agents[275]. Rao *et al*[276] in their study showed the development of lacrimal gland inflammation in a mouse model of herpes stromal keratitis underpinning the inflammatory origin of herpes stromal keratitis[276]. Ocular infestation of the HSV is usually secondary to an infection in another part of the body[277]. Buccal to ocular transmission is a common transfer route. HSV keratitis can cause cornea scars which can make it difficult for intraoperative procedures such as cataract surgery and implantation of intraocular lenses[278]. That said, a follow-up of 37 HSV-infected eyes undergoing cataract surgery returned reasonably good outcomes even though the authors advised cataract surgeons to ensure the disease is latent before surgery. Scarring secondary to HSK is thought to result from the infiltration of inflammatory bodies and angiogenesis[279,280]. Corneal subbasal nerve density reduces markedly even after keratitis lesion scarring in eyes infected with HSV[281,282]. Subbasal nerve density losses, and consequent corneal hypoesthesia, are less in cases of herpetic epithelial keratitis[282]. Stromal inflammation in HSK is believed to be Chemokine (specifically ACKR2) mediated[283]. Hence these chemokines can determine the amount of ocular involvement in an individual.

Yoshida *et al*[284] reported on a case of HSV in a 64-year-old immunocompetent female who developed corneal scarring even after the resolution of HSK[284]. A similar presentation managed by Pisitpayat *et al*[285] did not progress to corneal scarring and had better outcomes[285]. HSV has also been associated with a risk of developing Neurotrophic Keratopathy[286-289]. In children and neonates who have suffered HSK episode(s), the possibility of amblyopia and strabismus in the latter years is present[290].

Herpetic keratitis is the leading cause of cornea ulcer and corneal perforation in the world, recurrence of the condition predisposes the individual to developing cornea ulcer and perforation. HSK has been found to cause neurotrophic keratitis[291-293]. HSK may also be a causative factor of corneal graft failure[294,295]. Using OCT, Ichikawa *et al*[296] were able to show an increase in cornea densitometry in HSV-affected eyes[296]. Retrograde inflammation from the cornea to the anterior chamber can result in viral anterior uveitis[297]. This is usually characterized by granulomatous precipitates with/without cornea scarring. HSV may also lead to uveitis. Posterior uveitis has been postulated to occur following long periods of post-keratitis latency[298].

Testing an HSK-compromised eye presents unique challenges for the patient and the clinician. Tananuvat *et al*[299] reported corneal perforation secondary to non-contact tonometry in two cases with thin corneas secondary to HSK and scarring[299]. Rebound tonometry may be more appropriate as it presents less stress to the corneal tissues.

HSK has been found to get worse in areas where air pollution is more frequent[300].

HSK has been associated with cornea denervation, although a certain degree of cornea nerve regeneration occurs a lot of the nerves do not come back to normal[301]. Sensory neuronal voltage-gated ion channels were associated with pain propagation in HSV-1 infection[302]. Further investigations are needed to probe a possible association between HSK and limbal stem cell deficiency as a sequel[303]. This may account for cases of poor corneal re-epithelialization[304]. Limbal stem cells' loss in herpetic keratitis was associated with density alterations of central basal epithelia, and the subbasal

nerve plexus[305]. Moein *et al*[306], using a specific HSV-1 strain KOS-63 showed that the recurrence of HSK causes more denervation[306].

Chirapapaisan *et al*[307], demonstrated the reduction in corneal subbasal nerve density (CSND) using *in vivo* confocal microscopy denoting the reduction in CSND even in contralateral eyes that did not show any scar[307]. The location of the cornea scar has a role to play in the lower likelihood of cornea regeneration[308].

It has been discovered that METTL3 (Methyltransferase 3) promotes pathological angiogenesis through canonical Wnt and VEGF signaling[309]. Ultraviolet A light used to carry out corneal collagen cross-linking could cause or trigger reactivation of latent HSV in a patient without clinical symptoms[310]. HSK has been reported to predispose individuals to be affected by *Burkholderia cepacia* which usually affects people with cystic fibrosis or immunocompromised[311]. Montgomery *et al*[312] postulated that ocular glands could be affected by HSV infection or other bacterial infections of the cornea[312]. The mentioned underlying physiopathological mechanisms in HSK can give rise to corneal scarring, vision loss, intraocular pressure elevation, and glaucoma.

NOVEL THERAPEUTIC APPROACHES FOR HERPES SIMPLEX KERATITIS: CURRENT RESEARCH TRENDS

Stopping the reactivation of HSK is essential for the development of vaccine strategies against HSV-1[313]. The discovery of a vaccine for HSK has been plagued with concerns about their overall safety for the public, leading to non-licensure and eventually shutdown of these labs[314]. Carr *et al*[315] demonstrated that higher doses of their HSV-1 0ΔNLS vaccine were able to prevent HSV-mediated disease[315].

It has been discovered that intrastromal injection of Bevacizumab could result in the regression of neovascularization in patients with neurotrophic keratitis secondary to HSV infection[316]. Topical therapeutic management is plagued with many factors including corneal epithelial toxicity to antiviral drops[317] and the development of tolerance[318]. The HEDS study recommended a guideline for oral antiviral drugs as a safer method of managing this disease process[319, 320]. Intravenous acyclovir was reportedly therapeutic for herpetic stromal keratitis[321].

The use of Retinoic acid to stabilize regulatory T-cells which mediate inflammation and control the progression of stromal keratitis in an HSK model[322]. Wang *et al*[323] engineered a mouse with knocked-out signal peptide peptidase and demonstrated that these mice expressed reduced viral replication and reactivation as compared to control mice[323, 324]. Sodium polyanethol sulfonate has also been described to reduce the replication of HSV in corneal epithelial cells [325].

Antiviral therapy is the mainstay in the management of HSK, The development of vaccines against the HSK virus has met a roadblock due to therapeutic effects in humans which are controversial, even though several vaccine candidates are effective in animal models that would require testing in humans[326]. One such vaccine was developed and reported by Hasan *et al*[327] using selected proteins from ViralZone, however, it remains to be tested on animal models[327]. The use of certain chemical compounds that can modulate the chromatin state of the viral genome resulting in the enhancement of antiviral immunity or suppression of infection and recurrence is another option; novel therapeutic techniques such as CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) have significant potential to make changes in the latent viral DNA in sensory neurons and as such cure the neuronal location of the infection[328-333]. Wu *et al*[334] have also reported on AT-533, a heat shock protein-90 antagonist that expressed inhibitory effects on HSV keratitis.

The fermented extract of the Pomegranate fruit has been shown to alleviate inflammation and pain associated with HSK[335]. Similarly, Zannella *et al*[336] used a grape pomace extract to heal and prevent HSK lesions[336] successfully. Staying with herbal management options, Ribelato *et al*[337] reported that the Trichilia catigua extract shows promise as a therapeutic agent for the management of HSV/HSK[337]. Topical application of selected insulin formulations has also shown promise in accelerating re-epithelization in infective keratitis[338]. Extracts from the carnivorous purple pitcher plant have also been demonstrated to inhibit early viral transcription of HSV-1[339].

Maqsood *et al*[340] successfully used Omnilenz® to manage corneal epithelium defects successfully. They were able to adapt the contact lens to apply an amniotic membrane-derived cell matrix with 57% of recipients reporting complete resolution of lesions after treatment[340]. Varela-Garcia *et al*[341] also designed a special hydrogel contact lens to carry Acyclovir and Valacyclovir. They reported that the contact lenses so designed were able to carry Valacyclovir better than Acyclovir. They concluded that these special hydrogel contact lenses are a viable tool for extended delivery of antiviral therapy to infected eyes[341]. BostonSight developed the PROSE special contact lenses for clearing chronic corneal opacities. The Boston keratoprosthesis (KPro) is another example of an artificial cornea used in cases of HSK affecting corneal clarity[342]. Cressey *et al*[343] reported a case series where the PROSE was successfully used to clear corneal scarring in HSK patients[343]. In addition to contact lenses, ocular inserts are now being designed to provide continued delivery of the drug inside the eye[344]. Autologous bone marrow stem cells also show promise in managing immune-mediated corneal inflammation with little or no side effects[345,346].

Cryopreserved amniotic membrane has been used as adjuvant therapy[347]. Conversely, Insulin-Like Growth Factor Binding Protein-3 worsens HSK keratitis, hence its downregulation may mitigate HSK expression[348]. Treating human cornea explants that are infected with HSV with cold atmospheric plasma (CAP) has been shown to deactivate HSV-1 and lessen the severity[349]. Ointment-based matrix regenerating agent also offers some promise in the treatment of neurotrophic corneal ulcers secondary to HSK[350].

MiRNAs are novel discoveries that deepen our understanding of disease processes in the body[351]. Amniotic membrane transplantation is a good option for managing indolent epithelial keratitis and HSK in diabetic patients following cataract surgery[352]. Drops formulated from peripherally derived autologous blood may quicken the healing

of neurotrophic HSV keratitis[353]. miRNAs affect infection in the eyes by regulating the human immune system[354]. An attenuation of miR-155/miR-183/96/182 mitigated the intensity of PA keratitis in feline models as reported by Xu and Hazlett[355]. miRNAs have also been used to deliver HSV-1-erasing lentiviral particles, blocking the reoccurrence of HSV in at least three disease models[356].

The TBK1 and IKKε inhibitor, bx795 has been reported to suppress HSV-1 both *in-vivo* and *in-vitro*[357] In a study carried out by Zhang *et al*[358], they identified Ras-related C3 botulinum toxin as a potential target for treating HSV-1-related diseases using NSC23766 and Ehop016[358]. Following subconjunctival injection in mice, a novel SHIP-1 activator, AQX1125, reduced CD4+ lymphocyte infiltration *via* modulation of P13K signaling[359-360].

Diphenyleneiodonium (DPI), an inhibitor of NADPH Oxidase 2, yielded a reduction in ROS release from neutrophils; thus, DPI was hypothesized to ameliorate herpetic stromal keratitis *via* its reduction effects[361].

Harringtonine, isolated from *Cephalotaxus harringtonia*, showed potential as a novel viral entry inhibitor to strains (HSV-1 blue & HSV-1 153) with resistance to acyclovir by targeting herpesvirus entry mediator (HVEM)[362]. Fujimoto *et al*[363] suggested the use of HVEM and nectin-1 products as therapeutic and preventive drugs against HSV-1 and HSV-2 infection particularly nectin-1 Lg as an eyedrop[363].

Subconjunctival injection of PKHB1 peptide in murine eyes infected with HSV-1 triggered the release of antigen-presenting cells, CD8+ lymphocytes, and other immunodeficiency cascades which were attributed to the alleviation of viral antigens[364]. One percent dispirotripiperazine gel proved efficacious for the resolution of HSV keratitis in preclinical rabbit models[365]. Small interfering (siRNA) delivered to HSV-infected *in-vitro* cells with an adenovirus type-5 vehicle showed potential for prophylaxis *via* inhibition of herpesvirus replication[366]. Inhibiting effects on 'disruptor of telomeric silencing 1-like' (Dot1 L) by siRNA and EPZ ameliorated corneal inflammation *via* non-release of ROS both *in-vivo* & *in-vitro*[367]. Dendritic cell-based DNA vaccination relieved manifestations of primary and recurrent HSK in murine experiments[368]. The rare sugar, i-picose was identified as a promising novel therapeutic target in murine HSV-1-related diseases, including HSK[369].

Pigment Epithelium Derived Factor has been shown in mice to reduce the severity of HSK[370]. An atypical presentation of presumed herpetic stromal keratitis was reportedly controlled following inoculation with Staphylococcus aureus lysates[371]. Ke *et al*[372] demonstrated the role of the FAK/PI3/Akt signaling pathway and MMP-2 and MMP-9 play in the development of HSK[372]. In treating stromal keratitis, it is important to add topical steroids to quell inflammation[373].

Topical Tacrolimus has been shown to improve visual acuity and reduce cornea inflammation, neovascularization, and cornea scarring, thus it is possible to inculcate it into the armamentarium of HSK management[374]. Transplantation of acellular porcine corneal stroma was a viable short-term substitute in a sample of Chinese patients with HSK keratitis [375].

Gene therapy (using an adenovirus type-2 vector) *via* meganuclease delivery to HSV-1 infected rabbit cornea transplantation models led to reduced HSV expression and attenuated immune responses[376]. Metabolic reprogramming *via* intraperitoneal metformin in mice infected with ocular HSV led to reduced expression of HSK lesions; with marked limitation of CNS complications induced by attempted metabolic therapy with 2-deoxy-d-glucose[377]. Lipid mediators are suggested to mediate the induction and mitigation of inflammatory processes. Zhang *et al*[378] have suggested that the Lipid mediator 11(12)-EET is potentially able to treat HSK[378].

The use of Von Willebrand factor has been identified as a good anchor to help in the delivery of therapeutics to prevent scarring and poor vision secondary to damaged cornea surface[379].

Dhanushkodi *et al*[380] discovered the use of engineered fibroblast growth factor-1 as a novel technique to reduce primary and recurrent HSK[380,381]. Shan *et al*[382] further suggested a crucial role oleanolic acid plays in the treatment of HSV keratitis, especially skin lesions HSV zosteriform model[382]. γδ T cells in the cornea help in early immune defense against several infections including HSV, exploring further ways to boost its response to HSV could prove crucial in managing the condition[383].

Transcriptomic data and bioinformatic analysis could possibly provide clues into the detailed molecular mechanism of HSK action and the potential therapeutic targets[384]. Targeting the IL-27 which is a pro-inflammatory cytokine controlled CD4+ Foxp3+ Tregs (regulatory T-cells) could aid in treating HSV stromal keratitis[385,386].

Minegaki *et al*[387] in their study showed that tandem pentapeptides repeat a derivative of Major royal jelly proteins found in royal jelly has an anti-inflammatory ability which is beneficial in reducing IL-6 and TNF-α which are stimulated in HSV infection of the cornea[387].

In their work Rao and Suvas stated the role of hypoxia in the development of HSK lesions, they investigated the expression of hypoxia-associated glycolytic genes in HSV-1 infected corneas laying a great foundation for more research into inflammatory hypoxia and hypoxia associated genes and the possibility of targeting hypoxia-inducible factor[388]. Wang *et al*[389] suggested that blocking the interaction between glycoprotein K and signal peptide peptidase may have a therapeutic effect in the management of HSV-1-associated eye disease[389].

Jiang *et al*[390] in their research suggested that BMS-265246(CDK) which is a CDK ½ inhibitor is effective and potent against HSV-1 especially as it interferes with multiple steps in the replication of HSV-1[390]. Sumbria *et al*[391] in their study suggested a dietary change to increase levels of short-chain fatty acid as a possible modality to be in place to reduce the impact of herpes recurrence in humans[391]. Majmudar *et al*[392] in their work showed strong evidence to support that SPGG (sulfated pentagalloylglucoside) is a viral entry inhibitor against HSV infection of the eyes[392].

CONTACT LENS-RELATED HERPES SIMPLEX KERATITIS: RISK FACTORS AND PREVENTION

Contact lenses have been implicated in infectious processes[393]. Subramaniam *et al*[394] detailed a case report of HSK in a contact lens wearer that completely resolved on oral antiviral therapy of 800 mg of Acyclovir five times daily alone [394]. However, it should be noted that Acanthamoeba is more likely to cause keratitis in contact lens wearers than HSV [395-397]. AK is occasionally misdiagnosed as HSV in, especially, very early stages[398,399]. Toshida and Sadamatsu also reported an incidence of HSK in a myopic individual wearing contact lenses for orthokeratology[400].

Live-attenuated vaccines may also show promise in preventing outbreaks of HSK. These vaccines introduce a weakened strain of the virus to the body, thereby allowing the body to develop natural defenses to the disease[401].

PEDIATRIC HERPES SIMPLEX KERATITIS: UNIQUE CONSIDERATIONS AND MANAGEMENT

In childhood stromal keratitis is the most common cornea manifestation of HSV infection; it usually progresses with scarring, residual astigmatism, and amblyopia. The recurrence rate is higher in the pediatric population especially those with immunosuppression[402]. Autosomal recessive Tyrosinemia type II presents with pseudo-dendritic keratitis and palmoplantar hyperkeratosis in affected infants and young children[403]. It is a worthy differential in the pediatric population.

Identifying HSK and treatment in children is challenging as they are at high risk for developing visual morbidity and a more aggressive HSK course that results in the scarring of the cornea and possibly amblyopia[404]. HSV keratitis should be considered as a differential diagnosis in a pediatric patient with keratitis[405].

In the management of HSK in children, the use of oral acyclovir as prophylaxis is safe, and its efficacy is related to compliance with therapy[406]. The incidence of HSK in penetrating patients who had cornea refractive surgeries is higher than in the general population[407].

Pediatric patients who have undergone penetrating keratoplasty for HSK have been shown to experience graft rejection, this must be diagnosed to minimize permanent damage significantly[408,409]. Treatment of pediatric HSK usually involves acyclovir, which generally gives a good prognosis[410]. Suppressive oral therapy may however be needed in the future if a recrudescence occurs after initial topical therapy. Of worthy mention is the ability of the pediatric myelogenous leukemia drug 6-thioguanine to mitigate HSV both *in vitro* and *in vivo*[411].

HERPES SIMPLEX KERATITIS IN IMMUNOCOMPROMISED PATIENTS: CHALLENGES AND TREATMENT OPTIONS

The SARS-CoV-2[412] has been implicated in HSV Keratitis[413,414]. HSK has been reported to relapse post mRNA coronavirus disease 2019 vaccination[415]. Herpetic eye disease has been seen to result more from individuals who received the BNT162b2 vaccine than those who received the mRNA-1273 or Ad26.COV2.S vaccines[416].

An atypical type of HSV keratitis, Archipelago Keratitis has been identified in immunosuppressed persons[417,418]. Table 1 below summarizes further literature on HSV occurrence in immunocompromised individuals[419-434].

SURGICAL INTERVENTIONS FOR HERPES SIMPLEX KERATITIS: CORNEAL TRANSPLANTATION AND BEYOND

Descemet membrane endothelial keratoplasty has been identified as an effective option for treating cornea edema resulting from HSV-1-related endotheliitis[435-438]. Novel endothelium-free grafts with endothelial cell regenerative capability may improve outcomes for high-risk transplant cases secondary to chronic HSV endotheliitis[439]. Intensive antiviral prophylaxis could reduce the risk of graft failure and recurrence of the condition[440]. The use of topical steroids, antibiotics, and higher doses of oral acyclovir leads to better postoperative outcomes of deep anterior lamellar and penetrating keratoplasties for corneal scarring caused by HSK[441].

There are several surgical interventions for the management of HSK but it basically involves either the replacement of the infected tissue or support of the tissue to aid healing. Cornea neurotization is gaining more acceptance today[442-444]. Lin and Lai reported a novel technique where the supratrochlear nerve of the same side as the affected eye was tunneled to the cornea to re-innervate damaged trigeminal nerve fibers with good results[445]. Bourcier *et al*[446] also reported similar results using the Lateral Antebrachial Cutaneous Nerve *via* a minimally invasive cornea neurotization procedure[446].

Roberts *et al*[447], reported the usefulness of sutureless tectonic pul-through mini-DSAEK in the management of corneal perforations secondary to herpes simplex infection or other causes[447]. New Onset HSK after keratoplasty could be managed by antiviral medications or amniotic membrane transplantation[448].

A Bowman's layer onlay graft is relatively easier as it does not resolve to deeper keratoplasty, it has the potential to reduce superficial cornea scarring and/or anterior cornea abnormalities[449]. Amniotic membrane transplantation reduces ocular opacity and scarring by inhibiting the secretion of inflammatory cytokines and fibroblast proliferation[450, 451].

Table 1 Selected literature on herpes simplex virus occurrence in immunocompromised individuals

Ref.	Predisposing condition	Summary
Gupta *et al*[419]	Necrotizing fascitis	HZO was the first sign of reactivation of varicella-zoster
Murgova and Balchev[420]	COVID-19	COVID-19 vaccines caused a reactivation in HSV ocular diseases
Yildiz *et al*[421]	COVID-19	COVID-19 vaccines caused a reactivation in HSV ocular diseases
Huang *et al*[422]	COVID-19	HSK was the 3rd most common corneal complication after COVID-19 vaccination
Matharu *et al*[423]	Cancer	VZV and HSV cornea co-infection in a patient with systemic immunosuppression
Cohen *et al*[424]	COVID-19	Herpetic cornea infection may develop post SARS-CoV-2 vaccinations
Yoshida *et al* [425]	RA	Individuals with RA have the tendency to develop HSK which is usually more severe due to their immunocompromised state
Fei *et al*[426]	COVID-19	HSK could happen after vaccination with a possible preponderance
Al-Dwairi *et al*[427]	COVID-19	Reactivation of HSK on cornea graft after taking SARS-CoV-2 mRNA vaccine
Majtanova *et al*[428]	COVID-19	Five incidences of HSK after COVID-19 vaccination
Roberts *et al*[429]	COVID-19	Negative result of COVID-19 in the tears in a patient with recurring HSV Keratitis
Kuziez *et al*[430]	COVID-19	HSK was reviewed as an adverse effect occurring after COVID-19
Mohammadzadeh *et al*[431]	COVID-19	HSK-suspected reactivation resulted in corneal graft rejection
Ichhpujani *et al*[432]	COVID-19	HSK was implicated in a review of associated complications reported after vaccination
Ono *et al*[433]	Human herpes virus	Reactivation of HHV-6B infection
Sinha *et al*[434]	Psoriasis	HSV keratitis after taking secukinumab for the treatment of psoriasis

COVID-19: Coronavirus disease 2019; HSV: Herpes simplex virus; HSK: Herpes simplex keratitis; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; RA: Rheumatoid arthritis; HZO: Herpes zoster ophthalmicus; VZV: Varicella zoster virus; HHV-6B: Human herpes virus 6B.

Lamas-Francis *et al*[452] reported the use of amniotic membrane transplantation for corneal ulceration secondary to infectious causes, they reported a success rate of 62.8% with 37.2% requiring additional surgery[452]. Similarly, Hayek *et al*[453] reported using a lyophilized amniotic membrane for the treatment of a 2 mm wide perforating cornea ulcer and didn't need keratoplasty[453].

Corneal trauma during surgery poses special problems due to the possibility of recrudescence of latent HSV infections in carriers. Preventive medication before surgery has been suggested for HSV seropositive patients[454] Patients with co-morbid ocular conditions such as cataracts could undergo Penetrating Keratoplasty before the cataract surgery as it has been shown in the literature that this has fewer complications and higher graft survival rate[455].

Graft failure after Penetrating Keratoplasty is common in eyes with HSK, hence it is important that HSV-1 or VZV PCR testing is done on all explanted cornea[456]. High-dose antivirals with prolonged tapering steroid doses prior to performing mushroom keratoplasty on eyes with herpetic vascularized corneal scars resulted in lower rates of graft failure and immunologic rejection in a longitudinal study conducted by Yu *et al*[457]. There were also higher than normal rates of graft failure with the Boston type I KPro for eyes having prior corneal HSV infection[458]. Suzuki *et al*[228] considered lamellar graft patching a safe and effective option for managing corneal perforations secondary to HSK-associated neurotrophic keratopathy[228].

Tape splint Tarsorrhaphy has been identified as a useful inexpensive technique to treat Persistent corneal epithelial defects[459]. Hata-Mizuno *et al*[460] reported a case of conjunctival epithelial ingrowth after PKP in a patient with Herpetic corneal keratitis[460].

CONCLUSION

The quest for better diagnosis, prevention, and management of HSK was uppermost in the minds of sampled participants in a paper by Liu *et al*[461]. Novel corneal active storage mediums enable better study and research of *ex-vivo* disease patterns in herpetic keratitis[462]. In the future, target extraocular (maxillary) vaccination to inhibit ocular herpes simplex reactivation may improve the epidemiology of the disease[463].

FOOTNOTES

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MINIREVIEWS

Hepatitis B virus reactivation in patients treated with monoclonal antibodies

Silvia De Pauli, Martina Grando, Giovanni Miotti, Marco Zeppieri

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Abstract

Hepatitis B virus (HBV) reactivation poses a significant clinical challenge, especially in patients undergoing immunosuppressive therapies, including monoclonal antibody treatments. This manuscript briefly explores the complex relationship between monoclonal antibody therapy and HBV reactivation, drawing upon current literature and clinical case studies. It delves into the mechanisms underlying this phenomenon, highlighting the importance of risk assessment, monitoring, and prophylactic measures for patients at risk. The manuscript aims to enhance the understanding of HBV reactivation in the context of monoclonal antibody therapy, ultimately facilitating informed clinical decision-making and improved patient care. This paper will also briefly review the definition of HBV activation, assess the risks of reactivation, especially in patients treated with monoclonal antibodies, and consider management for patients with regard to screening, prophylaxis, and treatment. A better understanding of patients at risk can help clinicians provide optimum management to ensure successful patient outcomes and prevent morbidity.

Key Words: Hepatitis B virus; Reactivation; Acute infection; Chronic infection; Monoclonal antibodies

Core Tip: Reactivation of hepatitis B (HBV) induces a rapid and acute increase in viral replication in a patient with chronic HBV infection or prior HBV exposure. There is also an increased risk of HBV reactivation in patients treated with monoclonal antibodies. Organ damage can be due to various mechanisms and risk factors that activate the cascade of inflammatory responses, such as direct infection. It is of clinical importance to diagnose, manage, and treat individuals, especially those at risk. Patient outcomes, success of therapy, prevention of complications, and management of existing comorbidities depend on the correct multidisciplinary management in patients at risk.

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INTRODUCTION

Hepatitis B virus (HBV) infection can lead to acute or chronic infectious disease, particularly the latter when the infection is contracted during childhood. Chronic hepatitis B is estimated to affect 291 million people worldwide, of which almost 80% live in developing countries[1]. When the infection becomes chronic, the virus can remain in the organism in a latent form, thus acting as a reservoir for disease reactivation, which can also occur when there is evidence of recovery of the anti-HBV immune capacity (production of anti-HBs)[2].

When a patient is subjected to immunosuppressive therapy, HBV reactivation may occur. This condition is fairly common and can lead to serious consequences. Various treatments can induce viral reactivation, including oncological chemotherapies, checkpoint inhibitor therapies, stem cell treatments, immunobiological agents such as monoclonal antibodies (mAbs), *etc*[3]. The role of mAbs is fundamental in HBV reactivation.

DEFINITION OF HBV REACTIVATION

HBV reactivation can be considered as sudden and high viral replication in a patient with chronic HBV infection or previous viral exposure[4]. The primary HBV infection can be considered resolved by the presence and development of anti-HBs antibodies. However, as previously mentioned, HBV can remain latent in the body, acting as a reservoir for disease reactivation[2]. HBV reactivation can present with various symptoms. There are mild forms of hepatitis but also severe ones, which can lead to acute liver failure and death.

Following the 2018 American Association for the Study of Liver Diseases guidelines[5], the state of HBV reactivation in hepatitis B surface antigen (HBsAg)-positive and anti-HBc-positive patients can be defined according to the following parameters: (1) At least 2 log (or 100-fold) increase in HBV DNA compared to the baseline level; (2) HBV DNA at least 3 log (or 1000) IU/mL in a patient with previously undetectable HBV DNA; and (3) HBV DNA at least 4 log (or 10000) IU/mL if the baseline level is not available.

For HBsAg-negative and anti-HBc-positive patients, HBV reactivation is defined as detectable HBV DNA or reappearance of HBsAg[5].

RISK OF HBV REACTIVATION

The risk factors for HBV reactivation can be summarized into three general areas: (1) Host factors; (2) virologic factors; and (3) type and degree of immunosuppression[6]. Possible host risk factors include: The male sex, older age, presence of cirrhosis, and the type of disease needing immunosuppressive therapies[7]. Virologic factors include high baseline HBV DNA, HBeAg positivity, and chronic hepatitis B[8]. Referring to the risk factors related to immunosuppressive therapy, these depend above all on the type of underlying pathology and its involvement at a systemic level (*e.g.*, hematologic disease). As already mentioned, a variety of treatments may induce HBV reactivation, such as immunosuppressive and chemotherapies which have the greatest risk of causing HBV reactivation. The American Gastroenterology Association (AGA) categorizes drugs based on their potential to cause HBV reactivation[9].

When the risk of causing HBV reactivation is greater than 10%, the drug can be defined as high risk. Among these drugs, there are B-cell-depleting agents, anthracycline derivatives, moderate-dose corticosteroid therapy (*e.g.*, 10-20 mg prednisone daily), or high-dose corticosteroid therapy (*e.g.*, > 20 mg prednisone daily). Drugs classified as having a moderate risk (between 1 and 10%) of reactivation include tumor necrosis factor (TNF)-α inhibitors, cytokine or integrin inhibitors, and tyrosine kinase inhibitors. Lastly, those considered at low risk of HBV reactivation (less than 1%) include immunosuppressive agents (*e.g.*, azathioprine and methotrexate), and corticosteroid therapy[9].

MONOCLONAL ANTIBODIES AND RISK OF HBV REACTIVATION

In this mini-review, the risk of HBV reactivation in patients treated with mAbs is briefly discussed. In a recent review published in Antibodies[10], there is a warning of adverse liver reactions after the initiation of mAbs. Notably, among the mAbs at high risk of HBV reactivation are anti-CD20 agents; TNF-α inhibitors are instead considered by the authors to be moderate risk. The main results of the current literature are summarized in Table 1.

Anti-CD20 agents (ibritumomab, obinutuzumab, ofatumumab, rituximab)

These drugs are widely used today. Rituximab and ofatumumab are humanized antibodies whose function is to bind to the CD20 receptor present on the surface of B lymphocytes, blocking their response and consequently humoral immunity [11,12]. Historically, rituximab has been the most studied drug in this category. The first work that highlighted the association between this drug and viral reactivation was reported by the FDA MedWatch Database[13] where 118 cases of reactivation were reported to occur between 1997 and 2009. Driven by this evidence, in 2013 the manufacturers of these drugs (rituximab and ofatumumab) were required to add label warnings that highlighted the high risk of possible virus reactivation[12]. These data highlight an interesting aspect: the risk of HBV reactivation linked to depletion of CD20+ B lymphocytes by rituximab highlights how important immunity is in controlling the disease itself[14].

Tumor necrosis factor-α inhibitors (e.g., infliximab, etanercept, adalimumab, certolizumab, and golimumab)

Another highly used category of immunosuppressive drugs is anti-TNF alpha. They are widely used in treating rheumatological diseases such as rheumatoid arthritis and psoriasis, but also gastrointestinal diseases such as inflammatory bowel diseases. However, TNF is a fundamental element in countering HBV infection. Several studies have highlighted how it acts on two fronts: inhibiting virus replication and stimulating T cell immunity. The activated T-cells eliminate infected hepatocytes. Starting from this evidence, various authors have proposed that TNF inhibition through the above-mentioned drugs could lead to a slatentization of the virus, which would replicate, generating disease reactivation[15].

Other mAbs with possible risk of HBV reactivation

Several studies demonstrate that other mAbs are at risk of HBV reactivation. Secukinumab is a fully human monoclonal antibody targeting interleukin-17A and is used for psoriatic disease. A recent retrospective study by Megna *et al*[16] highlights how HBV reactivation is possible in patients undergoing treatment with Secukinumab without prophylaxis. Another multi-center prospective cohort study by Chiu *et al*[17] (63 patients involved with concurrent HBV/hepatitis C virus infection) showed that, without antiviral prophylaxis, 15.2% of HBV patients treated with secukinumab exhibited viral reactivation[17]. Considering the data reported in the literature and the evidence cited above, it appears possible, albeit in very limited cases, that B and C viruses can be reactivated in cases of therapy with these molecules without antiviral prophylaxis[16].

Another drug studied for its relation to cases of HBV reactivation is Ustekinumab. It is a human interleukin-12 (IL-12) and IL-23 antagonist used in adult patients affected by plaque psoriasis, active psoriatic arthritis, and moderate-to-severe active Crohn's disease where other therapies have failed (or in case of patients' intolerance). Several studies have reported cases of HBV reactivation in HBsAg-positive patients treated with ustekinumab[18,19].

SCREENING, PROPHYLAXIS, AND TREATMENT

The current approach, according to the indications of the most recent guidelines, suggests that all patients undergoing treatment with drugs at high and moderate risk of HBV reactivation should be subjected to screening (serum evaluation of HBsAg, anti-HBc, and anti-HBs)[6]. For a more complete screening, blood levels of HBeAg, HBV DNA, and aminotransferase should also be evaluated. According to the main guidelines, treatment must be based on two parameters, the levels of aminotransferase and HBV DNA in the serum (> 2000 IU per milliliter), as well as the severity of the liver disease [5,20].

Treatments with nucleoside analogue drugs such as entecavir or tenofovir are recommended in cases of chronic infection complicated by cirrhosis associated with the presence of HBV DNA in the blood[14]. Lamivudine can also be used in these cases but is burdened by high rates of drug resistance. For this reason, the use of entecavir and tenofovir should be preferred in cases where therapy with lamivudine has already been carried out, and tenofovir is to be preferred to entecavir, as explained by Ekpanyapong *et al*[21].

It is good practice to test all patients before starting immunosuppressive treatments. If serological HBV positivity is found, patients at high reactivation risk should undergo prophylactic treatment with anti-HBV nucleoside analogues. This approach saw the greatest chance of preventing viral reactivation[4]. For example, the American Gastroenterological Association recommends prophylaxis for patients undergoing high-risk and moderate-risk immunosuppressive therapy. This prophylactic treatment should be continued for at least 6 mo after the end of immunosuppressive therapy (12 mo if B-cell-depleting agents were used)[9].

There are currently several prophylaxis protocols studied and available. The most frequently used drugs are lamivudine, entecavir, adefovir, tenofovir disoproxil fumarate, and tenofovir alafenamide. All have demonstrated usefulness in preventing HBV reactivation, but entecavir has demonstrated the greatest efficacy in prophylactic treatment [4].

Future and ongoing research on HBV reactivation must evaluate the complexity of this illness. The risk of HBV reactivation can be associated with immunosuppressive therapy and reactivation. These drugs include those used to treat

Table 1 Current literature

Ref.	Type of study	Conclusions
Baldo *et al*[10], 2022	Review	Warning of adverse liver reactions after the initiation of mAbs. mAbs that are at high risk of HBV reactivation, TNF-α inhibitors are at moderate risk
Evens *et al*[13], 2011	Meta-Analysis	118 cases were reported to the US FDA in which rituximab was associated with HBV reactivation
Dusheiko *et al* [14], 2023	Review	B-cell–depleting therapy with rituximab highlights the contribution of memory B cells to HBV control
Nathan *et al*[15], 2006	Review	TNF inhibits hepatitis viral replication and stimulates HBV-specific T-cell responses to clear the virus from infected hepatocytes. TNF could cause increased expression of hepatitis B viral antigens
Megna *et al*[16], 2022	Prospective cohort study	Highlights the risk of HBV reactivation in patients with latent infection treated with secukinumab without prophylaxis
Chiu *et al*[17], 2018	Multicenter Study	Without antiviral prophylaxis, 7 of 46 (15.2%) patients with HBV exhibited viral reactivation during therapy with secukinumab
Chiu *et al*[18], 2013	Clinical Trial	Among 11 patients positive for hepatitis B surface antigen (HBsAg), two out of the seven (29%) patients who did not receive antiviral prophylaxis exhibited HBV reactivation
Ting *et al*[19], 2018	Prospective cohort study	Among the remaining 54 patients classified as inactive HBV carriers, resolved HBV infection, or isolated anti-HBc positivity, only 3 patients experienced virologic reactivation

HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; TNF: Tumor necrosis factor; US FDA: United States Food and Drug Administration.

autoimmune illnesses, organ transplants, and specific types of cancer. Research should examine how these treatments impact the host immune system and help latent HBV reactivate. Molecular processes and viral components may also significantly contribute. One of the main areas of research is understanding the molecular processes of HBV reactivation. This entails investigating how the virus endures in the liver and how certain circumstances can cause reactivation.

Innovative studies might concentrate on factors related to viruses, including modifications in viral gene expression, mutations, or adjustments in the viral life cycle that support reactivation. Genetic predisposition and host variables may also be significant. Studies may look at host variables that make people more vulnerable to HBV reactivation. This includes genetic variants that could impact the virus's ability to withstand immunological responses or maintain viral latency. Predicting which individuals are more likely to experience reactivation can be aided by identifying particular host variables. Antiviral prophylaxis, timing of therapies, and monitoring techniques are all part of clinical care and prevention. There is also ongoing research on the creation and assessment of preventive interventions such as immunization and antiviral medications.

CONCLUSION

HBV reactivation is a potentially fatal complication after immunosuppressive biological or targeted therapy. Despite monoclonal antibodies having target specificity, they are not free of adverse effects, including HBV reactivation. Reports from the literature demonstrate that this is more frequent in patients treated with anti-CD20 or anti-TNF. However, there are some case reports of other mAbs causing this adverse event. Many unanswered questions remain about the risk of HBV reactivation associated with recently introduced mAbs. These questions provide an opportunity for monitoring and research.

FOOTNOTES

Co-first authors: Silvia De Pauli and Martina Grando.

Author contributions: Grando M, De Pauli S and Zeppieri M wrote the outline; Grando M and De Pauli S performed the research and wrote the manuscript together as co-authors; Grando M, De Pauli S, Miotti G, and Zeppieri M assisted in the writing of the paper; Zeppieri M was responsible for the conception and design of the study and completed the English and scientific editing; Grando M, De Pauli S, Miotti G and Zeppieri M assisted in the editing and making critical revisions to the manuscript. Although authors are from different areas of specialization, all authors provided general information and details regarding Hepatitis B virus reactivation based on the literature review. Each author participated in the research and writing of the paper, even if not directly pertinent to the area of study, considering the multidisciplinary approach in managing these patients. All authors provided the final approval of the article.

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MINIREVIEWS

Cytomegalovirus infection in non-immunocompromised critically ill patients: A management perspective

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Abstract

Critically ill patients are a vulnerable group at high risk of developing secondary infections. High disease severity, prolonged intensive care unit (ICU) stay, sepsis, and multiple drugs with immunosuppressive activity make these patients prone to immuneparesis and increase the risk of various opportunistic infections, including cytomegalovirus (CMV). CMV seroconversion has been reported in up to 33% of ICU patients, but its impact on patient outcomes remains a matter of debate. Even though there are guidelines regarding the management of CMV infection in immunosuppressive patients with human immunodeficiency virus/acquired immuno deficiency syndrome, the need for treatment and therapeutic approaches in immunocompetent critically ill patients is still ambiguous. Even the diagnosis of CMV infection may be challenging in such patients due to non-specific symptoms and multiorgan involvement. Hence, a better understanding of the symptomatology, diagnostics, and treatment options may aid intensive care physicians in ensuring accurate diagnoses and instituting therapeutic interventions.

Key Words: Cytomegalovirus; Critically ill; Immunocompetent; Intensive care unit; Virus

Core Tip: Cytomegalovirus (CMV) reactivation in critically ill immunocompetent patients may lead to increased intensive care unit (ICU) and hospital mortality, prolonged mechanical ventilation, longer ICU stay and increased risk of secondary bacterial and fungal infections. Nevertheless, whether it is the cause of clinical deterioration or is just a marker of disease severity remains debatable. Hence, the need for any therapeutic intervention is a management conundrum. The data extrapolated from studies on immunocompromised patients may not apply to these otherwise immunocompetent patients. This warrants future large-scale prospective studies on CMV reactivation in immunocompetent critically ill patients.

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INTRODUCTION

Cytomegalovirus (CMV) infection is a known opportunistic infection in immunocompromised patients and a predictor of poor outcomes. It has been extensively studied in post-transplant patients, human immunodeficiency virus/acquired immunodeficiency syndrome and neonates. Critically ill patients represent a sick cohort with risk factors like multiple comorbidities, sepsis, high disease severity, prolonged intensive care unit (ICU) stay and medications with immunosuppressive effects. All these can cause immunoparesis, even in patients with no previous history of immunosuppression, making them prone to opportunistic infections.

A systematic review of 13 studies with 1258 critically ill immunocompetent patients showed the rate of active CMV infection to be 17% (95%CI, 11% to 24%). This review defined active CMV infection as a single positive result for polymerase chain reaction (PCR), CMV antigen (pp65) or viral culture[1]. The test used for defining active CMV infection has an impact on the prevalence. In a prospective study of 120 non-immunocompromised patients admitted in ICU who were CMV seropositive, the reactivation rate was 33% when real-time PCR was used, indicating a high disease burden in modern ICUs[2]. CMV reactivation was found to be associated with increased hospital stay or 30 d ICU mortality. Patients with severe sepsis and high disease severity had a CMV infection rate of 32% which was significantly higher to an average of 17% ($P < 0.0001$). Patients with active CMV infection also had a higher mortality rate with an odds ratio (OR) of 1.93 (95%CI, 1.29 to 2.88; $P < 0.001$)[1]. A meta-analysis which included 18 observational studies with almost 2400 immunocompetent critically ill patients, CMV reactivation rate was 31% (95%CI 24%-39%), with the OR for all-cause mortality rate with and without CMV infection being 2.16 (95%CI 1.70-2.74). However, the same study showed no effect on mortality when the analysis was limited to detecting CMV in blood[3]. This raises the dilemma of CMV positivity being a marker of severe illness carrying poor prognosis rather than a direct causative factor of increased mortality.

We conducted a systematic search from the databases of PubMed, Reference Citation Analysis (https://www.referencecitationanalysis.com/), EMBASE and Google Scholar from all the past studies till July 2023. The search terms included major MESH terms "Cytomegalovirus", "CMV", and "Non-immunocompromised" or "Immunocompetent". The results were filtered for the studies published in the English language and for adult patients (> 18 years). Studies with non-critically ill patients were also excluded. We manually screened the results and included the relevant literature.

PATHOPHYSIOLOGY

CMV is the commonest herpes viridae to infect humans. It is a double-stranded DNA virus with 165 genes which encode viral proteins that interact with host proteins. After an acute or primary infection, the virus enters a latent phase, which the presence of immunoglobulin G (IgG) antibodies can detect. The seroprevalence of CMV IgG antibodies in women of childbearing age in India is almost 80%–90%. In contrast, it is less than 50% in developed countries, showing a greater baseline prevalence in developing countries[4,5]. During the latent phase, CMV remains latent in dendritic cells and monocytes. The cytotoxic CD8+ T lymphocyte suppress viral gene replication. Secondary symptomatic disease occurs due to the reactivation of latent infection during a state of decreased immunity or secondary infection with a new strain.

Patients with severe sepsis or high severity of illness scores have high levels and inflammatory markers. However, a stress response may develop compensatory anti-inflammatory response syndrome in a few patients, producing immunoparesis[6]. As a result, the cytotoxic T lymphocyte-induced suppression of latent CMV is inhibited, and the virus enters the active lytic phase. Bacterial sepsis lead to endotoxin release and an increase in tumour necrosis factor (TNF) which can reactivate CMV[7]. Exogenously administered catecholamine infusions used rampantly in the ICU may also contribute to stimulating the CMV reactivation[8].

Another source of CMV could be blood transfusions, which are common in critically ill patients, leading to a de novo infection. The number of transfused units of packed red blood was found to be a significant risk factor (OR: 1.5, CI 1.06-2.13) for CMV infection[9]. Leucodepleted blood products are now a norm in post-transplant patients to prevent new infections with CMV. However, a sensitivity analysis of trials done during the meta analysis by Kalil *et al*[1] study showed that the rate of active CMV infection in studies using leucodepleted blood transfusions was similar to that who

did not use leucodepleted blood (19% *vs* 16%)[1].

Risk factors

A systematic review showed that the rate of CMV infection in mixed medico-surgical ICU patients was 8%, while the rate for primarily surgical ICUs was 23%. The cytokine storm occurring after a major surgery was suspected to be the plausible reason for this difference. Rate of CMV infection during the first five days of ICU stay (early screening) was 1%, which increased to 21% after day 5. This review defined high severity of disease as an Acute Physiology and Chronic Health Evaluation II score above 20, Simplified Acute Physiology Score above 40 or Sequential Organ Failure Assessment score of more than 10. The rate of infection for high and low disease severity was 32% (95%CI, 23% to 42%; $P < 0.001$) and 13% (95%CI, 6% to 27%; $P < 0.0001$), respectively[1].

Limaye *et al*[2] conducted a prospective study in 120 CMV seropositive immunocompetent patients. CMV plasma DNAemia was assessed by thrice weekly CMV PCR. Risk factors for CMV reactivation were male sex, ventilator at baseline and blood transfusions. The study compared CMV 7-d moving average area under the receiver operating characteristic between index day (1.3) and day 30 (2.3), which showed higher values on day 30 ($P < 0.0001$). This indicates that patients had a higher risk of CMV reactivation after 30 d of ICU stay than on admission[2]. In a prevalence study, patients who were serologically negative for CMV on admission were found to be positive on day 5 of ICU stay[1]. The delay in the development of active CMV infection can be due to the time taken by the virus to complete its lytic cycle and develop into a clinical disease. Also, most critically ill patients have a higher disease severity score on day 5 compared to admission, which shows worsening of patients with prolonged ICU stay.

Patients with higher levels of inflammation are more prone to CMV reactivation. A study showed higher C-reactive protein levels at admission as a risk factor[9]. Risk factors for CMV have been elaborated in Table 1[1,2,9-14].

