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EDITORIAL

Challenges and limitations of synthetic minority oversampling techniques in machine learning

Ibraheem M Alkhawaldeh, Ibrahem Albalkhi, Abdulqadir Jeprel Naswhan

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Abstract

Oversampling is the most utilized approach to deal with class-imbalanced datasets, as seen by the plethora of oversampling methods developed in the last two decades. We argue in the following editorial the issues with oversampling that stem from the possibility of overfitting and the generation of synthetic cases that might not accurately represent the minority class. These limitations should be considered when using oversampling techniques. We also propose several alternate strategies for dealing with imbalanced data, as well as a future work perspective.

Key Words: Machine learning; Class imbalance; Overfitting; Misdiagnosis

Core Tip: Addressing class imbalance in medical datasets, particularly in the context of machine learning applications, requires a cautious approach. While oversampling methods like synthetic minority oversampling technique are commonly used, it is crucial to recognize their limitations. They may introduce synthetic instances that do not accurately represent the minority class, potentially leading to overfitting and unreliable results in real-world medical scenarios. Instead, we can consider exploring alternative approaches such as Ensemble Learning-Based Methods like XGBoost and Easy Ensemble which have shown promise in mitigating bias and providing more robust performance. Collaborating with data science specialists and medical professionals to design and validate these techniques is essential to ensure their reliability and effectiveness in medical applications.

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INTRODUCTION

Imbalanced medical data may have a variety of issues that impede classification, such as the impact of noisy data and borderline samples, class overlapping, class imbalance, or the presence of small disjuncts. When training a dataset, an imbalanced class distribution can occur when one class has significantly more samples than the other, resulting in a majority and minority class. This class imbalance can lead to prediction bias in machine learning models, which often translates to poor performance in the minority class(es). To address this issue, several techniques have been proposed in the literature. These techniques include increasing the number of samples from the minority class by obtaining more data from the source, modifying the loss function to assign a higher cost to misclassifications in the minority class, oversampling the minority class by replicating or generating synthetic samples, undersampling the majority class by reducing the number of instances, or using a combination of these approaches. By employing these techniques, the aim is to mitigate the class imbalance problem and improve the performance of machine learning models on imbalanced datasets[1]. There are benefits and drawbacks to each strategy. Many publications have used oversampling, particularly synthetic minority oversampling technique (SMOTE) approaches to create artificial samples from minority samples, to address the issue of class imbalance in the medical, biomedical, and life sciences fields. This is evident from the abundance of oversampling methods developed in the last two decades. For instance, a PubMed search for the terms "oversampling" OR "SMOTE" produced 2157 results from publications between 2000 and 2022, whereas a Web of Science search produced 2185 hits (but only for medical-related topics) (Figure 1). Additionally, when comparing this to undersampling using a PubMed search for ("undersampling" and "machine learning") and ("oversampling" or "SMOTE" and "machine learning"), a noticeable difference is found (Figure 2). This essentially indicates the developing trend of oversampling research in the medical literature, which dealt with or simply discussed oversampling.

Although there has been a substantial increase, it is important to note that this does not automatically imply the effectiveness of the oversampling approach. The surge in oversampling research can be attributed to the significant prevalence of the class imbalance problem and the relative simplicity of oversampling solutions[2]. The concern regarding oversampling methods arises from their potential to artificially increase the number of minority-class instances by generating new ones based solely on their similarity to existing minority examples. This raises concerns about the possibility of overfitting during the learning process. While oversampling techniques may yield favorable results in machine learning experiments, this does not necessarily translate to practical success. Additionally, a more significant issue with oversampling is that the synthetic examples created may actually belong to a different class in the real world, despite their similarity to the minority class examples. This is due to the fact that there are instances from class A that are closer to examples from a different class B, regardless of their similarity to the minority class examples[3].

Multiple experimental papers have provided evidence to support the concerns regarding oversampling methods. Elreedy *et al*[4] conducted a study where they analyzed the probability distribution of the synthetic samples generated by the SMOTE method. Their findings led them to conclude that the synthetic data produced by SMOTE may not precisely match the original distribution of the minority class, which can have an impact on the classification performance. Similarly, Tarawneh *et al*[2] argue against the current forms and methodologies of oversampling, considering it a deceptive approach. They suggest that oversampling introduces falsified instances that are falsely classified as members of the minority class when they are more likely to belong to the majority class. Their conclusions were drawn from a recommended validation system that was applied to various class-imbalanced datasets, including medical datasets. The validation system involved hiding a number of majority examples and assessing the similarity between the synthesized examples generated by different oversampling methods and the hidden majority examples[3].

After conducting a detailed analysis of their findings, it becomes evident that all validated oversampling approaches suffer from errors in the synthesized samples. These approaches generate instances that are intended to represent the minority class but actually resemble the majority class or fall within the decision boundary of the majority class (Figure 3). The error rate varies across different validated methods and oversampled datasets, ranging from 0% to 100%. None of the strategies achieve zero error on all datasets, indicating their inability to accurately oversample medical records. The oversampling techniques attempt to fill the feature space gap by creating new instances that are similar to