CMV and sepsis

Bacterial sepsis can trigger CMV infection, as proved by murine models. This reactivation could result from TNF and nuclear factor-kß release[8]. A prospective study of 25 immunocompetent CMV seropositive patients with septic shock and an ICU stay of more than 7 d were monitored for CMV reactivation. Within 2 wk, 32% of patients showed reactivation, with the duration of ICU stay and mechanical ventilation being higher in these patients[11]. In another prospective, observational study of CMV-seropositive immunocompetent critically ill patients with sepsis due to bloodstream infection (BSI), weekly testing for CMV viraemia was performed. Twenty percent of patients developed CMV viraemia. Factors associated significantly with CMV viraemia were age ($P = 0.044$) and blood transfusions ($P = 0.022$). The primary endpoint (mortality and/or multiorgan failure) between patients with and without CMV viraemia was similar. However, patients with CMV viraemia had significantly fewer ICU-free days and fewer ventilator-free days. Patients who were in the ICU for more than 48 h before the onset of BSI had higher likelihood of developing CMV viraemia with a higher-grade of viraemia, fewer ICU-free days and ventilator-free days than those hospitalised for lesser than 48 h of BSI. Patients who developed sepsis when already in the ICU had a higher risk of CMV reactivation and worse outcomes than new ICU-bound patients, suggesting that patients with a prolonged ICU stay are more susceptible and should be considered for targeted interventions for CMV[12].

CMV and mechanical ventilation

More than two decades back, Papazian *et al*[15] reported CMV as an unexpected cause of ventilator-associated pneumonia. They conducted a prospective study over 5 years where autopsies were conducted on patients who succumbed to ventilator associated pneumonia with negative microbiological cultures. Immunocompromised patients were excluded. An open lung biopsy (OLB) was performed in few patients on invasive mechanical ventilation (IMV) with unexplained worsening of their respiratory status. Ventilator-associated CMV pneumonia was defined as an IMV duration of more than seven days with histopathological signs of CMV pneumonia (basophilic or eosinophilic inclusion body with a surrounding light halo within large nuclei suggestive of owl eye appearance). A total of 26 OLBs and 60 autopsies were performed. Twenty-five cases of CMV pneumonia were identified based on the above-described criteria. Histological studies were conducted 10–40 d after ICU admission. Interestingly, no bacteria were identified in 88% of lung cultures, with CMV being the sole identified pathogen in these cases[15]. This was in the pre-PCR era when molecular testing for respiratory pathogens was unavailable.

Stéphan *et al*[16] conducted a prospective study in 23 critically ill, mechanically ventilated, non-immunocompromised patients to assess the reactivation of latent CMV in blood or lungs who were seropositive. Viral cultures and PCR was used to evaluate the presence of CMV in blood and lung with 37 blood and 22 bronchoalveolar lavage (BAL) samples being examined. The tests were negative in all the 23 patients and also no CMV DNA could be amplified using PCR in blood or BAL samples indicating an absence of reactivation despite the high risk factors[16]. Hence, the dilemma of CMV being a causative pathogen or a chance finding continues.

A 5-year prospective study included 123 non-immunocompromised patients with severe acute respiratory distress syndrome requiring veno-venous extracorporeal membrane oxygenation (ECMO). Sixty-seven patients (54%) had human simplex virus (HSV) and/or CMV reactivation (20 viral co-infection, 40 HSV alone, and 7 CMV alone). HSV reactivation was earlier than CMV [11 (6–15) *vs* 19 (13–29) d, $P < 0.01$] and both were associated with a longer IMV duration and an increased hospital and ICU stay[17]. Patients on ECMO have increased volume of distribution, increased cytokine release and added stress to the system.

Effects of CMV reactivation on critical illness

CMV is known to worsen the state of immunoparesis, thereby increasing opportunistic infections, including bacteraemia

Table 1 Risk factors for cytomegalovirus reactivation

Risk factors for CMV reactivation	Ref.	Statistics
High disease severity	Kalil *et al*[1]	High disease severity (APACHE > 20, SAPS > 40 or SOFA > 10) 32% *vs* low disease severity (APACHE < 20, SAPS < 40 or SOFA < 10) 13% ($P < 0.0001$)
	Ong *et al*[10]	Mean APACHE IV 91 (71-113) *vs* 76 (62-99) ($P < 0.01$)
Prolonged ICU stay	Kalil *et al*[1]	1% < 5 d *vs* 21% at > 5 d ($P < 0.001$)
	von Müller *et al*[11]	32% by day 14
	Limaye *et al*[2]	33% by day 12
Sepsis, septic shock	Kalil *et al*[1]	Reactivation of CMV in patients with and without septic shock: 32% *vs* 15% ($P < 0.0001$)
	Osawa *et al*[12]	OR 4.62 ($P = 0.02$)
	Ong *et al*[10]	Reactivation of CMV in patients with and without septic shock 57% *vs* 41% ($P = 0.02$)
Previous seropositivity	Kalil *et al*[1]	Reactivation of CMV in patients with and without previous seropositivity for CMV: 31% *vs* 7% ($P < 0.0001$)
Mechanical ventilation	Osawa *et al*[12]	OR: 8.5 (95%CI 1.1 to 66.5 for high-grade CMV viremia, *i.e.* CMV PCR > 1000 copies/mL)
	Limaye *et al*[2]	OR 2.5 (0.9-7.3) ($P = 0.09$)
Multiple blood transfusions	Frantzeskaki *et al*[9]	OR 1.50 ($P = 0.02$)
	Chiche *et al*[13]	OR 3.31 ($P = 0.04$)
	Limaye *et al*[2]	OR 9.1 (1.0-84.7) ($P = 0.05$)
Surgical patients	Kalil *et al*[1]	Rate of CMV reactivation in medical ICUs: 8% *vs* surgical ICUs: 23% ($P < 0.001$)
Steroid use	Jaber *et al*[14]	CMV reactivation in patients with and without steroid use: 55% *vs* 33% ($P = 0.04$)
	Chiche *et al*[13]	OR 2.26 ($P = 0.08$)
Renal failure	Jaber *et al*[14]	58% *vs* 33% ($P = 0.02$)
	Ong *et al*[10]	16% *vs* 6% ($P < 0.01$)
Male	Limaye *et al*[2]	OR 3.6 ($P = 0.005$)
Raised CRP	Frantzeskaki *et al*[9]	OR 1.01 ($P = 0.02$)

CMV: Cytomegalovirus; APACHE: Acute physiology and chronic health evaluation; SAPS: Simplified acute physiology score; SOFA: Sequential organ failure assessment; ICU: Intensive care unit; OR: Odd's ratio, PCR: Polymerase chain reaction; CRP: C-reactive protein.

and fungemia[18,19]. It increases the proinflammatory and procoagulant states by changes in the levels of factor X, thrombin, von Willebrand factor and plasminogen inhibitor type 1. The all-cause mortality with active CMV infection is approximately twice compared to those without CMV infection[1,3,20,21]. CMV has been associated with prolonged mechanical ventilation and hospital and ICU stay[3,18,21]. Various studies with outcomes associated with CMV are elaborated in Table 2[1-3,7,9-18,22-29].

CLINICAL FEATURES

CMV presents with non-specific symptoms, affecting multiple organs making it difficult to suspect and identify in critically ill patients. Hence, the "CMV syndrome" described in post-transplant patients consists of fever, leukopenia and thrombocytopenia without other end-organ disease cannot be used to define CMV reactivation in this population[30].

CMV can present similarly to infective mononucleosis caused by the Ebstein-Barr virus (EBV). Fever and systemic symptoms are predominant, but cervical lymphadenopathy and tonsillitis are rarely seen compared to EBV. On a peripheral blood smear examination, the two defining hematologic abnormalities associated with mononucleosis are presence of more than 50 percent lymphocytes with greater than 10 percent being atypical lymphocytes[31].

Gastrointestinal manifestations include colitis, esophagitis and enteritis. Glucocorticoid use is associated with an increased risk of CMV colitis in otherwise immunocompetent adults. Diarrhoea, fever and abdominal pain are the common presenting symptoms[32]. Diarrhoea is usually bloody but can present as a profuse gastrointestinal haemorrhage. On endoscopy, well-demarcated ulceration without exudate (50%) is the most common appearance, followed by ulcero-infiltrative changes (25%) and pseudo membrane formation (25%)[33]. Pathology findings show inflammatory colitis with classical owl eye appearance or Cowdry inclusions typical of CMV disease. CMV can also cause granulomatous hepatitis, with subclinical transaminitis being the most common finding in immunocompetent patients

Table 2 Patient outcomes in studies in critically ill immunocompetent patients

Year of publication	Ref.	Study design	Patient population	Sample size	Prevalence of CMV (%)	Mortality rate (%) CMV positive *vs* negative	ICU stay	Ventilator duration	Other outcomes
1990	Domart *et al* [22]	Prospective, single center	Mediastinitis following cardiac surgery	115	25	55 *vs* 37 ($P < 0.01$)	69+/-36 *vs* 48+/-27 ($P < 0.05$)	ND	
1996	Stéphan *et al* [16]	Prospectivecase series, single center	Medico-surgical patients on mechanical ventilation	23	ND	52	ND	ND	
1996	Papazian *et al* [15]	Prospective single centre	Ventilator associated pneumonia	86	29	ND	ND	No difference ($P > 0.05$)	Severe hypoxemia CMV +/- (72 *vs* 95 mmHg, $P < 0.05$)
1998	Kutza *et al*[7]	Prospective longit-udinal, singles centre	Septic shock	34	32.4	ND	ND	ND	CMV active had higher TNFα, IL1ß, ALT
2006	Cook *et al*[23]	Prospective, singles centre	SICU	20		65 *vs* 33 ($P = 0.006$)	83.5 *vs* 36 ($P < 0.03$)	ND	92 *vs* 25 ($P < 0.004$)
2011	Heininger *et al*[24]	Prospective, singles center	Medical ICU with SAPS II > 40	56		55 *vs* 36	30 *vs* 23 ($P = 0.0375$)	ND	
2005	Jaber *et al*[14]	Retrospective matched case control study, single centre	Medico-surgical ICU patients	80		20 *vs* 11 ($P = 0.02$)	41 *vs* 31 ($P = 0.04$)	35 *vs* 24 ($P = 0.03$)	Bacteremia 15 *vs* 7 ($P = 0.05$)
2006	von Müller *et al*[11]	Prospective observa-tional study, single centre	Septic shock	38	18.4	57 *vs* 38 (NS)	54 *vs* 19 ($P = 0.0025$)	42 *vs* 16 ($P = 0.0025$)	
2008	Ziemann *et al* [25]	Retrospective study	Medical ICU	138	35	28.6 *vs* 10.9 ($P = 0.048$)	32.6 *vs* 22.1 ($P < 0.001$)		
2008	Limaye *et al* [2]	Prospective, multicentre	Mixed ICU	120	33	ND			
2009	Chiche *et al* [13]	Prospective study	Medical ICU on mechanical ventilator for > 2 d	242	16.1	54 *vs* 37 ($P = 0.082$)	32 *vs* 12 ($P < 0.001$)	27 *vs* 10 ($P < 0.001$)	Ventilator free days at 28 and 60, $P < 0.001$. Increased risk of bacteremia, $P < 0.033$, increased bacterial nosocomial pneumonia, $P < 0.001$
2010	Chilet *et al* [26]	Prospective observa-tional, single center	Surgical and trauma ICU	53	39.7	61 *vs* 46 ($P = 0.40$)	37 *vs* 11 ($P = 0.01$)	ND	TNF alpha, $P = 0.80$. CMV specific T cell response CD8+, $P = 0.05$. CD4, $P = 0.04$
2011	Heininger *et al*[24]	Prospective observa-tional study	Mixed ICU	86	40.6	37.1 *vs* 35.3, ($P = 0.861$)	30 *vs* 12 ($P < 0.001$)	22 *vs* 7.5 ($P = 0.003$)	
2009	Chiche *et al* [13]	Prospective, observation	Medical ICU	51	18	40 *vs* 13.3 ($P = 0.21$)	28 *vs* 14 ($P = 0.013$)	24 *vs* 8 ($P = 0.019$)	Bacterial VAP 40 *vs* 26.6 ($P = 0.70$)
2012	Coisel *et al*	Prospective study	Medical ICU	93	ND	55 *vs* 20 ($P < 0.01$)	25.5 *vs* 13 ($P =$	Bacteremia (%) 19.5 *vs* 10	VFD at 60 (d) median [IQR] 0 [0-

	[27]						0.037)	(P = 0.009)	25] *vs* 50 [11.5-58] (P = 0.001). Shock (%) 77 *vs* 30 (P = 0.001, acute renal failure (%) 50 *vs* 16 (P = 0.01)
2013	Clari *et al*[28]	Prospective observational, single center study	Surgical and trauma ICU	48	0.27	8 out of 17 (reactivation of CMV) *vs* 5 out of 14 (without CMV reactivation) (P = 0.523)			
2016	Ong *et al*[10]	Prospective, multicenter	ARDS patients on mechanically ventilated beyond day 4	271	27	Death by day 90 46 *vs* 28 (P < 0.01)	16 *vs* 9 (P < 0.01)	15 *vs* 8 (P < 0.01)	
2015	Frantzeskaki *et al*[9]	Prospective, observation, multicenter	Mixed ICU, Mechanical ventilated seropositive (anti CMV IgG) positive	80	14	18 *vs* 22 (P > 0.05), 28 D mortality rate	32 *vs* 21 (NS)	27.5 *vs* 18 (NS)	SOFA score higher with CMV reactivation (P < 0.006), 28 d survival no difference
2016	Osawa *et al* [12]	Prospective, multicentre	Septic patients with BSI	100	20	20 *vs* 15 (P = 0.585)	27 *vs* 20 (P = 0.07)		VFD 15 *vs* 25 (P = 0.05). ICU free days 7 *vs* 18 (P = 0.01)
2019	Hraiech *et al* [17]	Retrospective, observational, single center	ARDS on VV ECMO, assessed for HSV and CMV	123	21.9	52 *vs* 59 (P = 0.58)	ICU LOS 29 *vs* 16 P < 0.01. Hospital LOS 44 *vs* 24 (P < 0.01)	34 *vs* 17.5 (P < 0.01)	Duration of ECMO 15 *vs* 9 (P < 0.01)
2021	Zhang *et al* [29]	single-center, prospective observational study	Medical ICU patients on mechanical ventialtion	71	18.3	69.2 *vs* 19 (P < 0.01)	ICU LOS 27 *vs* 12 (P < 0.01)	25 *vs* 10 (P < 0.01)	Hospital expenses higher in patients with CMV reactivation (P < 0.02)
2009	Kalil *et al*[1]	Systematic review	Included patients in ICU, 9 prospective and 4 retrospective studies	1258	17	OR: 1.93 (1.29–2.88) (P = 0.01)	ND	ND	ND
2009	Osawa *et al* [21]	Systematic review	13 studies, 9 prospective, 4 retrospective	ND	0-33	CMV + 29 to 100 as compared with CMV – 11 to 74 (OR: 5.7)	33 to 69 d *vs* 22 to 48 d (P < 0.05)	21 to 39 d *vs* 13 to 24 d (P < 0.05)	75% *vs* 50% (P = 0.04)
2017	Lachance *et al* [18]	Systematic review and meta-analysis	22 studies, randomized trials, observational studies (either retrospective or prospective), or case-control studies	2199	9–71	CMV reactivation was associated with a 2.5-fold increase in ICU mortality with low heterogeneity (10 studies, n = 970 patients, OR = 2.55, 95%CI = 1.87–3.47; P < 0.001)	MD 6.60 d, 95%CI = 3.09–10.12; P = 0.0002, I^2 = 79%	ICU LOS was higher in CMV positive n (9 studies, n = 973 patients, MD 8.18 d, 95%CI = 6.14–10.22; P < 0.001)	Increase in nosocomial infections (OR 2.37-3.2) P < 0.05. Most common infections being ventilator-acquired pneumonia, bacteremsia, and fungal infections
2018	Li *et al*[3]	SR and MA	18 studies, mixed population	2398	CMV infection 27; CMV reactivation 31	All cause mortality OR: 2.16 (1.7-2.74)	ICU LOS stay (MD: 12 d)	9 d	

CMV: Cytomegalovirus; APACHE: Acute physiology and chronic health evaluation; SAPS: Simplified acute physiology score; SOFA: Sequential organ failure assessment; ICU: Intensive care unit; OR: Odd's ratio; PCR: Polymerase chain reaction; CRP: C-reactive protein; ND: No data; TNF: Tumor necrosis factor; IL: Interleukin; SICU: Surgical intensive care unit; VAP: Ventilator associated pneumonia; VFD: Ventilator free days; VV ECMO: Veno-venous extracorporeal membrane oxygenation; HSV: Herpes simplex virus; LOS: Length of stay; SR: Systematic review; MA: Meta analysis; ARDS: acute respiratory distress syndrome; IgG: Immunoglobulin G; IQR: interquartile range; ALT: alanine aminotransferase; NS: Not significant.

[34]. However, significant hepatic dysfunction and portal vein thrombosis are relatively rare[35].

The nervous system is the second most affected organ system in CMV infection in the immunocompetent host, leading to numerous clinical manifestations like meningoencephalitis, myelitis, Guillain-Barré syndrome (GBS), brachial plexus neuropathy, diffuse axonal peripheral neuropathy and transverse myelitis[36-40]. Meningoencephalitis is rare but can cause long-term residual neurological deficits. The incidence of CMV-related GBS is 0.6 to 2.2 cases per 1000 cases of primary CMV infection. A prospective observational study that included 506 patients with GBS found 63 (12.4%) had primary CMV infection, as detected by immunoglobulin M antibodies with IgG avidity combined with plasma CMV PCR [41]. In a case series of 42 patients with GBS and seropositivity for recent or past CMV infection, cerebrospinal fluid (CSF) showed the presence CMV DNA by PCR in one-third of cases[42]. Antibodies to ganglioside monosialic (GM)-2 are frequently positive in CMV-associated GBS and can aid in diagnosis[15].

The lung involvement by CMV is less conspicuous in critically ill patients, especially if they had any other concurrent pulmonary pathology. For BAL samples it is difficult to differentiate between a casual association with CMV positivity from a true infection. This is because the diagnosis depends on the quality of the BAL sample, the skillset of the pathologist and choice of diagnostic test. The gold standard diagnostic test is lung biopsy, which may not always be feasible in critically ill patients[15]. CMV has been known to cause pericarditis and myocarditis in immunocompetent patients, however, it is difficult to establish direct causality as it needs invasive endomyocardial biopsy. In a study of 40 patients with fatal myocarditis undergoing autopsy, CMV DNA was detected in 15 patients. In 67% of the patients for whom PCR was positive for CMV, *in situ* hybridisation revealed viral DNA in cardiomyocytes[43].

Haematological manifestations include mild to moderate haemolytic anaemia, thrombocytopenia, pancytopenia and disseminated intravascular coagulation. Laboratory investigations may show false positivity for cold agglutinins, rheumatoid factor and antinuclear antibodies[44,45].

Venous thrombosis including pulmonary embolism has been reported in immunocompetent patients with acute CMV infection. Deep vein thrombosis in lower limbs is a known complication of prolonged immobilisation in the ICU. However, development of thrombosis at unusual sites like internal jugular vein, portal vein, splanchnic vein, and mesenteric veins suggests an underlying procoagulant effect of CMV[46]. Other rarer manifestations of CMV are cystitis, nephritis and retinitis[47,48].

DIAGNOSIS

PCR is the most common test and can be used on serum, CSF and tissue samples. While qualitative PCR can be used to diagnose reactivation of infection, a quantitative test helps to determine the CMV DNA viral load.

Recently, the FDA has approved the Aptima CMV Quant Assay for quantitative testing of CMV. It is an in-vitro nucleic acid amplification test in human EDTA plasma performed on the fully automated Panther system. The indicated use is for solid organ and hematopoietic stem cell transplant patients. By performing serial DNA levels, it can also be used to assess the response to treatment in those receiving anti-CMV therapy. However, the Aptima CMV Quant Assay results should be interpreted with consideration to relevant clinical and laboratory findings. It has not been designed to serve as a screening assay for the presence of CMV in blood or blood products[49].

Nevertheless, this test's lack of widespread availability makes the CMV viral load test the only viable alternative. Laboratory-developed tests are tests developed or used by individual laboratory after validating them to the standard of the laboratory inspecting agencies. In the absence of standardised test across laboratories, each laboratory should establish independent cut off values as per the local population's viral load. A multicentre study that included 33 laboratories across United States, Europe and Canada demonstrated that for an individual sample the test variability ranged from 2.0 Log10 copies/mL to 4.3 Log10 copies/mL. This means 100000 copies/mL can be reported as 100 copies/mL from a different laboratory (3 Log10 difference)[50]. Hence, clinicians cannot compare results from two different laboratories. This poses a significant challenge in developing guidelines for managing CMV infection based on viral load cut-offs. There is significant heterogeneity in the type of tests used and threshold cut-offs used to define CMV DNAemia across various studies, as shown in Table 3[10,12,15,17,30,51].

On the day treatment for CMV is initiated, a baseline sample for quantitative test needs to be collected, followed by weekly monitoring throughout the therapy. This is due to CMV DNA having a half-life of 3–8 d in the plasma[52]. Therapy needs to be continued till viral load values are undetectable. The chances of resistant strains are higher if there is an increase in viral load after an initial drop, no decrease in viral load after two weeks of therapy and if there is a plateau in the rate of decline. Such cases should be evaluated for resistant strains done by sequencing UL54 and/or UL97 genes. However, this recommendation applies to post-transplant patients, and its generalisability to critically ill immunocompetent patients is questionable[53]. Most of the studies in these patients take a breakpoint of 500-1000 U/mL as a significant titre to begin therapy.

CMV DNA by PCR in BAL is a sensitive test to detect CMV in the respiratory tract. However, a prospective study of immunocompromised patients by Berengua *et al*[54] showed that only 34% of BAL samples positive for CMV by quantitative (qPCR) were also positive by culture. The probability for isolation of CMV by culture was 4.3% for a viral load cut-off of < 200 IU/mL and 100% for a viral load cut-off of > 900 IU/mL[54]. Vergara *et al*[55] conducted a prospective observational study of adult patients admitted to two ICUs within 24 h of presentation to the Department of Emergency. The study included both immunocompromised and immunocompetent patients. CMV in BAL, was detected in 35 of 133 ICU patients (26%), out of which 29% were immunocompetent. Factors significantly associated with positive CMV BAL test were immunosuppression (P = 0.017) and use of systemic corticosteroids (P = 0.002). CMV positivity was also associated with prolonged hospital stay (P = 0.017) and increased mortality rate (P = 0.024)[55]. Another prospective

Table 3 Polymerase chain reaction tests for cytomegalovirus and the cut offs used in various studies

Ref.	Test	Threshold copies/ml	Threshold as per IU/mL
Papazian *et al* [15]	PCR		500 IU/mL whole blood
Park *et al*[51]	RT-PCR	> 270 copies/mL in whole blood	
Hraiech *et al* [17]	PCR	Copy number > 500/mL CMV	"High reactivation" for viral loads greater than or equal to 1000 IU/mL or "low reactivation" for viral loads of 100–999 IU/mL
Zhang *et al*[29]	PCR	Copies > 500/mL	
Osawa *et al*[12]		Copies > 500 copies/mL	
Ong *et al*[10]			100 IU/mL

CMV: Cytomegalovirus; IU: International units; RT- PCR: Reverse transcription polymerase chain reaction.

study by Boeckh *et al*[56], in patients who had undergone haematopoietic stem cell transplant, found higher median viral loads in patients with CMV pneumonia. The control cohorts were divided into three groups. First were patients with radiological pneumonia but negative for standard virologic testing for CMV, second were patients with idiopathic pneumonia syndrome, and last was a cohort of asymptomatic patients. The study group included patients positive on standard CMV testing, shell culture or direct fluorescence assay. This study found a threshold of > 500 IU/mL to differentiate between true CMV pneumonia and pulmonary shedding[56]. A 500 IU/mL cut-off for BAL CMV is reasonable when associated with a relevant clinical picture. However, studies specific to immunocompetent critically ill patients are needed before we define a definite cut-off.

Other available tests are assays based on pp65 antigen in leukocytes. This is a less standard, labour-intensive manual procedure. As it detects antigens in human leukocytes, its sensitivity is poor in neutropenic patients. Tissue cultures are invasive, time-consuming and challenging to perform. However, histopathology examination remains the gold standard test to confirm end-organ disease in cases of pneumonia and colitis.

Serological tests are of limited benefit in highly endemic regions. The diagnosis of primary infection is ascertained when seroconversion is documented by the appearance of virus-specific IgG in the serum of a previously seronegative patient. Such an approach is feasible only when high-risk patients are identified and prospectively monitored, which may need to be more cost-effective. A study comparing the clinical outcomes between CMV seropositive and CMV seronegative critically ill, non-immunocompromised patients could not demonstrate an independent association between the CMV serostatus and ICU mortality. Secondary endpoints like time alive, rate of discharge from ICU or hospital, weaning rates and the requirement for renal replacement therapy were also comparable in both groups. Hence, merely testing for seropositivity is not recommended[53].

PROPHYLAXIS AND PRE-EMPTIVE THERAPY

The use of prophylaxis in high-risk critically ill patients may seem attractive because the treatment cost is significantly less than weekly surveillance of CMV. However, most patients in the ICU have risk factors for CMV. Hence, universal prophylaxis for all such patients exposes already critical patients to potentially toxic medications. Suboptimal antiviral therapy may also induce resistant CMV strains. The advantage of pre-emptive therapy is that it explicitly targets only patients with laboratory evidence of active CMV infection, leading to minimal exposure to antiviral drugs. Ganciclovir (GCV) is the drug of choice for pre-emptive therapy for CMV.

Cowley *et al*[57] conducted a single centre open-label randomised controlled trial (RCT), CMV Control in Critical Care (CCCC-trial), enrolling 124 non-immunosuppressed, seropositive for CMV and mechanically ventilated patients. The patients were randomised into three cohorts of 1:1:1 to Valacyclovir, Valganciclovir (450 mg per day) and no treatment. The primary outcome was CMV reactivation which was significantly lower in treatment groups *vs* control [Hazard ratio (HR) = 0.14; 95%CI 0.04 to 0.5]. However, the valacyclovir arm was prematurely terminated because of an increase in mortality rate. There were no differences between different arms in the levels of biomarkers [interleukin (IL)-6, TNFα] measured at days 14 and 28. Other secondary outcome measure like renal dysfunction or rate of platelet transfusions were not significant. Neutropenia or GM-CSF use was also not reported[57].

In a phase II trial by Limaye *et al*[58], GCV/valganciclovir was used to prevent CMV reactivation in the acute injury of the lung (GRAIL study). This study included nearly 160 non-immunocompromised, CMV seropositive, critically ill patients admitted with sepsis or trauma. Patients were randomised to receive prophylaxis with intravenous (IV) GCV for five days, followed by IV GCV or oral Valganciclovir, or to receive a placebo. Patients who received antiviral prophylaxis had decreased CMV reactivation as compared to the placebo arm (12% *vs* 39%). However, the primary outcome of IL-6 levels was not significantly different between both arms, nor were there any differences in the incidence of secondary infections including both bacteraemia or fungemia or the length of ICU stay. IL-6 is a proinflammatory cytokine, that was chosen as the primary outcome because increased levels have been shown to be associated with increased mortality. The

sepsis subset of GCV group had higher ventilator-free days (difference of median: 3 d, $P = 0.03$), had fewer mechanical ventilation days (difference of median: 1 d, $P = 0.06$) and a higher PaO_2:FiO_2 ratio during the initial week of ventilation. However, the mortality rate was comparable in both arms[58].

Given the small size of the current studies and the absence of any mortality benefit, universal prophylaxis for all immunocompetent critically ill patients cannot be recommended. A phase 3 trial (GRAIL 3 study) is underway with the target of randomly enrolling 500 acute respiratory failure patients to receive IV GCV or placebo[59]. This may shed more light on the therapeutic approach to managing these patients.

However, the benefit of a pre-emptive treatment (started based on seropositivity) is doubtful. The exact mechanisms of CMV reactivation are still not clear, and CMV reactivation could instead be a surrogate marker of primary disease severity. Therefore, giving antiviral drugs to these patients should be considered cautiously in terms of the benefit-risk ratio. A retrospective cohort study that included 136 adult non-immunocompromised patients with CMV DNAemia, had a cohort group of 66 GCV-treated patients (48.5%) and control group of 70 non-treated (51.5%) patients. There was no statistically significant difference for primary and secondary outcomes of 30-month survival (28.0% *vs* 38.9%) and 12-mo survival (40.3% *vs* 49.2%) respectively. In the subsequent multivariate analyses, GCV treatment was not associated with greater 30-mo survival (HR 1.307, 95%CI 0.759–2.251) and 12-mo survival (HR 1.533, 95%CI 0.895–2.624)[54]. Pre-emptive treatment based on CMV PCR copies was not beneficial. This was further substantiated by Papazian *et al*[60] through a double-blind, placebo-controlled RCT involving 19 ICUs in France to assess the effectiveness of pre-emptive antiviral therapy in mechanically ventilated patients. Seventy-six adults who had been on mechanical ventilation for at least 96 h, expected to remain so for ≥ 48 h and positive for CMV in blood were randomised to receive IV GCV at a dose of 5 mg/kg bid for 14 d ($n = 39$) or a matching placebo ($n = 37$). No significant difference was seen in ventilator-free days from randomisation to day 60 or 60-d mortality rate. However, no significant side effects like leukopenia or rise in creatinine were seen in the GCV arm. Based on the results of an interim analysis, the trial was stopped for futility. The sub-distribution hazard ratio for being alive and weaned from mechanical ventilation at day 60 was not significant (1.14, 95%CI of 0.63 to 2.06; $P = 0.66$). This trial showed no benefit in treating cases pre-emptively[60].

Treatment

Antiviral treatment is mandatory incase reactivation is associated with clinical CMV disease. It is reasonable that treatment for only significant CMV replication (blood or BAL) is not indicated unless it is associated with relevant clinical feature including lung infiltrates and at least two factors: Prolonged IMV, fever, diarrhoea, absence of bacterial diagnosis for the infiltrate, leukopenia, haemophagocytosis, hepatitis or hyperbilirubinemia. This points for CMV being a probable pathogen causing multiple organ dysfunction and not just a bystander or viral shedding[61].

The duration of treatment should be individualised. According to the third international consensus on the management of CMV in solid organ transplantation, the duration of therapy for CMV infection is determined by the fulfilment of the criteria below:

(1) Till CMV PCR or antigenemia becomes undetectable. Eradication of CMV is defined as below lower limit of quantification (LLOQ) on atleast one highly sensitive assay (LLOQ < 200 IU/mL) or two negative consecutive less sensitive assays. A completely undetectable viral load may not always be achievable when highly sensitive assays are used;

(2) Clinical evidence of the disease has resolved;

and (3) At least 2–3 wk of therapy[62].

CMV DNAemia does not accurately reflect the severity of clinical disease in all patients. Therefore, longer duration of treatment is essential in invasive diseases like pneumonitis in lung transplant recipients, tissue-invasive gastrointestinal disease and retinal or central nervous system infections. Secondary prophylaxis is not associated with fewer relapses after suppression of CMV DNA and is not routinely recommended in critically ill population. The available therapeutic options for treating CMV are summarised in Table 4[63-65].

CONCLUSION

CMV reactivation is prevalent in up to one-third of critical patients in the modern ICUs. The most common risk factors for CMV reactivation are previous seropositivity, higher disease severity, sepsis and septic shock and prolonged ICU stay. CMV reactivation may be associated with increased ICU and hospital mortality, prolonged mechanical ventilation, longer ICU stay and increased risk of secondary bacterial and fungal infections. There are a few challenges in treating CMV reactivation, as most of the studies in this field are observational. The 2 RCTs, the CCCC study[57] and GRAIL study[58], did not show any mortality benefit by treating CMV pre-emptively.

Further, the breakpoints to initiate therapy for pre-emptive treatment still need to be defined, and studies have considerable heterogeneity. Whenever the decision is made to treat, GCV remains the drug of choice. The patient monitoring using CMV DNA levels therapy is extrapolated from protocols from immunocompromised patients, especially solid organ transplant patients. This warrants validation from prospective studies in immunocompetent critically ill patients. Lastly, appropriate treatment duration and the role of secondary prophylaxis in patients who continue to be critically ill even after completing an anti-CMV regimen need to be investigated.

Table 4 Therapeutic options for cytomegalovirus

No.	Drug	Mechanism	Dose	Salient features	Adverse effects
1	Ganciclovir[1][63]	Nucleoside analog, needs intracellular phosphorylation to inhibit DNA polymerase, hence can develop resistance	5 mg/kg iv q12h	Preferable in life threatening disease, very high viral load and when there is a concern for inadequate gastrointestinal absorption	Severe neutropenia may become a therapy limiting adverse effect in up to 32% patients. May respond to G-CSF or GM-CSF
2	Valganciclovir[1]	Prodrug of ganciclovir	900 mg po q12h	Oral bioavailability is equivalent to iv ganciclovir, once-daily dosing and reduced risk of development of resistance	Neutropenia, thrombocytopenia, anemia, acute renal failure
3	Foscarnet or Phosphonoformate[1]	Does not require intracellular phosphorylation and therefore, retains activity against most GCV-resistant strains of CMV	90 mg/kg iv q12h	Intravenous PFA may be used under conditions of failure of GCV treatment, GCV resistance or excessive side effects such as leukopenia	PFA is nephrotoxic in 1/3rd patients, which limits its use in many critically ill patients
4	Cidofovir[1]	Acts directly on DNA polymerase	5 mg/kg iv once weekly	May be used as an alternative to PFA in case of GCV resistance. FDA approved only for CMV retinitis in HIV	Nephrotoxic on proximal tubular cells (Fanconi like syndrome). Pre-hydration and probenecid before the dose
5	Maribavir[1][64] (Livtencity, Takeda)	Inhibition of human CMV encoded kinase pUL97: Required for viral replication	400 mg po q12h	Used in resistance to GCV, PFA, CDV	No renal or hepatic dose adjustment required. It can cause nausea, vomiting, diarrhea and neutropenia
6	Letermovir[1][65] (Prevymis, Merck)	CMV viral terminase inhibitor	480 mg q24h po or iv	FDA approval for post HSCT and post renal transplant prophylaxis	Nausea, diarrhoea, vomiting, oedema. Various drug interactions requiring dose adjustments
7	Hyperimmune serum	Passive immune prophylaxis	400 U/kg on day 1, 4 and 8 and then 200 U/kg on day 12 and 16	As salvage therapy in severe recurrent CMV infections	High cost and heterogeneity of the preparation. Infusion related adverse effects like fever, shivering, rash

[1]United States Food and Drug Administration approved for treatment of cytomegalovirus in immunocompromised patients.
CMV: Cytomegalovirus; G-CSF: Granulocyte colony stimulation factor; GM-CSF: Granulocyte macrophage colony stimulation factor; GCV: Ganciclovir; CDV: Cidofovir; PFA: Phosphonoformate; DNA: Deoxy ribonucleic acid; FDA: Food and drug administration; HSCT: Hematopoietic stem cell transplantation; DNA: Deoxyribose nucleic acid; HIV: Human immunodeficiency virus; iv: Intravenous; po: Per oral.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Impact of metabolic dysfunction-associated steatotic liver disease on COVID-19 hospitalizations: A propensity-matched analysis of the United States

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Abstract

BACKGROUND

Metabolic dysfunction-associated steatotic liver disease (MASLD), formally known as nonalcoholic fatty liver disease, is the most common chronic liver disease in the United States. Patients with MASLD have been reported to be at a higher risk of developing severe coronavirus disease 2019 (COVID-19) and death. However, most studies are single-center studies, and nationwide data in the

United States is lacking.

AIM
To study the influence of MASLD on COVID-19 hospitalizations during the initial phase of the pandemic.

METHODS
We retrospectively analyzed the 2020 National Inpatient Sample (NIS) database to identify primary COVID-19 hospitalizations based on an underlying diagnosis of MASLD. A matched comparison cohort of COVID-19 hospitalizations without MASLD was identified from NIS after 1: N propensity score matching based on gender, race, and comorbidities, including hypertension, heart failure, diabetes, and cirrhosis. The primary outcomes included inpatient mortality, length of stay, and hospitalization costs. Secondary outcomes included the prevalence of systemic complications.

RESULTS
A total of 2210 hospitalizations with MASLD were matched to 2210 hospitalizations without MASLD, with a good comorbidity balance. Overall, there was a higher prevalence of severe disease with more intensive care unit admissions (9.5% *vs* 7.2%, $P = 0.007$), mechanical ventilation (7.2% *vs* 5.7%, $P = 0.03$), and septic shock (5.2% *vs* 2.7%, $P < 0.001$) in the MASLD cohort than in the non-MASLD cohort. However, there was no difference in mortality (8.6% *vs* 10%, $P = 0.49$), length of stay (5 d *vs* 5 d, $P = 0.25$), and hospitalization costs (42081.5 $ *vs* 38614$, $P = 0.15$) between the MASLD and non-MASLD cohorts.

CONCLUSION
The presence of MAFLD with or without liver cirrhosis was not associated with increased mortality in COVID-19 hospitalizations; however, there was an increased incidence of severe COVID-19 infection. This data (2020) predates the availability of COVID-19 vaccines, and many MASLD patients have since been vaccinated. It will be interesting to see if these trends are present in the subsequent years of the pandemic.

Key Words: COVID-19; Metabolic dysfunction-associated steatotic liver disease; Prevalence; Hospital charges; Inpatient resource utilization

Core Tip: This comprehensive study investigates the impact of metabolic dysfunction-associated steatotic liver disease (MASLD) on the severity of coronavirus disease 2019 (COVID-19) during the early stages of the pandemic, using the 2020 National Inpatient Sample database. It uniquely contrasts COVID-19 hospitalizations with and without MASLD, revealing that while MASLD is not linked to increased mortality, however, it is associated with a heightened risk of severe COVID-19 complications. This pivotal research offers valuable insights into the MASLD-COVID-19 relationship before the widespread availability of vaccines, setting the stage for further exploration into how these trends evolved in the later pandemic years.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an ongoing public health emergency with long-term effects on mortality and morbidity. As of November 2023, the World Health Organization has reported over 770 million confirmed cases, resulting in more than 6.9 million deaths worldwide[1]. Although preventive health measures and vaccinations have mitigated the risk of contracting COVID-19 and experiencing adverse outcomes to some extent, individuals with chronic diseases continue to face an elevated likelihood of poor[2]. Among these, chronic liver disease, particularly metabolic dysfunction-associated steatotic liver disease (MASLD), formally known as nonalcoholic fatty liver disease, is presumed to be a high-risk comorbid condition for severe COVID-19 owing to its inherent immune dysregulation[3-5]. MASLD, the hepatic manifestation of metabolic syndrome, comprises a spectrum of diseases ranging from hepatocellular steatosis and steatohepatitis to fibrosis and eventual cirrhosis[6]. MASLD has quickly become the most prevalent etiology of chronic liver disease in the United States[7,8]. MASLD has been linked to severe infections such as community-acquired pneumonia and a decline in lung function[9,10].

A recent case-control study revealed that MASLD did not correlate with increased in-hospital mortality rates, ventilatory assistance requirements, intensive care unit (ICU) admissions, or the total duration of hospital stay[11]. In contrast, other studies have indicated an association between MASLD and severe COVID-19. A recent meta-analysis of 14 studies of 1851 patients with MASLD by Singh *et al*[12] has shed light on the relationship between MASLD and COVID-19, revealing a significantly increased risk of severe COVID-19 outcomes in patients with MASLD. These studies have shown a 1.80-fold increase in the incidence of severe COVID-19 among MASLD patients, although they did not find a significant correlation with COVID-19 mortality. Another meta-analysis by Tao *et al*[13] reported an increased risk of severe infection and higher ICU admissions for MASLD patients, with no significant difference in mortality compared with non-MASLD patients. However, these meta-analyses predominantly included studies outside the United States and were often conducted in single-center settings with small sample sizes. These studies also had a high degree of variability owing to differences in the pandemic stage, data availability, sample sizes, study designs, and healthcare settings. This limitation raises concerns about the generalizability of their findings, especially in diverse populations, such as those in the United States.

Considering these contradictions in the literature, utilizing a large national database, our study aimed to provide a more comprehensive and nationally representative analysis of the impact of MASLD on COVID-19 hospitalizations in the United States.

MATERIALS AND METHODS

The present study utilized the National Inpatient Sample (NIS) 2020 database from the United States[14]. Detailed information on NIS's design and sampling methods is available at https://www.hcup-us.ahrq.gov. The NIS 2020 utilized the International Classification of Diseases (ICD) 10 coding system to store and report data. We used the "U07.1" ICD 10 code, introduced in March 2020 for COVID-19, to identify hospitalizations with a primary diagnosis (DX1) in the NIS 2020 database[15]. Hospitalizations were excluded if patients were < 18 years old, transferred, or had COVID-19 listed as a secondary diagnosis. Additionally, hospitalizations were excluded if there was any history of malignant neoplasms or liver and kidney transplant recipients, as these were deemed high-risk conditions that could confound the present analysis.

Outcome measures

The primary outcomes included inpatient mortality in COVID-19 patients with MASLD (with and without cirrhosis) and resource utilization, including length of stay and hospitalization costs. Secondary outcomes included the prevalence of systemic complications, including acute hypoxic respiratory failure, the need for mechanical ventilation, septic shock, and cardiac arrhythmias (including supraventricular tachyarrhythmias, ventricular tachyarrhythmias (VT), atrial fibrillation/flutter (Afib/Aflutter), and deep venous thromboembolism.

Statistical analysis

Statistical analysis was performed using statistical software for data science (STATA 16). We developed matched cohorts (MASLD and non-MASLD) using propensity score matching (PSM) to minimize the effects of hospital- and patient-level confounders. Propensity scores were generated with COVID-19 as the dependent variable and age, sex, cardiac comorbidities (heart failure and valvular dysfunction), hypertension, diabetes mellitus, and compensated and decompensated cirrhosis as independent variables. A 1:1 matching was performed using the command "clip match" (greedy matching) without replacement. Matched observations had a caliper width of 0.01 for the caliper matching variable (propensity scores). Cases and controls were matched 1:1 on age, sex, and Elixhauser comorbidities, including cardiac comorbidities (heart failure and valvular dysfunction), hypertension, diabetes, and cirrhosis (compensated and decompensated), as previously reported[16,17]. The covariate balance was visualized using the two-way plot shown in Figure 1. A two-sample Wilcoxon rank-sum (Mann-Whitney) test was used for continuous variables. The Chi-square test was used to compare categorical variables. The significance threshold was set at $P < 0.05$. For logistic regression, hierarchical models were designed using unbalanced variables in PSM (none), and outcomes were reported as odds ratios (OR) with 95% confidence intervals (95%CI) and P values. Patient consent and institutional review were not necessary, as the NIS is a de-identified, hospital-level, third-party database.

RESULTS

Baseline characteristics

Using propensity matching, 2210 hospitalizations with MASLD were matched to 2210 hospitalizations without MASLD. After matching, there was no significant difference in gender, race, hospital region, or hospital location (Table 1). Additionally, there were no differences in comorbidities, including heart failure ($P = 1.00$), arrhythmias ($P = 1.00$), valvular disease ($P = 1.00$), chronic obstructive pulmonary disease ($P = 1.00$), hypertension ($P = 1.00$), diabetes mellitus type 2 ($P = 1.00$), compensated cirrhosis ($P = 1.00$), and decompensated cirrhosis ($P = 1.00$) (Table 2). The median age at admission was significantly lower in the MASLD group (58 years) than in the non-MASLD group (66 years). The gender distribution was identical in both groups, with the majority of patients being female in both groups. Most patients in both groups were white (58.8%). The regional distribution of hospitalizations showed slight variations that were not statist-

Table 1 Baseline bio-demographic characteristics of coronavirus disease 2019 Hospitalizations with and without metabolic dysfunction-associated steatotic liver disease, *n* (%)

Characteristics	Non-MASLD	MASLD	*P* value
Total hospitalizations	2210	2210	
Age in years at admission, median (IQR)	66.0 (56.0, 76.0)	58.0 (47.0, 68.0)	< 0.001
Gender			1
Male	965 (43.7)	965 (43.7)	
Female	1245 (56.3)	1245 (56.3)	
Race			1
White	1300 (58.8)	1300 (58.8)	
Black	125 (5.7)	125 (5.7)	
Hispanic	615 (27.8)	615 (27.8)	
Asian	85 (3.8)	85 (3.8)	
Native American	20 (0.9)	20 (0.9)	
Other	65 (2.9)	65 (2.9)	
Region of the hospital			0.49
Northeast	375 (17.0)	340 (15.4)	
Midwest	535 (24.2)	610 (27.6)	
South	860 (38.9)	885 (40.0)	
West	440 (19.9)	375 (17.0)	
Location/Teaching status of the hospital			0.12
Rural	295 (13.3)	250 (11.3)	
Urban nonteaching	440 (19.9)	345 (15.6)	
Urban teaching	1475 (66.7)	1615 (73.1)	
Primary payer			< 0.001
Medicare	1120 (54.1)	820 (39.2)	
Medicaid	285 (13.8)	310 (14.8)	
Private	595 (28.7)	845 (40.4)	
Other	70 (3.4)	115 (5.5)	
Median household income national quartile for patient ZIP code			0.55
1st (0-25th)	710 (32.8)	760 (35.1)	
2nd (26th -50th)	575 (26.6)	615 (28.4)	
3rd (51st -75th)	540 (24.9)	515 (23.8)	
4th (76th -100th)	340 (15.7)	275 (12.7)	
Disposition of patient			0.28
Discharged to home or self-care (Routine discharge)	1320 (59.7)	1465 (66.3)	
Transfer to short-term hospital	90 (4.1)	50 (2.3)	
Transfer Other: Includes skilled nursing facility, intermediate care facility, Another Type of Facility	265 (12.0)	200 (9.0)	
Home health care	295 (13.3)	280 (12.7)	
Against medical advice	20 (0.9)	25 (1.1)	
Outcomes			
Inpatient mortality	220 (10.0)	190 (8.6)	0.49

Inpatient mortality in cirrhosis	418 (18.91)	226 (10.22)	0.05
Mechanical ventilation	150 (6.8)	220 (10.0)	< 0.001
ICU admission	160 (7.2)	210 (9.5)	0.007
Length of stay, median (IQR)	5.0 (3.0, 8.0)	5.0 (3.0, 8.0)	0.25
Total charges, median (IQR)	38614.0 (22040.5, 70258.5)	42081.5 (23021.0, 79820.5)	0.15

MASLD: Metabolic dysfunction-associated steatotic liver disease; ICU: Intensive care unit; IQR: Interquartile range.

Table 2 Inpatient outcomes of coronavirus disease 2019 hospitalizations with and without metabolic dysfunction-associated steatotic liver disease, *n* (%)

Secondary outcomes	No MASLD	MASLD	*P* value
Total hospitalizations	2210	2210	
ARDS	125 (5.7)	160 (7.2)	0.032
Hypoxic respiratory failure	1105 (50.0)	1220 (55.2)	< 0.001
Bacterial pneumonia	35 (1.6)	45 (2.0)	0.26
Diarrhea	185 (8.4)	145 (6.6)	0.022
Septic shock	60 (2.7)	115 (5.2)	< 0.001
Supraventricular tachycardia	25 (1.1)	30 (1.4)	0.5
Ventricular tachycardia	10 (0.5)	30 (1.4)	0.001
Atrial fibrillation	165 (7.5)	170 (7.7)	0.78
Aflutter	100 (4.5)	80 (3.6)	0.13
Pulmonary embolism	35 (1.6)	30 (1.4)	0.53
Deep venous thrombosis	75 (3.4)	65 (2.9)	0.39
Compensated cirrhosis	635 (28.7)	635 (28.7)	1
Decompensated cirrhosis	45 (2.0)	45 (2.0)	1

ARDS: Acute respiratory distress syndrome; MASLD: Metabolic dysfunction-associated steatotic liver disease.

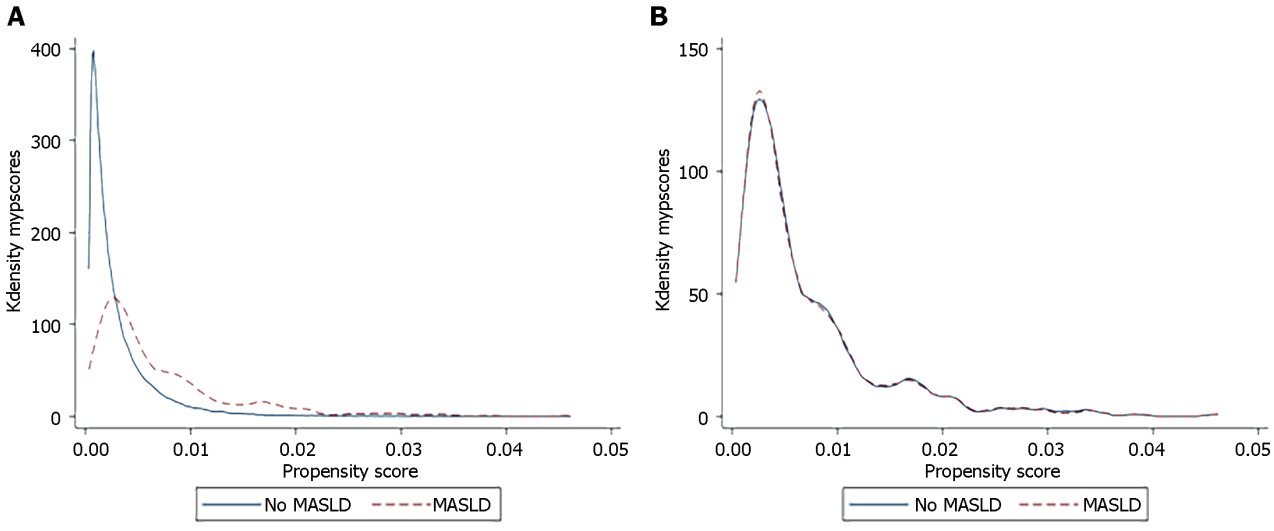

Figure 1 Two-way plot visualizing the covariate balance in our study. A: Before matching; B: After matching. MASLD: Metabolic dysfunction-associated steatotic liver disease.

ically significant, and most patients in both groups were admitted to urban teaching hospitals. Regarding primary payers, Medicare was the most common payer in the non-MASLD group (54.1%), followed by private payers (40.4%) in the MASLD group. In terms of patient disposition, a higher proportion of patients in both groups were discharged to their homes or self-care (66.3% in MASLD and 59.7% in non-MASLD) (Table 1). The list of the remaining comorbid conditions is presented in Supplementary Table 1.

Mortality outcomes and subgroup analyses

Overall, there was no difference in mortality between the MASLD and non-MASLD cohorts (8.6% *vs* 10%, $P = 0.49$) (OR 0.85, 95%CI: 0.53-1.34, $P = 0.48$). Subgroup analysis among patients without liver cirrhosis revealed no difference in mortality between the MASLD and non-MASLD cohorts (7.94% *vs* 6.35%, $P = 0.43$) (OR 1.27, 95%CI: 0.96-1.67, $P = 0.08$). However, subgroup analysis among patients with liver cirrhosis revealed higher mortality in non-MASLD patients (cirrhosis due to other causes such as alcohol-related liver disease and chronic viral hepatitis) than in the MASLD cohort (18.91% *vs* 10.22, $P = 0.05$) (OR 2.03, 95%CI: 1.12-4.25, $P = 0.05$).

The prevalence of ICU admission (9.5% *vs* 7.2%, $P = 0.007$), mechanical ventilation (7.2% *vs* 5.7%, $P = 0.03$), septic shock (5.2% *vs* 2.7%, $P < 0.001$), and VT (1.4% *vs* 0.5%, P 0.001) was higher in the MASLD cohort than in the non-MASLD cohort. The two cohorts showed no differences in the median hospital stay (5 d *vs* 5 d, $P = 0.25$) or hospitalization costs ($42081 in the MASLD group and $38614 in the non-MASLD group) (Table 1).

DISCUSSION

Our study is among the first in the United States to analyze the outcomes of COVID-19 among patients with MASLD at the national level. The results revealed that overall mortality did not significantly differ between the MASLD and non-MASLD cohorts in the initial phase of the pandemic. This trend persisted in subgroup analyses; among patients without liver cirrhosis, mortality rates were similar across both cohorts. However, among patients with liver cirrhosis, we found higher mortality in the non-MASLD group, which included patients with cirrhosis due to other causes such as chronic viral hepatitis, alcohol, or autoimmune diseases. Notably, the MASLD cohort exhibited a higher prevalence of ICU admission, mechanical ventilation, septic shock, and arrhythmias, indicating more severe disease. However, despite these differences in clinical outcomes, the two cohorts showed no significant differences in median hospital stay or hospitalization costs.

Our results align with those of several previous studies describing the relationship between MASLD and increased severity of COVID-19. Mahamid *et al*[5] showed that MASLD was associated with increased severity of COVID-19 pneumonia, irrespective of metabolic syndrome. Another retrospective study by Zheng *et al*[18] showed that obesity is associated with a six-fold higher risk of severe COVID-19 in patients with MASLD. This finding was consistent even after adjusting for factors such as age, gender, smoking habits, diabetes, hypertension, and dyslipidemia. In a study of 202 COVID-19 patients, Ji *et al*[19] discovered a correlation between advanced age, male gender, increased body mass index, the presence of MASLD, and a higher rate of comorbidities with the severity of COVID-19. There are conflicting reports on whether MAFLD is independently associated with mortality in COVID-19 patients. Kim *et al*[20] performed a retrospective study involving 867 patients and showed that MAFLD was not an independent predictor of mortality in COVID-19 patients [hazard ratio (HR) = 1.08; 95%CI: 0.59–1.97; $P = 0.80$]. Lopez-Mendez *et al*[21] reported higher mortality in patients with MASLD and COVID-19 (81.8% *vs* 18.2%, $P = 0.012$). In the initial phase of the COVID-19 pandemic, the available data were predominantly sourced from limited case series and observational studies, offering restricted insights. However, with the escalation of the pandemic, there has been a subsequent emergence of large-scale studies. These studies have contributed to a more robust understanding of the implications of COVID-19 on patients with MASLD. Two recent meta-analyses, Singh *et al*[12] and Tao *et al*[13], reported increased severity of COVID-19 in patients with MASLD. However, these studies did not find a significant difference in mortality between MASLD and non-MASLD patients, which is consistent with our results. However, both meta-analyses we examined primarily included studies from outside the United States. They also showed considerable variation because of differences in the pandemic stages, data availability, sample sizes, research methods, and healthcare settings. In contrast, using the largest national-level database in the United States, which encompasses a substantial sample size and diverse demographic coverage, increases the generalizability of our findings. In subgroup analysis, patients without liver cirrhosis showed no significant difference in mortality rates between the MASLD and non-MASLD cohorts. However, among patients with liver cirrhosis in the non-MASLD group (*e.g.*, alcohol-related liver disease and chronic viral hepatitis), there was a higher mortality rate than among those with MASLD-related cirrhosis. This could be explained by previously reported higher rates of complications and mortality among patients with alcohol-related liver diseases in the United States during the initial stages of the pandemic[22,23].

There is ongoing debate regarding the causes of poorer outcomes in certain patients, with various potential factors being studied. One theory suggests that MAFLD aggravates the cytokine storm associated with COVID-19 by promoting the release of inflammatory cytokines from the liver[24,25]. Conversely, it has been hypothesized that the shift in liver immune cells from pro-inflammatory M1 macrophages to pro-inflammatory M2 macrophages, which are regulatory, weakens innate immunity, thereby worsening the patient's condition[19,26]. Recent research supports both theories, showing that MAFLD patients exhibit elevated levels of specific inflammatory cytokines, such as interleukin-6, which plays a significant role in severe cases and treatment, and reduced levels of interferon-γ, which is essential for macrophage function[27]. Additionally, the upregulation of proteins facilitating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry, such as ACE2 and TMPRSS2, particularly in obese patients with MASLD, is another

factor[28]. The close association between fatty liver disease and metabolic syndrome suggests that similar harmful pathophysiological processes may contribute[29]. Our results also indicated that COVID-19 patients with MASLD are at a higher risk of cardiac arrhythmias, including VT. Several MASLD-related pathophysiological factors within the heart result in structural, electrical, and autonomic remodeling, causing arrhythmias[30]. Furthermore, cardiac arrhythmias place patients with COVID-19 at an increased risk of worse outcomes[31].

Regarding resource utilization in United States patients with MASLD and COVID-19 hospitalizations, despite a higher total hospitalization cost in the MASLD group than in the non-MASLD group (with costs of $42081 and $38614, respectively), this difference was not statistically significant (P = 0.15). Similarly, the mean length of hospital stay in both groups was similar at 5 d (P = 0.25). This apparent lack of significant cost disparity despite the presence of more complications in the MASLD group may be attributed to factors such as standardized treatment protocols and variations in cost calculation methodologies. Moreover, uniform insurance and billing practices and potential unaccounted-for confounding factors may have contributed to the unexpected similarity in hospitalization costs. Further investigations involving larger sample sizes and in-depth analyses are warranted to provide more precise insights. Our findings differ from those of previous studies on MASLD without COVID-19. For instance, Allen *et al*[32] reported annual care costs for MASLD patients with private insurance amounting to $7804 for new diagnoses and $3789 for long-term management, in contrast to the total annual cost of $2298 for matched controls without MASLD, underscoring the economic impact of this condition. Additionally, Adejumo *et al*[33] found differences in length of stay and total healthcare costs between genders, with females experiencing a shorter length of stay (4.55 *vs* 4.75 d) and lower total healthcare costs ($42848.00 *vs* $47026.00) compared to males. Unlike these studies, our study provides insights into the economic impact of COVID-19 on patients with MASLD. To the best of our knowledge, this is the first study to provide information on the costs associated with hospitalization for COVID-19 in individuals with MASLD across the United States.

While our findings contribute significantly to the current understanding of the relationship between MASLD and COVID-19, they also highlight the need to consider genetic variances as potential modulators of disease severity in patients with MASLD and COVID-19. Research indicates a significant genetic component in the severity of COVID-19, particularly in patients with MASLD. Genetic polymorphisms, such as PNPLA3 (rs738409), GCKR (rs780094), TM6SF2 (rs58542926), and LYPLAL1 (rs12137855), are associated with increased MAFLD risk and may influence COVID-19 outcomes. These genes are linked to lipid metabolism, glucose regulation, and liver function, which are vital for understanding COVID-19 severity in MAFLD patients with MAFLD. However, studies have reported mixed results regarding the impact of these genetic variants on COVID-19 severity, suggesting a complex relationship between these genetic factors and the disease[34]. A bioinformatics and systems biology approach also revealed differentially expressed genes shared between COVID-19 and MAFLD, highlighting the potential common pathogenesis and suggesting therapeutic targets for both conditions[35]. Future research should prioritize identifying genetic markers that predict severe COVID-19 in MASLD patients, aiming for more personalized treatment approaches to improve outcomes. Additionally, understanding the genetic interplay between MASLD and COVID-19 will aid in developing public health strategies and novel therapeutics for high-risk groups, ultimately easing healthcare burdens and enhancing patient care.

We acknowledge a few limitations to our study. We utilized the NIS database, which is subject to coding errors due to its dependence on the ICD-10 coding system. Second, the lack of information regarding medications and laboratory and radiological data has limited our ability to determine the specific baseline characteristics of patients with MASLD and individual patient management strategies. However, we used a validated tool for the Chronic Condition Indicator to assess the comorbidity burden of the patients as an essential prognostic factor. We also used PSM to minimize the effects of comorbid conditions in the comparison cohorts[36]. Third, the scope of this study was limited to inpatient data and did not include information on the outpatient outcomes of patients with MASLD who had non-severe COVID-19. In addition, the Agency of Healthcare Research and Quality recommends using an NIS to derive state-level data, owing to the relatively small contribution from several states. If state-level data were obtainable, it could have been helpful for policymakers to make certain public health efforts in certain states with more patients with MASLD and COVID-19 infection. Instead, we included geographical regions to study the outcome differences across the United States.

Despite some limitations, our study is the first to analyze and summarize national data on the impact of COVID-19-related hospitalizations in patients with MASLD during the early pandemic phase. The broad scope of our study, characterized by a large sample size and diverse range of participants, enhances the generalizability of our results. This diversity is reflected in the data from hospitals of varying sizes (small, medium, and large) and types (both teaching and non-teaching), as well as different settings, including rural and urban areas. Additionally, the variables included in the database allowed us to examine factors such as hospitalization costs, patient income, and other hospital characteristics, which are not often studied in single-center studies.

CONCLUSION

In conclusion, our study provides valuable insights into the existing and evolving body of evidence on the impact of COVID-19 in patients with MASLD. Our study showed that the presence of MASLD, with or without progression to liver cirrhosis, did not correlate with elevated mortality rates in patients hospitalized due to COVID-19. However, there was a notable increase in the severity of COVID-19 among this demographic group. It is important to note that this data, collected in 2020, predates the widespread distribution of COVID-19 vaccines. Since then, a significant proportion of patients with MASLD have received vaccination. Future analysis is required to determine whether these observed trends persist or are altered in the subsequent phases of the pandemic. This is important since MASLD is the most common cause of Chronic Liver disease in the US. Moreover, to mitigate the impact of COVID-19 on patients with MASLD, it is

critical to promote preventive measures, such as vaccination and booster doses, and provide timely treatment with advanced COVID-19 therapies.

ARTICLE HIGHLIGHTS

Research background

This study focused on the impact of metabolic dysfunction-associated steatotic liver disease (MASLD) on the severity and outcomes of coronavirus disease 2019 (COVID-19) hospitalizations. MASLD, formerly known as nonalcoholic fatty liver disease, is becoming increasingly prevalent and has been linked to more severe infectious diseases.

Research motivation

This study was motivated by a lack of comprehensive nationwide data on the relationship between the MASLD and COVID-19 outcomes in the United States. Previous studies have often been limited in scope, highlighting the need for more extensive research in this area.

Research objectives

The primary objective of this study was to analyze the impact of MASLD on the severity and outcomes of COVID-19 hospitalizations, particularly assessing the rates of intensive care unit (ICU) admission, mechanical ventilation, septic shock, and mortality in the United States.

Research methods

We conducted a retrospective analysis of the National Inpatient Sample 2020 database. This study utilized propensity score matching to compare COVID-19 hospitalizations with and without MASLD, controlling for demographics and comorbidities.

Research results

The study found that patients have a higher rate of severe COVID-19 outcomes, such as increased ICU admissions, need for mechanical ventilation, and septic shock. However, no significant difference in mortality rate was observed between patients with and without MASLD.

Research conclusions

MASLD is associated with an increased risk of severe COVID-19 complications but does not necessarily correlate with higher mortality. This finding is vital for healthcare providers in managing high-risk patient groups.

Research perspectives

Future research should investigate the genetic factors influencing MASLD's impact on COVID-19 to identify specific genetic markers that predict severe outcomes. This could lead to more personalized healthcare strategies and inform public health policies, particularly for high-risk groups. Further studies are needed to explore the economic impact and develop effective treatment protocols for MASLD patients with COVID-19.

FOOTNOTES

Author contributions: Sohail A, Ali H, Patel P, Dahiya DS, and Sohail AH were involved in the study's conception, design, data collection, results interpretation, initial drafting, and substantial revisions for the manuscript's intellectual content; Gangwani MK and Subramanium S played key roles in analyzing data, interpreting findings, and contributing to the manuscript's draft; Satapathy SK provided significant manuscript enhancements through thoughtful revisions and relevant suggestions; Furthermore, all authors engaged in a review, modification, and final approval of the manuscript, ensuring responsibility for every aspect of the work.

Institutional review board statement: The National Inpatient Sample is an anonymized database at the hospital level managed by a third party. It is specifically designed to maintain the confidentiality of patients, healthcare providers, and medical institutions. Since the data related to hospitalizations is devoid of any personal identifiers of patients, the requirement for patient consent was exempted from this study. Additionally, the nature of this de-identified data negated the need for approval from an institutional review board.

Informed consent statement: The National Inpatient Sample is an anonymized database at the hospital level, managed by a third party. It is specifically designed to maintain the confidentiality of patients, healthcare providers, and medical institutions. Since the data related to hospitalizations is devoid of any personal identifiers of patients, the requirement for patient consent was exempted for this study.

Conflict-of-interest statement: Conflict of Interest Statement: The authors of this article declare that they have no conflict of interest to report. None of the authors have received any fees for serving as speakers, consultants, or advisory board members for any organizations. There has been no receipt of research funding from any organizations. Additionally, none of the authors are employees of any organizations that may have an interest in the subject matter of this study.

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ORIGINAL ARTICLE

Observational Study

Chronic hepatitis B and occult infection in chemotherapy patients - evaluation in oncology and hemato-oncology settings: The CHOICE study

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Abstract

BACKGROUND

Reactivation of hepatitis B virus (HBV) infection is a well-known risk that can occur spontaneously or following immunosuppressive therapies, including cancer chemotherapy. HBV reactivation can cause significant morbidity and even mortality, which are preventable if at-risk individuals are identified through screening and started on antiviral prophylaxis.

AIM

To determine the prevalence of chronic HBV (CHB) and occult HBV infection (OBI) among oncology and hematology-oncology patients undergoing chemotherapy.

METHODS

In this observational study, the prevalence of CHB and OBI was assessed among patients receiving chemotherapy. Serological markers of HBV infection [hepatitis B surface antigen (HBsAg)/anti-hepatitis B core antigen (HBc)] were evaluated for all patients. HBV DNA levels were assessed in those who tested negative for HBsAg but positive for total anti-HBc.

RESULTS
The prevalence of CHB in the study cohort was determined to be 2.3% [95% confidence interval (95%CI): 1.0-4.2]. Additionally, the prevalence of OBI among the study participants was found to be 0.8% (95%CI: 0.2-2.3).

CONCLUSION
The findings of this study highlight the importance of screening for hepatitis B infection in oncology and hematology-oncology patients undergoing chemotherapy. Identifying individuals with CHB and OBI is crucial for implementing appropriate antiviral prophylaxis to prevent the reactivation of HBV infection, which can lead to increased morbidity and mortality.

Key Words: Hepatitis B virus; Chronic hepatitis B; Occult B infection; Oncology; Hepatitis B reactivation; Hematology-oncology

Core Tip: Hepatitis B virus (HBV) reactivation, a significant risk for individuals undergoing immunosuppressive therapy such as cancer chemotherapy, can lead to preventable morbidity and mortality. Our observational study determined the prevalence of chronic HBV (CHB) infection and occult HBV infection (OBI) in oncology and hematology-oncology patients receiving chemotherapy. Our results showed a 2.3% prevalence of CHB and 0.8% prevalence of OBI in our study cohort, underscoring the critical importance of routinely screening oncology and hematology-oncology patients for HBV infection. Identifying those with CHB and OBI is vital for promptly initiating antiviral prophylaxis, which can prevent the reactivation of HBV infection.

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INTRODUCTION

Reactivation of hepatitis B virus (HBV) infection is a well-known risk that can occur spontaneously or following immunosuppressive therapies, including cancer chemotherapy[1]. This reactivation causes significant morbidity and mortality, which is preventable if at-risk individuals are identified through screening and started on antiviral prophylaxis [1]. The prevalence of chronic hepatitis B (CHB) infection and occult hepatitis B infection (OBI) among oncology and hemato-oncology patients receiving chemotherapy is an important area of study. CHB refers to persistent HBV infection characterized by the presence of hepatitis B surface antigen (HBsAg) for more than 6 months. OBI, on the other hand, is defined as the presence of HBV DNA in the absence of detectable HBsAg[2]. Understanding the prevalence of CHB and OBI in this patient population is crucial for implementing appropriate preventive measures and antiviral prophylaxis to prevent HBV reactivation (HBVr). Previous studies have reported varying prevalence rates of CHB and OBI among cancer patients undergoing chemotherapy, highlighting the need for further investigation[3-5]. By determining the prevalence of CHB and OBI in this specific patient population, this study will contribute to the existing knowledge on HBV infection in the context of cancer chemotherapy. The findings will provide valuable insights into the need for routine screening, antiviral prophylaxis, and infection control measures to prevent HBVr and associated complications in oncology and hematology-oncology patients undergoing chemotherapy.

MATERIALS AND METHODS

Study design and population

This observational study estimated the prevalence of CHB and OBI in newly diagnosed oncological and hematology-oncology patients before starting chemotherapy. The study population included both male and female patients from urban and rural areas, aged 18 years or older, who were seeking treatment at a tertiary care oncology center. Patients with solid organ cancer, leukemia, and lymphoma, as well as those planning to undergo chemotherapy (standard chemotherapy protocols as per the cancer type) and hematopoietic stem cell transplant, were included in the study. Patients with a history of previous chemotherapy or immunosuppressive therapy, pre-existing CHB or chronic hepatitis C virus infection, or contraindications for antiviral therapy for CHB were excluded.

Data collection

Data were collected from a total of 400 patients over 2 years. All patients underwent screening for HBsAg and total anti-HBc. Patients who tested positive for either of these markers were further tested for HBV DNA quantification using polymerase chain reaction. All patients who were identified as CHB or OBI were started on antiviral prophylaxis with entecavir or tenofovir and followed up at 6 and 12 months with HBV DNA and liver function tests. The data collected from the patients were recorded on an Excel spreadsheet for further analysis.

Statistical analysis

The collected data were analyzed using appropriate statistical methods. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. The prevalence of CHB and OBI was estimated based on the number of patients testing positive for HBsAg, total anti-HBc, and HBV DNA. The statistical analysis determined the prevalence rates and associated confidence intervals (CIs) for CHB and OBI in the study population.

Ethical considerations

The study protocol was reviewed and approved by the relevant ethical committee. Informed consent was obtained from all participants before their inclusion in the study. Confidentiality and privacy of patient information were strictly maintained throughout the study.

RESULTS

In this observational study, we investigated the prevalence of CHB and OBI in a cohort of 400 patients visiting the oncology and hematology departments with different types of malignancies (Figure 1). Among the 400 subjects studied, 129 (32.3%) were females and 271 (67.8%) were males. The mean age of the study group was 51.34 years (95%CI: 49.83-52.85). Most of the participants were oncology patients (339, 84.8%), with only (61, 15.3%) patients with hematolymphoid malignancies (Figure 2).

A total of 9 patients (2.3%) tested positive for HBsAg (Figure 3) of whom 7 were above the age of 50 years and 2 were below 50 years (Figure 4). The distribution of cancer types among these patients included 5 with hepatocellular carcinoma, 2 with colon cancer, 1 with acute lymphoblastic leukemia, and 1 with pancreatic cancer. Five patients among them had a history of jaundice, of whom 3 were documented to have acute hepatitis B infection (Table 1). Only 2 of them had elevated liver enzymes. Two patients had high HBV DNA levels, which were undetectable at the 6- and 12-month follow-ups (Table 2). Among all HBsAg-negative cases, 3 patients tested positive for total anti-HBc. Among these patients, 1 had acute myeloid leukemia, 1 had a non-seminomatous germ cell tumor, and 1 had colon cancer (Table 3). Two of these patients had a history of jaundice in the past. None of the patients with OBI had detectable HBV DNA levels, and their liver enzymes were within the normal range (Table 4). All patients with CHB and OBI were started on antiviral prophylaxis (tenofovir or entecavir) and were followed up at 6 and 12 months. On follow-up, there was clearance of viral load and normalization of liver enzymes (Table 2).

DISCUSSION

This study identified a prevalence of CHB of 2.3% (95%CI: 1.0-4.2) and OBI of 0.8% (95%CI: 0.2-2.3) within the study group. Two patients in the CHB subgroup had high HBV-DNA levels with deranged liver enzymes wherein none of these patients knew their HBV status before our study. This reiterates the importance of such screening tests before immunosuppressive therapies. Anti-viral prophylaxis was initiated in both the CHB and OBI (moderate to severe risk) patients. These patients were followed up at 6 and 12 months, but no reactivation was noted. For those who had high viral load, 6 and 12-months follow-up revealed clearance of viral load.

These results provide valuable insights into the burden of hepatitis B infection in this specific patient population and have important implications for clinical management and preventive strategies. The prevalence of CHB in the study cohort is consistent with previous studies reporting a wide range of prevalence rates among cancer patients undergoing chemotherapy[3]. Furthermore, the prevalence of OBI in the study group was estimated to be 0.8%. Although OBI is often considered a low-level infection, it can still pose a risk of transmission, especially in immunocompromised individuals [5]. Identifying OBI in this patient population underscores the importance of infection control measures to prevent HBV transmission in healthcare settings. Among the patients who tested positive for HBsAg, the majority were above the age of 50 years. This is in line with previous studies that have shown an increased risk of CHB infection with advancing age [6]. It is noteworthy that none of the patients with OBI had detectable HBV DNA levels, and their liver function tests were within the normal range. This suggests that these patients may have resolved their HBV infection or have very low-level viral replication. However, it is important to monitor these individuals closely, as OBI can still pose a risk of reactivation under immunosuppressive conditions. Loss of immune control over HBV is a crucial event in HBVr, leading to an increase in HBV DNA levels among individuals previously exposed to HBV[7]. The immune system plays a role in partially controlling viral replication in these individuals, and this control can be disrupted by exposure to immunosuppressive therapy[8]. HBVr can occur due to the ability of HBV to remain latent in the liver as covalently closed circular DNA and its capacity to alter the immune system of infected individuals[9]. Weakening of cellular immune responses during immunosuppressive therapy or chemotherapy can increase HBV replication, leading to HBVr[10]. In a study from

Table 1 Patient demographics of the chronic hepatitis group

Patient demographics		Number of patients	Percentage
Sex	Female	1	11.1
	Male	8	88.9
Malignancy	ALL	1	11.1
	Colon	2	22.2
	HCC	5	55.6
	Pancreas	1	11.1
USG	Coarse liver	5	55.6
	Normal	4	44.4
Past history of jaundice	No	4	44.4
	Yes	5	55.6
Positive family history	No	8	88.9
	Yes	1	11.1
Past history of surgeries	No	8	88.9
	Yes	1	11.1
History of transfusion in the past	No	8	88.9
	Yes	1	11.1
History of acute hepatitis B in the past	No	6	66.6
	Yes	3	33.3

ALL: Acute lymphoblastic leukemia; HCC: Hepatocellular carcinoma; USG: Ultrasound sonography.

Table 2 Baseline hepatitis B virus DNA levels of the chronic hepatitis with follow-up at 6 and 12 months after the introduction of prophylaxis (tenofovir or entecavir)

Patient	0 months		6th months		12th months	
	HBV DNA	Liver enzymes	HBV DNA	Liver enzymes	HBV DNA	Liver enzymes
1	TND	Normal	TND	Normal	TND	Normal
2	TND	Normal	TND	Normal	TND	Normal
3	TND	Normal	TND	Normal	TND	Normal
4	TND	Normal	TND	Normal	TND	Normal
5	TND	Normal	TND	Normal	TND	Normal
6	TND	Normal	TND	Normal	TND	Normal
7	TND	Normal	TND	Normal	TND	Normal
8	HIGH	Elevated > 2X UNL	TND	Normal	TND	Normal
9	HIGH	Elevated > 2X UNL	TND	Normal	TND	Normal

HBV: Hepatitis B virus; TND: Target not detected; UNL: Upper limit of normal.

Hong Kong, among 104 patients with diffuse large B-cell lymphoma undergoing treatment 46 were found to be HBsAg-negative and anti-HBc-positive. Twenty-one of these patients were treated with R-CHOP and twenty-five were treated with CHOP alone. Of patients treated with R-CHOP, 5 (25%) developed HBVr. None of the patients treated with CHOP therapy developed HBVr[11].

In another study, 115 patients with non-Hodgkin's Lymphoma (NHL) who were receiving at least one dose of rituximab were examined for the risk of HBVr. Fifteen of these patients were HBsAg-positive, and ten of them did not receive antiviral prophylaxis during treatment. In all, 80% of patients who were HBsAg-positive and received rituximab

Table 3 Patient demographics of the occult hepatitis B infection group

Patient demographics		No. of patients	Percentage
Age	52 yr	1	33.3
	75 yr	1	33.3
	78 yr	1	33.3
Sex	Female	1	33.3
	Male	2	66.7
Malignancy	AML	1	33.3
	Colon	1	33.3
	NSGCT	1	33.3
USG	Normal	1	33.3
	Coarse liver	2	66.7
History of Jaundice	Yes	2	66.7
	No	1	33.3
History of blood transfusion in the past	Yes	1	33.3
	No	2	66.7
History of surgery in the past	Yes	1	33.3
	No	2	66.7
Family history	Yes	0	0.0
	No	3	100.0

AML: Acute myeloid leukemia; NSGCT: Non-seminomatous germ cell tumor; USG: Ultrasound sonography.

Table 4 Baseline hepatitis B virus DNA levels of occult hepatitis B infection group and follow-up at 6 and 12 months after the introduction of prophylaxis (tenofovir or entecavir)

Patient	0 months		6th months		12th months	
	HBV DNA	Liver enzymes	HBV DNA	Liver enzymes	HBV DNA	Liver enzymes
1	TND	Normal	TND	Normal	TND	Normal
2	TND	Normal	TND	Normal	TND	Normal
3	TND	Normal	TND	Normal	TND	Normal

HBV: Hepatitis B virus; TND: Target not detected.

therapy without antiviral prophylaxis experienced HBV-related hepatitis. Of the 95 patients with NHL who were HBsAg-negative, 4 developed HBV-related hepatitis of whom 2 died due to fulminant hepatic failure[12].

CONCLUSION

The findings of this study have important clinical implications. Routine screening for hepatitis B infection should be considered in oncology and hematology patients before initiating chemotherapy or immunosuppressive therapies. Identifying individuals with CHB and OBI allows for appropriate management strategies, including antiviral prophylaxis, to prevent reactivation and associated complications. Additionally, strict adherence to infection control measures is crucial to prevent HBV transmission in healthcare settings. A notable limitation of our study was that some patients (20 of 400) were lost to follow-up, potentially introducing bias and impacting prevalence estimates and while the study population of 400 patients was substantial, its single-center nature might limit its application to other healthcare settings, although the data support the existing guidelines and perhaps gives way to more work in this interesting clinical setting.

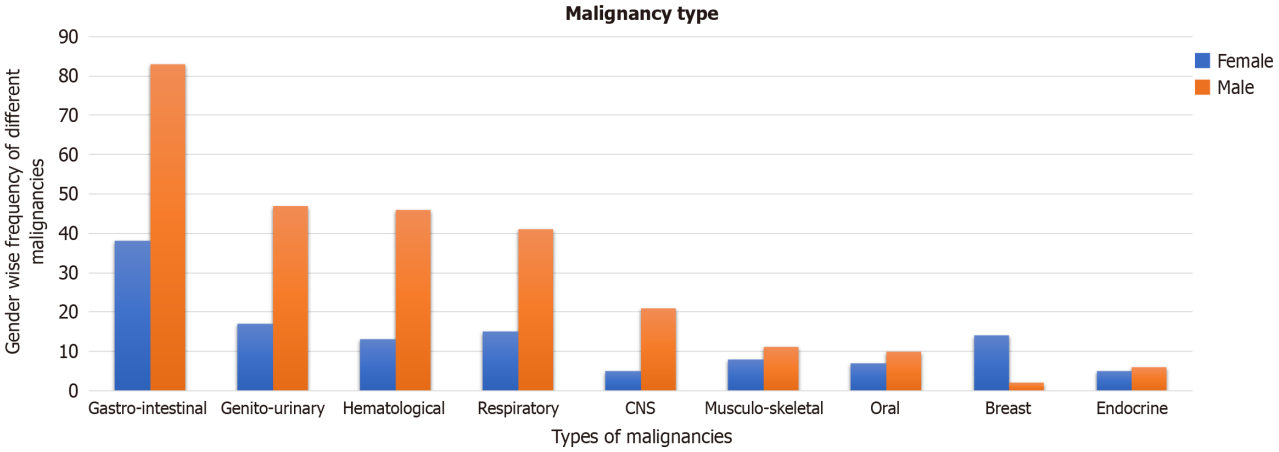

Figure 1 Types of malignancies in the study population.

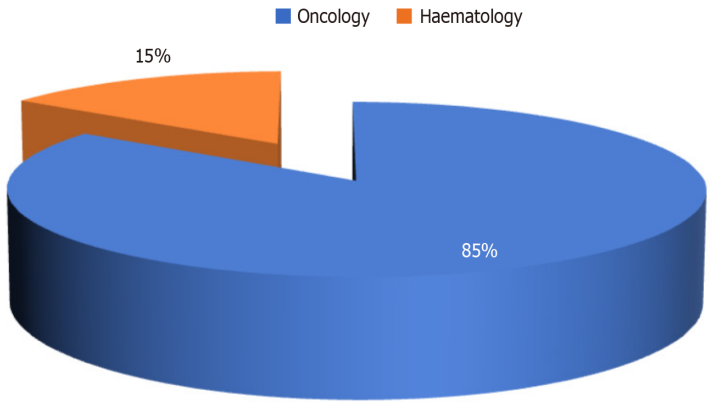

Figure 2 Pie chart depicting the distribution of malignancy among hematology and solid organ malignancy.

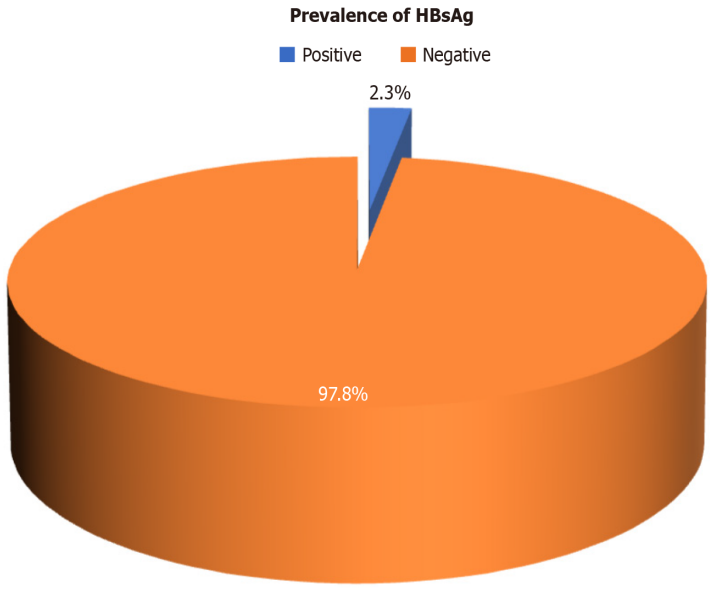

Figure 3 Prevalence of hepatitis B surface antigen in oncology and hemato-oncology patients. HBsAg: Hepatitis B surface antigen.

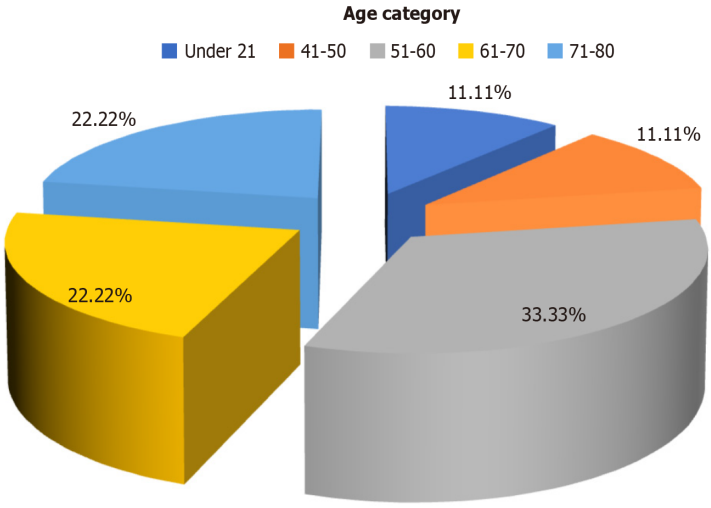

Figure 4 Age distribution of the chronic hepatitis subgroup.

ARTICLE HIGHLIGHTS

Research background

The issue of reactivation of hepatitis B virus (HBV) infection is often missed due to inadequate evaluation, especially for occult HBV infection (OBI). This reactivation following immunosuppression can lead to liver dysfunction, which in turn either impacts the continuation of therapy and/or increases the morbidity and mortality in an already immunocompromised patient. We attempted to study the same to know the current status of evaluation protocols and the prevalence of HBV infection as well as reactivation.

Research motivation

The protocols for the evaluation of patients with malignancy need to include checking for OBI as it carries risk of reactivation following chemotherapy during treatment. The presence of pre-existing HBV infection always involves a gastroenterology or hepatology consult regarding the consideration of antiviral therapy. On the other hand, OBI is not considered routinely in pretreatment evaluation and therefore any possible prophylaxis is delayed. More data are required in these specific situations to formulate better protocols for the future.

Research objectives

Our primary objective was to determine the prevalence of chronic HBV (CHB) and OBI among oncology and hematology-oncology patients undergoing chemotherapy. We followed up with patients for reactivation and initiated treatment/prophylaxis as and when indicated.

Research methods

In this observational study, the prevalence of CHB and OBI was assessed among patients receiving chemotherapy. Serological markers of HBV infection [hepatitis B surface antigen (HBsAg)/anti-hepatitis B core antigen (HBc)/anti-hepatitis B surface antibody] were evaluated for all participants. Those who tested negative for HBsAg but positive for total anti-HBc were tested for HBV DNA levels. Due ethical clearance was taken and data of 400 patients were collected over 2 years. Appropriate statistics were applied for analysis in this observational study.

Research results

In our study, the prevalence of CHB within the study cohort was determined to be 2.3% [95% confidence interval (95%CI): 1.0-4.2]. Additionally, the prevalence of OBI among the study participants was found to be 0.8% (95%CI: 0.2-2.3). Although the prevalence seems low, on consideration of the people affected by malignancy worldwide, the numbers may be significant.

Research conclusions

The findings of this study highlight the importance of screening for hepatitis B infection in oncology and hematology-oncology patients undergoing chemotherapy. Identifying individuals with CHB and OBI is crucial for implementing appropriate antiviral prophylaxis to prevent the reactivation of HBV infection, which can lead to increased morbidity and mortality.

Research perspectives

The direction of future research should be to actively look for OBI.

FOOTNOTES

Author contributions: Manrai M conceptualized and supervised the study and was involved with obtaining resources, data collection, editing, and validation; Sudevan N conducted the study and was involved in writing the manuscript; Tilak TVSVGK and Khurana H were involved with obtaining resources and data curation, Premdeep H was involved with data curation and editing.

Institutional review board statement: The study protocol was reviewed and approved by the relevant ethical committee of Armed Forces Medical College, Pune, Maharashtra, India. Informed consent was obtained from all participants before their inclusion in the study. Confidentiality and privacy of patient information were strictly maintained throughout the study.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrolment.

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ORIGINAL ARTICLE

Observational Study

Global trends in hepatitis C-related hepatocellular carcinoma mortality: A public database analysis (1999-2019)

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Abstract

BACKGROUND

Hepatitis C is the leading cause of chronic liver disease worldwide and it significantly contributes to the burden of hepatocellular carcinoma (HCC).

However, there are marked variations in the incidence and mortality rates of HCC across different geographical regions. With the advent of new widely available treatment modalities, such as direct-acting antivirals, it is becoming increasingly imperative to understand the temporal and geographical trends in HCC mortality associated with Hepatitis C. Furthermore, gender disparities in HCC mortality related to Hepatitis C are a crucial, yet underexplored aspect that adds to the disease's global impact. While some studies shed light on gender-specific trends, there is a lack of comprehensive data on global and regional mortality rates, particularly those highlighting gender disparities. This gap in knowledge hinders the development of targeted interventions and resource allocation strategies.

AIM

To understand the global and regional trends in Hepatitis C-related HCC mortality rates from 1990 to 2019, along with gender disparities.

METHODS

We utilized the Global Burden of Disease database, a comprehensive repository for global health metrics to age-standardized mortality rates due to Hepatitis C-related HCC from 1999 to 2019. Rates were evaluated per 100000 population and assessed by World Bank-defined regions. Temporal trends were determined using Joinpoint software and the Average Annual Percent Change (AAPC) method, and results were reported with 95% confidence intervals (CI).

RESULTS

From 1990 to 2019, overall, there was a significant decline in HCC-related mortality rates with an AAPC of -0.80% (95%CI: -0.83 to -0.77). Females demonstrated a marked decrease in mortality with an AAPC of -1.06% (95%CI: -1.09 to -1.03), whereas the male cohort had a lower AAPC of -0.52% (95%CI: -0.55 to -0.48). Regionally, East Asia and the Pacific demonstrated a significant decline with an AAPC of -2.05% (95%CI: -2.10 to -2.00), whereas Europe and Central Asia observed an uptrend with an AAPC of 0.72% (95%CI: 0.69 to 0.74). Latin America and the Caribbean also showed an uptrend with an AAPC of 0.06% (95%CI: 0.02 to 0.11). In the Middle East and North Africa, the AAPC was non-significant at 0.02% (95%CI: -0.09 to 0.12). North America, in contrast, displayed a significant upward trend with an AAPC of 2.63% (95%CI: 2.57 to 2.67). South Asia (AAPC -0.22%, 95%CI: -0.26 to -0.16) and Sub-Saharan Africa (AAPC -0.14%, 95%CI: -0.15 to -0.12) trends significantly declined over the study period.

CONCLUSION

Our study reports disparities in Hepatitis C-related HCC mortality between 1999 to 2019, both regionally and between genders. While East Asia and the Pacific regions showed a promising decline in mortality, North America has experienced a concerning rise in mortality. These regional variations highlight the need for healthcare policy-makers and practitioners to tailor public health strategies and interventions. The data serves as a call to action, particularly for regions where mortality rates are not improving, emphasizing the necessity for a nuanced, region-specific approach to combat the global challenge of HCC secondary to Hepatitis C.

Key Words: Carcinoma; Hepatocellular; Antiviral agents; Global Burden of Disease; Quality indicators; Health care; Liver neoplasms; Hepatitis C; Chronic hepatitis C

Core Tip: Hepatitis C virus (HCV) remains a crucial precursor for hepatocellular carcinoma (HCC), accounting for a significant proportion of HCC-related mortalities. Our study focused on trends from 1999 to 2019 and offers an extensive temporal analysis on the mortality rates in patients with HCV-related HCC. The data highlighted that despite advances in antiviral treatments for HCV, the mortality rates in HCC have not seen a corresponding decline. We also identified noticeable trends relating to gender, providing insights into demographic groups that are disproportionately affected. This study emphasizes the need for targeted interventions to reduce mortality rates in HCV-associated HCC, despite advancements in HCV treatment.

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INTRODUCTION

In the late 20th century, there was a significant shift in the etiology of chronic liver disease due to the advent of Hepatitis C infection, which also contributed to a rising trend in the incidence of liver-related malignancies[1]. Data from the World Health Organization (WHO) indicates that globally, around 58 million individuals are infected with the hepatitis C virus (HCV). Each year, there are about 1.5 million new cases of HCV, and the virus is responsible for roughly 290000 fatalities annually[2]. Based on 2020 data, hepatocellular carcinoma (HCC) ranks as the sixth most frequently diagnosed cancer globally and stands as the third leading cause of cancer-related deaths[3]. In Africa and East Asia, HBV infection is the predominant cause of HCC, responsible for up to 60% of cases. Conversely, in Western countries, chronic HCV infection emerges as the primary etiological factor for HCC, with about 34% of HCC cases in the United States (USA) attributed to it[4].