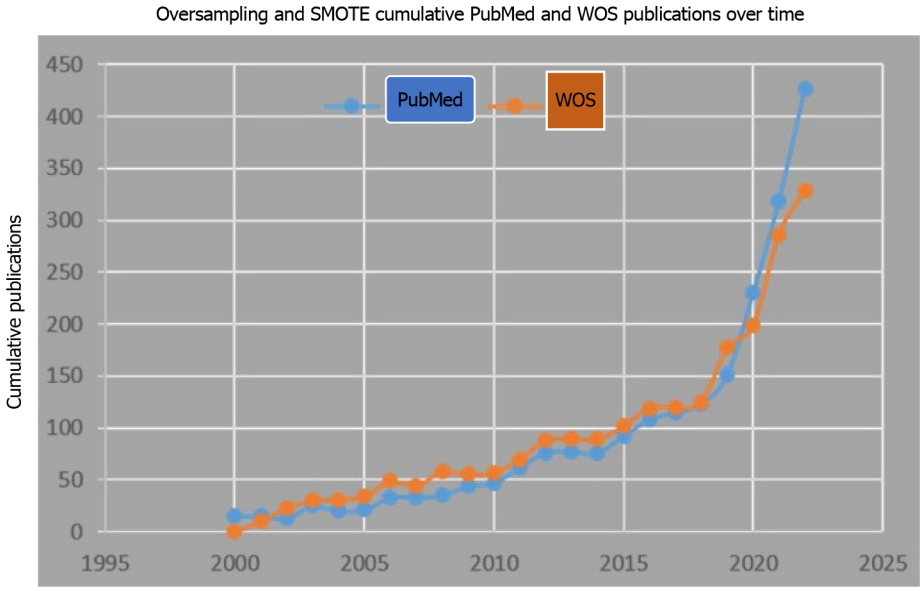

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Figure 1 The number of papers that discussed, utilized, or addressed oversampling or synthetic minority oversampling technique between 2000 and 2022.

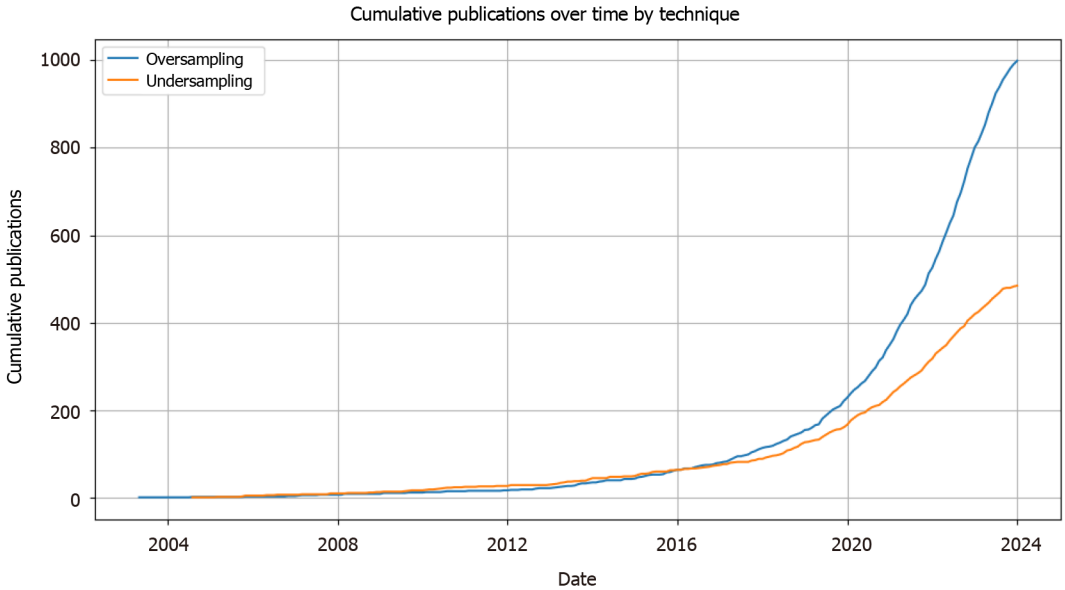

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Figure 2 Trend of PubMed publications on oversampling and undersampling techniques in machine learning over the years.

one or more minority instances. However, these techniques wrongly assume that the synthesized examples belong to the minority class without providing any guarantee. Consequently, the training of these instances becomes misguided, increasing the risk of overfitting the classifier on false data. This poses a significant threat as the entire machine learning system may fail when applied in real-world medical applications, where even a single incorrectly generated example can have severe consequences. Therefore, oversampling structured medical datasets by synthesizing new instances solely based on their resemblance to the minority examples is a questionable practice, particularly in the context of medical data. It is crucial to ensure that the additional samples truly fall within the minority class. Moreover, since the model itself is flawed, any external validation, subsequent analysis, or conclusions based on it should be critically examined. The potential harm and consequences of a misdiagnosis, inaccurate prediction, or prognosis can be particularly detrimental for cancer patients[1].

When it comes to the currently existing methods for dealing with class imbalance problems, researchers suggested various strategies for analyzing data, which are classified as data level, algorithm level, and hybrid (Figure 4). The methods are dependent on the size of the data collection, distribution, imbalance ratio, and model performance criteria[5].

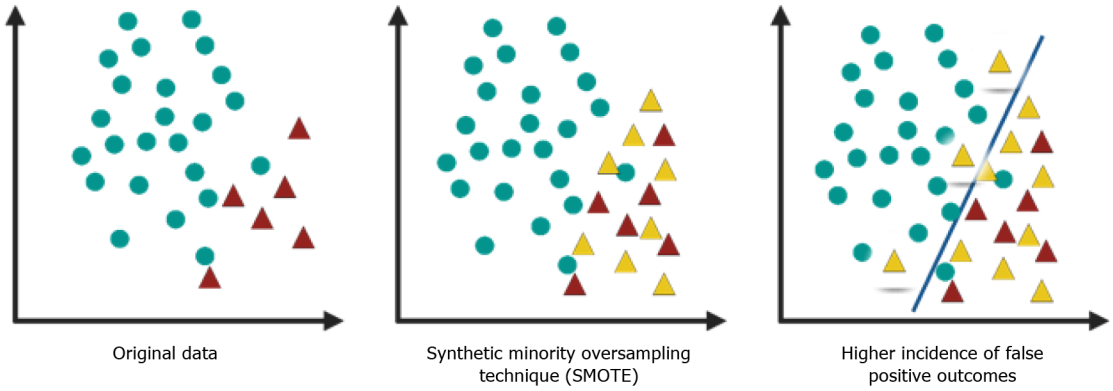

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Figure 3 Errors in the synthetic minority sample after synthetic minority oversampling technique application. SMOTE: Synthetic minority oversampling technique.