The annual incidence of HCC in patients with hepatitis C ranges from about 1-8%, being the highest in Japan (4%-8%), followed by Italy (2%-4%), and USA (1.4%)[5]. Various studies suggest that HCC might be the first complication to develop and a more common cause of death in patients with HCV-associated cirrhosis[6]. Most cases of HCC manifest in individuals with cirrhosis, which serves as an independent risk factor for developing HCC, irrespective of the underlying cause of the liver disease. Data suggests that about one-third of patients with cirrhosis will develop liver cancer during their lifetimes[7]. The annual incidence of HCC among cirrhotic patients ranges between 1%-8%, with specific rates varying based on the type of liver disease, and estimates indicate that patients infected with HCV face a 15 to 20 times higher risk of developing HCC. In those with cirrhosis, the annual incidence of HCC is projected to be between 1% and 4% over a period of 30 years[8]. In 2012, data indicated that around 170000 new cancer cases, which is approximately 7.8% of all the newly diagnosed cancers, were attributed to the HCV[9]. Over the last ten years, deaths attributable to HCV-related HCC have increased by 21.1%; however, at the same time, deaths from HCC secondary to causes other than HCV and alcohol have remained stable[10]. Hence, this escalating global burden of HCV infection-related HCC has gained attention among clinicians, public health professionals, and researchers.

There has been variability in the incidence of HCV-related HCC, based on ethnicity and geographic location. HCV is recognized as the primary cause of HCC in regions including the USA, Europe, Japan, and South America[10]. Delving deeper into country-specific data, the prevalence of HCV in Japan has been estimated to be 3%, and an estimated 85% of patients with HCC are infected with HCV[11]. In the USA, where the prevalence of HCV is about 1.8% of the population, around 50%-60% of HCC patients are HCV-positive[12]. Analyzing the demographic distribution within the USA, there are distinct variations of HCC by ethnicity and age. Particularly, Hispanic individuals and those born between 1945 and 1965 are at the highest risk of HCC[13]. A comprehensive study involving 150000 HCV-infected USA veterans revealed that the Hispanic demographic reported the most substantial yearly HCC incidence at 7.8%. This heightened incidence is believed to correlate with the increased prevalence of nonalcoholic fatty liver disease within this group[14]. Another study from a comparable cohort highlighted a 2.5-fold surge in HCC incidence, with a threefold increase in mortality since 2001, even after the introduction of direct-acting antivirals (DAAs)[15].

Despite the recent advancement in the development of antiviral therapies, including DAAs[16], which have, to an extent, revolutionized the treatment of HCV, there persists an ongoing debate on the long-term impact of the virus on liver health. Adequate treatment helps cure the active infection, but the possibility of progression to cirrhosis leading to HCC persists in some patients. Hence, it makes understanding the global mortality trends in HCV-related HCC of high academic interest and critical importance for public health policy and intervention strategies. In 2016, the World Health Assembly proposed eliminating viral hepatitis as a public health threat[17]. Still, most countries need to meet their expected goals by 2020 due to the impact of the coronavirus disease 2019 pandemic and various other factors[18]. Consequently, in 2021, the WHO established guidance featuring absolute incidence targets, specifying the number of new HCV infections per 100000 persons each year[19].

In our analysis, we aim to examine the change in trends in regional and gender-specific variances in mortality rates due to HCC secondary to hepatitis C. We aim to identify disparities that inform targeted interventions for at-risk populations. Prior studies have mainly analyzed global liver cancer trends without focusing on specific etiologies such as HCV. To our knowledge, no prior study has estimated the risk of HCV-related HCC mortality and HCC trends over time, covering all World Bank regions. This article aims to provide a comprehensive review of current mortality trends in Hepatitis C-related HCC. It will provide a better understanding of the global mortality burden and facilitate designs for policymakers by providing targeted measures to mitigate the impact of the increasingly prevalent malignancy.

MATERIALS AND METHODS

Study population and data collection

The primary data source for this study was the Global Burden of Disease (GBD) database, a comprehensive repository for global health metrics. Initiated in the early 1990s, the GBD database is continually updated to offer the most current insights into the global prevalence, incidence, and impact of diseases[20]. Compiled by the Institute for Health Metrics and Evaluation, the database strictly adheres to the Guidelines for Accurate and Transparent Health Estimates Reporting. The GBD database encompasses data from numerous countries and territories, capturing various health-related metrics, including mortality. Our analysis was divided by World Bank-defined regions, including Sub-Saharan Africa, East Asia and Pacific, Europe and Central Asia, Latin America and the Caribbean, Middle East and North Africa, and South Asia to provide a more comprehensive geographical perspective.

Measures of disease burden

This study focused on mortality related to HCC due to Hepatitis C to comprehensively understand the disease's long-term trends and status. Age-standardized estimates were employed to adjust for differences in the age distribution of different populations, aligning them to a standardized reference world population. This adjustment enabled the comparison of disease burdens across diverse populations. As in prior studies, rates were reported per 100000 population and were age-standardized to facilitate comparisons across different demographic structures[21].

Data analysis

We examined age-standardized mortality rates per 100000 population to analyze hepatitis C-related HCC mortality rates. Joinpoint software assessed temporal trends in the Average Percent Change (APC), representing the change in mortality during a specific period. Joinpoint identified time points where the trend changed significantly using a Monte Carlo permutation test and a *t*-test. Log-linear regression models were fitted to evaluate trends in age-standardized mortality rates. To enhance the reliability of our analysis, especially in the context of uncertain data distributions, we employed Joinpoint's Empirical Quantile Confidence Interval method. This approach provides robust and conservative 95% confidence intervals for APC, Average Annual Percent Change (AAPC), and the location of Joinpoints (tau). Unlike traditional methods that yield *P* values, these confidence intervals directly reflect the measurement level of the data, offering a more intuitive understanding of result reliability and significance[22]. We limited our models to a maximum of three Joinpoints to minimize bias. Statistical significance was determined based on these 95% confidence intervals, as the Empirical Quantile method does not compute traditional *P* values but offers a more reliable measure of significance[23].

RESULTS

World Bank regions

The overall mortality rate declined from 2.25/100000 in 1999 to 1.78/100000 in 2019, with an AAPC of -0.80% (95%CI: -0.83 to -0.77). The mortality rate displayed five segments: An increase from 1990-1995 (APC: 2.29, 95%CI: 2.05 to 2.54), a slight rise from 1995-1999 (APC: 0.38, 95%CI: 0.11 to 0.68), followed by sharp declines from 1999-2004 (APC: -4.61, 95%CI: -4.76 to -4.45), 2004-2012 (APC: -1.14, 95%CI: -1.32 to -1.03), and 2012-2019 (APC: -0.46, 95%CI: -0.62 to -0.22) (Figure 1).

For females, the rate declined from 2.08/100000 in 1999 to 1.53/100000 in 2019, with an AAPC of -1.06% (95%CI: -1.09 to -1.03). The trends showed an increase from 1990-1995 (APC: 1.36, 95%CI: 1.11 to 1.65) with subsequent declines, notably from 1999-2004 (APC: -4.92, 95%CI: -5.07 to -4.75) (Figure 1).

For males, the rate declined from 2.42/100000 in 1999 to 2.08/100000 in 2019, with an AAPC of -0.52% (95%CI: -0.55 to -0.48). There was an initial rise from 1990-1996 (APC: 3.02, 95%CI: 2.85 to 3.22) followed by varied declines, most sharply from 1999-2004 (APC: -4.19, 95%CI: -4.40 to -4.00) (Figure 1).

East Asia and the Pacific

The rate declined from 4.54/100000 to 2.50/100000 with an AAPC of -2.05% with a 95%CI of -2.10 to -2.00. The mortality rate displayed five segments: An increase from 1990-1995 (APC: 2.31, 95%CI: 1.92 to 2.81), a slight drop from 1995-1999 (APC: -0.06, 95%CI: -0.62 to 0.42), followed by sharp declines from 1999-2004 (APC: -7.83, 95%CI: -8.08 to -7.57), 2004-2012 (APC: -3.16, 95%CI: -3.38 to -2.98), and 2012-2019 (APC: -0.69, 95%CI: -0.94 to -0.34) (Figure 2).

For females, the rate declined from 4.35/100000 in 1999 to 2.19/100000 in 2019, with an AAPC of -2.39 (95%CI: -2.43 to -2.35). The trends showed an increase from 1990-1996 (APC: 1.16, 95%CI: 0.91 to 1.40), a sharp decline from 1996-2000 (APC: -2.53, 95%CI: -2.86 to -2.20) followed by further declines, notably from 2000-2004 (APC: -9.17, 95%CI: -9.45 to -8.90) (Figure 2).

For males, the rate declined from 4.73/100000 in 1999 to 2.96/100000 in 2019, with an AAPC of -1.60 (95%CI: -1.64 to -1.55). The trends showed an initial rise from 1990-1996 (APC: 3.19, 95%CI: 2.95 to 3.52) followed by varied declines, most sharply from 1999-2004 (APC: -7.07, 95%CI: -7.33 to -6.82) (Figure 2).

Europe and Central Asia

The overall population's mortality rate increased from 1.16/100000 in 1999 to 1.43/100000 in 2019, with an AAPC of 0.72, with a 95%CI of 0.69 to 0.74. The mortality rate exhibited five distinct segments: An increase from 1990-1994 (APC: 2.58, 95%CI: 2.44 to 2.73), a steady rise from 1994-2005 (APC: 0.56, 95%CI: 0.50 to 0.61), a further increase from 2005-2012 (APC: 1.23, 95%CI: 1.12 to 1.38), a negligible decline from 2012-2015, and a drop from 2015-2019 (APC: -0.89, 95%CI: -1.19 to -0.72) (Figure 3).

For females, the rate increased from 0.98/100000 in 1999 to 1.17/100000 in 2019, with an AAPC of 0.60% (95%CI: 0.59 to 0.62). Mortality trends indicated an increase from 1990-1994 (APC: 1.22, 95%CI: 1.07 to 1.39) with subsequent varied changes, a notable rise from 1998-2005 (APC: 1.02, 95%CI: 0.90 to 1.10), a significant increase from 2005-2008 (APC: 1.60, 95%CI: 1.33 to 1.73), a steady rise from 2008-2013 (APC: 0.74, 95%CI: 0.59 to 0.82), and a decline from 2013-2019 (APC: -0.58, 95%CI: -0.65 to -0.50) (Figure 3).

For males, the rate increased from 1.41/100000 in 1999 to 1.76/100000 in 2019, with an AAPC of 0.77% (95%CI: 0.75 to 0.80). The segments displayed an initial rise from 1990-1994 (APC: 4.10, 95%CI: 3.91 to 4.30), growth from 1994-1999 (APC: 0.65, 95%CI: 0.50 to 0.86), a plateau from 1999-2006, an increase from 2006-2012 (APC: 1.33, 95%CI: 1.20 to 1.54), a slight drop from 2012-2015, and a decline from 2015-2019 (APC: -1.19, 95%CI: -1.50 to -1.01) (Figure 3).

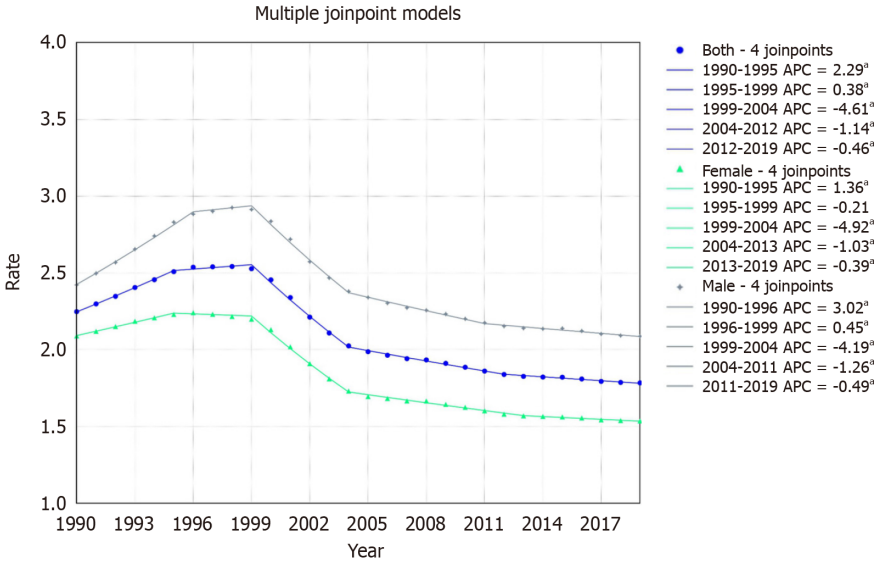

Figure 1 Hepatitis C-related hepatocellular carcinoma mortality rates per 100000 from 1999 to 2019 globally (World Bank regions). [a]*P* value < 0.05, significant trend.

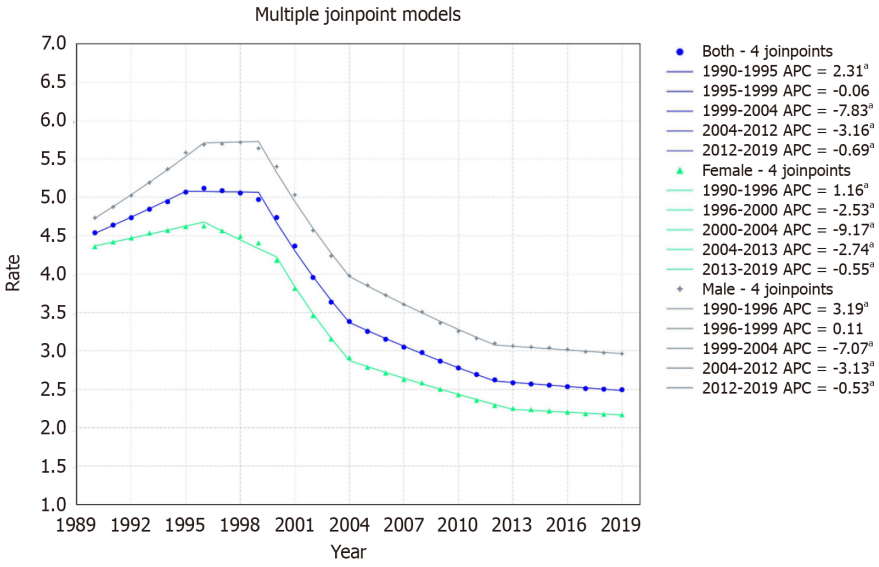

Figure 2 Hepatitis C-related hepatocellular carcinoma mortality rates per 100000 from 1999 to 2019 in East Asia and Pacific region. [a]*P* value < 0.05, significant trend.

Latin America and the Caribbean

For Latin America & Caribbean, a minor increase was noted from 1.06/100000 in 1999 to 1.09/100000 in 2019 with an AAPC of 0.06 with a 95%CI of 0.02 to 0.11. Mortality trends displayed six segments: An initial decline from 1990-1993 (APC: -0.91, 95%CI: -1.40 to -0.26), a further decrease from 1993-2000 (APC: -2.40, 95%CI: -2.56 to -2.26), an increase from 2000-2006 (APC: 1.11, 95%CI: 0.83 to 1.29), a pronounced rise from 2006-2012 (APC: 2.37, 95%CI: 2.11 to 2.81), a steady increase from 2012-2016 (APC: 0.66, 95%CI: 0.35 to 1.45), and a decline from 2016-2019 (APC: -0.59, 95%CI: -1.34 to -0.12) (Figure 4).

For females, the rate declined from 1.22/100000 in 1999 to 1.05/100000 in 2019, with an AAPC of -0.52% (95%CI: -0.56 to -0.48). The trends displayed six segments: A decline from 1990-1993 (APC: -1.31, 95%CI: -1.86 to -0.79), a sharp decrease from 1993-2000 (APC: -3.23, 95%CI: -3.36 to -3.10), a slight increase from 2000-2007 (APC: 0.47, 95%CI: 0.26 to 0.60), a significant rise from 2007-2010 (APC: 2.33, 95%CI: 1.76 to 2.63), a steady increase from 2010-2015 (APC: 0.77, 95%CI: 0.46 to 1.00), and a decline from 2015-2019 (APC: -0.56, 95%CI: -1.10 to -0.24) (Figure 4).

For males, the rate increased from 0.87/100000 in 1999 to 1.12/100000 in 2019, with an AAPC of 0.88% (95%CI: 0.82 to 0.94). Mortality trends highlighted an initial negligible change from 1990-1993, a decline from 1993-2000 (APC: -1.17, 95%CI: -1.66 to -0.97), a pronounced rise from 2000-2006 (APC: 2.06, 95%CI: 1.52 to 2.41), a further significant increase from 2006-2012 (APC: 3.24, 95%CI: 2.89 to 3.89), an increase from 2012-2016 (APC: 1.11, 95%CI: 0.70 to 2.08), and a slight decline from 2016-2019 (APC: -0.47) though it was not statistically significant (Figure 4).

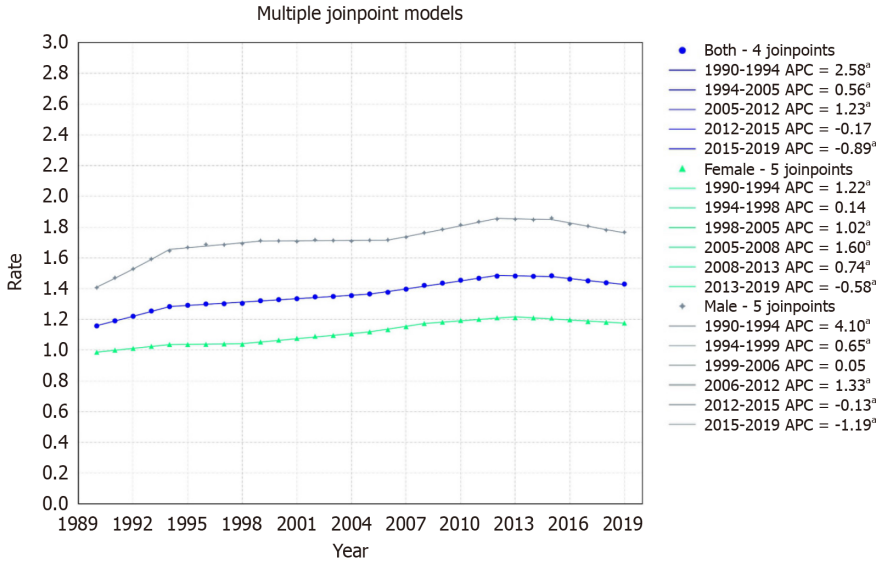

Figure 3 Hepatitis C-related hepatocellular carcinoma mortality rates per 100000 from 1999 to 2019 in Europe and Central Asia region. [a]*P* value < 0.05, significant trend.

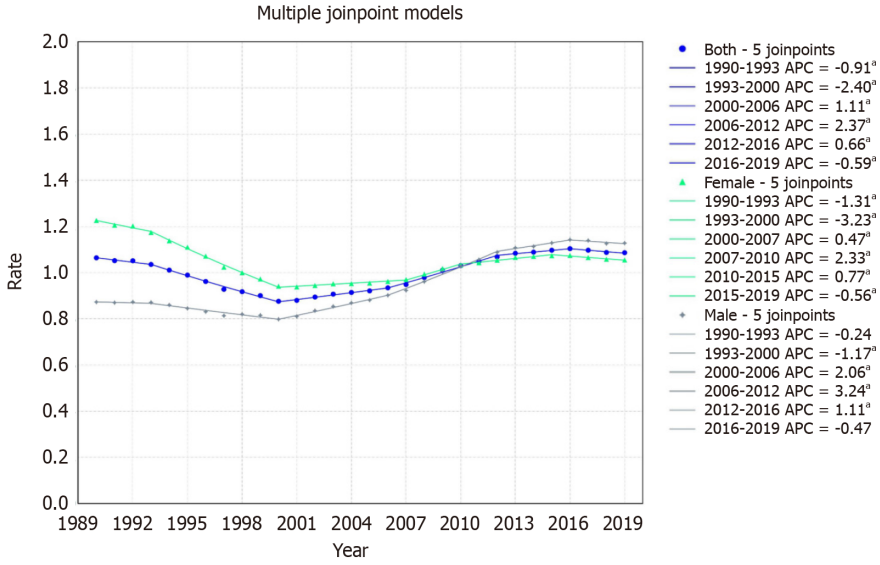

Figure 4 Hepatitis C-related hepatocellular carcinoma mortality rates per 100000 from 1999 to 2019 in Latin America and the Caribbean region. [a]*P* value < 0.05, significant trend.

Middle East and North Africa

In the Middle East & North Africa, the rate slightly decreased from 3.81/100000 to 3.74/100000 with an AAPC of 0.02 with a 95%CI of -0.09 to 0.12. The mortality rate presented three segments: An initial decline from 1990-1999 (APC: -1.10, 95%CI: -1.64 to -0.69), a substantial rise from 1999-2011 (APC: 2.10, 95%CI: 1.80 to 2.49), and a decrease from 2011-2019 (APC: -1.77, 95%CI: -2.35 to -1.27) (Figure 5).

For females, the rate declined from 2.36/100000 in 1999 to 2.12/100000 in 2019, with an AAPC of -0.38% (95%CI: -0.44 to -0.33). The trends indicated six segments: A decline from 1990-2000 (APC: -1.79, 95%CI: -2.11 to -1.61), a transient increase from 2000-2003 (APC: 2.58, not statistically significant), a mild rise from 2003-2006 (APC: 0.91, 95%CI: 0.29 to 1.88), a pronounced increase from 2006-2009 (APC: 4.14, 95%CI: 2.91 to 4.73), a decrease from 2009-2015 (APC: -0.87, 95%CI: -1.20 to -0.45), and a significant decline from 2015-2019 (APC: -2.51, 95%CI: -3.39 to -2.00) (Figure 5).

For males, the rate increased from 5.20/100000 in 1999 to 5.25/100000 in 2019, with an AAPC of 0.12% (95%CI: -0.02 to 0.24). The trends showed three segments: An initial decline from 1990-1998 (APC: -1.10, 95%CI: -2.02 to -0.49), a substantial increase from 1998-2011 (APC: 2.04, 95%CI: 1.74 to 2.51), and a decline from 2011-2019 (APC: -1.74, 95%CI: -2.42 to -1.18) (Figure 5).

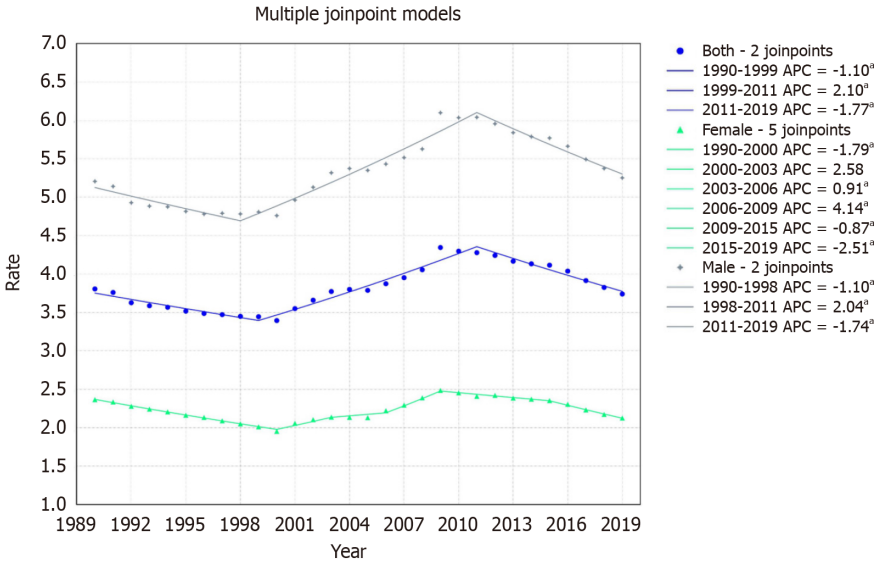

Figure 5 Hepatitis C-related hepatocellular carcinoma mortality rates per 100000 from 1999 to 2019 in the Middle East and North Africa region. [a]*P* value < 0.05, significant trend.

North America

In North America, the mortality rate increased from 0.72/100000 to 1.53/100000 with an AAPC of 2.63, with a 95%CI of 2.57 to 2.67. The mortality trends showcased four distinct segments: A rise from 1990-1999 (APC: 4.02, 95%CI: 3.86 to 4.21), an increase from 1999-2010 (APC: 2.87, 95%CI: 2.74 to 3.01), an uptick from 2010-2015 (APC: 1.60, 95%CI: 1.20 to 2.10), and a minor rise from 2015-2019 (APC: 0.16, not statistically significant) (Figure 6).

For females, the rate increased from 0.61/100000 in 1999 to 1.17/100000 in 2019, with an AAPC of 2.21% (95%CI: 2.18 to 2.25). The mortality trends illustrated five segments: An increase from 1990-2000 (APC: 3.68, 95%CI: 3.58 to 3.79), a rise from 2000-2004 (APC: 1.50, 95%CI: 1.00 to 1.95), a pronounced growth from 2004-2009 (APC: 3.13, 95%CI: 2.83 to 3.69), an increase from 2009-2015 (APC: 1.32, 95%CI: 1.06 to 1.59), and a decline from 2015-2019 (APC: -0.48, 95%CI: -0.97 to -0.09) (Figure 6).

For males, the rate increased from 0.84/100000 in 1999 to 1.94/100000 in 2019, with an AAPC of 2.90% (95%CI: 2.84 to 2.95). The trends presented four segments: An initial rise from 1990-1998 (APC: 4.33, 95%CI: 4.12 to 4.61), growth from 1998-2008 (APC: 3.26, 95%CI: 3.10 to 3.47), an increase from 2008-2014 (APC: 2.20, 95%CI: 1.81 to 2.59), and an uptick from 2014-2019 (APC: 0.76, 95%CI: 0.17 to 1.10) (Figure 6).

South Asia

In South Asia, rates decreased from 0.86/100000 to 0.81/100000 with an AAPC of -0.22 with a 95%CI of -0.26 to -0.16. The mortality trends revealed four segments: An initial increase from 1990-2001 (APC: 0.27, 95%CI: 0.14 to 0.54), a decrease from 2001-2008 (APC: -0.37, 95%CI: -0.77 to -0.05), a sharp decline from 2008-2012 (APC: -1.82, 95%CI: -2.54 to -1.16), and a minor uptick from 2012-2019 (APC: 0.10, 95%CI: -0.17 to 0.57) (Figure 7).

For females, the rate declined from 0.98/100000 in 1999 to 0.88/100000 in 2019, with an AAPC of -0.37% (95%CI: -0.42 to -0.31). The mortality trends showcased four segments: A slight increase from 1990-2001 (APC: 0.13, 95%CI: -0.01 to 0.39), a decline from 2001-2008 (APC: -0.69, 95%CI: -1.07 to -0.29), a sharp drop from 2008-2012 (APC: -2.12, 95%CI: -2.94 to -1.40), and a mild rise from 2012-2019 (APC: 0.20, 95%CI: -0.11 to 0.67) (Figure 7).

For males, the rate declined from 0.75/100000 in 1999 to 0.72/100000 in 2019 with an AAPC of -0.09 (95%CI: -0.13 to -0.05). The trends indicated six segments: An initial minor decline from 1990-1994 (APC: -0.17, 95%CI: -0.89 to 0.14), a surge from 1994-1997 (APC: 1.19, 95%CI: 0.67 to 1.51), a stagnant phase from 1997-2008 (APC: 0.01, 95%CI: -0.31 to 0.11), a decline from 2008-2012 (APC: -1.41, 95%CI: -1.82 to 0.30), a decrease from 2012-2016 (APC: -0.37, 95%CI: -1.57 to 0.00), and an increase from 2016-2019 (APC: 0.54, 95%CI: -0.01 to 1.23) (Figure 7).

Sub-Saharan Africa

Sub-Saharan Africa mortality rates decreased from 1.30/100000 to 1.25/100000 with an AAPC of -0.14 with a 95%CI of -0.15 to -0.12. The mortality trends portrayed five segments: An initial surge from 1990-1998 (APC: 1.73, 95%CI: 1.65 to 1.79), a decline from 1998-2004 (APC: -0.91, 95%CI: -0.97 to 1.70), a sharper drop from 2004-2007 (APC: -1.30, 95%CI: -1.44 to -0.99), followed by declines from 2007-2014 (APC: -0.47, 95%CI: -0.56 to -0.30), and 2014-2019 (APC: -1.00, 95%CI: -1.20 to -0.87) (Figure 8).

For females, the rate declined from 1.40/100000 in 1999 to 1.24/100000 in 2019, with an AAPC of -0.41% (95%CI: -0.43 to -0.38). The trends demonstrated four segments: An increase from 1990-1998 (APC: 0.70, 95%CI: 0.60 to 0.80), followed by declines from 1998-2007 (APC: -0.87, 95%CI: -1.05 to -0.79), 2007-2014 (APC: -0.45, 95%CI: -0.58 to -0.07), and 2014-2019 (APC: -1.27, 95%CI: -1.53 to -1.08) (Figure 8).

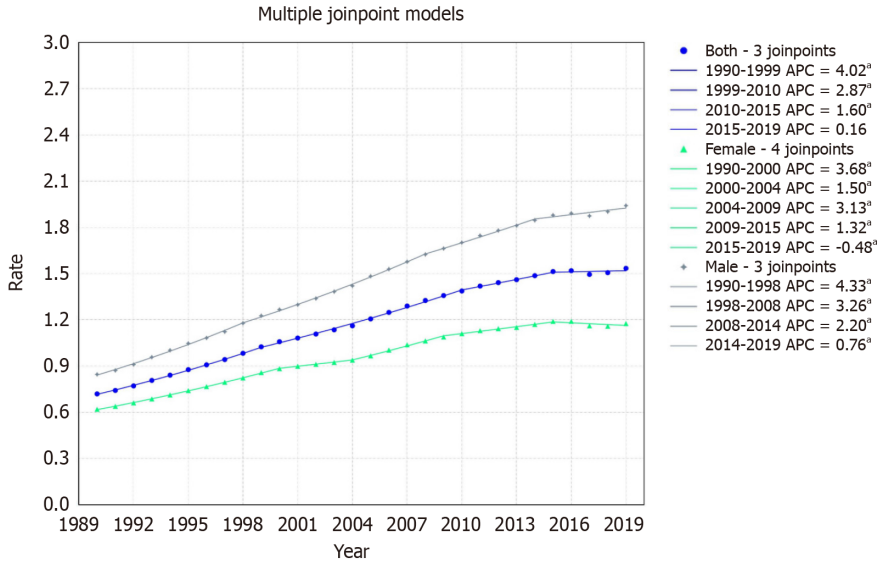

Figure 6 Hepatitis C-related hepatocellular carcinoma mortality rates per 100,000 from 1999 to 2019 in the North America region. [a]*P* value < 0.05, significant trend.

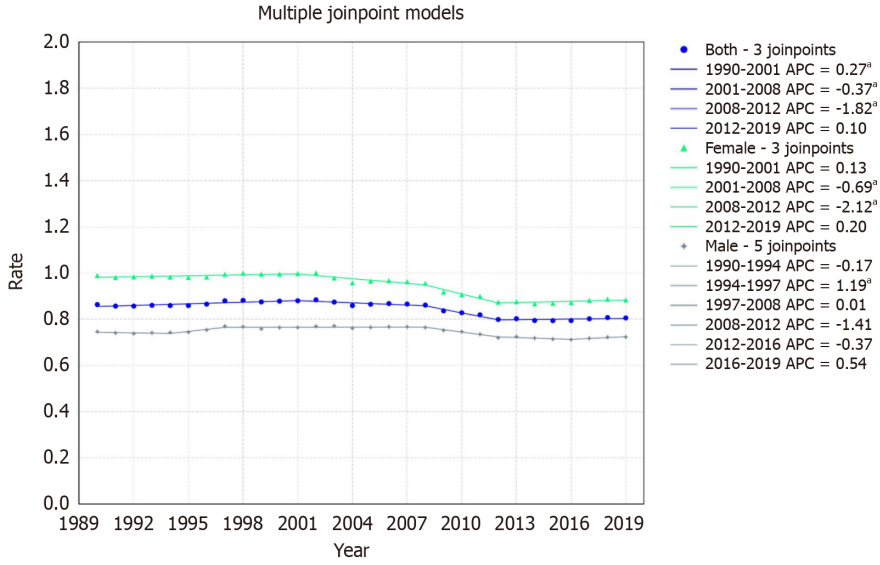

Figure 7 Hepatitis C-related hepatocellular carcinoma mortality rates per 100000 from 1999 to 2019 in the South Asia region. [a]*P* value < 0.05, significant trend.

For males, the rate increased from 1.17/100000 in 1999 to 1.25/100000 in 2019, with an AAPC of 0.23% (95%CI: 0.21 to 0.26). The patterns illustrated four segments: A pronounced rise from 1990-1997 (APC: 3.26, 95%CI: 3.15 to 3.40), followed by minor growth from 1997-2000 (APC: 0.16, 95%CI: -0.23 to 0.48), a decline from 2000-2008 (APC: -1.23, 95%CI: -1.44 to -1.13), and another decrease from 2008-2019 (APC: -0.58, 95%CI: -0.65 to -0.49) (Figure 8).

DISCUSSION

The results of our study show an overall decline in the mortality rates of patients with hepatitis C-related HCC over the last two decades. Notably, females exhibited a remarkable decrease in mortality compared to males. Regionally, East Asia and the Pacific displayed a significant decline in mortality, while Europe and Central Asia witnessed an upward trend. Latin America and the Caribbean also experienced an increase in mortality rates. However, no significant difference was observed in the Middle East and North Africa. North America exhibited a notable upward trend. South Asia and Sub-Saharan Africa significantly declined throughout the study period. This raises the hope of identifying areas for implementing more targeted resources. Despite some progress, multiple challenges remain in meeting the WHO 2030 goal of eliminating viral hepatitis[24].

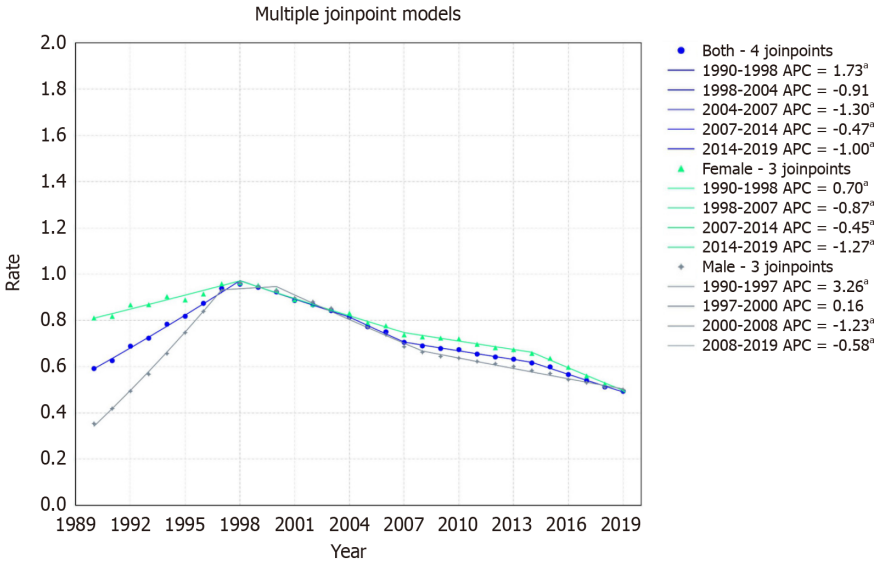

Figure 8 Hepatitis C-related hepatocellular carcinoma mortality rates per 100000 from 1999 to 2019 in the Sub Saharan African region. [a]*P* value < 0.05, significant trend.

Globally, over 170 million people are affected by chronic HCV, with 50%-80% of these cases eventually leading to cirrhosis and HCC[25]. Despite ongoing challenges in creating and implementing preventive vaccines for HCV, recent advancements in treatment using specific drugs have significantly lowered the morbidity and mortality associated with HCC[26-28]. The declining trends in deaths due to HCV-related HCC are likely influenced by the cumulative impact of hepatitis C viral suppression achieved through new-generation antiviral agents[29]. The most rapid decline in mortality occurred between 1999-2004. At the same time, the overall trends of death caused by HCC declined slowly, probably due to population growth and aging[30,31].

Females demonstrated a marked decrease in mortality as compared to the male cohort. This finding has been reported previously, with most studies involving Western cohorts and two studies being in Asian cohorts[32-37]. In a large multi-ethnic Asian patient cohort, it was found that females with HCC were significantly more adherent to surveillance protocols. As a result, they often presented with less advanced stages of HCC, leading to correspondingly better overall survival rates compared to males[38]. A higher proportion of females had HCC diagnosed during regular surveillance, resulting in detection at an earlier BCLC stage, with significantly smaller tumor sizes and lower incidences of portal vein tumor invasion and extrahepatic involvement. Being diagnosed earlier allows for better therapeutic options, as seen by the trend of more women receiving curative treatments[38]. Studies have hypothesized that sex hormones may play a role in the pathogenesis of HCC, suggesting a protective effect of estrogen against the development of HCC and an increased risk associated with testosterone[39,40]. The male predominance in HCC mortality is traditionally attributed to factors such as a higher prevalence of HCV infection in males and gender differences in high-risk lifestyle behaviors, including heavier alcohol consumption and smoking among males[41].

Decreasing mortality in Eastern Asia may be related to long-term vision, cost-effective interventions, and effective medical-care systems in high-risk countries (*e.g.*, China)[42,43]. However, HCC-related mortality showed the largest increasing trend in Central Asia. Uzbekistan has high mortality due to HCC associated with the seroprevalence of HCV infections, and the transmission of HCV was standard in medical treatment and drug abusers[44]. In Europe, there is an increasing trend in mortality, as patients successfully treated for HCV exhibit high rates of drug and liver-related mortality. Notably, overall mortality rates in these patients are significantly higher than in the general population, including for those without cirrhosis at the time of successful HCV treatment[45].

Additionally, we did not observe a decreasing trend for HCC-related mortality in American countries. Data on the prevalence, incidence, and risk factors for HCC in Latin America is limited[46]. Mendez-Sanchez N. reported that in Mexico, the cause-specific mortality rate for this condition was 4.1 per 100000 in 2000, and it rose to 4.7 per 100000 by 2006 [47]. The primary causes of liver cancer in Argentina or Brazil include HCV and HBV infection, alcohol abuse, cryptogenic cirrhosis, and schistosomiasis[48,49]. However, North America showed a significant uptrend in mortality, although the results of previous literature are conflicting. Ramani *et al*[50-52] noted a decline in the death rate from HCV-related HCC since 2011, attributing this to various factors: (1) The introduction of Direct-acting Antivirals (DAAs); (2) Enhanced screening and better access to HCC therapies; (3) Aging of the HCV-infected birth cohort alongside competing risks from other causes of death; and (4) Inherent differences in the remaining HCV patients from the birth cohort, leading to a reduced risk of developing HCV cirrhosis and HCC. However, Younossi *et al*[53] showed a statistically significant increase in in-hospital mortality for HCV admissions from 2005-2009.

The mortality trend decreased in South Asian countries; however, in the past twenty years, the incidence of HCC in India has increased, especially in Mumbai, Chennai, and Bangalore[54]. Despite data showing that the incidence of HCV-related HCC in Asia has decreased since 2006, the potential risk of HCV infection cannot be ignored[55]. HCV cure is associated with a decreased risk of HCC in Sub-Saharan Africa; however, even after achieving a sustained virological response, patients with cirrhosis continue to face a risk for HCC, underscoring the necessity for ongoing surveillance[56].

Prevention of HCV *via* vaccination is currently unfeasible due to its extreme genetic variability, the absence of small animal models, and the complexity of its glycoproteins[57]. Prevention of HCV can be achieved through measures such as screening of blood products, using disposable needles, stringent sterilization of medical instruments, and strict measures against illegal drug use. While immediate medical intervention may not be necessary for new HCV infections, the WHO strongly recommends treatments such as pan-genotypic DAAs and interferon (IFN) for chronic cases. Sofosbuvir/velpatasvir has been shown to be effective and safe in a phase III trial for chronic HCV in the Asian population, and elbasvir/grazoprevir has also been proven effective in a phase III trial among Asia-Pacific/Russian participants[2,58,59]. These new medications offer a higher rate of sustained virological response, require shorter treatment durations, and cause fewer toxic side effects. It is widely recognized that while the risk of HCC occurrence decreases, it does not completely disappear after viral eradication achieved by either DAAs or IFN therapies[60]. Developing new therapies could minimize progress from chronic HCV infection to HCC. However, patients with advanced liver fibrosis still require regular surveillance after HCV eradication. The use of DAAs for the management of HCV is relatively recent. More evidence is needed to show that effective treatment of HCV by DAAs can decrease the risk of developing HCV-related HCC. These medications can be costly; however, some DAAs are available at a much lower price in a few Asian countries than in the West[61]. Nevertheless, the cost may decrease as more pan-genotypic drugs are introduced, allowing for greater accessibility and a subsequent decrease in HCV-related mortality. One meta-analysis indicated no significant increase in HCC occurrence with DAA therapy compared to IFN-based treatment (RR 0.68)[62]. Another analysis showed similar risks for HCC occurrence and recurrence with DAAs and IFN-based treatments. These findings suggest comparable HCC risks between DAA and IFN therapies[63].

While this study offers a comprehensive analysis of global mortality trends in Hepatitis C-related HCC across various demographics and regions, it has several limitations. The study relies on data from the GBD database, which may have inherent biases or inaccuracies. The study does not account for the impact of healthcare access, socioeconomic factors, or comorbidities on mortality rates, potentially affecting the interpretation of the results. The study focuses solely on mortality rates, not considering other important clinical outcomes such as quality of life or disease progression. The study does not explore the potential impact of new therapeutic interventions on mortality trends. Lastly, the age-standardized estimates may only partially capture the complexities of age-related risk in different populations. These limitations should be considered when interpreting the findings and should guide future research in this area.

CONCLUSION

In conclusion, we offer a comprehensive analysis of global mortality trends associated with hepatitis C-related HCC. The data reveals significant disparities in mortality rates across different regions and demographic groups, emphasizing the critical need for targeted interventions. Advances in hepatitis C treatment have shown promise, yet the persistently high mortality rates in certain areas and among specific populations call for a multi-faceted approach. This should encompass not only medical treatment but also broader strategies like improving healthcare access, raising public awareness, and promoting early diagnosis. The findings provide a robust foundation for future research and policy initiatives to mitigate the global impact of hepatitis C-related HCC.

ARTICLE HIGHLIGHTS

Research background

This research delves into the evolving global landscape of hepatocellular carcinoma (HCC) mortality, specifically focusing on its correlation with hepatitis C virus (HCV) infection. Historically, HCV has significantly influenced the etiology of chronic liver disease and liver-related malignancies, notably HCC. The study highlights the increasing global burden of HCV-related HCC, a concerning trend noted across various regions worldwide. It emphasizes the need to understand these trends in the context of recent advancements in HCV treatment and changing demographic patterns, particularly given the significant public health implications and the challenges in meeting World Health Organization's goals for viral hepatitis elimination.

Research motivation

There is an urgent need to address the rising global burden of HCC secondary to HCV infection. Despite advancements in treatment, HCV remains a leading cause of HCC, with varying impacts across different regions and demographics. This study aims to identify and understand these disparities to inform targeted healthcare interventions. Addressing this issue is crucial for future research and public health policy, as it directly contributes to the World Health Organization's goal of eliminating viral hepatitis as a public health threat. Understanding the regional and demographic variations in HCC mortality rates due to HCV is essential for developing effective prevention and treatment strategies, ultimately reducing the global HCC burden.

Research objectives

This study's principal objective is to comprehensively analyze the trends in HCC mortality associated with HCV infection across various World Bank regions. The study aims to dissect these trends by gender and geographic location, offering insights into regional and demographic disparities. A critical goal is to identify areas with rising or declining mortality

rates, which could signify the effectiveness of current interventions or indicate areas needing more focused attention. Realizing these objectives is significant for future research as it provides a detailed understanding of the global landscape of HCV-related HCC. This knowledge is crucial for guiding public health policies, designing targeted interventions for at-risk populations, and shaping future studies to reduce the global burden of HCC.

Research methods

Our study utilized the Global Burden of Disease database to examine HCC mortality due to HCV, focusing on different World Bank regions. We employed age-standardized mortality rates for precise demographic comparisons, analyzing these rates with Joinpoint regression software to detect trends and changes. Additionally, we used the Empirical Quantile Confidence Interval method for reliable results, despite uncertain data distributions. Our approach stands out for its regional focus and advanced statistical techniques, offering a detailed understanding of HCC mortality trends linked to HCV.

Research results

The study identified distinct regional and gender-specific trends in HCC mortality due to HCV. Key findings include a global decline in HCC mortality, with notable regional variations and gender disparities. The impact of advanced treatments like Direct-acting Antivirals (DAAs) coincided with mortality rate declines. However, North America showed an increasing trend, highlighting the need for region-specific strategies. The study underscores the importance of targeted interventions and further research to address unresolved issues in HCC mortality trends.

Research conclusions

This study introduces a theory that regional and demographic factors significantly impact HCC mortality rates from HCV infection. It postulates that global mortality decline in some regions is due to effective DAAs use, while increases in other areas might result from varying healthcare access, public health policies, and socio-economic conditions.

Research perspectives

Future research from this study should focus on: (1) Investigating the causes behind regional and gender disparities in HCC mortality from HCV, considering healthcare access and socio-economic factors; (2) Assessing the long-term efficacy of DAAs in preventing HCC in chronic HCV patients; (3) Evaluating and enhancing public health strategies in regions with increasing HCC mortality; (4) Continuing comprehensive data analysis for identifying new trends; and (5) Studying the socioeconomic impact of HCV and HCC, including treatment cost-effectiveness.

FOOTNOTES

Author contributions: Author Contributions: Ali H, Sohail AH, Dahiya DS, and Vikash F were responsible for the conceptualization, methodology, software development, data curation, validation, and drafting the original manuscript; Ali H, Dahiya DS, Sohail AH, Khalid F, Moond V, and Gangwani MK contributed to the critical review and editing of the manuscript and took on roles in project administration; Jamil AR, Patel P, and Satapathy SK were involved in reviewing and editing the article and provided supervision throughout the project.

Institutional review board statement: The present study did not require institutional review board oversight because Global Burden of Disease Study 2019 database is de-identified and freely accessible. It does not identify hospitals, health care providers, or patients.

Informed consent statement: Participants were not required to give informed consent to this retrospective study since the analysis of baseline characteristics used publicly available anonymized clinical data. Please contact me for any queries.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: The data used in this study is publicly available at Global Burden of Disease Study 2019 (GBD 2019) (https://ghdx.healthdata.org/gbd-2019).

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ORIGINAL ARTICLE

Basic Study

Country-based modelling of COVID-19 case fatality rate: A multiple regression analysis

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Abstract

BACKGROUND

The spread of the severe acute respiratory syndrome coronavirus 2 outbreak worldwide has caused concern regarding the mortality rate caused by the infection. The determinants of mortality on a global scale cannot be fully understood due to lack of information.

AIM

To identify key factors that may explain the variability in case lethality across countries.

METHODS

We identified 21 Potential risk factors for coronavirus disease 2019 (COVID-19) case fatality rate for all the countries with available data. We examined univariate relationships of each variable with case fatality rate (CFR), and all independent variables to identify candidate variables for our final multiple model. Multiple regression analysis technique was used to assess the strength of relationship.

RESULTS

The mean of COVID-19 mortality was 1.52 ± 1.72%. There was a statistically significant inverse correlation between health expenditure, and number of computed tomography scanners per 1 million with CFR, and significant direct

correlation was found between literacy, and air pollution with CFR. This final model can predict approximately 97% of the changes in CFR.

CONCLUSION

The current study recommends some new predictors explaining affect mortality rate. Thus, it could help decision-makers develop health policies to fight COVID-19.

Key Words: COVID-19; SARS-CoV-2; Case fatality rate; Predictive model; Multiple regression

Core Tip: The current study recommends some new predictors explaining affect mortality rate. Thus, it could help decision-makers develop health policies to fight coronavirus disease 2019.

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INTRODUCTION

Coronavirus Disease 2019 (COVID-19), an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to tremendous worldwide effects on the lives of people around the world, including large-scale morbidity and mortality and limited access to healthcare services (covid19.who.int)[1]. The worldwide case fatality rate (CFR) of COVID-19 has been estimated to be around 1.5% as of now[2]. Different variants of the SARS-CoV-2 virus have been discovered to cause the disease. The clinical presentation of the disease ranges from asymptomatic status to upper respiratory tract symptoms, mild pneumonia, severe respiratory symptoms, acute respiratory distress syndrome, extrapulmonary manifestations, and death[1,3].

Various risk factors for COVID-19 mortality have been named in the literature, including age, male gender, comorbidities (such as chronic kidney disease (CKD), cardiovascular disease, chronic obstructive pulmonary disease (COPD), diabetes mellitus, malignancy, underlying autoimmune disease, and hypertension), ethnicity, vaccination status, smoking history, obesity, and socioeconomic status[4-12] On the other hand, some factors have been proven to be protective, which may reduce the severity or mortality of COVID-19 infection, as among which vaccination[13], efficiently staffed facilities, particularly by registered nurses[14,15] corticosteroid treatment[16], and healthy diet[17] are the most notable. Gathering updated information from international data sources could throw light on the protective or potential risk factors to avoid COVID's severe morbidities and mortality.

This study aims to assess the correlation between different known risk factors or protective measures and the COVID-19 CFR, described by the number of deaths relative to number of confirmed cases. A similar study was performed in 2020 on 39 countries[18]. Our study is an update to the former one.

MATERIALS AND METHODS

Data collection

In this modeling publicly available register-based ecological study, we started with a literature review focused on the potential risk factors of COVID-19 mortality through Our World in Data, and COVID-related mortality risk factors through World Bank, Our World in Data, Statistica, OECD Database, and World Population Review. Our approach was consistent with a study done by Jennifer Pan, a Modelling study of factors causing death variation estimation by country.

We included all the countries with available COVID-19 CFR data, which enclosed 188 countries. There were 17 countries that had not reported any mortality data until the last date of our data collection (June 24, 2022); therefore, they were not included in the study. We did not have any exclusion criteria. We determined 21 risk factors for the worthwhile COVID-19 deaths, including GDP per capita, Population density, health expenditure per capita, Age, Obesity, Diabetes, human immunodeficiency viruses (HIV), Tobacco Users, Life expectancy, General death rate per 1000, Hospital beds per 1000, Physicians per 1000, Radiologist per 100 K, computed tomography (CT) scanners per million, Air pollution, Literacy rate, Human development index, case fatality rate, Tests per 1 million, Doses per 100 people, Given 1+ dose, Percent Fully vaccinated. In calculating the CFR, we used the total number of confirmed cases and fatalities for a particular country from Our World in Data[19]. Therefore, the CFR formula would be as follows: CFR in % = *Number of deaths from Corona/ Number of confirmed cases of Corona* ∗ 100[20].

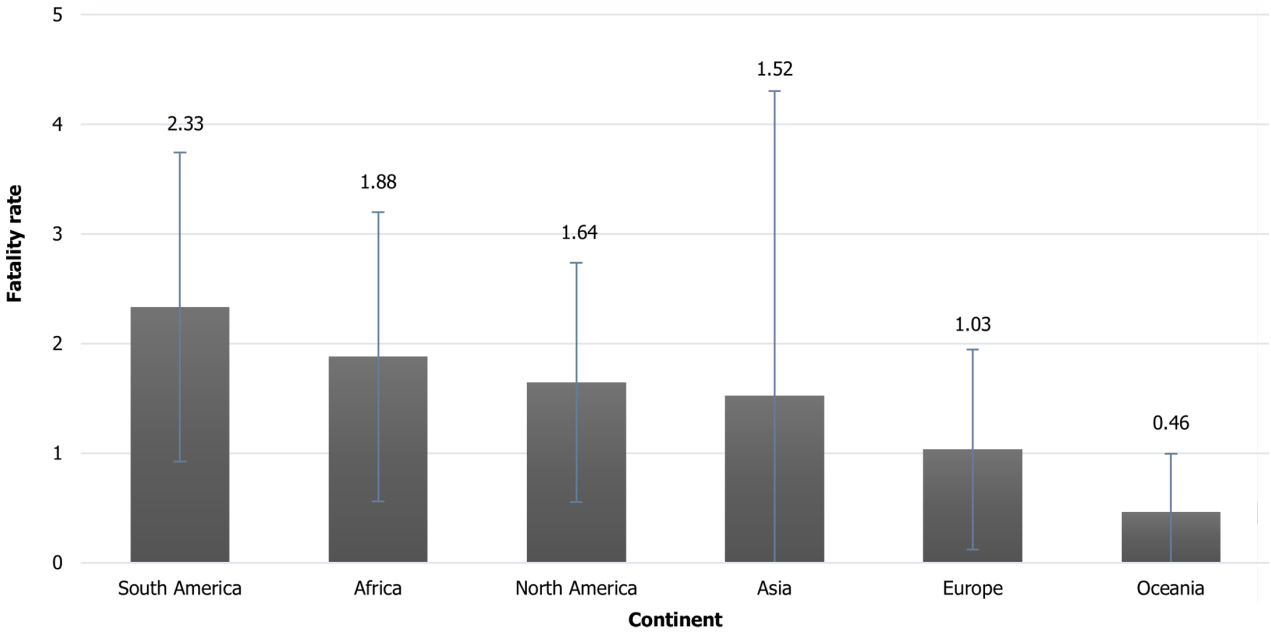

Figure 1 Coronavirus disease 2019 fatality rate by continent.

In regard to vaccination, people are placed in fully vaccination group two weeks after a second dose of mRNA COVID-19 vaccine, two weeks after receiving a second dose of the Novavax COVID-19 vaccine, or two weeks after they get a single dose of the Janssen/Johnson & Johnson COVID-19 vaccine. They are considered in given 1+ group if they have received at least one dose of COVID-19 vaccine, including fully vaccinated people[21]. Therefore, fully vaccinated people are those who have received two doses for most vaccines, or one or three doses for a few manufacturers[22].

Statistical analysis

A descriptive analysis of variables has been carried out. Data were presented as mean ± standard deviation for quantitative variables, and numbers (percentage) for qualitative data. We found that GDP, Health Expenditure per capita, Percent HIV, Air pollution, and tests per 1 million were highly positively skewed. In order to reduce the influence of extreme observations, we processed these variables at the logarithm scale.

As our dependent variable, we looked at the univariable relationships between case fatality rates in the next step, and all independent variables to identify candidate variables for our final multiple model.

Multiple regression analysis technique was used to assess the strength of the relationship between an outcome (the dependent variable) and several predictor variables as well as the importance of each of the predictors to the relationship, often with the effect of other predictors statistically eliminated by using the ordinary least squares[23], and the ability to identify outliers we found the multiple models for case fatality rate to be appropriate.

We pointed out the significance of each variable in these univariable models and finally selected variables for our multiple model at P value < 0.05. To detect the severity of multicollinearity, we focused on variance inflation factor (VIF) [24,25]. The model was designed by including all univariable candidate predictors and further developed by adding all significant interaction terms. Our preliminary final model included all 16 variable predictors significant at P values < 0.05. All reported P values were 2-sided with a 0.05 significance level. All variables screening in multiple regression methods led to choosing the most important variables that contribute to the response variable. Statistical analyses were conducted using SPSS version 28 and GraphPad Prism 8.

RESULTS

All 188 countries were included in the analysis. The total number of registered COVID-19 cases was 541937600 with a total mortality number of 6317644 patients. The mean of COVID-19 mortality was 1.52 ± 1.72%, with a range between a minimum of 0.03% (Bhutan country) to a maximum of 18.2% (Yemen country). Evaluation of CFR based on the continents, revealed the minimum fatality rate which was in Oceania continent (0.46%) and the maximum which was in South America (2.33%). It was mentioned that 50% of the studied countries had the case fatality rate > 1.15% (median) (Figure 1).

The mean population density was 447.6 ± 2113 person/Km^2. The percentage of population > 70 years old was 5.42 ± 4.22% (min: 0.53, United Arab Emirates, max: 18.5, Japan). The mean GDP per capita in 2020 was 15389.61 ± 23441.43. The lowest life expectancy rate was in Central African Republic (53.6 years) and the highest was in Hong Kong (85.39 years). There were 2.35 ± 1.93 physicians per 1000, and hospital beds per 1000, with the highest for Cuba (8.3) and Japan (13.05) respectively. The lowest number of conducted tests per one million population was in Algeria (5083) and the highest was

Table 1 Descriptive statistics (mean ± SD) for candidate predictor variables, R square, coefficient (SE) for univariate regression between predictors and case

Variable	Mean (SD)	R^2	Beta (SD)	*P* value
Population density[1]	300.96 (1539.00)	0.027	-0.116 (0.051)	0.025
GDP per capita in 2020[1]	15389.61 (23441.43)	0.000	0.013 (0.053)	0.812
Health Expenditure per capita[1]	1161.25 (1865.17)	0.176	-0.251 (0.041)	< 0.001
Population ages ≥ 70 yr	5.42 (4.22)	0.062	-0.059 (0.017)	< 0.001
Percent Obese	18.93 (9.93)	0.042	-0.021 (0.007)	0.006
Percent Diabetes	7.92 (4.09)	0.030	-0.042 (0.018)	0.018
Percent HIV[1]	1.91 (4.33)	0.001	0.022 (0.066)	0.743
Percent Tobacco Users	20.05 (9.55)	0.016	-0.013 (0.008)	0.116
Life expectancy at birth	72.53 (7.31)	0.157	-0.054 (0.009)	< 0.001
General death rate per 1000	7.87 (2.97)	0.017	0.044 (0.025)	0.075
Hospital beds per 1000	3.29 (2.44)	0.115	-0.145 (0.041)	< 0.001
Physicians per 1000	2.35 (1.93)	0.064	-0.197 (0.074)	0.009
Radiologist per 100 K	13.56 (5.52)	0.005	0.008 (0.026)	0.759
CT scanners per million	29.05 (20.02)	0.353	-0.932 (0.213)	< 0.001
Air pollution[1]	22.31 (14.73)	0.066	0.416 (0165)	0.014
Literacy rate	82.25 (17.33)	0.067	0.013 (0.006)	0.023
Human development index	0.72 (0.15)	0.178	-2.787 (0.445)	< 0.001
Tests per 1 million[1]	1654560.98 (2994282.33)	0.234	-0.266 (0.036)	< 0.001
Doses per 100 people	133.56 (79.68)	0.356	-0.007 (0.001)	< 0.001
Given 1+ dose	57.71 (26.97)	0.319	-0.020 (0.002)	< 0.001
Percent Fully vaccinated	52.49 (26.82)	0.341	-0.021 (0.002)	< 0.001

[1]Ln Transformed.

HIV: Human immunodeficiency viruses; CT scanner: Computed tomography scanner GPD: Gross Domestic Product.

in Denmark (21880771). Regarding the vaccination rate, the lowest percentage of fully vaccinated rate was in Algeria (5083) and the highest was in Denmark (21880771). It was mentioned that 50.8% of the studied countries given 1+ dose vaccination rate < 65.4 (median), and 50.5% given full vaccinated rate < 60.7 (median).

With regards to the number of confirmed cases and performed COVID tests, the minimum and maximum rates were from Africa and Europe, respectively. The minimum and maximum vaccination rates associated with Africa and South America, respectively.

Means and standard deviations of candidate predictors along with the univariate regression coefficient for each individual predictor are shown in Table 1. In these univariate regression models, our analysis demonstrated a statistically significant inverse correlation between CFR and logarithm of population density (P = 0.025), logarithm of health expenditure per capita (P < 0.001), Population ages ≥ 70 Years (P < 0.001), percent obese (P = 0.006), percent diabetes (P = 0.018), life expectancy at birth and hospital beds per 1000 (P < 0.001), physicians per 1000 (P = 0.009), CT scanners per 1 million, Human Development Index (HDI), logarithm of tests per 1 million, given greater than one vaccination, and percent of fully vaccinated people (P < 0.001). Conversely, it revealed a statistically significant direct correlation between logarithm of air pollution (P = 0.014) and literacy rate (P = 0.023) with CFR (Table 1).

There were also several cases of collinearity. For instance, there were strong positive correlations between the general death rate and Prevalence of current tobacco use (r = 0.333, P < 0.001), Hospital beds per 1000 (r = 0.598, P < 0.001), Physicians per 1000 (r = 0.467, P < 0.001), HDI (r = 0.176, P = 0.018), Current health expenditure per capita (r = 0.154, P = 0.042), and inverse correlation with Diabetes prevalence (r = -0.299, P < 0.001), and Air pollution (r = -0.317, P = 0.002). Also we find a significant positive correlation between CT scanners per 1M and Current health expenditure per capita (r = 0.337, P = 0.041), Hospital beds per 1000 (r = 0.479, P = 0.003), Human development index (r = 0.358, P = 0.029), and inverse correlation with obesity rate (r = -0.422, P = 0.009), and there was a significant direct correlation between HDI and Obesity Rates (r = 0.533, P < 0.001) and Diabetes (r = 0.224, P = 0.002).

In the following, we have multiple predictors without a statistically significant association with the response (all variable with P > 0.05 in Table 1). VIF values were < 5 which represents a medium level of collinearity, therefore we decided to keep all variables, thus we used model reduction (reduce the model by removing terms one at a time) to

Table 2 Multiple regression results with forward method for all candidate statistically significant correlated independent variables

Model	Variable	Beta (SD)	Standardized beta	*P* value	R square (*P* value)
1	(Constant)	7.80 (1.30)		< 0.001	0.799 (< 0.001)
	Health expenditure per capita	-1.06 (0.17)	-0.894	< 0.001	
2	(Constant)	3.42 (1.82)		0.093	0.895 (< 0.001)
	Health expenditure per capita	-0.79 (0.16)	-0.667	< 0.001	
	Air pollution	0.93 (0.32)	0.384	0.018	
3	(Constant)	4.29 (1.40)		0.016	0.947 (< 0.001)
	health Expenditure per capita	-0.69 (0.12)	-0.580	< 0.001	
	Air pollution	0.91 (0.24)	0.377	0.006	
	Number of CT scanners (1 million people)	-0.51 (0.18)	-0.246	0.023	
4	(Constant)	3.10 (1.14)		0.030	0.974 (< 0.001)
	Health expenditure per capita	-0.58 (0.10)	-0.486	< 0.001	
	Air pollution	1.01 (0.19)	0.415	< 0.001	
	Number of CT scanners (per 1 million people)	-0.74 (0.16)	-0.357	0.002	
	Literacy rate	0.01 (0.00)	0.199	0.031	

CT scanner: Computed tomography scanner.

increases the precision of predictions from the model. To use the statistical significance criterion, after set the significance level on 0.05 we tried different variables to find a model with as many statistically significant terms as possible but with no statistically insignificant terms. We applied the statistical significance criterion manually, with stepwise regression algorithm.

It has been observed that first "Current Health expenditure per capita "with $P < 0.001$ was entered into the model (model 1), then the variable "Air pollution" has been added to the model with level of significance of 0.018 (model 2), in the third model, "number of CT scanners per 1000 people" has been added to the model and it has been observed that the entry of these changes the Air pollution level of significance from 0.018 (model 2) to 0.006 (model 3). In the fourth model, after entering the "Literacy rate" variable, the regression coefficients and *P* values of all three previous variables have changed in the model, but the direction of their relationship has not changed. After entering the last variable (literacy rate) into the other model, none of the candidate predictors had the criteria to enter the model, therefor the final model will be the fourth model, with the final multiple regression equation: Y = 3.1 - 0.58 (Health Expenditure per capita) + 1.01 (Air pollution) – 0.74 (Number of CT scanners) + 0.01 (Literacy rate).

Base on this model, we could claim that this final model can predict approximately 97% of the changes in CFR. Therefore, it suggested an optimal model (Table 2, Figure 2). In this model, there was a statistically significant inverse correlation between health expenditure ($P < 0.001$) and number of CT scanners per 1 million ($P = 0.002$) with CFR. In addition, a statistically significant direct correlation was found between literacy rate ($P = 0.038$) and air pollution ($P < 0.001$) with CFR.

The correlation between estimated model and CFR is shown in Figure 2. As mentioned before, despite the fact that the model has shown an appropriate fit, due to the lack of data in some variables, including the CT variable, we have lost a lot of data.

DISCUSSION

Out study provides new population-based insights regarding the risk factors of COVID-19 mortality. This is an update on our previous efforts to identify indices and predictors of fatal outcomes in different populations based on their socioeconomic status and healthcare system resources.

In previous studies there was a direct association between CFR and increasing age with or without comorbidities[7-9,16-18,26,27]; however, in this study, age 70 and above showed a negative correlation with CFR. This finding can be explained by variance factors such as priority in receiving jabs, strict isolation and social distancing for seniors and lower threshold of intensive care unit (ICU) admission. A higher development index in the countries with a higher median age was also reported as another explanation[19].

With regard to the association between CFR and smoking, there have been discrepant results in the literature. A meta-analysis by Hou *et al*[28] revealed that smoking was independently associated with increased mortality rate in COVID-19 patients, particularly in former smokers. A review article by World Health Organization (WHO) in 2020 demonstrated an increased severity and mortality of COVID-19 in hospitalized smoking patients[29]. Conversely, in a review article in

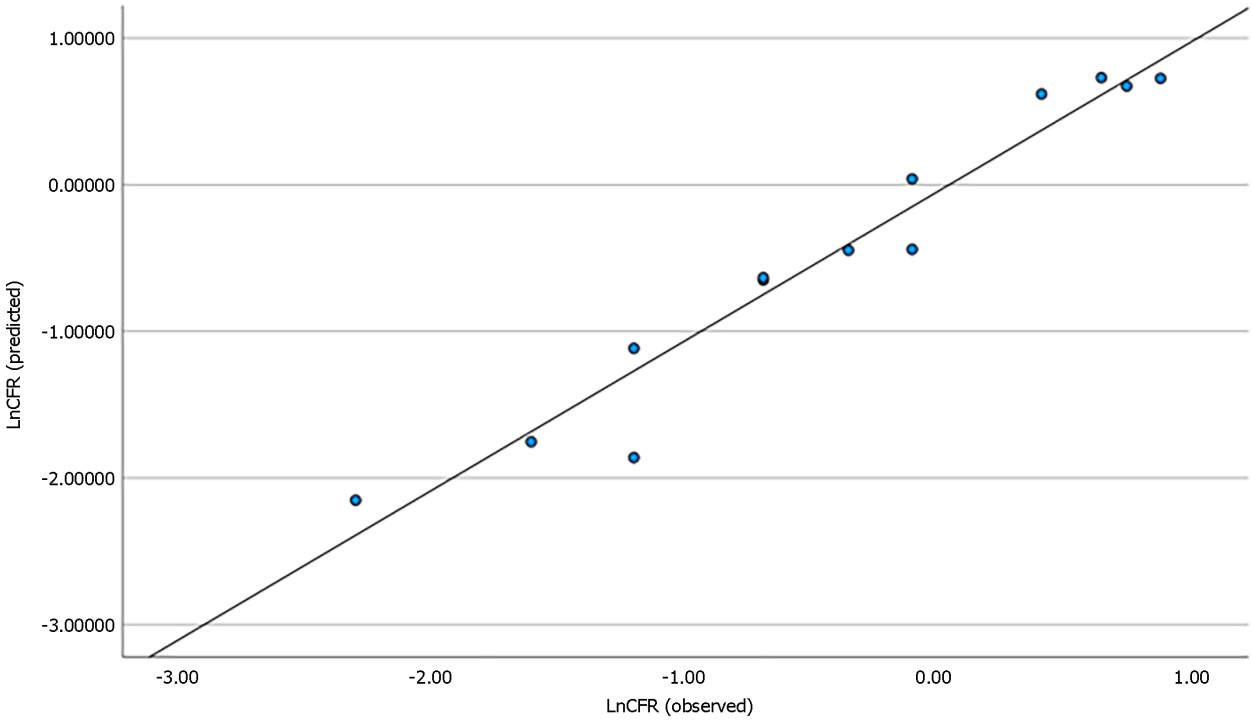

Figure 2 In this scatter plot, the relationship between the observed case fatality rate values and the estimated values obtained by the model can be seen. Despite the fact that the model has shown a favorable fit, but due to the small number of data related to some variables, including the computed tomography scanners variable, we have lost a lot of data regarding case fatality rate. CFR: Case fatality rate.

Alberta, there was no significant increase in severity of COVID-19 in smokers[12]. Comparably, smoking did not have statistically significant correlation with CFR in our study. The reasonable explanation for this controversy could be a lower threshold to use antivirals in these patients or admit them to an ICU.

Life expectancy at birth is described by the average number of years that a newborn is expected to live, considering the age- and sex-specific rates at the time of birth[30]. It is an indicator of the overall mortality level of a population which summarizes the mortality pattern including all age groups - children and adolescents, adults, and the elderly. In our study, countries with a higher COVID-19 CFR had a lower life expectancy at birth which have a latitude of explanations such as better health service coverage and higher health literacy rate in those countries with longer life expectancy. This finding was in agreement of the results of life expectancy evaluation in several countries, including United States and Canada, during the COVID-19 pandemic[31-35].

In a study by Sen-Crowe *et al*[36] on 183 countries, there was a weakly positive significant association between the number of ICU beds per 100000 population and COVID-19 mortality. Though, there was no significant association between the number of hospital beds or acute care beds per 100000 populations and COVID-19 mortality. In other studies, a negative association between hospital capacity, especially hospital beds, and COVID-19 mortality has been confirmed[18,37,38]. Similarly, our study revealed lower COVID-19 CFR in the countries with a higher number of hospital beds per 1000 population, which potentially can be explained by the higher level of care for COVID-19 patients in these countries. Similar findings were noted in association of COVID-19 CFR and the number of physicians per 1000 population, which was in line with the cross-sectional study by Tchicaya *et al*[39] in France in 2020. Increased staffing, particularly with Registered Nurses, has been reported as a protective factor leading to decreased mortality[15]. In another study by Stephen Rocks in 2020 on the effects of hospital capacity on COVID-19 mortality in 33 countries, a negative association between hospital capacity, especially hospital beds, and COVID-19 mortality rate was found. However, they realized that the main determinants in these countries were mostly variables other than treatment capacity per se, including earlier lock-down restrictive measures, which limited the number of new cases. Moreover, they found that low hospital capacity can affect mortality by detracting other sources from non-health sections leading to indirect effects on COVID-19 mortality rather than a direct effect[37].

With regards to COVID-19 testing, the more COVID tests were performed, the lower was the CFR. This finding was in agreement with the previous studies in which a negative association between COVID-19 testing and mortality was demonstrated[40-42], particularly among low-income countries and those with fewer hospital beds[41]. Timely testing can lead to earlier isolation and effectively limit the disease spread, as well as appropriate treatment interventions, if required[43,44].

HDI is defined by having a standard living with a healthy and long life and access to education (Our World in Data). Studies on the association between HDI and COVID-19 CFR, found a negative correlation[45-48]. However, there have been a few studies that have reported positive correlation which was explained by a higher infection rate as a result of higher rate of chronic disease, higher number of performed COVID-19 tests and older population in these high HDI countries[14,49,50]. Our study demonstrated less CFR in countries with a higher HDI worldwide. This also could be due

to more access to education in these countries.

Several types of COVID vaccines have been produced from inactivated or weakened virus vaccines to RNA and DNA vaccines[51]. To date, based on the WHO vaccine tracker, 169 and 198 vaccines have been under clinical and pre-clinical development, respectively. Vaccination by COVID-19 vaccine has been mentioned as a protective factor against the disease severity and mortality in the literature[9,17]. In a meta-analysis by Huang *et al*[13] in 2021, it was found that all types of vaccines, compared with no vaccine status, were effective to decrease the frequency of severe cases and consequently, to decrease the mortality rate. In a longitudinal study on 90 countries, 10% increase in vaccine coverage led to 7.6% decrease in CFR[52]. Our study also revealed a negative association between the number of COVID-19 vaccine doses, receiving at least one dose vaccine and fully vaccination with CFR.

With regard to the correlation between comorbidities such as diabetes and obesity with CFR, there have been various results in the literature. In a meta-analysis of 87 studies on the association between COVID-19 mortality and the comorbidities, diabetes mellitus was one of the most important factors associated with mortality[53]. Similar findings have been reported in the literature[54,55]. Comparably, there has been an increased COVID-19 mortality in obese patients (body mass index over 30 kg/m^2), both in adults and pediatrics[17,56,57]; however, in an evidence-based review in Alberta, there has not been a consistent association between obesity and mortality. Moreover, there was not a high strength association between diabetes and COVID-19 mortality[12]. Our study revealed a negative correlation between diabetes and obesity with CFR indicating decreased CFR in diabetics or obese patients (Table 1). The higher prevalence of obesity and diabetes in countries with higher HDI[11,14,45,47,48] which can explain the lower CFR in this population.

Our regression analysis modeling was able to define the effects of the above given variables on the CFR, when considering them together. Our subsequent modeling and regression analysis revealed that the following variables have the highest connection with CFR: Health expenditure per capita, CT scanner per 1 million, air pollution, and literacy rate.

Health expenditure means all expenditures required for the planning of health services, family planning activities, nutrition activities and emergency aid designated for health, except for the provision of drinking water and sanitation (WHO). There has been a negative correlation between health expenditure and rigorous policies regarding COVID-19, including closure of the schools, universities and working places, home confinement, as well as internal and international travel restrictions[58]. Despite the thought that higher health expenses should decrease mortality due to better patient care, a positive correlation between health expenditure and COVID-19 mortality is reported in the literature[59]. This finding is in agreement with our study. It could be the result of lower efficacy of the health care in regions with higher health expenses.

In a literature review by Pan *et al*[18] on 39 countries in 2020, the number of CT scanners per one million was associated with decreased CFR. We also found statistically significant association between the number of CT scanners per one million and COVID-19 CFR. This can be explained by earlier detection of lung involvement through CT scan which leads to timely treatment[60,61], particularly in low- to mid-income countries with less availability of COVID-19 tests , earlier in the course of pandemic[62] which can prevent more severe disease and subsequently decreases mortality.

Several studies have considered air pollution as a risk factor which increases morbidity and mortality of COVID-19[43,44,63]. This was in agreement with our study, both in univariate regression and multiple regression analysis. This could be because of the detrimental cardiovascular effects of air pollution, both short- and long-term, through oxidative stress and inflammation in lungs and heart vessels, leading to reduced lung function, atherosclerosis, and acute thrombotic complications[64].

There are many studies indicating negative correlation of health literacy with COVID-19 morbidity and mortality[65,66]. To the best of our knowledge, the correlation between COVID-19 CFR and literacy rate has not been reviewed in the literature. This study revealed a negative correlation between CFR and literacy rate, based on the WHO's definition of literacy rate. High health literacy rate may originate from a better literacy rate leading to lower mortality due to healthier behavior.

CONCLUSION

This study revealed controversial findings pertaining to the different variables analysis per se. However, adding multiple variables in a model increased the accuracy of the evaluation of those risk factors. Our multiple-model regression analysis explained a higher percentage of changes in CFR in relation with health expenditure, number of CT scanners per one million, air pollution, as well as literacy rate.

Limitations

There were some limitations to this study. Firstly, source of the analysed data in our study was based on the reports from different countries; therefore, the true CFR could be more than the reported one. Moreover, in this study, we could not include all countries due to inadequate recording of data on CFR. Secondly, we could not evaluate or achieve statistically significant correlation between CFR and some variables due to incomplete data on some variables that have been already mentioned in some isolated reports.

ARTICLE HIGHLIGHTS

Research background

The worldwide case fatality rate (CFR) of coronavirus disease 2019 (COVID-19) has been estimated to be around 1.5% as of now. Different variants of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus have been discovered to cause the disease. The clinical presentation of the disease ranges from asymptomatic status to upper respiratory tract symptoms, mild pneumonia, severe respiratory symptoms, acute respiratory distress syndrome, extrapulmonary manifestations, and death. Various risk factors for COVID-19 mortality including age, male gender, comorbidities (such as chronic kidney disease, cardiovascular disease, chronic obstructive pulmonary disease), diabetes mellitus, malignancy, underlying autoimmune disease, and hypertension), ethnicity, vaccination status, smoking history, obesity, and socioeconomic status. On the other hand, some factors have been proven to be protective, which may reduce the severity or mortality of COVID-19 infection, as among which vaccination, efficiently staffed facilities, particularly by registered nurses corticosteroid treatment, and healthy diet are the most notable. Gathering updated information from international data sources could throw light on the protective or potential risk factors to avoid COVID's severe morbidities and mortality.

Research motivation

We find it interesting the topic trend analysis in the COVID-19 literature, and the success of modeling studies in the field of predicting disease behavior was a turning point for us.

Research objectives

The objective motivation for doing this study is to assess the correlation between different known risk factors or protective measures and the COVID-19 case fatality rate and we decided to design and conduct a new study based on modeling.

Research methods

Twenty-one potential risk factors were identified for COVID-19 case fatality rate for all the countries with available data. Univariate relationships of each variable with case fatality rate, and all independent variables to identify candidate variables for our final multiple model were examined. Finally multiple regression analysis technique was used to assess the strength of relationship between case fatality rate and several predictors' variables as well as the importance of each predictor to the relationship.

Research results

There was a statistically significant inverse correlation between health expenditure, and number of computed tomography scanners per 1 million with case fatality rate, and a significant direct correlation was found between literacy, and air pollution with case fatality rate, this final model can predict approximately 97% of the changes in case fatality rate, conclusion: The current study recommends some new predictors explaining affect mortality rate. Thus, it could help decision-makers develop health policies to fight COVID-19.

Research conclusions

I suggest to do the same study with the updated data and compare the results. Multiple regression analysis technique is used as the most wrong and reliable method.

Research perspectives

Considering that global vaccination has been carried out, it is suggested that the approach of future realizations is to investigate the effectiveness of vaccines and compare the performance of vaccines with each other.

FOOTNOTES

Author contributions: Gholamrezanezhad A and Sagheb S conceived of the presented idea, and set the first draft of manuscript; Karami M and Pavlovic E developed the theory and worked out almost all of the technical details, and data extraction; Sagheb S and Fakhrzadegan M designed the model and the computational framework and analysed the data; language editing and revising the manuscript has done by Pavlovic E; All authors discussed the results and contributed to the final manuscript.

Institutional review board statement: In this modeling publicly available register-based ecological study as a population study, all data are available on open data sources like Our World in Data, World Bank, Statistics, OECD Database, and World Population Review. We included no private patients and no private data, and everything is clear in the references. As the research involves information freely available in the public domain, this study doesn't need to ethics code or institute approval.

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ORIGINAL ARTICLE

Basic Study

Development of a multiplex polymerase chain reaction assay for detection of hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1

Waleed Abdelgaber Nemr, Radwan K Nashwa

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Abstract

BACKGROUND

Hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus 1 (HIV-1) are the most epidemic blood-borne viruses, posing threats to human health and causing economic losses to nations for combating the infection transmission. The diagnostic methodologies that depend on the detection of viral nucleic acids are much more expensive, but they are more accurate than serological testing.

AIM

To develop a rapid, cost-effective, and accurate diagnostic multiplex polymerase chain reaction (PCR) assay for simultaneous detection of HCV, HBV, and HIV-1.

METHODS

The design of the proposed PCR assay targets the amplification of a short conserved region featured with a distinguishable melting profile and electrophoretic molecular weight inside each viral genome. Therefore, this diagnostic method will be appropriate for application in both conventional (combined with electrophoresis) and real-time PCR facilities. Confirmatory *in silico* investigations were conducted to prove the capability of the approached PCR assay to detect variants of each virus. Then, Egyptian isolates of each virus were subjected to the wet lab examination using the given diagnostic assay.

RESULTS

The *in silico* investigations confirmed that the PCR primers can match many viral variants in a multiplex PCR assay. The wet lab experiment proved the efficiency

of the assay in distinguishing each viral type through high-resolution melting analysis. Compared to related published assays, the proposed assay in the current study is more sensitive and competitive with many expensive PCR assays.

CONCLUSION

This study provides a simple, cost-effective, and sensitive diagnostic PCR assay facilitating the detection of the most epidemic blood-borne viruses; this makes the proposed assay promising to be substitutive for the mistakable and cheap serological-based assays.

Key Words: Diagnosis; Blood-borne viruses; Multiplex polymerase chain reaction; High-resolution melting

Core Tip: The current study approaches a cost-effective diagnostic assay to detect the most common blood-borne viruses (hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1) in a single test of multiplex polymerase chain reaction (PCR). This article includes the procedures in computational biology to achieve the PCR design and the practical examination of the given assay for detecting the targeted viruses with the interpretation of the results.

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DOI: https://dx.doi.org/10.5501/wjv.v13.i1.88164

INTRODUCTION

The primary concerned blood-borne pathogens are hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus 1 (HIV-1). They can be transmitted by contacting the viral particles with wounded skin, mucous membranes, or blood during unsafe medical treatment, blood transfusion, or unprotected sex actions. The World Health Organization (WHO) reported that the inflammation of the liver (hepatitis) may lead to liver cirrhosis and cancer. Viral hepatitis is one of the major reasons of mortality worldwide; more than 350 million patients are affected by the infection with HCV or HBV. They cause death for more than 1.1 million people every year. If the spreading of the hepatitis viruses will not be addressed, it is expected that more than 3 million people will be newly infected every year. Accordingly, United States $150 million has been allocated to fund international programs specialized in infection control and treatment of hepatitis viruses (https://www.who.int/news/item/17-05-2023-high-level-resource-mobilization-conference-to-eliminate-viral-hepatitis).

On the other hand, HIV attacks the body's immune system, making the body more vulnerable to microbial infection and cancer. The recent WHO records reported that HIV caused infection for more than 39 million people and death for 630000 people until 2022 (https://www.who.int/health-topics/hiv-aids#tab=tab_1).

Given that the first line to combat the infection transmission of these pathogens is the accurate screening for infected patients, this makes the invention of precise diagnostic assays highly interesting. Serological assays and nucleic acid amplification testing (NAT) are the most common methods for viral diagnosis. Serological methods can detect viral antigens or their specific antibodies in blood specimens, while NAT identifies viral genomes in any biological sample (such as tissues and body liquids).

The selection of a suitable diagnostic system depends on the ability of the assay to detect a wide range of viral variants with the same precision. This requires the knowledge of the viral biodiversity in the targeted geographic region. Many different subtypes diverged from HCV genotypes 1-3 are globally epidemic. They are sub-classified according to the divergence in the viral genome sequence. Africa shows the highest prevalence rate of HCV (5.3%), while Egypt recorded the highest burden of HCV genotype 4 (prevalence rate = 17.5%) until 2013[1]. However, recent records revealed a decrease in this percentage in Egypt after a national program for antiviral medications for HCV-infected patients[2].

Although serological tests are cheaper and faster, they are less accurate and may result in false positive or false negative reactions. The false negative may be exhibited due to the inability to detect the viral markers in samples with a low viral load (which may occur in recently infected patients)[3], or the difficulty of finding antibodies in samples derived from immune-suppressed patients and patients with current HIV infection[4]. On the other hand, the false positive may be exhibited in samples derived from patients who received interfering medications such as immunoglobulin[5], patients with rheumatoid arthritis[6], and patients who recovered from a previous infection[7], or due to the cross-reactivity of some antibodies with similar viral antigens[4]. Furthermore, antibody-targeted serological assays cannot differentiate between active- or past-infected patients. Also, these assays are inappropriate for examining recently infected patients because the detectable level of specific antibodies cannot be achieved before at least 2 mo of the viral infection[8].

Despite the cost, the NAT-based diagnostic methods [such as polymerase chain reaction (PCR)] are more effective and accurate than the mistakable serological methods[7]. Furthermore, PCR methodologies are easy to establish into highly throughput automation systems with the benefits of low risk of contamination, regardless of whether during reaction preparation or postoperative chemical disposal[9].

The current study aimed to develop a rapid, cost-effective, and accurate diagnostic multiplex PCR assay, which depends on the simultaneous detection of HCV, HBV, and HIV-1. Such cost-effective NAT-diagnostic assays will be promising to be a good substitute for cheap serological assays, especially in low-income countries.

MATERIALS AND METHODS

Genomics analysis

Viral genomic data were retrieved for different isolates of HCV, HBV, and HIV-1 from the National Center for Biotechnology Information (NCBI) website. Previously published specific primers for each virus were computationally evaluated for the best matching with a wide range of different isolates. Therefore, ClustalW multiple sequence alignment was carried out to determine the lowest variability region among the whole genome sequences of different geographic strains or genotypes of each virus (Table 1). This is to verify that the selected primers are located and surround a universally conserved region that shares homologous sequences among different aligned sequences. The ClustalW alignment and the entropy analysis of aligned sequences were conducted using BioEdit software[10]. Then, amplicons located inside genomic regions that showed the lowest entropy values were selected for further analyses.

PCR primer evaluations and harmonization

Best-matched primers with the universally conserved regions, inside variables' genomes of each virus, were examined for some specific criteria. The capability of primer pairs to work in a combined mixture was evaluated, as multiplex PCR primers, by predicting their ability to form dimers *in silico*, using AutoDimer software at a minimal score (= 4)[11].

Particularly, the selection of suitable primers was based on avoiding any primer that could form stable hairpins or dimers (whether by inter- or intra-oligos) having $\Delta G < -9$ kcal/mol, or if their estimated melting temperature (Tm) is close to the expected primer annealing temperature (if the difference of Tm < 10 °C), according to primer design guidelines[12].

The harmonization of the selected primers was carried out to adjust the Tm of all primers at a suitable unique Tm value. This was done by adjusting the nucleotide length and the percentage of guanine and cytosine (%GC) inside each primer. Hence, these primers will be able to work at similar annealing temperatures during the triplex PCR run. Consequently, some selected primers were modified to equalize their melting temperature which was standardized at 60.095 ± 0.5 °C.

Therefore, primers' melting temperature and their predicted amplicon sequences were determined using the NCBI Primer-BLAST web tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/)[13]. The melting profile of each amplicon sequence was predicted by utilizing the uMELT web tool (https://www.dna-utah.org/umelt/quartz/um.php)[14].

Laboratory experiment

Positive human plasma samples for HCV (genotype 4), HBV (genotype D), and HIV-1 were obtained from the Microbiology Reference Lab of the National Blood Transfusion Service, Ministry of Health, Egypt. These positive samples were tested to validate the theoretical design of the triplex PCR. Viral genomes were extracted using the PREP-NA-S DNA/RNA extraction kit (product# P-007-N, DNA-Technology LLC., Moscow) according to manufacturer's instructions. The viral load of each sample was determined using commercial real-time PCR quantitative kits manufactured by DNA-Technology LLC (Moscow), including Hepatitis C Virus Quantitative Real-Time PCR Kit (#Q4-P603-24), Hepatitis B Virus Quantitative PCR Kit (#Q2-P602-24), and Human Immunodeficiency Virus Quantitative PCR Kit (#R3-P609-S3). Plasma samples were diluted with normal saline solution to serial logarithmic concentrations till achieving the viral load equivalent to 100 IU/mL. Then, further gradual dilutions were made, which ranged from 10 to 90 IU/mL. The amplification efficiency (E) and the limit of detection (LOD) of both monoplex and triplex PCRs were determined according to previously published guidelines[15]. The E values were calculated by the following equation: $E = 10^{-(1/n)}$.

Where n is the slope of the regression line of the calibration curve which is plotted by [log of concentration, quantification cycle (Cq value)] as (x, y) values. Then, the percentage of PCR amplification efficiency was determined by: $\%E = (E - 1) \times 100$.

The LOD was estimated as the lowest concentration of viral load that could be determined by the assay with a sensitivity and specificity both ≥ 90% (95% confidence interval). The sensitivity and specificity of the test were estimated according to Wang *et al*[16] by the following calculations: Sensititvity = *Ture positivity*/(*Ture positivity* + *false negativity*) × 100, Specificiy = *Ture negativity*/(*False positivity* + *ture negativity*) × 100.

The statistical evaluation of the diagnostic assay was conducted using MedCalc statistical software.

Practically, viral genomes were extracted from the diluted samples by the same extraction kit. Purified viral genomes (whether RNA or DNA) were added as templates with 10 pmol of each primer in reverse transcription (RT) reaction which was carried out using Invitroge SuperScript™ III Reverse Transcriptase according to the manufacturer's instructions (Fisher Scientific Ltd., United Kingdom), to produce a complementary DNA (cDNA) amplicon from each viral RNA genome (HCV and HIV-1). An amount of 10 µL of the obtained RT reaction volume was transferred to a real-time PCR tube containing 10 pmol of each primer (Table 2) in iQ™ SYBR® Green Supermix reaction mix (Bio-Rad Laboratories, United States), according to the guide manual. The real-time PCR run was performed on a Rotor-gene Q 5plex high-resolution melting (HRM) machine (QIAGEN, United States) and was analyzed using its built-in software. The

Table 1 List of different National Center for Biotechnology Information records of different geographic strains for hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1 that are involved in ClustalW and entropy analyses

Virus	Taxon ID	Classification	Country of isolation	NCBI accession number
HCV	2847144 (genotype 1a)	Isolate ZS30	China	KC844049
	11103 (genotype 1b)	Isolate 2000621	Israel	MT632133
	31649 (genotype 2)	Subtype 2a, isolate PR63	China	KF676351
	356426 (genotype 3)	Subtype 3a	India	GQ275355
	356418 (genotype 4)	Subtype 4a, strain ED43	Egypt	GU814265.1
	33746 (genotype 5)	Subtype 5a	United Kingdom	NC_009826
	356469 (genotype 6)	Subtype 6k, isolate KM41	China	DQ278893
HBV	489455 (genotype A)	Strain AON	Japan	LC488828
	489460 (genotype B)	Isolate 4265-Viet12	Viet Nam	LC064379
	2764122 (genotype C)	Isolate C173334	Cambodia	LC535933
	2847137 (genotype D)	Isolate B-H10-Ban	Bangladesh	LC519824
	2847138 (genotype E)	Isolate Mart-B84	Martinique	HE974384
	2847139 (genotype F)	Isolate VHB-PER036	France	LT935669
	2847140 (genotype G)	Isolate MEX918M	Mexico	AB625342
	2847141 (genotype H)	Isolate Itabashi	Japan	LC491577
	2847142 (genotype I)	Isolate 8290	Viet Nam	AF241411
HIV	11676 (HIV-1)	Isolate 99SE-MP1299 (subtype O)	Senegal	AJ302646
		Isolate 5104_SEB_AIM_E3	United States	MT190832
		Isolate 01AETH04BKM	Thailand	DQ314732
		Isolate RBF168	France	GU111555
		Clone pCMO2.3	Cameroon	AY618998
		Isolate 01ZATM45 (subtype: C)	South Africa	AY228557
		Isolate 193008 (subtype: AD)	Uganda	MW006063
		Isolate BI	Belgium	MN486005
		Isolate 99GR303	Greece	AY046058
		Isolate HK002 (subtype: B)	Hong Kong	FJ460499
		Isolate SE8646	Sweden	AY352654
		Isolate 01BRRJUD508 (subtype: F1)	Brazil	MG365771
		Isolate 04KBH8 (subtype: D)	South Korea	DQ054367
		Isolate D9451 (subtype: URF)	Japan	MN187301
		isolate C.IN.05.NIRT333.1 (subtype: C)	India	KF766540
		Isolate 98UA0116 (subtype: A; group: M)	Ukraine	AF413987
		Isolate M61	Spain	DQ854714
		Isolate MtBs.18	Russia	MK984159
		Isolate IIIB	United Kingdom	KJ925006
		Isolate MBC200	Australia	AF042100
		Isolate 60000 (subtype: A1 variant)	Italy	EU861977
		Isolate TV721	Canada	HM215249
		Isolate pXJDC6291-2-6 (subtype: CRF07_BC)	China	KC503852

HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV-1: Human immunodeficiency virus 1; NCBI: National Center for Biotechnology Information.