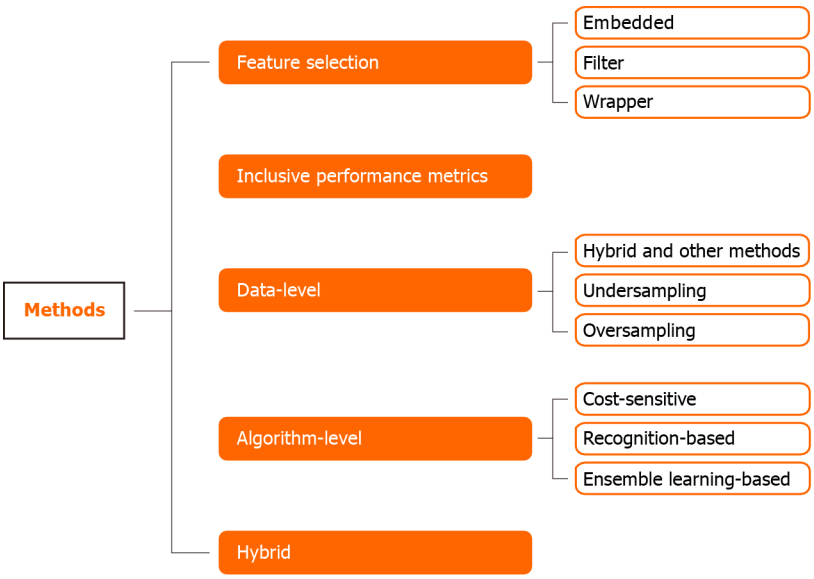

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Figure 4 Overview of the existing methods for the class imbalance problem.

Undersampling approaches, like oversampling, have some drawbacks at the data level, such as the loss of critical information for data distribution. Undersampling can result in the loss of relevant information by removing valuable and significant patterns. Oversampling and undersampling approaches can be used independently or in hybrid methods. Because they are based on existing techniques, these hybrid methods built on them share the same limitations[5]. Recent research suggested random partitioning of data with a voting rule, a resampling method that works by randomly splitting the imbalanced dataset into a number of smaller balanced sub-datasets. On each sub-dataset, a machine-learning model is subsequently trained. The final prediction is made by applying a voting mechanism to the individual model forecasts. Other resampling strategies were outperformed by this strategy. When tested using several machine learning classifiers on 33 benchmark class-imbalanced datasets, this approach has the potential to overcome the present limitations [6].

Hybrid techniques combine methods at multiple levels. For example, when data-level methods are used to process data externally and distribute classes to instances, the learning process is then carried out internally using algorithm-level methods. You can read for a thorough explanation of these techniques[7]. In algorithm-level methods, researchers have the ability to modify conventional machine learning models by assigning weights or costs to classifiers in order to mitigate bias towards the majority class. This approach ensures that the learning model remains unaffected by the class distribution. These methods can be categorized as recognition-based, cost-sensitive, or ensemble learning-based techniques.

In the absence of non-target class instances, recognition-based approaches such as one-class learning are employed. They model the classifier on the representation of the minority class and proceed to learn primarily from minority class instances rather than attempting to distinguish dissimilar patterns from majority class and minority class examples. One-class classification includes features such as outlier identification and novelty discovery. This approach performs well, particularly with high-dimensional data. One-class learning may be used to build many models, including support vector

machines and isolation forests; however, it cannot be used to build other models, such as decision trees and Nave Bayes [5].

Cost-sensitive methods are crucial in medical applications because of the significance of minimizing false positive and false negative instances. These methods involve adjusting the misclassification cost to achieve a balance between the majority and minority classes. For instance, assigning a higher weight or cost to false negative predictions compared to false positive predictions can be an effective approach. This practical solution enables cost-sensitive learning in the context of medical applications. In the literature, there are several cost-based approaches available to address class imbalance in data, including weighted cross-entropy, multiclass dice loss function, and focal loss. There are other recent methods such as weighted extreme learning machine, cost-sensitive decision tree ensemble methods, and cost-sensitive deep neural networks[5,8].

Ensemble learning methods have gained significant attention in various fields, including machine learning and medical applications. These methods combine multiple weak learners to create a more robust model with improved performance. Some popular ensemble learning techniques including voting and boosting ensemble learning models, such as XGBoost and Easy Ensemble, have been found to outperform individual learning models and exhibit greater resistance to noise and outliers[3,8]. However, it is important to consider the potential drawbacks of ensemble learning. These models often require significant training time and may be prone to overfitting in certain scenarios. To address the limitations of ensemble learning, researchers have introduced new techniques. Ensemble pruning methods aim to reduce the complexity and training time of ensemble models while maintaining their performance. Regularization techniques, such as dropout and bagging, have also been incorporated into ensemble models to mitigate the risk of overfitting[8].

The use of feature selection approaches is growing in addressing data imbalance. These techniques reduce computational and storage costs, eliminate redundant information, and facilitate data visualization. Feature selection methods can be grouped into filter, wrapper, and embedded methods. Filter methods select variables using statistical measures, while wrapper methods assess features based on model performance and selection criteria. Embedded methods, like least absolute shrinkage and selection operator regression, perform feature selection as an integral part of the learning process [5]. Additionally, it is important to consider diverse performance metrics, such as the area under the precision-recall curve, Matthew's correlation coefficient, F-score, and geometric mean, to effectively evaluate model performance in the presence of class imbalance. These metrics provide a comprehensive assessment of model performance, taking into account precision, recall, and the balance between sensitivity and specificity[5,9,10].

To effectively address class imbalance, it is advisable to consider various approaches at different levels, including data-level, algorithm-level, and hybrid-level techniques. These approaches aim to mitigate bias and improve classifier algorithms by combining different methods. There is a growing body of research focusing on hybridization techniques that integrate sampling, feature selection, and classifier building to gain a better understanding of class representation and achieve more accurate classification results. For instance, evolutionary computing can be employed in feature selection, while ensembles can be constructed to tackle the challenges associated with class imbalance. By adopting these practical and reliable solutions, the issue of class imbalance in medical datasets can be significantly improved, as these approaches are based on sound assumptions[5,9].

To gain insights into the impact of oversampling and other methods on real-world medical applications, it is crucial to collaborate with data science specialists and medical professionals. By working together, these experts can create and evaluate these approaches, taking into account their unique perspectives and expertise. This collaboration can provide valuable guidance and influence the selection or adaptation of appropriate techniques. The joint efforts of medical doctors and data scientists can lead to the development of more reliable and efficient solutions in the field of healthcare.

CONCLUSION

It is recommended to address class imbalance at different levels considering data-level, algorithm-level, and hybrid-level approaches that can mitigate bias. At the algorithmic level, Ensemble Learning-Based Methods such as XGBoost and Easy Ensemble, have proved to have a better performance than individual learning models, and they provide more resistance to noise/outliers. Another hybrid method is Random Undersampling Boost which is not without limitations either, noting that there is not a one-size-fits-all approach and exercising caution should be taken when addressing class imbalances. The adoption of such more practical and trustworthy solutions would improve the class imbalance issue in medical datasets more because these approaches have no wrong assumptions[2-6]. It is vital to understand how oversampling and other methods may affect real-world medical applications. Collaborating with data science specialists and medical professionals can enhance the development and testing of reliable and effective solutions, as their insights can provide valuable advice and influence the selection or modification of relevant techniques.

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EDITORIAL

Current protocol to achieve dental movement acceleration and pain control with Photo-biomodulation

Angela Dominguez

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Abstract

When designing a study on dental movement acceleration or pain control during orthodontic treatment, it is crucial to consider effective parameters. The objective of this editorial is to compile the most effective parameters supported by evidence that should be considered in future studies to achieve complete parameter homogenization. The protocol currently recommended to homogenize the parameters and facilitate the development of further meta-analysis in terms of acceleration of movement and pain control in orthodontics is Wavelength: 810 nm, 2.2 J per surface, 0.1 W in continuous mode/0.1 W average power in a super-pulsed, sweeping movement, 1mm from the mucosa, 22 seconds along the vestibular surface and 22 seconds along the lingual surface, the recommended speed of movement is 2 mm/sec, 1 application during each orthodontic control, to achieve dental movement acceleration and repeat the dose at 24 h to ensure pain elimination. The energy density and power density will depend on the spot size used in the equipment and the distance from the mucosa. It will strengthen the evidence of photobiomodulation as the best therapy to accelerate tooth movement and at the same time control the pain produced by orthodontic treatments.

Key Words: Photobiomodulation; Laser-assisted orthodontics; Dental movement acceleration; Pain control; Diode laser

Core Tip: Several Systematic Reviews and Meta-Analyses have been published to evaluate the effectiveness of photobiomodulation (PBM) in accelerating dental movement and pain control during orthodontic treatment. These studies suggest that PBM is an effective method to achieve these objectives. However, all reports show a lack of standardization in the ideal parameters required.

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INTRODUCTION

It is very common to find in randomized controlled clinical trials that evaluate the speed of tooth movement during orthodontic treatments or pain control, diverse protocols of photobiomodulation (PBM).

This means that at the time of developing a meta-analysis, or even a systematic review, the authors report that there needs to be a consensus in respect of guidance for clinical use, due to the lack of consistency of wavelengths and parameters applied.

The research question is no longer whether PBM controls pain and accelerates tooth movement when applied during orthodontic treatment. At the moment several systematic reviews and meta-analyses are in support of its efficacy[1-7].

The current question is how can we obtain the greatest effectiveness in PBM when we want to achieve those objectives.

To accomplish this goal, studies should focus on determining the necessary parameters such as wavelength, energy, power, application time, energy density, application points, and number of sessions.

This editorial aims to synthesize the parameters that are currently accepted to be effective in achieving these objectives during orthodontic treatment:

Pain control and dental movement acceleration.

Employing standardized parameters for accelerating movement and pain control will allow clinicians to be more effective in achieving these goals and will allow researchers to have adequate reproducibility in randomized controlled clinical trials that will later form part of systematic reviews and meta-analyses.

Wavelength

It is not enough to reproduce parameters such as energy, power, time, and energy density if we select a wavelength that is not the most effective to accelerate the movement.

Do we know what it is? The answer is: Yes.

In 2020, the first systematic review was conducted to establish an effective range of low-level laser therapy wavelengths for accelerating tooth movement in orthodontic treatment[8]. That review concluded that most randomized controlled trials related to accelerating tooth movement in orthodontic treatments are ideally between 780 and 830 nm wavelengths. The mean increase in speed of the dental movement calculated as a percentage of the control group was 24%.

Three years later, a systematic review and meta-analysis were performed with the same objective[9]. The authors concluded that ≤ 810 was determined as the wavelength associated with faster orthodontic tooth movement.

A range that fluctuates between 730 nm to 830 nm stimulates cell activity better[10,11]. It has been reported that wavelengths over 900 nm attract melanin and hemoglobin, resulting in a superficial energy uptake in soft tissue and insufficient energy in bone tissue. Shorter wavelengths have a greater ability to pass through soft tissue. Additionally, wavelengths within the range of 618 to 405 nm may not effectively stimulate orthodontic movement[12].

As for the effective wavelength to control pain during orthodontic treatments, the evidence has shown that pain reduction after 24 h is not significantly dependent on wavelength[13].

This indicates that if we are applying PBM to control pain and it disappears, we are not necessarily using the most effective wavelength to accelerate movement, as we considered in the years before these publications.

Emission mode

Until a few years ago, most studies were carried out to obtain the benefits of PBM in continuous mode. Most of the literature, before 2015, where we referred to Low-Level laser Therapy, provided studies with therapeutic equipment that emitted in continuous or pulsed mode. Currently, we have super-pulsed lasers available. However, the emission mode in laser-assisted orthodontics is not decisive. After selecting a device with the recommended wavelength, it can be continuous, pulsed, or super-pulsed. It is essential to take this into account when calculating the total energy delivered.