Table 2 *In silico* evaluation of selected primer-pairs that demonstrated their suitability for simultaneous detection of a wide range of subtypes for hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1 using triplex real-time polymerase chain reaction amplification

Virus	Primers		Primer Tm[1] (°C)	Intended matches	Amplicon size (bp)	Amplicon Tm[2] (°C)	Ref.
HCV	Forward	GGTGCACGGTCTACGAGAC	60.15	HCV genotype 1 subtypes: 1a, 1b, 1c, 1g, and 1e. HCV genotype 2 subtypes: 2a, 2b, 2c, 2e, 2f, 2k, and 2m. HCV genotype 3 subtypes: 3a, 3b, 3g, 3i, and 3k. HCV genotype 4 subtypes: 4a, 4d, 4f, 4g, 4l, 4m, 4n, 4o, 4r, and 4v. HCV genotype 5 subtype: 5a. HCV genotype 6 subtypes: 6a, 6e, 6h, 6k, 6l, 6m, 6n, and 6r. HCV genotype 7 subtype: QC69. Unclassified HCV subtypes: 08.40.072, 08.80.075, 08.80.014, 08.80.070, 2b/1a, 2k/1b, and M2123. Recombinant HCV viruses	64	86.5	Chen *et al*[28], with modifications
	Reverse	GCCTTGTGGTACTGCCTGAT	60.04				Chen *et al*[28]
HBV	Forward	CTTCATCCTGCTGCTATGCCT	60.20	HBV genotypes A, A1, A2, and A3. HBV genotype B. HBV genotypes C and C1. HBV genotypes D and D4. HBV genotype E, including the relative Egyptian isolates AC# KU736891 and KU736892. HBV genotypes F, F2, and F4. HBV genotype G. HBV genotype H. HBV recombinant A/E. HBV recombinant B/C	71	80.5	Kishk *et al*[29]
	Reverse	GACAAACGGGCAACATACCTT	59.79				Prakash *et al* [30], with modifications
HIV	Forward	GCCTCAATAAAGCTTGCCTTGA	59.51	HIV type 1. Simian immunodeficiency virus	121	85.5	Rouet *et al*[31]
	Reverse	GGCGCCACTGCTAGAGATTTT	61.01				

[1]Primer melting temperature (Tm) and the amplicon size were determined using National Center for Biotechnology Information Primer-BLAST web-tool.
[2]Amplicon melting temperature was predicted using uMELT wed-based tool.
HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV-1: Human immunodeficiency virus 1.

thermal profile of the PCR included 35 cycles of 95 °C for 30 s (denaturation), 58 °C for 15 s (annealing), and 72 °C for 30 s (extension) which was followed by optical fluorescent emission reading at the green channel. PCR product melting profile was obtained using end-point HRM analysis at 0.5 °C resolution. Additionally, PCR products were separated on 1.5% agarose gel electrophoresis to verify the accuracy of the experiment at the expected molecular weights.

RESULTS

Bioinformatics findings

The current study assigned four important *in silico* parameters as successful criteria to reach the suitable combination of triplex PCR primers. This is to improve the accuracy of the detection for all targeted viral genomes (HCV, HBV, and HIV-1) in a single PCR test. The first parameter is the suitability of the selected primer to match a wide range of subtypes of each virus. Thus, primers that surround the lowest entropy regions along the aligned whole genome sequences of each virus were selected as genus-specific universal primers (Figure 1). Therefore, the produced amplicon was predicted to be homologous among all genotypes of each virus. This is desirable for obtaining a universal PCR product specific to each type of virus. As shown in Table 2, the set of the selected primers could detect HCV genotypes 1-7 including 44 subtypes and their recombinant strains, HBV genotypes (A-G) including the recombinant strains A/E and B/C, and almost all HIV type 1 subtypes plus simian strains.

The second parameter is to obtain a similar annealing temperature for all primers. Therefore, some primers were modified to obtain a similar Tm value (60.095 ± 0.5 °C) among all the selected primers. The third parameter is to select primers that exhibit lower stable dimers or hairpin forms to increase their efficacy to match their targets during PCR. AutoDimer software outputs revealed that there are no hairpins and three low stable-primer dimers which may be formed at the maximum temperature < 22.3 °C. The first dimer is formed by HIV reverse primer with itself (ΔG = -6.23 kcal/mole), and there are two self dimers formed by HCV forward primer (ΔG = -5.37 and -1.76 kcal/mole) (Figure 2). However, these dimers will not be efficient at annealing temperatures higher than 33 °C which prevents their stability and dimerization.

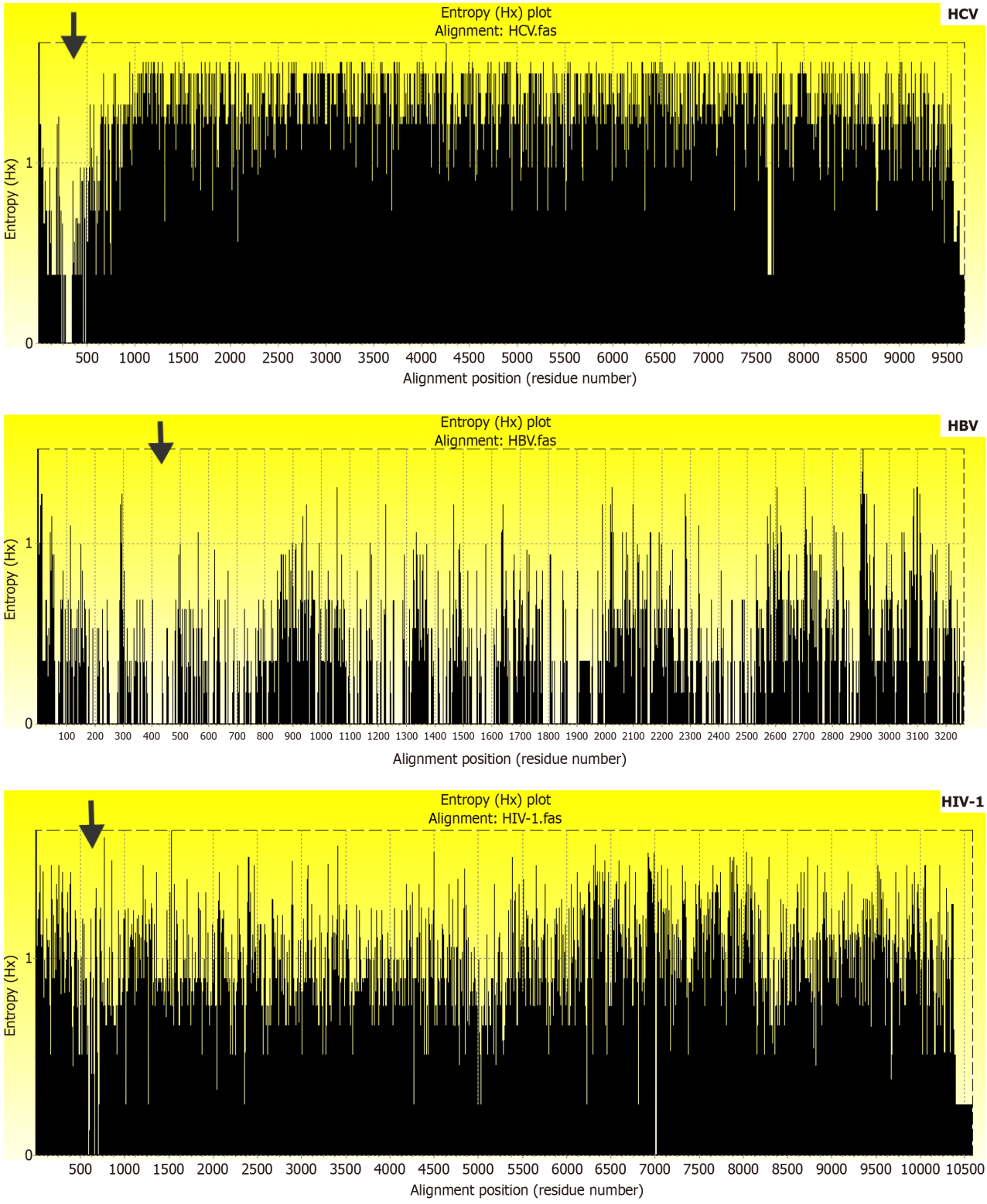

Figure 1 Entropy plot of aligned whole-genome sequences using BioEdit software. Arrows indicate the lowest variability regions used for locating conserved amplicons for each type of virus. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV-1: Human immunodeficiency virus 1.

The fourth parameter is based on the ability to discriminate each amplicon of each virus by a distinguished melting profile using HRM. Therefore, the predicted melting curve of each amplicon revealed a distinguishable single melting point which is represented by a single peak, as shown in uMELT analysis data for each viral PCR product (Figure 3, Table 2).

Real-time PCR experimental analysis

According to the *in silico* evaluations, a single PCR product will be obtained from both triplex and monoplex PCRs for each viral genome. This was verified by the visualization of a single band on gel electrophoresis at the expected molecular

```
HCV fd GGTGCACGGTCTACGAGAC versus HCV fd GGTGCACGGTCTACGAGAC
Matches = 6
Score = 4
NGTGCACN
est. tm =  10.7 oC
DeltaG 37 degrees =  -5.37 kcal/mole

                    5'-GGTGCACGGTCTACGAGAC-3'
                       x||||||x
        3'-CAGAGCATCTGGCACGTGG-5'

HCV fd GGTGCACGGTCTACGAGAC versus HCV fd GGTGCACGGTCTACGAGAC
Matches = 8
Score = 5
GTCTNNNAGAC
est. tm =  less than zero
DeltaG 37 degrees =  -1.76 kcal/mole

                    3'-CAGAGCATCTGGCACGTGG-5'
                       ||||xxx||||
            5'-GGTGCACGGTCTACGAGAC-3'

HIV rv GGCGCCACTGCTAGAGATTTT versus HIV rv GGCGCCACTGCTAGAGATTTT
Matches = 6
Score = 6
GGCGCC
est. tm =  22.3 oC
DeltaG 37 degrees =  -6.23 kcal/mole

                      5'-GGCGCCACTGCTAGAGATTTT-3'
                         ||||||
      3'-TTTTAGAGATCGTCACCGCGG-5'

NO HAIRPIN HITS FOR THIS Score NUMBER!!!
```

Figure 2 Screenshot of AutoDimer output evaluating all selected polymerase chain reaction primers for dimer and hairpin formations (score number = 4).

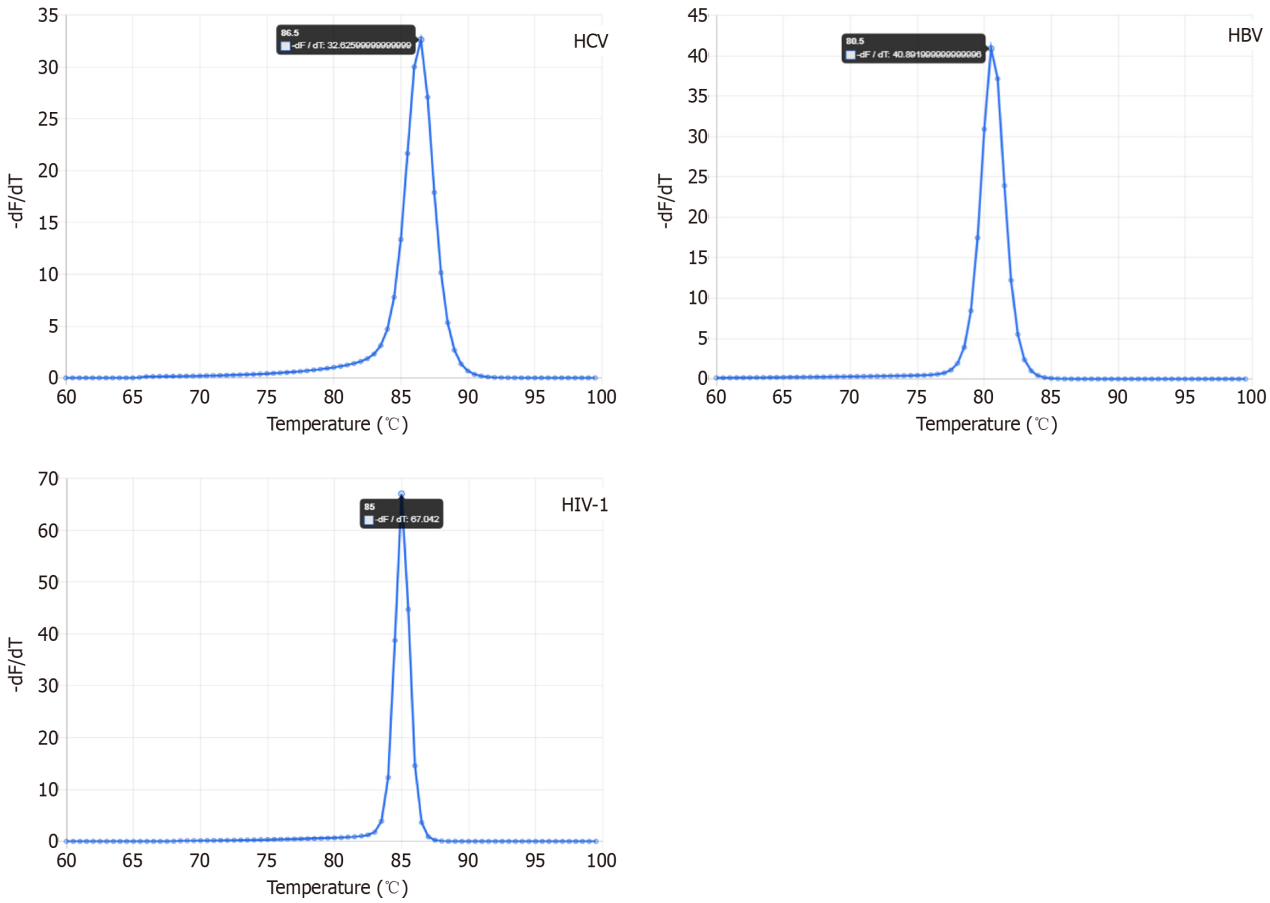

Figure 3 Screenshots of predicted melting profiles of hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1 amplicons using the uMELT web-based tool. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV-1: Human immunodeficiency virus 1.

weight and a distinguished single melting curve peak for each virus (Figures 4 and 5). In detail, electrophoresis migration of PCR products resulted in a single band for HCV at 65 bp (base pairs), HBV at 71 bp, and HIV at 121 bp, without the appearance of non-specific amplifications or primer dimers in the gel.

Accordingly, HRM analysis revealed that the formation of oligonucleotides dimers or non-specific amplicons was negligible; a tiny melt-curve peak emerged at a lower melting temperature (76.2 °C), and this signal rose from unintended noises of non-reacted nucleic acids. These noises did not interfere with the peaks of each viral amplicon (82 °C for HBV, 84.8 °C for HIV, and 86.7 °C for HCV). Accurately, each viral amplicon showed similar and reliable melting profiles, even by using monoplex or triplex primer sets. When both of these PCR sets were run with the LOD viral copies, HRM showed that the peak height of monoplex PCRs was somewhat greater than the analogous triplex ones (Figure 5). This agreed with the estimated rational median of %E which was only higher as 3.19% and the LOD was decreased to -15% for monoplex PCRs over the opposite triplex PCRs (Figure 6). Overall, the obtained LOD values satisfy 100% for specificity and more than 90% for sensitivity at 95% confidence interval. Statistically, the correlation between %E and LOD equals -0.966 which is significant ($P < 0.002$) at the 0.01 level (2-tailed Pearson correlation).

DISCUSSION

The development of accurate and cost-effective detection methods for diagnosing epidemic viruses attracts the concern of many scientists and manufacturers. Commercially, serological-based diagnostic kits are lower priced than NAT-based ones. However, many previous studies praised the accuracy of NAT over serological assay[17-20]. Such diagnostic methodologies are important for screening blood samples to confirm the absence of blood-borne viruses before transplant or blood donation. This is important to ensure the safety of biological sample transplantation and to besiege the spreading of epidemic viruses.

Because RNA viruses have a better ability for mutation than DNA viruses, many variants were generated with multiple mutated locations in their RNA genomes. Thus, this may result in false negative PCR detection when the primer or the probe cannot match mutated targets[21]. Consequently, many *in silico* investigations in this study were conducted to evaluate the conservation of primer matching sites; this is to decrease the possibility of mismatching with their targeted genomes and to ensure the capability of the given PCR test to detect a wide range of variants for each virus. In contrast, many similar published studies lack enough evidence in the same regard[3,16,22]. Additionally, there is a lack of guiding information in the literature on how to design new universal primers for many variants of a particular organism. This information should also include a demonstration of how to use computer simulations to evaluate the specificity of the primers in multiplexed PCRs.

Therefore, in the current study, the methodology of the primer design was clarified with the interpretation of primer analysis. Particularly, the PCR assay utilizes a fluorescent intercalating dye (Sybr-green) to recognize the new synthetic double-stranded PCR products in the reaction pool. Accordingly, the addition of TaqMan probes, as fluorescent reporters, is not needed; this reduces the cost per run and reduces the possibility of variable oligonucleotide cross-reactivity which may cause interference in the multiplex PCR pool. Given that the PCR efficiency in Sybr-green PCRs and TaqMan is comparable[23], this increases the benefit of using Sybr-green PCRs. However, the possible formation of primer dimers may cause overlapped Syber-green emission signals. Therefore, it is important to calculate the Tm value of the expected amplicon sequence to specify its fluorescent signals through the HRM analysis[24]. In addition, the sequence of some primers was modified in the current study to enhance the accuracy of primers annealing to their intended targets at a uniformed Tm lower than each amplicon's Tm, as proved in the *in silico* evaluations. These evaluations also proved the suitability of the selected primers to detect a wide range of variants for each virus in a multiplex PCR without detectable cross-reactivity.

The evaluations of the practical laboratory experiment revealed that the %E value was greater in HBV (having DNA genome) than in HCV and HIV (having RNA genome). This may be due to the limited sensitivity of the cDNA synthesis, which is an important step to provide PCR with a DNA template synthesized from a template of RNA sequence. Consequently, the LOD value in HBV-PCRs was lower than that in PCRs detecting RNA viruses. Therefore, this correlation between %E and LOD reflects the importance of improving the %E of the PCR to increase the sensitivity of the diagnostic assay. Accordingly, further developmental trials are required to enhance the sensitivity of cDNA synthesis by trying more sensitive reverse transcriptases or using single-step RT-PCR systems.

Referring to the global hepatitis program report of the WHO (https://www.who.int/hepatitis/publications/annex_4-7.pdf), the most sensitive PCR-based qualitative detection kits for HCV (as a representative example for the other targeted viruses) have lower LOD values (up to 60 IU/mL) than serological kits which have LOD values greater than 1000 IU/mL. However, the cost price per test of such PCR kits ranges from $20 to $100, depending on whether the kit is manual- or automated-based technology. Furthermore, the PCR cycling time to perform a single run for each virus ranges from 2-3 h, in addition to more than 1 h for the viral nucleic acid extraction procedure. Hence, to determine three types of viruses by separately specific PCR tests, the cost and the running time will increase three times, besides the effort, extra time labor cost, and instrument maintenance cost. However, this study revealed that triplex PCRs have fewer amplification efficiencies than analogous monoplexes, but they still maintain the benefit of the competitive sensitivity with the compensation of their less cost and time. This reflects the good impact of the proposed multiplex PCR assay for potential application especially in low-income countries.

The approached methodology in the present study reduces the cost of determining the three viruses simultaneously to $15 (price in January 2022) in a single tube, and this also reduces the work and time to achieve the final results within 2.5 h. In addition, the ability to detect a wide range of subtypes of each targeted virus increases the benefits of this assay.

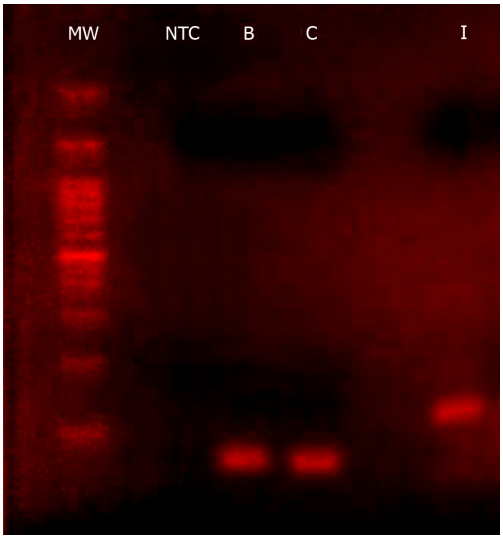

Figure 4 Agarose gel electrophoresis photograph of migrated polymerase chain reaction products for hepatitis B virus (B), hepatitis C virus (C), and human immunodeficiency virus 1 (I). The amplification was done using a triplex primers set. The lane indicated by (MW) was loaded with 100-bp DNA ladder, and NTC indicates the no-template control sample. Agarose gel was made at a concentration of 2% (W/V) of molecular screening agarose (Roche Diagnostics GmbH).

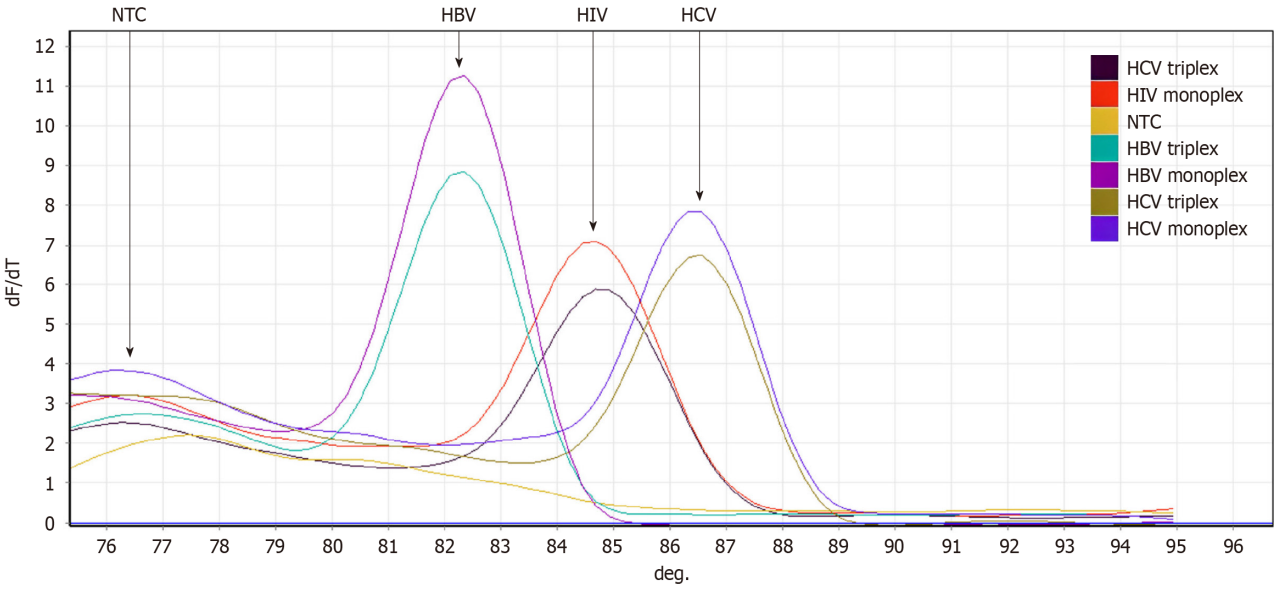

Figure 5 End-point analysis of monoplex and triplex polymerase chain reactions using high-resolution melting which differentiated between hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1 amplicons according to the melt profile. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV-1: Human immunodeficiency virus 1; NTC: No template control.

Moreover, the LOD in the present assay is greater than the LOD of the serological tests and is competitive with the expensive TaqMan-based assays. In detail, as described above, the LOD of most HCV-serological kits is greater than 1000 IU/mL (according to the previously mentioned WHO report), while the present assay of the current study exhibited LOD values at 61.5 IU/mL for HCV, 7 IU/mL for HBV, and 98.3 IU/mL for HIV-1 in the monoplex reaction. These values were increased to 67.2, 8.8, and 116 IU/mL, respectively, in the multiplex reaction.

The achieved LODs in a related study, which aimed at developing Sybr-green-based duplex PCR to detect HCV and HIV-1, were equivalent to 568 and 232.6 IU/mL, respectively[25]. In another study, the achieved LODs reached 114 IU/mL for HCV and 291 IU/mL for HIV-1[26]. Compared to a TaqMan-based assay conducted by Meng *et al*[27], the automated-complete system (performs the nucleic acid extraction and multiplex PCR run in a closed system) for detecting the HCV, HBV, and HIV-1 exhibited LOD values equivalent to 77 IU/mL, 5.4 IU/mL, and 24.7 IU/mL, respectively.

Accordingly, the assay of the present study showed greater sensitivity than the published Sybr-green-based assays and is a good competitive to the expensive automated TaqMan systems. Generally, the development of combined automated diagnostic systems improves the sensitivity of the assay; this is due to the reduction of the loss to viral templates-copy number through the extraction and cDNA synthesis steps. This indicates that the further development of the proposed

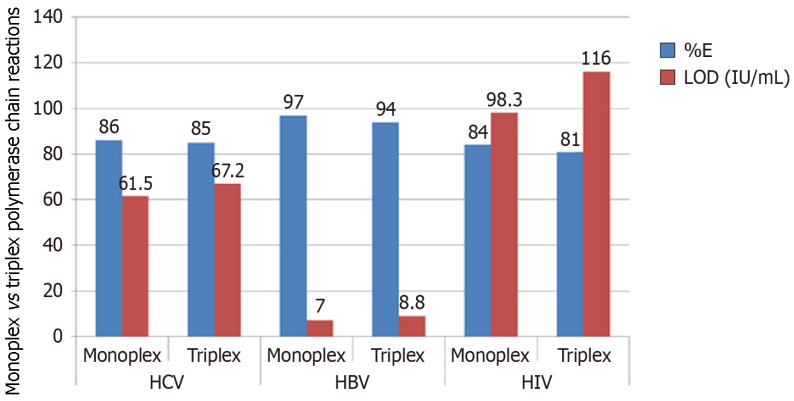

Figure 6 Amplification efficiency and limit of detection of monoplex *vs* triplex polymerase chain reactions targeting hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1 genomes. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV-1: Human immunodeficiency virus 1; LOD: Limit of detection.

PCR assay in this study to apply it in an automated-complete system will result in promising outcomes and further save time and cost. This is because the using of fluorescent intercalating dyes (such as Sybr-green) as reporter dyes in real-time PCR systems, eliminates the need to add expensive fluorescent-labeled probes. However, the specifying of Sybr-green-fluorescent signals to a particular amplicon in the multiplex PCR is urgently required through the HRM analysis to distinguish the detected virus.

CONCLUSION

The current study approached a rapid, cost-effective, and sensitive PCR-based diagnostic assay for the simultaneous detection of HCV, HBV, and HIV-1 in a single tube. *In silico* and *in vitro* evaluations proved the eligibility of this assay for application in large-scale screening of blood samples. Further developmental studies are recommended to translate this work into a commercial product.

ARTICLE HIGHLIGHTS

Research background

The most epidemic blood-borne viruses are hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus 1 (HIV-1). They cause mortality for millions of people worldwide. Although serology diagnostic methods are less accurate than nucleic acid amplification testing (NAT) for detecting blood-borne viruses in blood samples, they are commonly used to save money and time.

Research motivation

The innovation of rapid and cost-effective NAT assays will allow us to substitute the mistakable serologoical assays, and this can be achieved through a multiplex polymerase chain reaction (PCR) assay for the simultaneous detection of blood-borne viruses in a single test.

Research objectives

The present study focused on developing a new multiplex PCR assay for simultaneous detection of HCV, HBV, and HIV-1 in a single tube.

Research methods

The *in silico* design of the PCR assay targets conserved sequences in each viral genome among all variants. This was evaluated by multiple sequence alignment and finding the lowest entropy regions. The selected primers were evaluated also to avoid the possibility of forming stable dimers or hairpins. All primers were harmonized in the melting temperature to anneal at the same temperature during PCR. A practical experiment was conducted to prove the feasibility of the present assay.

Research results

The *in silico* evaluations proved the worthiness of the selected primers to match with many variants of each virus with negligible ability to form dimers, this ensures the efficiency of the proposed PCR assay. Consequently, the sensitivity of this assay showed the ability to detect HCV at a limit of detection (LOD) of 61.5 IU/mL. Furthermore, the LOD value is 7

IU/mL for HBV and 98.3 IU/mL for HIV-1.

Research conclusions

The proposed cost-effective PCR assay of the current study achieved a competitive sensitivity with the analogous multiplex PCR assays.

Research perspectives

The findings of the current study encourage further developmental studies to apply this assay in an automated system for large-scale virology screening of blood samples.

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FOOTNOTES

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SYSTEMATIC REVIEWS

Outcomes of liver resection in hepatitis C virus-related intrahepatic cholangiocarcinoma: A systematic review and meta-analysis

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Abstract

BACKGROUND

Cholangiocarcinoma is the second most common primary liver malignancy. Its incidence and mortality rates have been increasing in recent years. Hepatitis C virus (HCV) infection is a risk factor for development of cirrhosis and cholangiocarcinoma. Currently, surgical resection remains the only curative treatment option for cholangiocarcinoma. We aim to study the impact of HCV infection on outcomes of liver resection (LR) in intrahepatic cholangiocarcinoma (ICC).

AIM

To study the outcomes of curative resection of ICC in patients with HCV (*i.e.*, HCV+) compared to patients without HCV (*i.e.*, HCV-).

METHODS

We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) and observational studies to assess the outcomes of LR in ICC in HCV+ patients compared to HCV- patients in tertiary care hospitals. PubMed, EMBASE, The Cochrane Library and Scopus were systematically searched from inception till August 2023. Included studies were RCTs and non-RCTs on patients ≥ 18 years old with a diagnosis of ICC who underwent LR, and compared outcomes between patients with HCV+ *vs* HCV-. The primary outcomes were overall survival (OS) and recurrence-free survival. Secondary outcomes include perioperative mortality, operation duration, blood loss, intrahepatic and extrahepatic recurrence.

RESULTS

Seven articles, published between 2004 and 2021, fulfilled the selection criteria. All of the studies were retrospective studies. Age, incidence of male patients, albumin, bilirubin, platelets, tumor size, incidence of multiple tumors, vascular

invasion, bile duct invasion, lymph node metastases, and stage 4 disease were comparable between HCV+ and HCV- group. Alanine transaminase [MD 22.20, 95%confidence interval (CI): 13.75, 30.65, $P < 0.00001$] and aspartate transaminase levels (MD 27.27, 95%CI: 20.20, 34.34, $P < 0.00001$) were significantly higher in HCV+ group compared to HCV- group. Incidence of cirrhosis was significantly higher in HCV+ group [odds ratio (OR) 5.78, 95%CI: 1.38, 24.14, $P = 0.02$] compared to HCV- group. Incidence of poorly differentiated disease was significantly higher in HCV+ group (OR 2.55, 95%CI: 1.34, 4.82, $P = 0.004$) compared to HCV- group. Incidence of simultaneous hepatocellular carcinoma lesions was significantly higher in HCV+ group (OR 8.31, 95%CI: 2.36, 29.26, $P = 0.001$) compared to HCV- group. OS was significantly worse in the HCV+ group (hazard ratio 2.05, 95%CI: 1.46, 2.88, $P < 0.0001$) compared to HCV- group.

CONCLUSION

This meta-analysis demonstrated significantly worse OS in HCV+ patients with ICC who underwent curative resection compared to HCV- patients.

Key Words: Cholangiocarcinoma; Bile duct cancer; Hepatitis C; Surgical resection; Hepatectomy

Core Tip: Impact of hepatitis C virus (HCV) infection on survival outcomes in patients with intrahepatic cholangiocarcinoma (ICC) undergoing curative resection remains unclear. This is the first systematic review and meta-analysis comparing outcomes of surgical resection of ICC in HCV-positive patients *vs* HCV-negative patients. Our primary outcomes include overall survival (OS) and recurrence-free survival; secondary outcomes include perioperative mortality, operation duration, blood loss and recurrence. Our review and analysis demonstrated worse OS in HCV-positive patients compared to HCV-negative patients.

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INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver malignancy accounting for 15% of all primary liver malignancy, after hepatocellular carcinoma (HCC)[1]. Though rare, its incidence and mortality rates have increased in recent years[2,3]. Incidence amongst males increased from 1.51 per 100000 in 1993-1997 to 4.07 per 100000 in 2013-2017 and incidence amongst females increased from 1.73 per 100000 to 2.95 per 100000 respectively[4]. Mortality rates in cholangiocarcinoma have been reported to be up to 2 deaths per 100,000 in the United States, with mortality rates 3 times higher in Asia, and are still increasing[5]. Surgery remains the only potentially curative treatment modality in resectable ICC. However, the presentation for ICC is non-specific and patients may be diagnosed late; a retrospective study on patients with ICC demonstrated that 54% of ICCs were unresectable at diagnosis[6].

Common causes of ICC include cirrhosis, alcohol, hepatotoxins, chronic viral hepatitis, hepatolithiasis and liver fluke infections[7,8]. Patients with hepatitis C virus (HCV) have a 2-fold increase in risk of developing ICC compared to the general population[9]. To add on, a meta-analysis by Wang *et al*[10] in 2016 on 2842 patients with ICC showed that HCV was associated with worse survival [hazard ratio (HR) 2.64, 95% confidence interval (CI): 1.77-3.93] compared to controls. However, their study included patients who received various forms of treatment, ranging from curative surgery to palliative treatment. In 50 patients who received liver resection (LR) for ICC, Hai *et al*[11] however showed that HCV was not a predictor of survival following LR. While HCV is a significant risk factor for ICC, the prognostic significance of HCV remains uncertain for patients with ICC following LR. This study aims to perform a systematic review and meta-analysis to compare the survival between patients with HCV infection (*i.e.* HCV+) *vs* those without HCV (*i.e.* HCV-) in ICC following LR.

MATERIALS AND METHODS

Study selection and search strategy

This systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines[12]. The protocol for this systematic review and meta-analysis was registered at PROSPERO (Ref no: CRD42023459605). A systematic search of the following databases (PubMed, EMBASE, The Cochrane Library and Scopus) was conducted for studies published from inception to 19th August 2023. A

combination of the following search terms was used: "cholangiocarcinoma" or "bile duct cancer", and "hepatectomy" or "liver resection", and "hepatitis C" or "HCV". The search was restricted to the title, abstract and keywords. The complete search strategy is appended in Supplementary Table 1. Search strategies for other databases were modified from the initial search strategy done on PubMed based on the database requirements.

Included studies were randomised controlled trials (RCTs) and non-RCTs on patients ≥ 18 years old with a diagnosis of ICC who underwent LR, and compared outcomes between patients with HCV+ *vs* HCV-. Exclusion criteria were studies: (1) On other types of liver malignancies (*e.g.*, HCC) or underwent liver transplantation (LT); (2) single-arm studies without comparison; (3) which did not include our outcome of interest; (4) on the same cohort of patients; and (5) based on article type (non-English studies, conference abstracts, case report or series, editorials, expert opinions, and review articles without original data). There were no studies which reported on the same cohort of patients. LR was defined as any form of surgical resection of the liver, including wedge resection, anatomical resection such as minor LR and major LR, and non-anatomical resection. HCV+ was defined as presence of anti-HCV antibodies detected on serology.

All cross-references were screened for potentially relevant studies not identified by the initial literature search. After removing duplicates, two authors screened abstracts for potential inclusion screening independently (Cheo FY and Chan KS). The included studies' full texts were reviewed and selected based on the inclusion and exclusion criteria. All discrepancies were resolved after review by the senior author (Shelat VG).

Data extraction

Data extraction was independently performed by two authors (Cheo FY and Chan KS). The following variables were extracted from each study: Publication details (name of first author, publication year and country), study characteristics (sample size, sex, age, Child-Pugh score, presence of cirrhosis, baseline tumor markers (alpha-fetoprotein, carbohydrate antigen 19-9, carcinoembryonic antigen, and tumor size). Our primary outcomes were overall survival (OS) and recurrence free survival (RFS). Our secondary outcomes were perioperative outcomes, including mortality, operation duration, blood loss, and tumor recurrence.

Assessment of study quality

Two authors (Cheo FY and Chan KS) independently assessed the included studies' quality. Observational studies were assessed using the modified Newcastle-Ottawa scale (Supplementary Table 2)[13]. No RCTs were included in this study. Only observational studies with sufficient quality (articles with a score >6) were included. Disagreements between authors were resolved by discussion with the senior author (Shelat VG).

Statistical analysis

Study variables were extracted to Microsoft Excel 365 (Microsoft®, Washington, United States). Categorical variables were described as *n* (%), and continuous variables were expressed as mean ± SD, or median [interquartile range (IQR)] unless otherwise specified. For continuous variables expressed only in median and range or IQR, mean and SD were estimated from median and range values using methods described by Wan *et al*[14]. Meta-analysis was performed using RevMan 5.4 (Review Manager 5.4, The Nordic Cochrane Centre, Copenhagen, Denmark). For cumulative OS and RFS, HR and standard error (SE) were estimated indirectly according to the methods described by Parmar *et al*[15]. Pooled HR was calculated through the inverse-variance method using the natural logarithm of HR [ln(HR)] and SE[16]. For studies that used univariate and multivariate analysis to assess the impact of HCV infection, the effect size from the multivariate analysis was used in our pooled analysis. Dichotomous outcomes were pooled and calculated using the Mantel-Haenszel method and expressed as odds ratio (OR) with 95%CI. Continuous outcomes were pooled and calculated using the inverse variance method and expressed as mean difference (MD) with 95%CI. Heterogeneity was assessed using Cochrane's Q and quantified by I^2. If data was heterogenous (defined as $I^2 > 50\%$), a random-effects model using the DerSimonian and Laird approach was used. Statistical significance was defined as $P < 0.05$. Publication bias was investigated using funnel plots. Due to low sample size, quantitative analysis was not performed for short-term intra-operative and post-operative outcomes.

RESULTS

The systematic search identified 697 articles from the four databases. There were 492 articles after removal of the duplicates. Titles and abstracts of all the identified articles were screened. The remaining 53 articles underwent full-text review, of which seven articles were included in the final analysis[11,17-22]. The PRISMA diagram for the study selection process is appended in Figure 1. The funnel plots are appended in Supplementary Figure 1.

Study characteristics

There were seven studies with 1181 patients (HCV+ *n* = 205, HCV- *n* = 976)[11,17-22]. Kaibori *et al*[19] performed propensity score matching (PSM) analysis to derive their cohorts; only the PSM cohort was analysed in our study. Uenishi *et al*[20] performed univariate and multivariate analyses on the impact of HCV infection on outcomes of surgical resection in cholangiocarcinoma, of which outcomes of the multivariate analysis was included in our study. Yang *et al*[21] reported on OS of HCV+ and HCV- groups in the early relapse subgroup (within 24 mo), which we excluded from our quantitative analysis of OS due to selection bias and misrepresentation of the entire cohort of ICC. While the study by Hai *et al*[11] performed Kaplan-Meier analysis on OS, HR and SE could not be estimated due to the lack of clarity of the Kaplan-Meier curve; clinical demographics and other outcomes were still included. The study by Terakawa *et al*[22] was

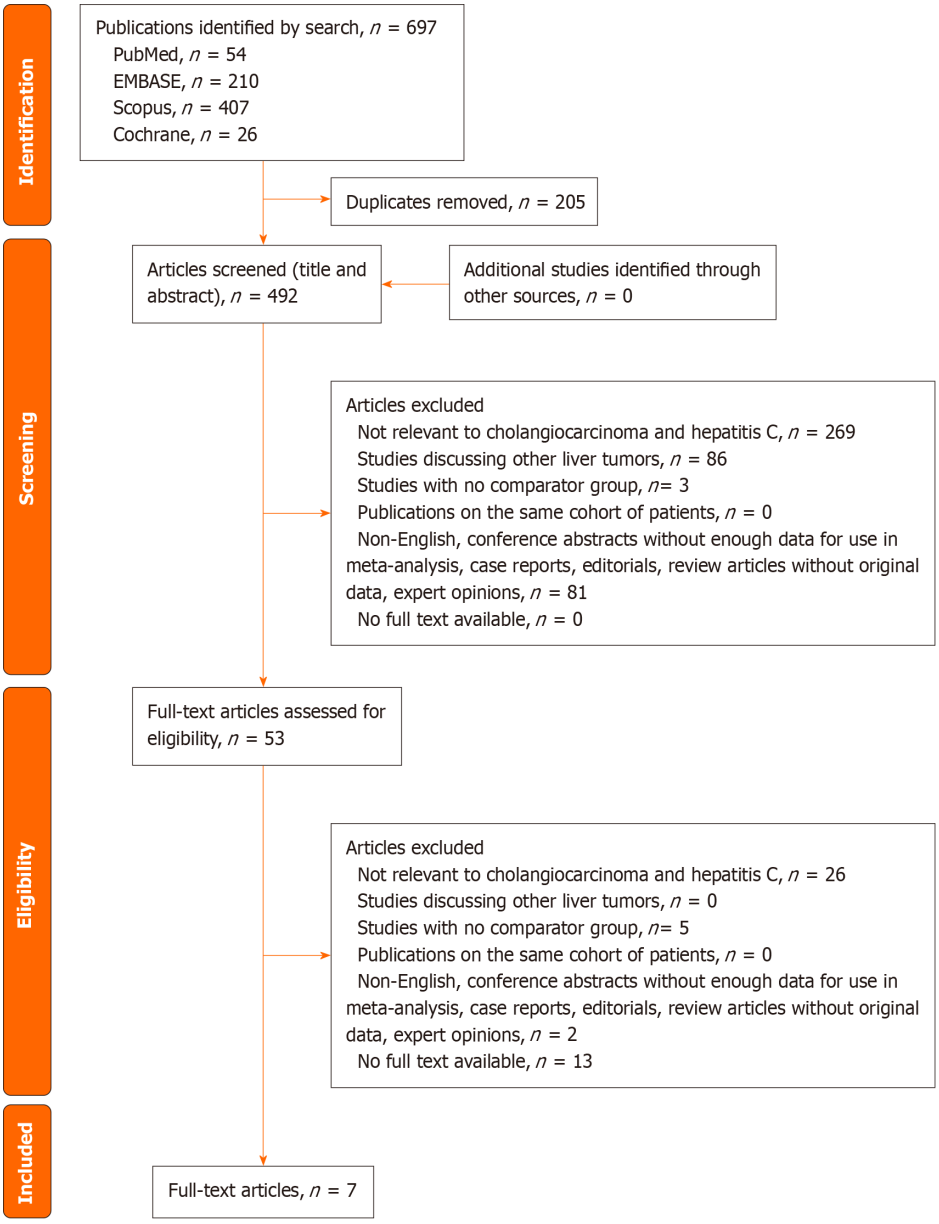

Figure 1 Preferred Reporting Items for Systematic reviews and Meta-Analyses flowchart for study selection.

reported in Japanese; however, as the tables and figures were in English, relevant data such as survival outcomes were included in our study to avoid dilution to power and sample size.

Clinical demographics

The study characteristics and patient demographics of individual studies were summarized in Table 1. The overall mean age was 65.0 years, and 17.2% (n = 46/267) patients had cirrhosis. There were 17.7% (n = 55/311) patients with multiple tumors on diagnosis, and the mean tumor size ranged from 3.6-6.4 cm. 12.5% of patients (n = 16/128) had synchronous HCC lesions. Pooled analysis showed that age, incidence of male patients, albumin, bilirubin, platelets, tumor size, incidence of multiple tumors, vascular invasion, bile duct invasion, lymph node metastases, stage 4 disease were comparable between HCV+ and HCV- (Table 2). However, alanine transaminase (ALT) and aspartate transaminase (AST) levels were significantly higher in HCV+ (ALT: MD +22.2, 95%CI: 13.75, 30.65; AST: MD +27.27, 95%CI: 20.20, 34.34) compared with HCV-. There were also more patients with cirrhosis (OR 5.78, 95%CI: 1.38, 24.14, P = 0.02), poorly differentiated disease (OR 2.55, 95%CI: 1.34, 4.82, P = 0.004), and concomitant HCC (OR 8.31, 95%CI: 2.36, 29.26, P = 0.001) in the HCV+ group compared to HCV- group.