Power

Several studies report 100 mW (0.1 W)[14-18], however, this varies greatly depending on the equipment used. Nowadays there is equipment that operates in pulsed or continuous mode, but its minimum power is 0.2 W, so it can be pulsed at 50% to obtain an adequate amount of total energy. We also have super-pulsed diodes in which it is advisable to select 0.2 W.

Total energy

Since 2014 thanks to the first systematic review that evaluated these two objectives[19], we know that the most common and effective energy input is in the range of 0.2-2.2 J per point/2-8 J per tooth, to accelerate orthodontic tooth movement and control pain, the recommended energy per point ranged from 1-2 J when a single tooth was irradiated to 0.5-2.25 J per point when all teeth in the dental arch were irradiated.

Currently, 2-4 J per tooth is suggested to obtain both effects.

Energy density

Energy density is the most important parameter in laser PBM, as it estimates the actual energy received by the target tissue. It is often referred to as fluence (J/cm^2), meaning dose; however, some scholars also refer to energy in joules as dose[20].

The energy density is frequently mentioned in the dental literature, but the area of the spot in the tissue is often omitted. This error makes it impossible to verify their results or to see how they calculated the critical energy density information. Inconsistency in reporting these parameters is a major source of conflicting results in research and has contributed greatly to hindering acceptance of the effects of PBM[21].

The energy density is equal to the total energy if the tip or handpiece used in the equipment measures 1 cm 2, however, the dental equipment used for intraoral applications has round tips and in many cases, very small used without activation to allow the energy to reach the tissue and obtaining much higher energy densities, which are not always reported and make it difficult to standardize the parameters at the time of meta-analysis. This is why the ranges are extensive and energy density intervals range from 4.25 to 80 J/cm^2; depending on the tip used to achieve movement acceleration and pain control.

Equipment tips are not interchangeable between manufacturers.

To adapt the protocol in the clinic, depending on the equipment that the practitioner has, he can adjust the energy density in 80 J/cm^2 by applying the formula:

$$DE = \frac{P(W) \times T(s)}{A\ (cm^2)}$$

DE: Energy density;

T: Time in seconds;

A: Area in cm^2 in cm^2;

It is important to take into account that manufacturers report the diameter of the tip. To find the area in cm^2, the following formula should be applied:

$A = \pi \times r^2$

Examples: For a 7 mm diameter tip the area is 0.38 cm^2; For a 3 mm diameter tip the area is 0.07 cm^2.

Ideally, spot size (The area that reaches the tissue) measurements would be preferred, but due to the complexity of clinical practice due to is an infrared laser, it is recommended to standardize applications based on tip area, which is the closest alternative.

Power density

Aside from energy density, a second important parameter in laser measurement is power density[22]. Few studies have reported on power density, thus it is not extensively covered in the literature: 20 mW/cm^2[23] and 6.37 W/cm^2[24].

The energy density is not adequately reported because the distance from the mucosa to which the laser is applied is not standardized and this parameter depends on this distance.

Currently, it is suggested at 1mm from the mucosa.

Future studies should report without exception the energy density to facilitate a later consensus on both energy density and power density (fluence and irradiance) when looking for either of the two therapeutic objectives during orthodontic treatment.

Punctual application or scanning movement

The average increase in speed movement calculated as a percentage of the control group found in 2020 was 24%. Many authors recommended punctual application and divided the root into thirds, for example, Limpanichkul *et al*[14] and Doshi-Mehta *et al*[16], and some up to 5 points per surface[25,26]. This type of application is highly recommended in research cases to calculate the energy density per point in a precise way. However, it is complicated to reproduce it in clinical practice and it is easier to make a scanning movement. The recommended speed of movement is 2 mm/sec[27]. During orthodontic treatment, this movement is performed along the buccal and lingual surface of each tooth which requires acceleration for 22 s to obtain 2.2 J per surface, at 100 mW (0.1 W)[28] obtaining an average 30% acceleration of the movement. These parameters are also used for pain control.

Number of sessions

Studies differ greatly in the number of applications. There are reports of several acceleration appointments between orthodontic controls[29-31]. It is advisable to apply PBM in the orthodontic control appointment without additional sessions; repeated doses between control appointments do not increase the average acceleration.

The increase in the number of sessions does not exceed 30% acceleration of the movement which is the percentage by which the speed is increased if applied just in the controls. Therefore, it is not justifiable to increase the number of appointments between them.

To guarantee a pain-free orthodontic treatment, it is necessary to repeat the dose 24 hours after the orthodontic adjustment[32]. The suggested single monthly dose that allows acceleration of movement is insufficient to eliminate pain.

CONCLUSION

The protocol currently recommended to homogenize the parameters and facilitate the development of further meta-analysis in terms of acceleration of movement and pain control in orthodontics is Wavelength: 810 nm, 2.2 J per surface, 0.1 W in continuous mode/0.1 W average power in a super-pulsed, sweeping movement, 1mm from the mucosa, 22 seconds along the vestibular surface and 22 seconds along the lingual surface, the recommended speed of movement is 2 mm/sec, 1 application during each orthodontic control, to achieve dental movement acceleration and repeat the dose at 24 h to ensure pain elimination. The energy density and power density will depend on the spot size used in the equipment and the distance from the mucosa.