Oncological outcomes

Table 3 summarizes the survival outcomes reported in individual studies. Four studies involving 841 patients (HCV+ n = 145, HCV- n = 696) reported on OS[18-20,22]. Pooled HR showed statistically significantly worse OS in the HCV+ group (HR 2.05, 95%CI: 1.46, 2.88, P < 0.0001) (Figure 2). Heterogeneity was not significant among the studies (I^2 = 0%, P = 0.56). The study by Cai *et al*[18] had very few HCV+ patients compared with HCV- patients (HCV+ n = 3, HCV- n = 527). In

Table 1 Baseline characteristics and patient demographics of the included studies (*n* = 7)

No.	Ref.	Study design	Study period	Country	Sample size, *n* (%)	Age, yr	Males, *n* (%)	Tumor size, cm	Cirrhosis, *n*	Tumor stage	Tumor grade
1	Hai *et al* [11], 2005	Retrospective	Jan 1997-Dec 2002	Japan	HCV+: 17; HCV-: 21	HCV+: 69.0 ± 4.9; HCV-: 60.6 ± 12.4	HCV+: 10; HCV-: 13	HCV+: 3.6 ± 2.3; HCV-: 6.4 ± 4.5	NR	HCV+:I: 4, II: 4, III: 3, IV: 6; HCV-: I: 0, II: 6, III: 7, IV: 8	NR
2	Kaibori *et al*[19], 2021[1]	Retrospective	Jan 2000-Dec 2007	Japan	HCV+: 102; HCV-: 102	HCV+: ≥ 70: 56/102; HCV-: ≥ 70: 59/102	HCV+: 64; HCV-: 74	HCV+: ≥ 3.5cm: 69/102; HCV-: ≥ 3.5cm: 61/102	HCV+: 24; HCV-: 8	NR	HCV+:well: 14, moderate: 49, poor: 27; HCV-: well: 21, moderate: 53, poor: 11
3	Uenishi *et al*[20], 2014	Retrospective	Jan 2000-Dec 2011	Japan	HCV+: 33 HCV-: 57	HCV+: 66.9 ± 9.0; HCV-: 64.3 ± 11.2	HCV+: 23; HCV-: 38	HCV+: 4.7 ± 1.7; HCV-: 4.8 ± 2.6	HCV+: 14; HCV-: 3	HCV+:I: 12, II: 7, III: 5, IV: 9; HCV-: I: 18, II: 9, III: 5, IV: 25	HCV+: poor: 7; HCV-: poor: 7
4	Cai *et al* [18], 2021	Retrospective	Dec 2008-Dec 2017	China	HCV+: 3; HCV-: 527	NR	NR	NR	NR	NR	NR
5	Ariizumi *et al*[17], 2011	Retrospective	1989-2008	Japan	HCV+: 42; HCV-: 92	NR	NR	NR	NR	NR	NR
6	Yang *et al* [21], 2019	Retrospective	Jan 2005-Dec 2011	China	HCV+: 1; HCV-: 167	NR	NR	NR	NR	NR	NR
7	Terakawa *et al*[22], 2004	Retrospective	Jan 1992-Dec 2001	Japan	HCV+: 7; HCV-: 10	HCV+:64.0 ± 3.0; HCV-: 66.0 ± 3.0	HCV+: 4; HCV-: 8	HCV+: 5.0 ± 1.2; HCV-: 5.1 ± 1.0	NR	HCV+:II: 1, III: 3, IV: 3HCV-: II: 2, III: 4, IV: 4	HCV+:well: 1, moderate: 4; HCV-: well: 1, moderate: 5

[1]Values included in this study is obtained after propensity score matching. HCV: Hepatitis C Virus; NR: Not reported.

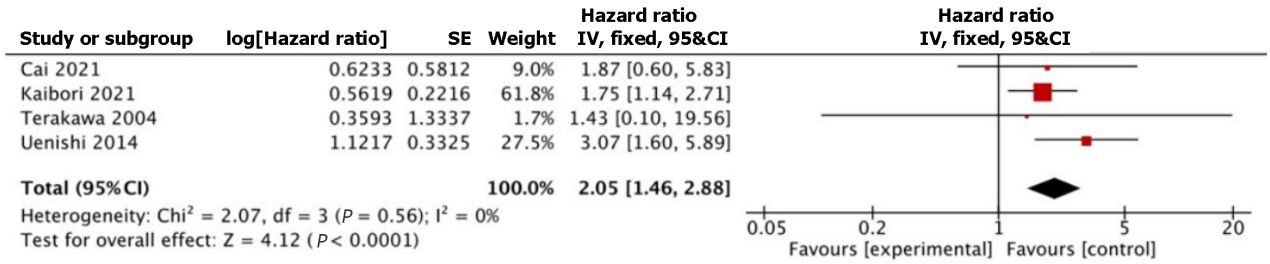

Figure 2 Comparison of overall survival between hepatitis C virus-positive group and hepatitis C virus-negative group in patients with intrahepatic cholangiocarcinoma post-liver resection. CI: Confidence interval.

view of this, sensitivity analysis was performed to exclude their study; OS remained significantly worse in HCV+ group (HR 2.07, 95%CI: 1.45, 2.96, $P < 0.0001$) compared to HCV- group.

There were 2 studies involving 294 patients (HCV+ $n = 135$, HCV- $n = 159$) which reported on RFS[19,20]. Meta-analysis was not performed for RFS due to the small sample size and limitations in interpretation. Kaibori *et al*[19] reported significantly worse RFS in the HCV+ group (HR 1.61, 95%CI: 1.09, 2.38, $P = 0.016$) compared to the HCV- group. Uenishi *et al*[20] reported comparable RFS between the HCV+ group and HCV- group (HR 1.59, 95%CI: 0.74, 3.41, $P = 0.24$).

Secondary outcomes

There was one study which reported on incidence of post-operative mortality. Uenishi *et al*[20] reported in-hospital mortality of 13% in HCV+ group ($n = 3/33$) and 4.8% in HCV- group ($n = 1/57$). However this did not reach statistical significance ($P = 0.609$).

Table 2 Summary of effect size of different study variables and outcomes between hepatitis C virus-positive group and hepatitis C virus-negative group

No.	Study variables and/or outcomes	No. of data sets	Total number of patients, *n* (HCV+/HCV-)	No. of patients (%)		Effect size, OR (95%CI)/MD (95%CI)/HR (95%CI)[1]	*P* value	I^2, %	Model used
				HCV+	HCV-				
Demographics and histopathological findings									
1	Age, yr	4	349 (159/190)	NA		2.55 (-3.09, 8.20)	0.38	82	RE
2	Male	4	349 (159/190)	101 (63.5)	133 (70.0)	0.74 (0.47, 1.17)	0.20	0	FE
3	ALT, IU/L	2	294 (135/159)	NA		22.20 (13.75, 30.65)	< 0.00001[a]	0	FE
4	AST, IU/L	2	294 (135/159)	NA		27.27 (20.20, 34.34)	< 0.00001[a]	0	FE
5	Albumin, g/L	2	294 (135/159)	NA		-0.11 (-0.34, 0.12)	0.34	71	RE
6	Bilirubin, umol/L	2	294 (135/159)	NA		-0.07 (-0.33, 0.19)	0.61	84	RE
7	Platelets, $10^4/mm^3$	2	294 (135/159)	NA		-1.96 (-5.88, 1.96)	0.33	71	RE
8	Tumor size, cm	3	145 (57/88)	NA		-0.61 (-1.79, 0.57)	0.31	62	RE
9	Multiple tumors	3	311 (142/169)	25 (17.6)	30 (17.8)	1.12 (0.61, 2.06)	0.70	0	FE
10	Cirrhosis	2	294 (135/159)	35 (25.9)	11 (6.9)	5.78 (1.38, 24.14)	0.02**a**	69	RE
11	Vascular invasion	3	145 (57/88)	20 (35.1)	36 (40.9)	0.76 (0.37, 1.54)	0.45	0	FE
12	Bile duct invasion	2	294 (135/159)	53 (39.3)	64 (40.3)	0.86 (0.53, 1.39)	0.53	0	FE
13	Lymph node metastases	4	349 (159/190)	41 (25.8)	54 (28.4)	0.85 (0.53, 1.37)	0.51	15	FE
14	Stage 4	3	145 (57/88)	18 (31.6)	37 (42.0)	0.64 (0.32, 1.28)	0.21	0	FE
15	Poorly differentiated	2	273 (127/146)	34 (26.8)	18 (12.3)	2.55 (1.34, 4.82)	0.004[a]	0	FE
16	Simultaneous HCC lesions	2	128 (50/78)	13 (26.0)	3 (3.8)	8.31 (2.36, 29.26)	0.001[a]	0	FE
Outcomes									
17	Overall survival	4	841 (145/696)	NA		2.05 (1.46, 2.88)	<0.0001[a]	0	FE

[1]Odds ratio and 95% confidence interval (CI) was used for dichotomous outcomes, mean difference and 95%CI was used for continuous outcomes, and hazards ratio and 95%CI was used for time-to-event outcomes.

[a]Data with statistical significance ($P < 0.05$).

ALT: Alanine transaminase; AST: Aspartate transaminase; CI: Confidence interval; FE: Fixed-effects; HCV: Hepatitis C virus; HR: Hazards ratio; I^2: Heterogeneity; MD: Mean difference; NA: Not applicable; OR: Odds ratio; RE: Random-effects.

There was one study which reported on operative time and intraoperative blood loss. Terakawaet al[22] reported mean operative duration of 359 ± 74 min in the HCV+ group compared to 336 ± 34 min in the HCV- group. They additionally reported mean intraoperative blood loss of 2037 ± 577 mL in HCV+ group compared to 1226 ± 269 mL in HCV- group. However, no comparative statistical analysis was performed to compare between HCV+ and HCV- groups[22].

There were two studies which reported on tumor recurrence post-LR; Yang *et al*[21] reported comparable tumor recurrence (both intrahepatic and extrahepatic) in HCV+ group and HCV- group (HR 3.28, 95%CI: 0.80, 13.51, $P = 0.098$) . Kaibori *et al*[19] showed comparable incidence of intrahepatic recurrence [HCV+: 36% ($n = 33/92$), HCV-: 30% ($n = 28/94$); $P = 0.467$] and extrahepatic recurrence [HCV+: 36% ($n = 25/69$) *vs* HCV-: 27% ($n = 18/67$); $P = 0.322$] between HCV+ and HCV- groups.

DISCUSSION

Hepatitis B virus (HBV) and HCV infection are significant risk factors involved in the pathogenesis of cholangiocar-

Table 3 Survival outcomes reported in the included studies (*n* = 7)

No.	Ref.	1-yr OS, %	1-yr DFS, %	3-yr OS, %	3-yr RFS, %	3-yr DFS, %	5-yr OS, %	5-yr RFS, %
1	Hai *et al*[11], 2005	HCV+: 70.9; HCV-: 75.6	HCV+: 55.7; HCV-: 49.0	HCV+: 41.4; HCV-: 30.1	NR	HCV+: 27.9; HCV-: 32.7	NR	NR
2	Kaibori *et al*[19], 2021[1]	NR	NR	NR	NR	NR	HCV+: 32.2; HCV-: 44.7	HCV+: 25.0; HCV-: 31.3
3	Uenishi *et al*[20], 2014	NR	NR	HCV+: 30.6; HCV-: 65.6	HCV+: 29.9; HCV-: 31.4	NR	HCV+: 21.9; HCV-: 32.8	HCV+: 22.4; HCV-: 20.6
4	Cai *et al*[18], 2021	NR	NR	NR	NR	NR	NR	NR
5	Ariizumi *et al* [17], 2011	NR	NR	NR	NR	NR	HCV+: 53; HCV-: 32	NR
6	Yang *et al*[21], 2019	NR	NR	NR	NR	NR	NR	NR
7	Terakawa *et al* [22], 2004	NR	NR	NR	NR	NR	NR	NR

[1]Values included is this study is obtained after propensity score matching.
DFS: Disease-free survival; HCV: Hepatitis C virus; NR: Not reported; OS: Overall survival; RFS: Recurrence-free survival.

cinoma. Interestingly, while HBV infection has been shown to provide favourable prognosis for patients with cholangiocarcinoma, HCV+ is associated with shorter OS compared to HCV- patients[10]. Our study similarly showed that HCV+ is associated with worse OS in ICC following LR. However, there is limited data on peri-operative outcomes.

Risk factors for ICC include biliary tract diseases such as primary sclerosing cholangitis, recurrent pyogenic cholangitis, primary biliary cirrhosis, congenital malformations of the bile duct (*i.e* choledochal cysts), cirrhosis and chemical exposure[23]. Incidence of HCV has been reported to be 13.8%-23.1% in ICC[24,25]. Various mechanisms have been proposed on the role of HCV in the pathogenesis of ICC[26]. One postulation is that cholangiocytes and hepatocytes share the same liver progenitor cell; cholangiocytes express receptors which are susceptible to HCV infection[27]. Another postulation is that the initial HCV infection of hepatocytes result in transdifferentiation of hepatocytes into cholangiocytes [28]. Interaction of cholangiocytes with HCV protein induces chronic biliary inflammation with resulting development of ICC.

HCV is a significant risk factor in the development of cholangiocarcinoma[29]. Globally, HCV is strongly associated with cholangiocarcinoma, especially in the Western populations[9]. Therefore, an understanding of its impact on outcomes helps to guide clinical decisions and development of treatment pathways. A previous meta-analysis by Wang *et al*[10] explored the impact of HCV infection on survival outcomes in patients with ICC, regardless of treatment modality, and showed poorer prognosis in HCV+ patients. However, we wish to understand the implications of HCV on long-term outcomes following curative LR in ICC. Since then, more studies comparing LR outcomes in ICC between HCV+ and HCV- groups have been published. This updated meta-analysis included five new studies with 1053 patients; we showed that HCV+ patients had worse OS compared to HCV- in patients who received curative LR for ICC[17-19,21,22]. We hypothesize potential reasons for these observations which are discussed below.

Several factors prognosticate OS and RFS in ICC following LR, including cirrhosis, positive surgical margins, tumor morphology patterns, tumor size, nodal involvement, and vascular invasion[30,31]. Chronic HCV is recognised as a significant precursor to liver cirrhosis, due to its process of chronic hepatocellular injury leading to chronic inflammation, resulting in scarring and fibrosis[32]. Cirrhosis has been associated with worse short-term and long-term survival; for instance, Zaydfudim *et al*[33] reported higher postoperative mortality (OR = 2.24; 95%CI: 1.16, 4.34, $P = 0.016$) in patients with cirrhosis; Sasaki *et al*[34] reported worse 5-year disease-specific survival (75.4% in patients with normal liver function *vs* 59.1% in patients with cirrhosis, $P = 0.04$) in cirrhotic patients as well. Liver cirrhosis is also a risk factor of tumor recurrence in cholangiocarcinoma; Tsilimigras *et al*[35] reported a significant association between cirrhosis and very early recurrence (within 6 mo after resection) of ICC post-LR (OR 2.06, 95%CI: 1.25, 3.40, $P = 0.005$) and Zhang *et al* [36] reported a significant association between cirrhosis and late intrahepatic recurrence (more than 24 mo after resection) (HR 1.99, 95%CI: 1.11, 3.56, $P = 0.019$). This may be due to the increased carcinogenic potential of remnant cirrhotic liver and biliary system which predisposes to neocarcinogenesis, resulting in de novo recurrence of cholangiocarcinoma[26]. While our meta-analysis showed that HCV+ group had worse OS compared to HCV- group, there was also increased incidence of liver cirrhosis in the HCV+ group (OR 5.78, 95%CI: 1.38, 24.14, $P = 0.02$). Liver cirrhosis may be a confounding factor for worse OS in HCV+ ICC as discussed above, rather than HCV alone.

An important consideration in surgical candidates is the risk of post hepatectomy liver failure (PHLF)[37]; Lei *et al*[38] showed that patients with PHLF diagnosed using the 50-50 criteria was independently associated with higher 90-d mortality (HR 8.63, 95%CI: 3.33-22.35, $P < 0.001$). Clinically relevant PHLF (grade B/C) has been reported to be associated with postoperative 90-d mortality (OR 7.26, 95%CI: 2.90, 18.17) and significantly worse long-term survival outcomes (HR 1.90, 95%CI:1.32, 2.71)[37]. Post-LR, adequate functional liver remnant (FLR) is required to sustain the body's metabolic, synthetic and detoxifying requirements[39]. Due to chronic hepatocellular injury leading to scarring and fibrosis in cirrhotic livers, these functions are greatly reduced, predisposing to liver failure[40]. Current guidelines recommend FLR

of > 30% in patients with liver steatosis and > 40% in patients with cirrhosis to reduce risk of PHLF[41,42]. One of the possible reasons for worse OS in HCV+ ICC may be due to PHLF in the HCV+ group due to higher incidence of cirrhosis. Unfortunately, in our review, none of the included studies described the incidence of PHLF; this remains a postulation to be validated, and correlation cannot be drawn. Nevertheless, other markers have been used to predict risk of PHLF, such as the use of indocyanine green retention rate at 15 minutes (ICGR15). Makuuchi's criteria serve as a guide to assess the extent of hepatectomy based on ICGR15 to reduce risk of PHLF[43]. In our review, there was a mix of studies reporting either comparable ICGR15 between HCV+ and HCV- groups (such as the study by Hai *et al*[11] with comparable incidence of ICGR15 > 10% in HCV+ group (n = 11/17, 64.7%) compared to HCV- group (n = 4/21, 19.0%), P = 0.0656), or higher ICGR15 in HCV+ compared to HCV- (such as the study by Kaibori *et al*[19] with 71% with ICGR15 ≥10% in HCV+ compared to 48% in HCV-). Whether or not PHLF is a cause of worse OS in HCV+ ICC following LR remains to be answered.

Another possible reason for poorer prognosis in HCV+ patients may be attributed to synchronous or metachronous HCC in HCV+ patients. Chronic HCV infection is the leading cause of HCC in Western countries. HCV is also associated with a large proportion of HCC in certain Asian and African countries[44,45]. Carcinogenesis of HCC and cholangiocarcinoma in the background of chronic HCV-induced cirrhosis share similarities and has been postulated to be associated with the occurrence of synchronous or metachronous HCC and cholangiocarcinoma lesions[46]. A literature review of reported synchronous HCC and cholangiocarcinoma cases by Watanabe *et al*[47] found that 72.7% of cases were positive for HCV. Survival outcomes in patients with synchronous or metachronous HCC and cholangiocarcinoma are generally poorer and may distort survival outcomes in HCV+ group[48]. In our study, incidence of simultaneous HCC lesions found on pathologic studies is significantly higher in HCV+ group compared to HCV- group (OR 8.31, 95%CI: 2.36, 29.26, P = 0.001), which may confound and contribute to worse outcomes in the HCV+ group.

Tumor biology is another important consideration in survival. Higher tumor grade and poorly differentiated tumors confer a worse prognosis on survival. A retrospective study by Mao *et al*[49] identified tumor differentiation as an independent predictor of higher postoperative mortality in cholangiocarcinoma (relative risk 1.356, 95%CI: 1.081, 1.699, P = 0.008). Nickkholgh *et al*[50] reported that high grade tumor (defined as Grade 3-4) was an independent determinant of recurrence in ICC post-resection (HR 1.63, 95%CI: 1.04, 2.55, P = 0.034). HCV-induced development and progression of liver fibrosis involve epithelial-mesenchymal transition (EMT) of cholangiocytes, resulting in reduced expression of E-cadherin, which is associated with poor tumor differentiation in cholangiocarcinoma[26,51,52]. In our study, incidence of poorly differentiated ICC was significantly greater in HCV+ group compared to HCV- group (OR 2.55, 95%CI: 1.34, 4.82, P = 0.004). This may have consequently resulted in worse OS in the HCV+ group.

Advanced tumor stage and metastatic disease are poor prognostic factors in cholangiocarcinoma[53]. In advanced tumors, several factors contribute to more aggressive tumor behavior. Notably, presence of vascular invasion increases the risk of haematogenous spread of tumor cells, and tumor multiplicity provide additional nidus for tumor to grow and spread from[54-56]. Expectedly as well, nodal disease has been shown to be associated with worse survival (22.9 mo *vs* 30.1 mo, P = 0.03)[57]. The question lies in whether HCV+ increases the risk of more advanced disease or nodal metastases, since HCV infection results in EMT as described above[58]. This question remains unanswered based on our findings, but may be due to the low sample size of the included studies.

The advent of direct-acting antivirals (DAAs) have revolutionized the treatment of HCV, where it is possible to achieve a cure for hepatitis C[59]. The American Association for the Study of Liver Diseases recommends first-line therapy with glecaprevir/pibrentasvir and sofosbuvir/velpatasvir for 8 wk and 12 wk respectively for treatment-naïve adults[60]. However, there are no guidelines on antiviral therapy duration for patients with HCV+ HCC or ICC. In HCV-related HCC, HCV eradication therapy has been proven to improve long-term outcomes of HCC undergoing curative treatment [61-63]. Further meta-analyses suggest benefits of HCV treatment on long-term HCC survival outcomes[64,65]. While the literature on the utility of DAAs in HCV+ ICC is scarce, the oncogenesis of ICC is similar to that of HCC. Hence, we theorize similar benefits of HCV eradication therapy in the ICC population.

Adjuvant chemotherapy is recommended for patients with resected cholangiocarcinoma[66]. The American Society of Clinical Oncology recommends the use of adjuvant capecitabine as first-line therapy for 6 mo[67]. However, certain chemotherapy agents are hepatotoxic and may exacerbate or accelerate fibrosis in HCV+ patients with chronic liver inflammation. Studies have suggested that treatment of HCV infection may also reverse cirrhosis in some group of patients (*e.g.* those without decompensated liver cirrhosis), allowing for the use of adjunct treatment such as chemotherapy[68]. Unfortunately, the use of adjuvant chemotherapy and underlying liver function was not discussed in the included studies and this falls beyond the scope of our study. The combined role of DAAs and adjuvant chemotherapy on underlying liver function and long-term survival should be evaluated.

While our study excluded patients who underwent LT, the use of LT in treating ICC is worth exploring. LT was previously contraindicated in managing ICC due to poor outcomes and high recurrence post-LT. Initial studies reported 3-year OS post-LT ranging from 4.9%-39.0% without receiving pre-transplant treatment and 3-year RFS rate of 28.8-35.0% [69-71]. However, recent studies have reported reasonable outcomes in certain groups of patients with ICC who received LT, with 3-year OS rates post-LT ranging from 47.9%-83.3% and 5-year OS rates ranging from 31.3%-83.3%. 3-year RFS rates also ranged from 41.7%-52.0% in newer studies[72-74]. Transplant outcomes have improved drastically due to improved effectiveness of neoadjuvant therapy such as gemcitabine-based systemic chemotherapy and locoregional therapy including trans-arterial chemoembolization and radiofrequency ablation, in addition to protocols to determine eligibility for LT in patients who demonstrate disease stability or pathological response to these pre-transplant treatment modalities[73-75]. With these improvements in preoperative treatment and a more stringent organ recipient selection process, LT may provide an alternative treatment of cure as standard of care for ICC in the future. Additionally, LT also deals with the problem of cirrhosis and PHLF that comes with LR, which may be a contributing factor to worse survival. With ongoing trials assessing outcomes of LT in ICC currently underway, we anticipate treatment of cholangiocarcinoma

to evolve in the future[76-78]. Although not explored in our study, subsequent studies could analyse the impact of HCV infection on outcomes following other treatment modalities in ICC.

There are a few limitations in our study. All the included studies were retrospective observational studies which have inherent selection bias. The absence of high-quality evidence from RCTs and prospective studies may limit interpretation of the outcomes from our analysis. Subsequent studies should employ methods such as PSM and RCTs to reduce bias for more conclusive results. Nevertheless, quality assessment was performed for the included studies and all the included studies had at least moderate quality evidence. The number of studies included in this meta-analysis is relatively small due to our strict inclusion criteria of studies comparing post-hepatectomy outcomes of ICC in HCV+ and HCV- subgroups. All included studies were conducted in Asia, namely Japan and China, despite including ICC globally, hence causing possible limitations in the generalizability of our results. Global incidence of cholangiocarcinoma is highest in Asia, especially Japan[79]. However, incidence of cholangiocarcinoma is rising in Western countries over the past decade, of which their population is underrepresented in our study[80]. Prevalence of chronic HCV infection share a different distribution globally, with middle-low-income countries in the Eastern Mediterranean and European regions suffering the highest burden of disease[81]. Ideally, a more heterogenous sample including these populations would produce results that may be more representative of the global population, hence future studies involving patients from regions of high HCV and cholangiocarcinoma prevalence will provide more insight. We could not perform meta-analysis on RFS and our secondary outcomes due to inadequate data from our included studies. Lastly, this study did not include subgroup analyses of tumors undergoing major hepatectomy. Performing major hepatectomy on a background cirrhotic liver or chronically HCV-infected liver has its additional risks (*e.g.* PHLF and post-operative mortality). Thus, a separate analysis focusing on this subgroup may provide valuable insight and guidance in management.

CONCLUSION

Our meta-analysis demonstrated that HCV infection is associated with significantly worse OS in ICC patients undergoing LR with curative intent. Further studies of the underlying mechanisms of oncogenesis of the biliary tree in HCV infection, including genetic and basic science studies are warranted to understand its disease process. More prospective studies with PSM-derived cohorts including analysis of other aspects of treatment such as PHLF and liver augmentation strategies should be conducted to validate our findings.

ARTICLE HIGHLIGHTS

Research background

Incidence of intrahepatic cholangiocarcinoma (ICC) has been rising over the past decade. Hepatitis C virus (HCV) infection is an important risk factor in the development of ICC. Currently, liver resection (LR) remains the only curative treatment modality for ICC. Our study aims to study the outcomes of LR in ICC patients with HCV-positive (HCV+) compared to HCV-negative (HCV-) ICC patients.

Research motivation

Long-term outcomes of curative LR in ICC can be affected by patient and tumor characteristics. The impact of HCV infection on post-LR outcomes should be reviewed and quantitatively concluded.

Research objectives

We aim to identify HCV+ patients as a high-risk subgroup amongst ICC patients undergoing curative LR. Our analysis concluded that HCV+ patients had worse overall survival compared to HCV- patients following LR. Our findings act as a stepping stone for future studies to validate our findings, to determine a cause for this outcome, as well as to devise strategies to improve outcomes in HCV+ ICC patients undergoing curative LR.

Research methods

Four databases (PubMed, EMBASE, Scopus and The Cochrane Library) were systematically searched for relevant studies, which were subsequently screened for inclusion in our study based on our inclusion criteria. We assessed the quality of included observational studies using the modified Newcastle-Ottawa Scale. There were no randomised controlled trials included in our study. Our primary outcomes were overall survival (OS) and recurrence-free survival. Secondary outcomes include perioperative mortality, operation duration, blood loss, intrahepatic and extrahepatic recurrence. Study variables, primary and secondary outcomes were extracted from included studies. Pooled hazard ratio (HR) was calculated through the inverse-variance method using the natural logarithm of HR [ln (HR)] and standard error. Dichotomous outcomes were pooled and calculated using the Mantel-Haenszel method and expressed as odds ratio (OR) with 95% confidence interval (CI). Continuous outcomes were pooled and calculated using the inverse variance method and expressed as mean difference with 95%CI.

Research results

Our meta-analysis demonstrated significantly worse OS in HCV+ patients with ICC that underwent curative resection

compared to HCV- patients (HR 2.05, 95%CI: 1.46, 2.88, $P < 0.0001$). Our analysis also showed increased incidence of cirrhosis (OR 5.78, 95%CI: 1.38, 24.14, $P = 0.02$), poorly differentiated tumors (OR 2.55, 95%CI: 1.34, 4.82, $P = 0.004$), as well as simultaneous hepatocellular carcinoma (HCC) lesions in HCV+ patients (OR 8.31, 95%CI: 2.36, 29.26, $P = 0.001$) compared with HCV- patients. Our findings identify HCV infection as a significant poor prognostic factor in ICC patients undergoing curative LR and as a significant risk factor of liver cirrhosis, poor tumor differentiation and incidence of simultaneous HCC lesions. However, the presence of increased liver cirrhosis and poor tumor differentiation may be confounding factors for worse OS in HCV+ patients. No statistically significant differences were noted between HCV+ and tumor stage, tumor invasion and metastases in our study.

Research conclusions

Our study concluded that HCV infection is associated with significantly worse OS outcomes in ICC post-LR. This may be confounded by increased incidence of cirrhosis and poorly differentiated tumors with HCV infection. The exact pathophysiology and confirmation of our findings ought to be explored in future well-designed prospective studies. The role of viral eradication therapy and chemotherapy in this subgroup of patients should also be explored.

Research perspectives

Future research should be performed with randomized controlled trials or propensity score matched cohorts to validate our findings. Further studies should also explore the role of adjuncts such as anti-viral therapy and adjuvant chemotherapy in HCV+ ICC patients who underwent curative LR.

FOOTNOTES

Author contributions: Cheo FY conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, revising the article, final approval; Chan KS conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, critical revision, final approval; Shelat VG interpretation of data, revising the article, critical revision, final approval.

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META-ANALYSIS

Efficacy and safety of bamlanivimab in patients with COVID-19: A systematic review and meta-analysis

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Abstract

BACKGROUND

Monoclonal antibodies (mAbs) have shown clinical benefits against coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several studies have reported the use of bamlanivimab as a promising treatment option for COVID-19.

AIM

To synthesize the latest evidence for the efficacy and safety of bamlanivimab alone in the treatment of adult patients with COVID-19.

METHODS

A literature search was conducted in PubMed, Cochrane Library, Web of Science, medRxiv, and Google Scholar using "SARS-CoV-2", "COVID-19", "LY-CoV555", and "Bamlanivimab" keywords up to January 25, 2023. The quality of included studies was assessed using the Cochrane bias tools. The Comprehensive Meta-Analysis software version 3.0 was used to analyze the data.

RESULTS

A total of 30 studies involving 47368 patients were included. A significant

difference was observed between the bamlanivimab and standard of care/placebo groups in terms of mortality rate [risk ratio (RR) = 50, 95% confidence interval (CI): 0.36-0.70], hospitalization rate (RR = 0.51; 95%CI: 0.39-0.68), and emergency department (ED) visits (RR = 0.69; 95%CI: 0.47-0.99); while the two groups exhibited no significant difference in terms of intensive care unit (ICU) admission ($P > 0.05$). Compared to other mAbs, bamlanivimab was associated with a higher rate of hospitalization (RR = 1.44; 95%CI: 1.07-1.94). However, no significant difference was detected between the bamlanivimab and other mAbs groups in terms of mortality rate, ICU admission, and ED ($P > 0.05$). The incidence of any adverse events was similar between the bamlanivimab and control groups ($P > 0.05$).

CONCLUSION

Although the results suggest the efficacy and safety of bamlanivimab in COVID-19 patients, further research is required to confirm the efficacy of this drug for the current circulating SARS-CoV-2 variants.

Key Words: SARS-CoV-2; COVID-19; Bamlanivimab; Monoclonal antibody; Meta-analysis

Core Tip: The present study is the most comprehensive systematic review and meta-analysis on the efficacy and safety of bamlanivimab in the treatment of coronavirus disease 2019 (COVID-19). A significant difference was observed between the bamlanivimab and standard of care/placebo groups in terms of mortality rate, hospitalization rate, and emergency department visits. While the two groups exhibited no significant difference in terms of intensive care unit admission. The present results suggested that bamlanivimab might be effective and safe for the treatment of COVID-19.

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INTRODUCTION

Despite a high rate of vaccination, cases of breakthrough coronavirus disease 2019 (COVID-19) have been reported worldwide[1]. Consequently, numerous pharmaceutical interventions have been proposed to prevent and manage severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the primary cause of COVID-19[2]. Numerous clinical studies have demonstrated that various monoclonal antibodies (mAbs), including sotrovimab[3], casirivimab/imdevimab[3,4], cilgavimab/tixagevimab[5], regdanvimab[6], bamlanivimab/etesevimab[7], and bamlanivimab[8] could be potentially effective in reducing mortality and morbidity in patients with mild to moderate COVID-19. These interventions specifically target the spike protein of the SARS-CoV-2 virus, thereby, inhibiting its activity[9]. In particular, bamlanivimab has been approved by the United States Food and Drug Administration (FDA) for the treatment of non-hospitalized patients with mild to moderate COVID-19[10]. It is worth noting that the FDA has recently revoked the authorization of bamlanivimab for the treatment of COVID-19 due to the emergence of SARS-CoV-2 variants that are resistant to this particular mAbs[11]. However, bamlanivimab is still used in combination with etesevimab for the management of mild to moderate COVID-19 in individuals at high risk of developing severe symptoms[12]. Multiple studies have shown that the administration of bamlanivimab is strongly associated with a notable decrease in the risk of mortality, lower hospitalization rates, and a decreased likelihood of intensive care unit (ICU) admission compared to treatment options that do not include mAbs[8,13,14]. However, the effectiveness of anti-SARS-CoV-2 mAb agents against the Omicron variant of the virus has some concerns[15]. Therefore, the objective of this study is to compile and analyze the available evidence regarding the effectiveness and safety of bamlanivimab in the treatment of patients with COVID-19.

MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses was utilized as a reporting guideline for conducting this systematic review and meta-analysis of primary studies[16].

Literature search

An extensive literature search was conducted to gather relevant evidence. The search was performed in PubMed, Cochrane Library, Web of Science, medRxiv, and Google Scholar, up to January 25, 2023. Apart from the database search, the reference lists of the included studies were also examined to identify any additional relevant records. No language restrictions were applied. The search strategy in PubMed included keywords such as "Coronavirus", "COVID-19",

"SARS-CoV-2", "Bamlanivimab", and "LY-CoV555". The specific search strategy in PubMed was as follows: (((((((Coronavirus[Title/Abstract]) OR (Coronavirus[MeSH Terms])) OR (COVID-19 [Title/Abstract])) OR (SARS-CoV-2 [Title/Abstract])) OR (COVID-19[MeSH Terms])) OR (SARS-CoV-2 [MeSH Terms])) OR (2019 novel coronavirus infection[Title/Abstract])) OR (2019-nCoV infection[Title/Abstract])) AND ((Bamlanivimab [Title/Abstract] OR (LY-CoV555 [Title/Abstract]).

Selection study

To be included in the study, the selected studies had to meet the following criteria: (1) Addressing adult patients who with positive COVID-19 results based on the polymerase chain reaction test; (2) Bamlanivimab alone as treatment; (3) Using placebo (PBO), standard of care (SOC), and other therapeutic interventions as the control group; and (4) Addressing mortality rate, hospitalization rate, emergency department (ED) visits, ICU admission rate, and incidence of adverse events as the measures of efficacy and safety. Animal studies, case reports, letters to the editor, and studies that did not report relevant outcomes were excluded from the analysis.

Risk of bias assessment

Two authors independently assessed the bias risk in observational studies and randomized controlled trials (RCTs) using Nonrandomized Studies of Interventions (ROBINS-I) tool and Cochrane Risk of Bias (ROB) tool, respectively[17,18].

Data extraction

Two authors independently extracted the following data from the included studies: (1) Study characteristics including information such as the name of the first author, year of publication, location of the study, and study design; (2) Participant characteristics such as sample size, sex distribution, and the mean age of the participants; (3) Intervention and control including details on the sample size of both intervention and control groups, and the treatment dosage and duration; and (4) Efficacy and safety outcomes consisted of the reported efficacy (*i.e.*, mortality rate, hospitalization rate, ED visits, ICU admission rate, and the incidence of adverse events). By independently extracting this data, the authors ensured a thorough and accurate collection of information for analysis.

Data analysis

The Comprehensive Meta-Analysis software was employed to compare the efficacy and safety between bamlanivimab and the control groups. The risk ratio (RR), along with a 95% confidence interval (CI), was employed to analyze the dichotomous variables. The level of heterogeneity was assessed using the I^2 statistic, with a value greater than 50% or a *P*-value less than 0.1, indicating high heterogeneity ($I^2 > 50\%$ or $P < 0.1$). A random-effects model was employed in highly heterogeneous studies, while a fixed-effects model was used for studies with low heterogeneity. Both RCTs and observational studies were analyzed together to estimate the effect size. Subgroup analyses were conducted based on the age of patients (less than 65 years or 65 and over), sample size, and study design. Moreover, a sensitivity analysis was performed by excluding studies with remarkable risk of bias for outcomes of mortality rate and hospitalization rate. Publication bias was assessed by Begg's test and Egger's test.

RESULTS

Figure 1 depicts the study selection process, starting from the initial literature search, removal of duplicates, and screening based on title, abstract, and full-text. Out of the initial 584 studies identified after removing duplicates, 49 full-text studies were considered for eligibility assessment. Ultimately, a total of 30 studies with 47368 patients were included in the meta-analysis[8,10,11,13,14,19-43]. Excluded studies are presented in Figure 1 along with their corresponding reason. The majority of the included studies were of retrospective nature and conducted in the United States. Furthermore, most of the studies were published in 2021, coinciding with the SARS-CoV-2 Delta wave. COVID-19 vaccination status was reported in a few number of studies. Studies mainly evaluated the efficacy of bamlanivimab in patients with mild-to-moderate COVID-19 infection. In most studies, bamlanivimab was administered at a dose of 700 mg. In some studies, however, patients received doses of 2800 and 7000 mg. More detailed information on the characteristics of the included studies is listed in Table 1.

Risk of bias assessment

Supplementary Tables 1 and 2 respectively show the risk of bias assessment determined by ROB and ROBINS-I tools. Accordingly, the included studies had acceptable quality.

Efficacy outcomes

Mortality rate: The pooled estimate revealed a significant difference in mortality rate of the bamlanivimab compared to the SOC/PBO groups (RR = 0.50; 95%CI: 0.36-0.70, $P < 0.05$, $I^2 = 15\%$) (Figure 2A). However, no significant difference was observed between bamlanivimab and other mAbs in terms of mortality rate (RR = 1.71; 95%CI: 0.85-3.44, $P = 0.12$, $I^2 = 0\%$) (Supplementary Figure 1).

Hospitalization rate: A significant difference was observed in the hospitalization rate of bamlanivimab-receiving patients compared to those treated with SOC/PBO (RR = 0.51; 95%CI: 0.39-0.68, $P < 0.05$, $I^2 = 80\%$) (Figure 2B). Moreover, a

Table 1 Main characteristic of included studies

Ref.	Country	Design	Sample size	Male %	Severity of COVID-19	Bamlanivimab			Comparison(s)			
						n	Mean age	Comorbidity[1]	Name	*n*	Mean age	Comorbidity[1]
Alam *et al*[19], 2021	United States	RS	264	44	MM	160	81	58.1	SOC	86	84	51.2
Bariola *et al*[13], 2021	United States	RS	1392	44.39	MM	232	67.3	75.9	SOC	1160	67.1	73.6
Brock *et al*[20], 2021	United States	RS	108	NA	MM	58	NA	100	SOC	58	NA	100
Chen *et al*[21], 2021	United States	RCT	24	54	MC	18	NA	NA	PBO	6	43.2	NA
Chen *et al*[21], 2021	United States	RCT	452	44.9	MM	309	45	69.6	PBO	143	46	66.4
Chew *et al*[23], 2022	United States	RCT	317	51.1	MS	159	NA	NA	PBO	158	NA	NA
Cooper *et al*[24], 2021	United States	RS	5758	45.13	NA	1718	60	56.3	SOC, B/E, C/I	4040	NA	> 50
Corwin *et al*[25], 2021	United States	RS	6117	42.7	MM	780	62.6	68.1	SOC	5337	56.7	47.1
Destache *et al*[14], 2021	United States	RS	234	47	MM	117	72	69.2	SOC	117	72	63.3
Djuric *et al*[26], 2022	Serbia	RS	31	67.74	MS	13	62.2	30.8	SOC	18	65.9	38.9
Farcy *et al*[11], 2022	United States	PS	321	60.12	MM	201	64.2	56.2	C/I	120	66.3	58.3
San Filippo *et al*[39], 2022	United States	RS	453	47.01	MM	183	66.9	44.8	C/I	270	63.4	51.9
Ganesh *et al*[27], 2021	United States	RS	4670	50.62	MM	2335	63	54.2	SOC	2335	63	55.1
Ganesh *et al*[28], 2021	United States	RS	3596	50.02	MM	2747	NA	53.3	C/I	849	NA	48.3
Gottlieb *et al*[29], 2021	United States	RCT	577	45.40	MM	309	NA	NR	PBO, B/E	156	NA	NA
Heller *et al*[31], 2023	Germany	RS	26	45	MM	10	81	NR	SOC, C/I	23	NA	NA
Iqbal *et al*[8], 2021	United States	RS	284	NA	MM	144	NR	10.3	SOC	140	NA	63.60
Karr *et al*[10], 2022	United States	RS	46	63.04	MM	40	69	65	SOC	6	69	50
Kumar *et al*[32], 2022	United States	RS	403	52.10	MM	218	66	50.5	SOC	185	62	43.8
ACTIV-3/TICO LY-CoV555 Study Group *et al*[30], 2021	United States	RCT	314	57.32	NA	163	63	72	PBO	151	59	65
McCreary *et al*[33], 2021	United States	RCT	1935	46.20	MM	128	57	47	B/E, C/I	1807	NA	NA
Monday *et al*[34], 2022	United States	RCT	643	42.76	MM	294	61	72.8	B/E	349	55	72.4
Murillo *et al*[35], 2022	United States	RS	107	42.99	MM	39	NA	NA	SOC	63	NA	NA
Priest *et al*[36], 2022	United States	RS	758	49	MM	379	NA	88	SOC	379	NA	88

Quenzer *et al*[37], 2022	United States	RS	270	51.85	MM	134	60.3	92.5	SOC	136	63.3	69.1
Rubin *et al*[38], 2021	United States	RS	1257	43.75	NA	191	64	NR	SOC	1066	64.6	NA
Savoldi *et al*[40], 2022	Italy	PS	635	61.57	MM	161	63	72.7	B/E, C/I	474	NR	NA
Sridhara *et al*[41], 2023	United States	RS	2182	42.98	NA	1099	64	52.8	SOC	1091	46	20.9
Voelker and Jerath[42], 2022	United States	PS	678	43.65	NA	380	NA	NA	C/I	298	NA	NA
Webb *et al*[43], 2021	United States	QES	13534	55.19	NA	479	65	90.8	SOC, C/I	5651	NA	NA

[1]The percentage of patients with at least one comorbidity.

B/E: Bamlanivimab/etesevimab; C/I: Casirivimab/imdevimab; QES: Quasi-experimental study; MM: Mild to moderate; MS: Mild to severe; MC: Mild to critical; mAb: Monoclonal antibody; N: Number; NA: Not acquired; PBO: Placebo; PS: Prospective study; RCT: Randomized clinical trial; RS: Retrospective study; SOC: Standard of care.

significant difference was detected between the hospitalization rate of the bamlanivimab group compared to mAbs one (RR = 1.44; 95%CI: 1.07-1.94, P = 01, I^2 = 53%) (Supplementary Figure 2).

ED visits: The combined analysis of these studies revealed a significant difference in the frequency of ED visits between bamlanivimab-treated patients and those receiving SOC (RR = 0.69; 95%CI: 0.47-0.99, P = 0.04, I^2 = 58%). No significant difference was observed between bamlanivimab and other mAbs in terms of ED visits (RR = 0.96; 95%CI: 0.76-1.20, P = 0.74, I^2 = 0%) (Figure 2C and Supplementary Figure 3).

ICU admission: The result of meta-analysis showed no significant difference in the ICU admission rate of the bamlanivimab-treated patients and those receiving SOC (RR = 0.82; 95%CI: 0.57-1.18, P = 0.29, I^2 = 42%) (Figure 2D). No significant difference was observed between bamlanivimab and other mAbs in terms of ICU admission (RR = 1.60; 95%CI: 0.86-2.98, P = 0.13, I^2 = 0%) (Supplementary Figure 4).

Safety outcomes

Any adverse events: The pooled estimate of included studies showed no significant difference in adverse events between the bamlanivimab and SOC/PBO groups (RR = 1.01; 95%CI: 0.81-1.26, P = 0.88, I^2 = 0%) (Figure 2E). Moreover, no significant difference was observed in adverse events between the bamlanivimab and other mAb groups (RR = 6.13; 95%CI: 0.71-52.72, P = 0.09, I^2 = 1%) (Supplementary Figure 5).

Publication bias: No evidence of publication bias was detected for pooled estimate of mortality rate (P = 0.24) and hospitalization rate (P = 0.11) based on Begg test. However, Egger's test indicated a publication bias for pooled estimates of mortality rate (P = 0.01) and hospitalization rate (P = 0.004) (Supplementary Figures 6 and 7).

Subgroup and sensitivity analyses: The subgroup analysis showed no significance difference in mortality rate and hospitalization rate by mean age of patients treated with bamlanivimab compared to SOC/PBO and by sample size (Table 2). Sensitivity analysis also exhibited no significant change compared to the excluded studies (Table 2).

Table 2 Subgroup and sensitivity analyses for efficacy and safety outcomes

Analysis	Studies, *n*	Sample size, *n*	Point estimate (95%CI)	*P* value	Heterogeneity		
					Q value	*P* value	I^2
Sensitivity analysis							
Mortality rate soc (excluding Brock 2021and Djuric 2021)	16	29091	0.52 (0.37-0.73)	< 0.001	18.45	0.24	18.71
Hospitalization rate (excluding Brock 2021)	17	26565	0.55 (0.42-0.71)	< 0.001	69.65	0.21	77.02
Hospitalization rate (excluding Voelker 2022)	7	8177	1.38 (1.00-1.92)	0.04	14.25	0.02	57.91
ICU admission (excluding Brock 2021)	6	15759	0.88 (0.60-1.29)	0.52	7.79	0.16	35.81
Subgroup analysis							
Hospitalization rate by design, BAM *vs* SOC/PBO							
OS	16	25904	0.67 (0.60-0.75)	< 0.001	82.27	< 0.001	81.76
RCT	2	769	0.44 (0.21-0.94)	0.03	1.95	0.16	48.80
Hospitalization rate by sample size, BAM *vs* SOC/PBO							
< 1000	11	23453	0.51 (0.43-0.61)	< 0.001	50.51	< 0.001	80.20
≥ 1000	7	3220	0.77 (0.67-0.89)	< 0.001	22.22	0.001	73.00
Hospitalization rate by mean age, BAM *vs* SOC/PBO							
< 65	8	22783	0.77 (0.67-0.88)	< 0.001	26.91	< 0.001	73.99
≥ 65	4	1918	0.46 (0.32-0.66)	< 0.001	0.30	0.96	< 0.001
Mortality rate by design, BAM *vs* SOC/PBO							
OS	17	28916	0.44 (0.31-0.62)	< 0.001	14.67	0.54	0.00
RCT	1	314	1.67 (0.57-4.86)	0.34	0.00	1.00	0.00
Mortality rate by sample size, BAM *vs* SOC/PBO							
< 1000	12	4030	0.48 (0.31-0.76)	0.002	15.55	0.15	29.29
≥ 1000	6	25200	0.51 (0.31-0.84)	0.009	4.43	0.48	0.00
Mortality rate by mean age, BAM *vs* SOC/PBO							
< 65	8	25639	0.60 (0.37-0.96)	0.037	9.89	0.19	29.26
≥ 65	5	2189	0.40 (0.20-0.79)	0.008	5.16	0.27	22.53

CI: Confidence interval; ED: Emergency department; mAb: Monoclonal antibody; PBO: Placebo; RCT: Randomized clinical trial; OS: Observational study; SOC: Standard of care.