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EDITORIAL

New evidence-based practice: Artificial intelligence as a barrier breaker

Ricardo Maia Ferreira

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Abstract

The concept of evidence-based practice has persisted over several years and remains a cornerstone in clinical practice, representing the gold standard for optimal patient care. However, despite widespread recognition of its significance, practical application faces various challenges and barriers, including a lack of skills in interpreting studies, limited resources, time constraints, linguistic competencies, and more. Recently, we have witnessed the emergence of a ground-breaking technological revolution known as artificial intelligence. Although artificial intelligence has become increasingly integrated into our daily lives, some reluctance persists among certain segments of the public. This article explores the potential of artificial intelligence as a solution to some of the main barriers encountered in the application of evidence-based practice. It highlights how artificial intelligence can assist in staying updated with the latest evidence, enhancing clinical decision-making, addressing patient misinformation, and mitigating time constraints in clinical practice. The integration of artificial intelligence into evidence-based practice has the potential to revolutionize healthcare, leading to more precise diagnoses, personalized treatment plans, and improved doctor-patient interactions. This proposed synergy between evidence-based practice and artificial intelligence may necessitate adjustments to its core concept, heralding a new era in healthcare.

Key Words: Evidence; Clinicians; Patients; Artificial intelligence; Evidence-based practice

Core Tip: Evidence-based practice principles remain crucial in clinical care. However, practical application faces challenges. The recent emergence of artificial intelligence offers solutions for the main barriers. Artificial intelligence can swiftly provide evidence, enhances clinical decision-making, combat patient misinformation, and improve clinical consultations. The integration of artificial intelligence into evidence-based practice represents a potential paradigm shift, requiring some adjustments to the core concept of evidence-based practice.

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INTRODUCTION

The evidence-based practice (EBP) principles are several years old[1], yet their importance remains as relevant as ever. EBP continues to be considered the gold standard for the best clinical practice[2]. As it was developed some years ago, it has undergone some changes (*e.g.*, evidence-informed practice), but its foundation principles persist, with the best evidence, clinical expertise, and patient preferences playing central roles[3]. However, despite the widespread acknowledgment of its importance in the daily clinical practice, its practical application can be challenging and some barriers to its implementation arise. Some of these barriers include[4]: Lack of skills to understand studies; insufficient resources and funding; time constraints; lack of informatics and linguistic competencies; overtasking, heavy workload and competing priorities; inadequate training and stakeholders support; patient personal characteristics and health illiteracy; lack of motivation, confidence, interest, and commitment to change; evidence "unrealistic", inaccessible, "unreadable", conflicting, and massive.

While the concept is not new[5-9], we have only recently witnessed the emergence, interest, and adoption of a new technological revolution known as artificial intelligence (AI)[5,10]. AI encompasses the creation of machine learning technology, capable of performing high-level executive functions that typically require human intelligence (such as reasoning, learning, planning, and creativity)[7,11-17]. We observe applications in techs (*e.g.*, smart cars, smart homes, smartphones, computers, robots, and drones), navigation systems, code writing, facial recognition, chatbots, image and data analysis, translators, audio output, and more[11-13,18-23]. Like any other technological tool, AI has incrementally become a part of our lives, despite the reluctance of some public[13,16,24,25]. The AI use brings both advantages and disadvantages. Its advantages and disadvantages are summarized in (Table 1)[7,14,24,26-35].

By understanding and carefully evaluating its pros and cons, AI could be considered as a potential solution for overcoming some of the main barriers encountered in the application and implementation of EBP. Here are some examples: (1) Inability to stay updated with the best evidence. As it may be known, the pace of scientific production is currently at its peak. The number of articles is growing exponentially[36], making it nearly humanly impossible to search for and read all the information published every day. AI can assist in the search process by summarizing the latest literature within seconds, thus saving clinicians time for other tasks[24,26,31,37-39]. However, as it currently stands, AI still has some "bugs" (known as *AI Hallucinations*) and is not yet able to critically analyze it[12,13,21,34,37,39-51], making it essential that the clinician continues to do their own "homework"[51,52]. In addition, there is another evidence-related barrier – comprehension. Studies often employ a specialized scientific language, with English as the predominant language[53,54]. Many clinicians still do not have a satisfactory scientific and linguistic understanding to stay updated [55]. AI is already capable of providing definitions, complete document translations, (re)writing, and summarizing[13,18,21,22,25,37,39,54,56-61], overcoming this barrier; (2) Enhanced clinical decision-making. During a clinical session, it is up to the clinician to assess the patient's clinical situation and, based on the results, present the best intervention plan to their patients, considering the best and most recent literature along with their clinical experience[62]. Therefore, the first phase of clinical reasoning heavily relies on the clinician's "isolated" judgment. AI has already evolved to the point where it can integrate information from imaging and clinical findings, typical disease progression patterns, treatment responses (risk and benefits), and scientific information[7,8,12,16,23,24,35,38,39,57,63-70]. Consequently, AI can act as a second clinician in the decision-making process, where the human clinical expert can interact with the "artificial clinical expert", leading to more accurate decisions and presenting more precise diagnoses, prognoses, and personalized/tailored interventions plans for their patients[6,14,16,23,52,57,65,69-73]. This human-machine interaction may be particularly valuable for those just starting out their careers. As explored, one of the foundations for better clinical decision-making involves clinical experience. However, those who are just starting out in the profession do not yet have enough clinical experience to be experts in the field, and often have to make the first stage of the decision solely based on scientific evidence[74]. Therefore, AI could act as the expert in this situation, helping novice clinicians with their clinical decision-making; (3) Patient misinformation. While patients often do not actively participate in clinical decision-making, their beliefs and preferences should, in accordance with the principles of EBP, be considered when devising an intervention plan[1]. In this way, there is a mutual partnership between the clinician and the patient, as their beliefs and preferences can help

Table 1 Example of artificial intelligence advantages and disadvantages

Advantages	Disadvantages
Efficiency	Job displacement
Accuracy	Bias
Cost reduction	Lack of empathy
Constant availability	Complexity
Data analysis	Security risks
Customization	Lack of transparency
Scalability	Fairness
Natural language processing	Regulation
Automation	Ethical concerns
Productivity	
Accessibility	