DISCUSSION

The objective of this study was to analyze and synthesize the most recent evidence on the effectiveness and safety of bamlanivimab, a mAb intervention, during the prevalence of the SARS-CoV-2 Omicron variant. Despite the protective role of vaccines against SARS-CoV-2 infection, effective treatments are still required to manage COVID-19 disease, particularly with the emergence of new variants[44]. The results demonstrated the efficacy of bamlanivimab in achieving positive clinical outcomes among patients diagnosed with COVID-19.

The results of the meta-analysis revealed a significantly lower mortality rate in the bamlanivimab-receiving individuals compared to those treated with SOC/PBO. However, this difference was not significant between the bamlanivimab and other mAb groups. Clinical studies showed the similar efficacy of mAb treatments in reducing COVID-19-induced death [11,40]. Consistent to our findings, meta-analyses conducted on the efficacy of bamlanivimab revealed that treatment with bamlanivimab is significantly associated with a lower mortality rate compared to the control group[45,46]. In general, the clinical evidence suggests that mAb treatments may contribute to a reduction in the mortality rate among patients with COVID-19[4,30,34,42,47]. Targeting the spike protein of the SARS-CoV-2 virus with anti-SARS-CoV-2 mAbs may serve as a potential mechanism for reducing the mortality rate of COVID-19 patients[48].

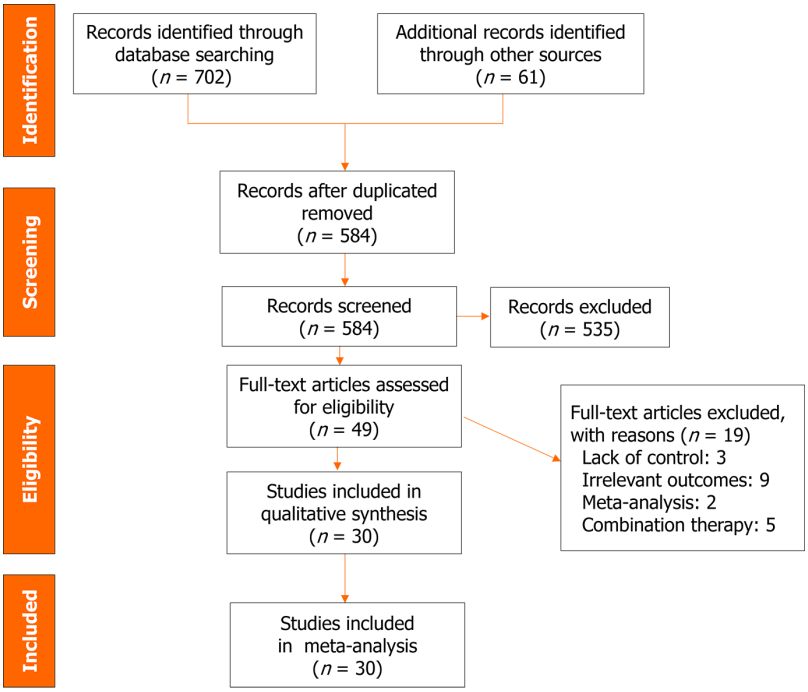


Figure 1 PRISMA flow diagram of study selection process.

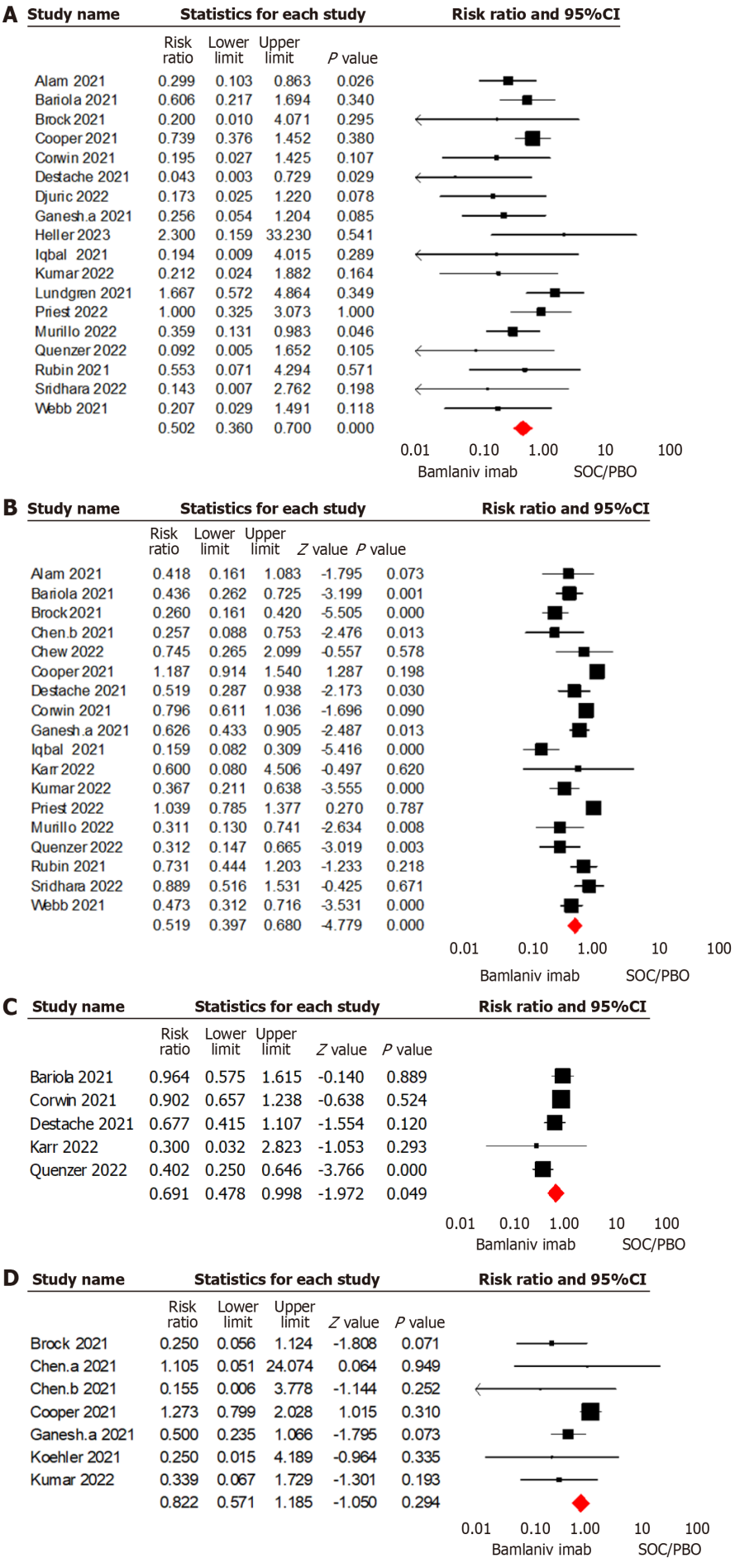
According to results of the present meta-analysis, bamlanivimab-treated patients had a lower likelihood of being admitted to the hospital compared to those receiving SOC/PBO. However, hospitalization rate was higher in the bamlanivimab group than the other mAbs group. Bamlanivimab treatment may contribute to a reduced rate of hospitalization among COVID-19 patients. Consistent with the mentioned finding, other meta-analyses[45,46] on the efficacy of this drug also demonstrated that treatment with bamlanivimab is associated with a lower rate of hospitalization in patients with mild to moderate COVID-19 compared to control groups. This further supports the potential effectiveness of bamlanivimab in reducing the hospital admission in individuals with COVID-19. Indeed, real-world studies demonstrated that therapeutic mAbs, including bamlanivimab[38,40], sotrovimab, casirivimab/imdevimab[4,40], and bamlanivimab/etesevimab[40] can significantly reduce the rate of COVID-19-related hospitalization. According to these studies, the use of these mAbs can effectively lower the severity of the disease and decrease the need for hospitalization in individuals affected by COVID-19.

The results of the present study demonstrate a significant positive effect of bamlanivimab on reducing the need for ED visits in patients with COVID-19 compared to SOC/PBO. However, this difference was not significant between the bamlanivimab and other mAb groups. A meta-analysis of RCTs comparing mAbs-receiving patients with PBO group indicated a significant association of mAbs with a lower rate of ED visits[49]. A possible explanation for this difference could be due to differences in the type of mAb treatments as intervention or included in the study design.

According to the present meta-analysis, treatment with bamlanivimab was not significantly associated with a lower rate of admission to ICU compared to SOC/PBO or mAbs. On the contrary, a meta-analysis by Xiang *et al*[45] showed a significant association of bamlanivimab with reduced ICU admission rate compared to the controls. This difference can be due to the number of studies included in the quantitative analysis. Compared to Xiang *et al*[45], the present research identified and included more studies in the meta-analysis of data on ICU admission rate.

Consistent with previously published meta-analyses[45] on the safety profile of bamlanivimab, the present study found similar incidence of adverse events in both the bamlanivimab and control groups. In general, the bamlanivimab-related incidence of adverse events in COVID-19 patients was mild and well-tolerated[11,19,39]. The most frequent adverse events in studies included nausea, diarrhea, headache, and respiratory distress[21,29]. In terms of severe adverse events, no significant difference was observed between the bamlanivimab and control groups. Chen *et al*[21] found no cases of discontinuations due to adverse events in bamlanivimab-treated patients at different doses (700, 2800, and 7000 mg). Gottlieb *et al*[29] also found similar results in COVID-19 patients receiving bamlanivimab doses of 700, 2800, and 7000 mg.

Two important points should be considered in the interpretation of the present results. First, several studies have documented evidence of post-COVID-19 condition among individuals after the initial SARS-CoV-2 infection which is a serious problem for many recovered COVID-19 patients[50-52]. Given the importance of post-COVID-19 conditions in designing effective treatments for COVID-19, and considering the lack of validated treatment for these conditions, it is crucial to conduct longitudinal monitoring of COVID-19 patients. This monitoring is vital for the development of effective therapeutic agents[52]. Second, studies have shown the resistance of some SARS-COV-2 variants to bamlanivimab. Hoffmann *et al*[53] reported the resistance of SARS-CoV-2 variant B.1.1.7 to bamlanivimab. A study conducted by Peiffer-Smadja *et al*[54] showed the emergence of resistance mutants in bamlanivimab-receiving COVID-19 patients. A RCT conducted by Choudhary *et al*[55] reported the emergence of SARS-CoV-2 escape mutations in COVID-19 patients during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Study name
Statistics for each study
Risk ratio and 95%CI
Risk ratio
Lower limit
Upper limit
P value
Alam 2021 0.299 0.103 0.863 0.026
Bariola 2021 0.606 0.217 1.694 0.340
Brock 2021 0.200 0.010 4.071 0.295
Cooper 2021 0.739 0.376 1.452 0.380
Corwin 2021 0.195 0.027 1.425 0.107
Destache 2021 0.043 0.003 0.729 0.029
Djuric 2022 0.173 0.025 1.220 0.078
Ganesh.a 2021 0.256 0.054 1.204 0.085
Heller 2023 2.300 0.159 33.230 0.541
Iqbal 2021 0.194 0.009 4.015 0.289
Kumar 2022 0.212 0.024 1.882 0.164
Lundgren 2021 1.667 0.572 4.864 0.349
Priest 2022 1.000 0.325 3.073 1.000
Murillo 2022 0.359 0.131 0.983 0.046
Quenzer 2022 0.092 0.005 1.652 0.105
Rubin 2021 0.553 0.071 4.294 0.571
Sridhara 2022 0.143 0.007 2.762 0.198
Webb 2021 0.207 0.029 1.491 0.118
0.502 0.360 0.700 0.000
0.01 0.10 1.00 10 100
Bamlaniv imab SOC/PBO
B
Study name
Statistics for each study
Risk ratio and 95%CI
Risk ratio
Lower limit
Upper limit
Z value
P value
Alam 2021 0.418 0.161 1.083 -1.795 0.073
Bariola 2021 0.436 0.262 0.725 -3.199 0.001
Brock 2021 0.260 0.161 0.420 -5.505 0.000
Chen.b 2021 0.257 0.088 0.753 -2.476 0.013
Chew 2022 0.745 0.265 2.099 -0.557 0.578
Cooper 2021 1.187 0.914 1.540 1.287 0.198
Destache 2021 0.519 0.287 0.938 -2.173 0.030
Corwin 2021 0.796 0.611 1.036 -1.696 0.090
Ganesh.a 2021 0.626 0.433 0.905 -2.487 0.013
Iqbal 2021 0.159 0.082 0.309 -5.416 0.000
Karr 2022 0.600 0.080 4.506 -0.497 0.620
Kumar 2022 0.367 0.211 0.638 -3.555 0.000
Priest 2022 1.039 0.785 1.377 0.270 0.787
Murillo 2022 0.311 0.130 0.741 -2.634 0.008
Quenzer 2022 0.312 0.147 0.665 -3.019 0.003
Rubin 2021 0.731 0.444 1.203 -1.233 0.218
Sridhara 2022 0.889 0.516 1.531 -0.425 0.671
Webb 2021 0.473 0.312 0.716 -3.531 0.000
0.519 0.397 0.680 -4.779 0.000
0.01 0.10 1.00 10 100
Bamlaniv imab SOC/PBO
C
Study name
Statistics for each study
Risk ratio and 95%CI
Risk ratio
Lower limit
Upper limit
Z value
P value
Bariola 2021 0.964 0.575 1.615 -0.140 0.889
Corwin 2021 0.902 0.657 1.238 -0.638 0.524
Destache 2021 0.677 0.415 1.107 -1.554 0.120
Karr 2022 0.300 0.032 2.823 -1.053 0.293
Quenzer 2022 0.402 0.250 0.646 -3.766 0.000
0.691 0.478 0.998 -1.972 0.049
0.01 0.10 1.00 10 100
Bamlaniv imab SOC/PBO
D
Study name
Statistics for each study
Risk ratio and 95%CI
Risk ratio
Lower limit
Upper limit
Z value
P value
Brock 2021 0.250 0.056 1.124 -1.808 0.071
Chen.a 2021 1.105 0.051 24.074 0.064 0.949
Chen.b 2021 0.155 0.006 3.778 -1.144 0.252
Cooper 2021 1.273 0.799 2.028 1.015 0.310
Ganesh.a 2021 0.500 0.235 1.066 -1.795 0.073
Koehler 2021 0.250 0.015 4.189 -0.964 0.335
Kumar 2022 0.339 0.067 1.729 -1.301 0.193
0.822 0.571 1.185 -1.050 0.294
0.01 0.10 1.00 10 100
Bamlaniv imab SOC/PBO

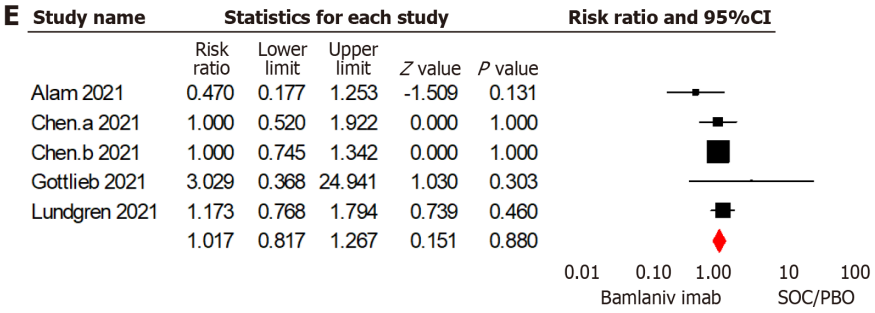

Figure 2 Forest plot analysis. A: Forest plot analysis for the outcome of mortality rate of bamlanivimab *vs* standard of care/placebo (SOC/PBO) in patients with coronavirus disease 2019 (COVID-19); B: Forest plot analysis for the outcome of hospitalization rate of bamlanivimab *vs* SOC/PBO in patients with COVID-19; C: Forest plot analysis for the outcome of emergency department visit of bamlanivimab *vs* SOC/PBO in patients with COVID-19; D: Forest plot analysis for the outcome of intensive care unit admission of bamlanivimab *vs* SOC/PBO in patients with COVID-19; E: Forest plot analysis for the outcome of adverse events of bamlanivimab *vs* SOC/PBO in patients with COVID-19. CI: Confidence interval.

treatment with bamlanivimab (700 mg). However, no resistance mutations were identified in patients treated with 7000 mg bamlanivimab. These findings highlight the importance of viral resistance during the development of treatments for COVID-19 patients. SARS-CoV-2 mutations may also lower the effectiveness of current preventive therapies in individuals, including vaccines. The SARS-CoV-2 variant B.1.351 could significantly reduce the efficacy of Novavax COVID-19 vaccine[56].

The present study has some remarkable limitations. Firstly, the included studies did not report the type of SARS-CoV-2 variant. Therefore, the present findings may not be applicable to some SARS-COV-2 variants of interest. Secondly, the majority of studies included in the meta-analysis were retrospective, causing an inherent risk of bias. Moreover, many of these retrospective studies did not utilize propensity score matching to minimize selection bias and confounding variables. Thirdly, we could not perform subgroup analyses based on these variables as the information on the comorbidity percentage and COVID-19 vaccine status of the studies was not complete. Therefore, the present results cannot be generalized to patients with unknown COVID-19 vaccine status. Finally, the present results should be interpreted with caution due to the presence of potential publication bias in several outcomes.

CONCLUSION

The present meta-analysis demonstrated the association of bamlanivimab treatment with a reduction in the mortality rate, hospitalization rate, and ED visits in patients with COVID-19 compared to SOC-receiving group. However, it did not show a significant efficacy in improving clinical outcomes compared to other mAb treatments. In terms of safety, bamlanivimab was safe and well-tolerated in patients with COVID-19. However, studies did not report the specific type of SARS-CoV-2 variants. Therefore, the findings may not be directly applicable to patients with current SARS-CoV-2 variants. Future research should be focused on the efficacy of bamlanivimab against the current SARS-CoV-2 variants, especially in immunocompromised patients who are more susceptible to the new SARS-CoV-2 variants in terms of mutations and resistance to treatment with mAbs. Moreover, the comorbidity percentage and COVID-19 vaccination rate should be considered in evaluating the efficacy of bamlanivimab in COVID-19 patients.

ARTICLE HIGHLIGHTS

Research background

Bamlanivimab, a monoclonal antibody (mAb), has been used as a therapeutic agent for patients with coronavirus disease 2019 (COVID-19). Previous studies have shown that bamlanivimab may be effective in treating COVID-19 patients.

Research motivation

Despite several studies evaluating the clinical benefit of bamlanivimab in COVID-19 patients, there is currently no comprehensive systematic review and meta-analysis assessing its efficacy and safety as a treatment.

Research objectives

This study aims to evaluate the use of bamlanivimab in improving efficacy outcomes compared to other treatments in COVID-19 patients. Additionally, the safety profile of bamlanivimab is compared to control groups.

Research methods

A thorough search was conducted in PubMed, Cochrane Library, Web of Science, medRxiv, and Google Scholar up to January 25, 2023. Cochrane bias tools were utilized to assess the risk of bias in the included studies. Data analysis was

performed using Comprehensive Meta-Analysis software (version 3).

Research results

A total of 30 studies were identified and included in the meta-analysis. The meta-analysis revealed a significant difference between the bamlanivimab and standard of care/placebo groups in terms of mortality rate, hospitalization rate, and emergency department (ED) visits. However, there was no significant difference between the two groups regarding intensive care unit (ICU) admission. When compared to other mAbs, bamlanivimab did not demonstrate superior efficacy in terms of hospitalization rate, mortality rate, ICU admission, and ED visits. No significant difference was observed between the treatment groups in terms of adverse events.

Research conclusions

Although the present results demonstrate the efficacy and safety of bamlanivimab in treating COVID-19, further research is necessary to confirm its effectiveness against novel circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants.

Research perspectives

In the future, studies should be focused on the efficacy of bamlanivimab against the current SARS-CoV-2 variants, especially in immunocompromised patients who are more susceptible to the new SARS-CoV-2 variants in terms of mutations and resistance to treatment with mAbs. Moreover, the comorbidity percentage and COVID-19 vaccination rate should be considered in evaluating the efficacy of bamlanivimab in COVID-19 patients.

FOOTNOTES

Author contributions: Amani B designed and administrated the study, and drafted the manuscript; Khodavirdilou L and Kardan Moghaddam V carried out the literature search; Kardan Moghaddam V and Akbarzadeh A performed the data extraction; Rajabkhah K and Kardan Moghaddam V were involved in assessing the quality of studies; Amani B and Akbarzadeh A performed the data analysis; Amani B and Khodavirdilou L performed the writing, review & editing; and all authors have read and approved the final manuscript.

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META-ANALYSIS

Dengue induced acute liver failure: A meta summary of case reports

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Abstract

BACKGROUND

Dengue fever is the most common cause of viral hemorrhagic fever, with more than 400 million cases being reported annually, worldwide. Even though hepatic involvement is common, acute liver failure (ALF) is a rare complication of dengue fever.

AIM

To analyze the demographic profile, symptomology, hospital course and outcomes of patients presenting with ALF secondary to dengue infection by reviewing the published case reports.

METHODS

A systematic search was performed from multiple databases including PubMed, *Reference Citation Analysis*, Science Direct, and Google Scholar. The search terms used were "dengue" OR "severe dengue" OR "dengue shock syndrome" OR "dengue haemorrhagic syndrome" OR "dengue fever" AND "acute liver failure" OR "hepatic failure" OR "liver injury". The inclusion criteria were: (1) Case reports or case series with individual patient details; (2) Reported acute liver failure secondary to dengue infection; and (3) Published in English language and on adult humans. The data were extracted for patient demographics, clinical symptomatology, clinical interventions, hospital and intensive care unit course, need for organ support and clinical outcomes.

RESULTS

Data from 19 case reports fulfilling the predefined inclusion criteria were included. The median age of patients was 38 years (inter quartile range: Q3-Q1 26.5 years) with a female preponderance (52.6%). The median days from diagnosis

of dengue to development of ALF was 4.5 d. The increase in aspartate aminotransferase was higher than that in alanine aminotransferase (median 4625 U/L *vs* 3100 U/L). All the patients had one or more organ failure, with neurological failure present in 73.7% cases. 42.1% patients required vasopressor support and hepatic encephalopathy was the most reported complication in 13 (68.4%) cases. Most of the patients were managed conservatively and 2 patients were taken up for liver transplantation. Only 1 death was reported (5.3%).

CONCLUSION

Dengue infection may rarely lead to ALF. These patients may frequently require intensive care and organ support. Even though most of these patients may improve with supportive care, liver transplantation may be a therapeutic option in refractory cases.

Key Words: Dengue fever; Acute liver failure; Dengue induced hepatitis; Hepatic failure; Fulminant hepatitis; Severe dengue

Core Tip: Dengue infection frequently affects liver function but, in most cases, it exhibits transient and mild increase of transaminases. Rarely, it may lead to severe liver injury and development of acute liver failure (ALF). As there is no specific therapy, most of these patients are managed conservatively and provided with organ support. N-acetyl cysteine is increasingly been used in the management of non-paracetamol induced ALF. However, its utility in ALF secondary to dengue is still limited to small case series and case reports. Even liver transplantation has been rarely attempted in these patients because of high incidence of underlying multi-organ failure and increased risk of bleeding. However, clinical outcomes in these patients may be improved with early recognition and timely supportive care.

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INTRODUCTION

Dengue is the most common cause of viral hemorrhagic fever, globally. It is endemic in many tropical countries, but in the last few years, cases have also been frequently reported from non-endemic regions[1,2]. As per the current estimates, worldwide, more than 5 billion people are at risk of getting affected with dengue, and more than 400 million cases are being reported annually[2].

Traditionally, dengue was classified as non-classical dengue fever (DF), classical DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS)[3]. However, the modified dengue classification by the World Health Organisation divides it into three categories: Dengue without warning signs, dengue with warning signs and severe dengue. Patients with severe capillary leak, hypotension, severe bleeding or severe organ involvement are all classified as severe dengue [4].

Classically, dengue patients present with fever and rash and in severe cases with bleeding and shock. Liver injury is commonly reported in patients with dengue, and various phases of liver dysfunction have been described as secondary to dengue infection. In most patients, the liver enzymes have transient mild elevation[5]. Marked elevation of transaminases by more than ten times has also been described, and termed as dengue–induced severe hepatitis (DISH), which may occur in 4%-15% of the dengue cases[5-7]. However, the progression of DISH to acute liver failure (ALF) is rare and is reported in less than 1% of cases[5].

Patients with liver involvement generally present with gastrointestinal symptoms like nausea, vomiting, abdominal pain and anorexia, along with yellowish discoloration of the eyes and skin. Hepatomegaly has been reported to be present in 4%-79% of the patients[7-10]. However, in patients with severe disease, the presence of complications or multiple organ involvement may complicate the clinical picture. Patients with severe dengue may frequently require intensive care unit (ICU) admission and usually a multi-organ support[11]. Such patients have significant morbidity and mortality. Further, there is a substantial difference in mortality rates between DISH and ALF secondary to dengue, making it essential to recognize it early and institute supportive care[5].

As ALF secondary to dengue is rare and there is a dearth of data from large trials, we aimed to analyze the demographic profile, symptomology, hospital course and outcomes of patients presenting with ALF secondary to dengue infection by reviewing the published case reports and case series.

MATERIALS AND METHODS

For the present meta-summary, a systematic search was performed from multiple databases including, PubMed, Reference Citation Analysis, Science Direct, and Google Scholar. The search terms used were "dengue" OR "severe dengue" OR "dengue shock syndrome" OR "dengue haemorrhagic syndrome" OR "dengue fever" AND "acute liver failure" OR "hepatic failure" OR "liver injury". The inclusion criteria were: (1) Case reports or case series with individual patient details; and (2) reported acute liver failure secondary to dengue infection. Further, it was filtered for the literature published in English and on adult (> 18 years) humans. We excluded: (1) Conference abstracts; and (2) case reports or series that did not have individual biochemical data. All reports published till September 30, 2023 were included. The authors manually screened the results to include only the relevant literature and removed the duplicate articles.

All the selected case reports and case series were evaluated. The data were extracted for patient demographics, clinical symptomatology, clinical interventions, hospital and ICU course, need for organ support and clinical outcomes. A datasheet for evaluation was further prepared.

Statistical analysis

Excel and Microsoft Office 2019 were used to analyze the prepared datasheet. Categorical variables were presented as frequency and percentage. For continuous variables, mean [standard deviation (SD)] or median [interquartile range (IQR)] were calculated, as appropriate. SPSS (version 25.0, IBM SPSS Inc., Chicago, IL, United States) was used for statistical analysis. For tabulation and final documentation, Microsoft (MS) Office software (MS Office 2019, Microsoft Corp, WA, United States) was used.

RESULTS

The present meta-summary was performed using the PRISMA 2009 checklist (Figure 1). Eventually, data from 19 case reports fulfilling the predefined inclusion criteria were included in the analysis (Table 1)[12-30]. The median age of patients was 38 years (IQR: Q3-Q1 26.5 years) with a female preponderance (52.6%), as shown in Table 2. Most cases were reported from India (7, 36.8%) and Sri Lanka (5, 26.3%). The median days from diagnosis of dengue to development of ALF was 4.5 d. The baseline laboratory reports and hospital course are given in Table 3. The increase in aspartate aminotransferase (AST) was higher than that in alanine aminotransferase (ALT) (median 4625 U/L *vs* 3100 U/L). All the patients had one or more organ failure, with neurological dysfunction being most commonly reported in 14 patients (74%). The most common organ support required was cardiac, with 42.1% requiring vasopressors to maintain blood pressure. Most patients recovered with supportive therapy and two patients had undergone liver transplantation. The patients reported to require ICU stay had a median ICU stay of 6 days. 42.1% of patients required vasopressor support. Hepatic encephalopathy was the most commonly reported complication in 13 (68.4%) cases. Only one death was reported (mortality rate 5.3%).

DISCUSSION

Dengue infection may rarely lead to ALF. In the present meta-summary, all the patients had severe dengue, with 79% being diagnosed with DSS. The rise in AST was more than ALT, along with an increase in other liver function parameters. The median international normalized ratio (INR) value was 2.13, and the most common reported complication was hepatic encephalopathy (68.4%). Only 1 death was reported in our cohort of patients.

Dengue infection commonly leads to deranged liver functions, but ALF is rarely reported. Liver injury in patients with dengue may be multifactorial. The direct cytopathic effect of dengue virus may lead to liver injury. Further, the cytokine storm associated with severe dengue fever may cause immune-mediated hepatic injury and may progress to ALF. Severe hypotension associated with DSS, may also lead to hepatic hypoperfusion and contributes to liver injury. Additionally, frequent use of hepatotoxic drugs (paracetamol, nonsteroidal anti-inflammatory drugs, antibiotics) may contributes to liver injury (Figure 2)[5,6,31].

Even though DEN-1 and DEN-3 types of dengue virus have been shown to have more prominent liver tropism, all 4 serotypes (DEN-1 to DEN-4) have been shown to affect the liver and may cause fulminant hepatitis[32-34]. Most of the cases in our summary were reported from the Indian subcontinent (India 37% and Sri Lanka 26%). This is reasonable as these are tropical countries, where dengue is endemic and all four serotypes are prevalent. In India alone, more than 63000 dengue cases were reported in 2022[35].

The median days to develop ALF from the diagnosis of dengue was 4.5 d. This is consistent with the previous reports which have shown that there is a gradual increase in transaminase levels which peak around seven days of illness[5]. Even though the increase in transaminases is the most common liver function abnormality associated with dengue fever, there may be derangement of other parameters including, bilirubin, alkaline phosphatase and INR levels, especially in severe disease. This was also evidenced in our report, where we observed higher median levels of bilirubin (5.5 mg/dL), alkaline phosphatase (191 IU/L) and INR (2.13).

Transaminases are more frequently raised in patients with severe forms of dengue. Even the level of increase in transaminases depends on the severity of dengue[36,37]. As per a recent meta-analysis, AST may be raised in 75% of cases of DF as compared to 80% of patients with DHF. Similarly, ALT was raised in 52% of patients with DF and 54% of patients with

Table 1 Details of published case reports

Number	Ref.	Country	Age	Sex	Comorbidities	MELD score	Complications	Organ support	Outcome
1	Subramanian *et al* [28], 2005	India	35	M	None	17	None	None	Alive
2	Vinodh *et al*[29], 2005	India	19	M	None	30	Shock	Vasopressors	Alive
3	Penafiel *et al*[25], 2006	Singapore	69	M	None	NA	GI bleed, HE	RRT, IMV, MARS	Alive
4	Ling *et al*[22], 2007	Singapore	55	M	None	NA	None	None	Alive
5	Gasperino *et al* [19], 2007	United States	69	F	CAD	30	HE	Vasopressors, RRT, IMV	Alive
6	Osorio *et al*[23], 2008	Canada	31	F	None	NA	MODS	NA	Dead
7	Sedhain *et al*[27], 2011	Nepal	20	F	None	31	ARF	None	Alive
8	Agarwal *et al*[13], 2011	India	33	M	None	25	HE, seizures	None	Alive
9	Jhamb *et al*[21], 2011	India	19	M	None	27	HE, shock, GI bleed	IMV	Alive
10	Abeysekera *et al* [12], 2012	Sri Lanka	52	F	Hypertension	NA	HE	None	Alive
11	Arora *et al*[14], 2015	India	22	M	None	10	Minor bleeding, HE	None	Alive
12	Dalugama *et al* [16], 2017	Sri Lanka	53	M	None	16	None	None	Alive
13	Dalugama *et al* [17], 2018	Sri Lanka	43	F	Diabetes, dyslip-idaemia	NA	Shock, HE, AKI, GI bleed	RRT	Alive
14	Samarasekara *et al* [26], 2018	Sri Lanka	38	F	None	26	DVT, AKI	None	Alive
15	Galante *et al*[18], 2019	Canada	49	F	None	NA	Shock, AKI, HE	Vasopressors, RRT, IMV	Alive
16	Paul *et al*[24], 2020	India	50	M	Alcoholic liver disease	29	HE	None	Alive
17	Lewis *et al*[30], 2020	United States	23	F	None	NA	HE	None	Alive
18	Chikkala *et al*[15], 2021	India	29	F	None	38	HE	Vasopressors, RRT, IMV	Alive
19	Gunasekera *et al* [20], 2022	Sri Lanka	54	F	Hypertension, diabetes	36	Bleeding, HE, AKI	Vasopressors, RRT, IMV	Alive

AKI: Acute kidney injury; CAD: Coronary artery disease; DVT: Deep venous thrombosis; F: Female; GI: Gastrointestinal; HE: Hepatic encephalopathy; IMV: Invasive mechanical ventilation; M: Male; MODS: Multi organ dysfunction syndrome; NA: Not available; RRT: Renal replacement therapy.

DHF[38]. The increase in AST levels is greater as compared to ALT levels. It can partly be due to release of AST from the muscular injury secondary to dengue[39]. In most cases, the transaminase levels return to their baseline by day 21 of illness, with ALT levels taking longer duration to normalize due to their longer half-life[5]. Again, the coagulopathic derangement is also dependent on the severity of dengue. Greater increase in INR has been reported in patients with DSS (1.53) as compared to DHF (1.27), while it remained normal in patients with DF[9].

The treatment of ALF associated with dengue fever is largely supportive. Although no specific treatment is recommended, there is increasing interest in using intravenous N-acetylcysteine (NAC) for managing such cases. Even in our cohort, 6 (31.6%) patients were administered NAC for ALF, albeit in different doses and wide variation of duration [12,15,16,17,20,30]. NAC is the recommended antidote for managing ALF secondary to paracetamol overdose[40], but is increasingly been used in managing non-paracetamol related ALF[41]. In ALF secondary to dengue infection, small case series have shown improved survival with early NAC administration in patients with grade 1 and 2 encephalopathy[6,42]. The exact mechanism of action remains unknown, but it is postulated that NAC administration may help restore hepatic anti-oxidants, scavenge oxygen free radicals and improve oxygen delivery due to its vasodilatory effect[6,41,42].

Table 2 Baseline patient parameters

Parameter of interest	Frequency (%)
Age (yr), median	38 (IQR 26.5)
Sex	Females 10 (52.6%)
Country of origin	
India	7 (36.8)
Sri Lanka	5 (26.3)
Canada	2 (10.5)
Singapore	2 (10.5)
United States of America	2 (10.5)
Nepal	1 (5.3)
Diagnostic methodology	
Only serology (IgM)	14 (73.7)
Only antigen (NS1 Ag)	3 (15.8)
Both antigen (NS1 Ag) and serology (IgM)	2 (10.5)
Capillary leak syndrome	11 (57.9)
Dengue shock syndrome	15 (78.9)
Diagnosis of dengue to ALF in days, median (IQR Q3-Q1)	4.5 (1)

ALF: Acute liver failure; IQR: Interquartile range; IgM: Immunoglobin M; Ag: Antigen.

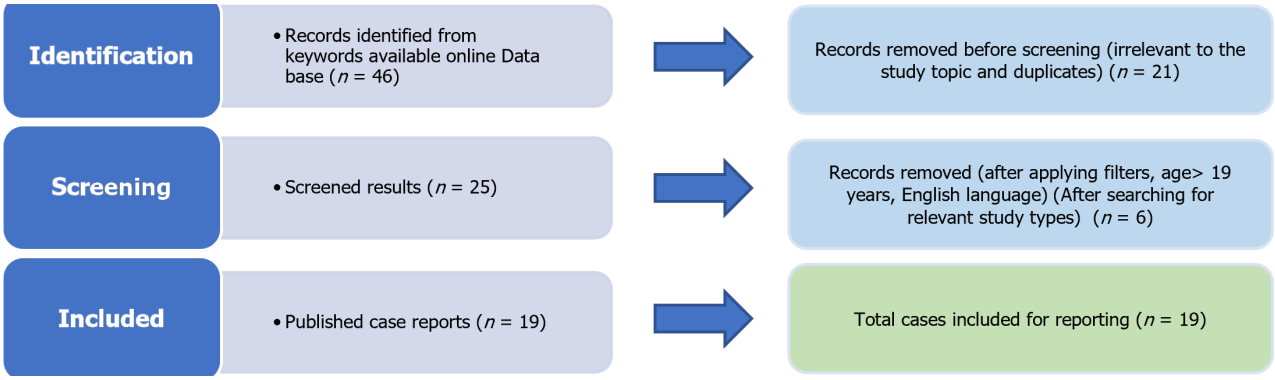

Figure 1 PRISMA flow diagram of the selected literature for the meta-summary.

As dengue induced ALF is rare, the data regarding utility of NAC has been extrapolated from studies in acetaminophen and non-acetaminophen induced ALF. Earlier reports suggested that NAC may be more useful in preventing rather than treating hepatic injury and hence, it was recommended to start NAC early (within 8-12 h) of acetaminophen overdose[43]. However, it is difficult to determine the exact time of hepatic insult in patients with non-acetaminophen induced liver failure and hence, it is recommended to initiate NAC in patients with significant acute liver injury as soon as ALF is detected[44]. Further, it may not be beneficial in later stages of the disease, when liver injury is advanced[42, 45]. Hence, NAC may be a useful adjunct in managing patients with severe liver injury, if initiated early. Further, large scale studies need to be performed to evaluate its efficacy, dose and duration of therapy in patients with ALF secondary to dengue infection.

Among the other therapies, corticosteroids have also been shown to be beneficial. Corticosteroids may improve outcomes in patients with severe dengue, but their role in ALF secondary to dengue has not been evaluated[46]. Further, most of these patients may require organ support in the form of renal replacement therapy, invasive mechanical ventilation or vasopressors. Therapies like cytokine filtration, plasma exchange and molecular adsorbent recirculating system have also been used in patients with severe ALF. However, large scale data is missing to recommend their routine use[20,25].

Although liver transplantation is considered as the ultimate therapeutic intervention in patients with ALF, it may be challenging in ALF secondary to dengue due to presence of hemodynamic instability, high risk of bleeding and

Table 3 Baseline laboratory parameters and hospital course

Parameter of interest	Frequency (%)
Hematocrit, median	42.60 (IQR 15.15)
Total leucocyte count (per µL), median	5800 (IQR 5800)
Platelet count (per µL), median	25500 (IQR 28250)
AST (U/L), median	4625 (IQR 11902.5)
ALT (U/L), median	3100 (IQR 2607)
AST/ALT ratio, median	2.45 (IQR 1.75)
Alkaline phosphatase (IU/L), median	191 (IQR 117)
Total bilirubin (mg/dL), median	5.5 (IQR 5.63)
Direct bilirubin (mg/dL), median	2.55 (IQR 2.75)
INR, median	2.13 (IQR 1.79)
Albumin (g/L), median	2.85 (IQR 0.48)
Creatinine (mg/dL), median	1.63 (IQR 2.12)
MELD score, median	27.5 (IQR 5)
Days of ICU stay, median	6 (IQR 2)
Specific therapies	
N-acetyl cysteine	6 (31.6)
Types of organ support	
Vasopressors	8 (42.1)
Renal replacement therapy	6 (31.6)
Ventilation	6 (31.6)
MARS	1 (5.3)
Organ failure	14 (73.7)
Neurological	10 (52.6)
Cardiac	8 (42.1)
Renal	7 (36.8)
Respiratory	
Complications	
Hepatic encephalopathy	13 (68.4)
Bleedings	3 (15.8)
DVT	1 (5.3)
Liver transplantation	2 (10.5)
Death	1 (5.3)

AST: Aspartate aminotransferase; ALT: Alanine transaminase; MARS: Molecular adsorbent recirculating system; ICU: Intensive care unit; DVT: Deep venous thrombosis; MELD: Model for end-stage liver disease.

underlying organ dysfunction. Hence, till date it has been successfully conducted in only a few cases[15,18].

Outcome of dengue patients with liver involvement depends on the severity of liver injury. Most patients with mild increase in transaminases show complete recovery and even those with DISH, have low reported mortality rates of less than 1%. However, ALF secondary to dengue is associated with high mortality rates ranging from 58.8%-66.7%[5,47]. In our cohort, only one death was reported, which may be attributed to selective reporting[23].

Strength and limitations

The present meta-summary compiled 19 case reports of ALF secondary to dengue infection from across the world, and is first of its kind. Moreover, we included those case reports and series which had individual patient details to compare

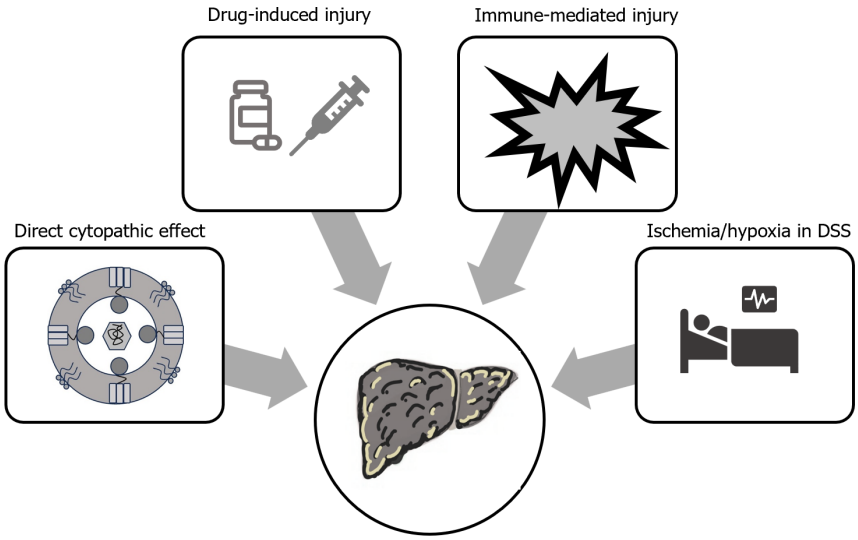

Figure 2 Mechanism of acute liver injury in patients with dengue fever. IL: Interleukin; TNF: Tumor necrosis factor; DSS: Dengue shock syndrome.

patient demographics, clinical course, and outcomes. However, the included studies were only case reports without any control arm. As these reports were heterogeneous, they are prone to high risk of bias and missing data, which may affect the generalizability of the results.

CONCLUSION

Dengue infection may rarely lead to ALF. These patients may frequently require intensive care and organ support. There is no specific therapy, but intravenous NAC therapy, if initiated early, maybe beneficial. Even though most of these patients may improve with supportive care, liver transplantation may be a therapeutic option in refractory cases. Early recognition is important for institution of supportive care, prognostication and timely referral for liver transplantation.

ARTICLE HIGHLIGHTS

Research perspectives

This research sheds light on the complexities of dengue-induced acute liver failure (ALF) and provides a foundation for further investigations and targeted interventions.

Research conclusions

ALF secondary to dengue infection is a rare but critical manifestation, requiring intensive care and organ support. Early recognition is vital for prognostication and timely referral for potential liver transplantation. Intravenous N-acetyl-cysteine shows promise as a supportive therapy, but large-scale studies are needed to validate its efficacy, dosage, and duration. Despite the challenges associated with liver transplantation in these cases, it remains a therapeutic option in refractory situations.

Research results

Nineteen case reports met the inclusion criteria, revealing a median age of 38 years, female preponderance (52.6%), and a median of 4.5 d from dengue diagnosis to ALF development. Most cases originated from India (36.8%) and Sri Lanka (26.3%). Elevated transaminases, neurological dysfunction, and cardiac support were common. Notably, only one death was reported (5.3% mortality), and most patients recovered with supportive therapy, while two underwent liver transplantation.

Research methods

A systematic search of multiple databases, including PubMed, Reference Citation Analysis, Science Direct, and Google Scholar, was conducted using specific keywords. Inclusion criteria comprised case reports or series with individual patient details and acute liver failure secondary to dengue infection. Data extracted from selected reports included patient demographics, clinical interventions, organ support requirements, and clinical outcomes. Statistical analysis was performed using SPSS, and the PRISMA 2009 checklist guided the meta-summary.

Research objectives

This meta-summary aims to analyze the demographic profile, symptomatology, hospital course, and outcomes of patients with ALF secondary to dengue infection. By reviewing published case reports and case series, we seek to delineate the patterns of liver involvement, identify factors influencing disease severity, and explore potential therapeutic strategies.

Research motivation

The motivation behind this study arises from the scarcity of large-scale data on ALF secondary to dengue, its varied clinical presentations, and the need for tailored therapeutic interventions. Given the rising frequency of dengue cases in both endemic and non-endemic areas, insights into ALF dynamics become crucial for effective management and prognosis.

Research background

Dengue, a prevalent cause of viral hemorrhagic fever, has witnessed an increasing global impact, extending beyond tropical regions. With over five billion people at risk and 400 million annual cases, the spectrum of dengue manifestations has expanded. Though liver involvement in dengue is common, ALF is rare, necessitating a comprehensive understanding of its demographics, clinical course, and outcomes.

FOOTNOTES

Author contributions: Nasa P acquisition of data, analysis and interpretation of data, drafting the article, final approval; Juneja D acquisition of data, analysis and interpretation of data, drafting the article, final approval; Jain R interpretation of data, revising the article, final approval.

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