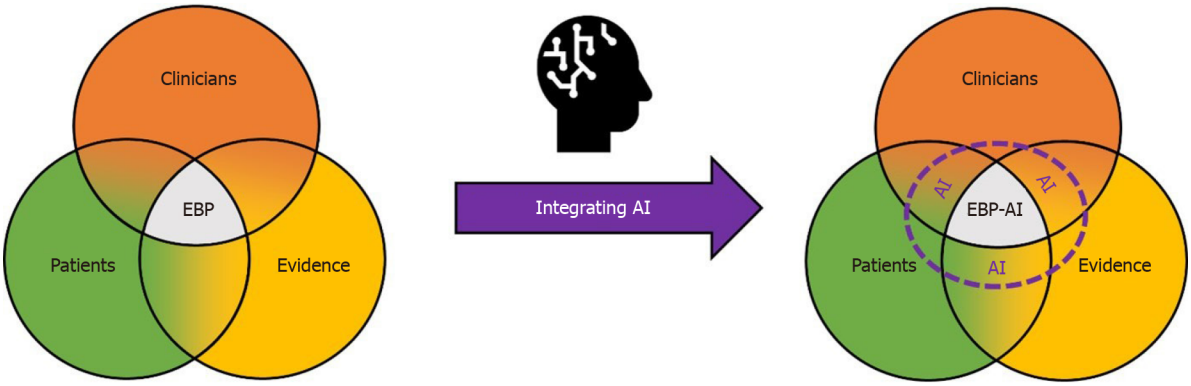

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Figure 1 Evidence-based practice artificial intelligence. EBP: Evidence-based practice; AI: Artificial intelligence.

(positively) limit the number of possible interventions/therapies/treatments, making a treatment plan easier to adhere [75]. However, patients' beliefs and preferences can sometimes pose a barrier. Patients frequently arrive at clinical appointments misinformed about their clinical condition and some of the interventions/therapies/treatments[76]. AI, if less biased[34,40], could correctly provide information to patients about their clinical condition and the most appropriate interventions/therapies/treatments[14,71,77]. This will greatly facilitate the doctor-patient interaction, thus enhancing the quality of intervention planning and clinical management[71,77]; and (4) Time constraints in clinical practice and consultations. As is well known, many clinicians spend a substantial amount of time on bureaucratic and administrative tasks, leaving them with insufficient time for proper patient care[7,38,75]. AI can assist in scheduling, triage, filling out forms, billing, monitoring, and responding to routine tasks (almost like an artificial assistant or secretary)[7,28,38,69,73,78]. This would allow clinicians to free up more time for tasks that involve essential human interaction, simply by issuing a few basic and quick commands[31,38,52].

CONCLUSION

As explored, AI can be considered a useful tool in clinical management, encompassing various aspects such as time management, assessment, interaction, prescription, monitoring, decision-making, information processing, and more. This could potentially usher in a paradigm-shift in EBP, requiring minor adjustments to its core concept. Consequently, the new EBP-AI proposal is presented in (Figure 1).

FOOTNOTES

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OPINION REVIEW

Evidence-based literature review: De-duplication a cornerstone for quality

Barbara Hammer, Elettra Virgili, Federico Bilotta

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Abstract

Evidence-based literature reviews play a vital role in contemporary research, facilitating the synthesis of knowledge from multiple sources to inform decision-making and scientific advancements. Within this framework, de-duplication emerges as a part of the process for ensuring the integrity and reliability of evidence extraction. This opinion review delves into the evolution of de-duplication, highlights its importance in evidence synthesis, explores various de-duplication methods, discusses evolving technologies, and proposes best practices. By addressing ethical considerations this paper emphasizes the significance of de-duplication as a cornerstone for quality in evidence-based literature reviews.

Key Words: Duplicate publications as topic; Databases; Bibliographic; Artificial intelligence; Systematic reviews as topic; Review literature as topic; De-duplication; Duplicate references; Reference management software

Core Tip: Effective de-duplication is crucial for maintaining the quality and credibility of systematic reviews. It ensures data accuracy, eliminates bias, reduces workload, and enhances trust in findings. However, challenges such as variability in data, database indexing, and resource constraints exist. Best practices include clear documentation, the use of reference management software, manual review when necessary, handling multiple versions of the same paper, addressing non-journal sources, and ethical considerations. Advancements like Deduklick and Automated Systematic Search Deduplicator offer promise for more accurate and efficient de-duplication methods. De-duplication is a fundamental step in evidence synthesis, contributing to transparent and reproducible research in systematic reviews.

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INTRODUCTION

Evidence-based literature reviews are essential for informed decision-making in research and practice. However, without proper de-duplication, duplicated records may skew findings, leading to biased conclusions. This opinion review aims to shed light on the importance of de-duplication in evidence synthesis and its evolution over time and to give a big picture of what's available as a solution so research teams can make an informative decision on what's best for the project when it comes to de-duplication.

What is de-duplication?

In the world of computing, database de-duplication refers to the technique of ensuring that specific information is only stated once. One way to find information that is consistent across multiple sources (such as data files, books, websites, and databases) is through record linkage (RL). Data matching, RL, data linkage, entity resolution, and many other terms are focused on finding such records and eliminating duplicates. "RL is necessary when joining different data sets based on entities that may or may not share a common identifier (*e.g.*, database key, URI, National identification number), which may be due to differences in record shape, storage location, or curator style or preference"[1]. The term de-duplication, in medical scientific writing, refers to the process of identifying and removing duplicate citations from the search results retrieved from various databases. "Record de-duplication is of great advantage for de-duplicating citation in bibliographic databases"[2]. However, the identification of duplicated citations is not a trivial task. "Records are usually not identical, because they may come from different databases and may differ in the treatment of authors' names of journal titles, indexing, and special field"[3]. Duplicated citations are the result of the standard in evidence synthesis studies like systematic reviews which require comprehensive searching in multiple databases to identify eligible studies. Duplicates can arise due to multiple factors, such as the same study being indexed in multiple databases and because "certain types of information are recorded differently (and inconsistently) in the different databases"[4].

As stated by the research team from the Pain Research, Nuffield Department of Anaesthetics, Churchill Hospital in Oxford: "if duplicate records are not removed effectively, reviewers can waste time screening the same records for inclusion and run the risk of accidentally including same paper more than once in their meta-analyses, leading to inaccurate conclusions"[5]. Hence removing duplicate citations is an important and necessary step between searching and screening in a process of the systematic review.

In practice, de-duplication is available *via* search platforms, reference management software (Table 1), and screening assistance tools (Table 2). However, automation does not entirely solve de-duplication issues. The process typically involves exporting search results into the library in one of the research management tools, merging data sets and identifying duplicated citations. Duplicates are identified by comparing various bibliographic elements such as titles, authors, publication dates, journal names, *etc.* Once potential duplicates are identified, the research team reviews these records to confirm if they are indeed duplicates. Confirmed duplicates are removed from the data set, ensuring that only unique study is kept and counted only once in the systematic review. Then the de-duplicated data set forms the basis for subsequent stages of the systematic review: study selection, data extraction, and data synthesis.

EVALUATION OF THE DE-DUPLICATION - METHODS, TECHNIQUES, AND TOOLS

De-duplication can be traced back to various stages in the development of information management and technology. Over time, de-duplication methods have evolved from manual to sophisticated automated techniques. However, to this day effective de-duplication methods may involve a combination of automated and manual approaches to ensure accurate and reliable results, as the race to create the ultimate tool continues. "In the early days of bibliographic record-keeping manual cataloging was the only process of creating metadata representing information resources, such as books, sound recordings, moving images, *etc.* Cataloging provided information such as author's names, titles, and subject terms that describe resources, typically through the creation of bibliographic records"[6]. Librarians and researchers manually

Table 1 Reference management software

Paid	Free
EndNote	Mendeley
RefWorks	Zotero
	Bib TeX

Table 2 Systematic review tools that offer de-duplication

Paid	Free
Covidence	Rayyan
DistillerSR	Systematic Review Assistant-De-duplication Module
Deduklick	Automated Systematic Search Deduplicator

reviewed catalogue cards or printed bibliographies to identify and eliminate duplicate entries. "The introduction of the term database coincided with the availability of direct-access storage from the mid-1960s onwards. As computers grew in speed and capability, several general-purpose database systems emerged; by the mid-1960s a number of such systems had come into commercial use"[7]. "Since the 1970s metadata were in machine-readable form and were indexed by information retrieval tools, such as bibliographic databases or search engines"[6].

With the rise of electronic databases, de-duplication has become more complex. Databases allowed for the storage and retrieval of vast amounts of bibliographic information, leading to an increased need for automated methods in de-duplication. There are several existing de-duplication tools, methods, and techniques that have been developed to address the challenges of identifying and eliminating duplicates from datasets. "Reference management software has been a useful tool for researchers since the 1980s"[8]. Within a brief timeframe, a marketplace was established, and commercial products were produced. The development of *e.g.*, EndNote[9], Zotero[10], or Mendeley[11], *etc.* provided researchers with tools to manage and de-duplicate their collections of references. This type of tool introduced automated (default) de-duplication features.

The introduction of Digital Object Identifiers (DOIs) "in the late 1990s, and implementation in the early 2000s"[12], has greatly facilitated de-duplication. DOIs provide a standardized and unique identifier for each publication, making it easier to track and manage duplicates. Screening assistance tools like, *e.g.*, Covidence[13] and Rayyan[14] were developed specifically for systematic reviews. These tools integrated automated de-duplication, collaboration features, and support for the review process. "Modern data matching algorithms utilize advanced techniques, including tokenization, stemming, and phonetic algorithms, to handle variations in text data and improve the accuracy of matching.

The following terms explain the various types of de-duplication processes: (1) Exact match de-duplication: This method examines precise matches in key fields, such as unique identifiers or customer IDs. If the same information is shown on multiple records, these duplicates are removed; (2) Fuzzy match de-duplication: Fuzzy de-duplication techniques use algorithms to determine the similarity between records, even if they do not have exact matches in key fields, allowing for the recognition of duplicates with slight differences or misspellings; and (3) Rule-based de-duplication: Rule-based de-duplication involves defining specific rules or criteria to identify duplicates. These rules can be based on data patterns, business logic, or specific requirements"[15].

Practical solutions for effective de-duplication constantly evolve. In 2013 research team from Fourth Military Medical University in China described a pragmatic strategy of combining automated and manual searching duplicates in a systematic review[16]. This paper evaluates the extensiveness and characteristics of duplicates in the PubMed, EMBASE, and Cochrane Library databases. Identifies two types of duplicates: Type-I (duplicates among different databases) and type-II (duplicate publications in different journals/issues). Results showed that most type-I duplicates are identified by the auto-searching method, while nearly all type-II duplicates are identified by the hand-searching method. The hand-searching approach has a substantially greater incidence of incorrect items in type-I duplicates, most of which come from the EMBASE database. The authors recommend employing a combined strategy of auto-and-hand-searching methods to find duplicates in the systematic review due to the insufficiency of a single strategy.

In 2015, Canadian researchers explored and compared the effectiveness of various de-duplication features, specifically in the Ovid and EBSCO database platforms and three selected reference management software packages: RefWorks, EndNote, and Mendeley[17]. The authors recorded the time taken to de-duplicate each option, the number of false positives, and the false negatives, and in conclusion, recommended different de-duplication options based on the skill of the searcher and the reason for de-duplication. Overall, the results of the study highlight the variation in time and effectiveness of different de-duplication options, providing insights for researchers to choose the most suitable option based on their needs and expertise. Same year research team from Bond University in Australia developed a de-duplication program - The Systematic Review Assistant-De-duplication Module (SRA-DM) to improve the effectiveness of duplicate detection[18]. The paper presents the evaluation of the SRA-DM against EndNote's default de-duplication process, comparing their sensitivity and specificity in detecting duplicates. The goal of the study was to determine the reliability and effectiveness of the SRA-DM in removing duplicate records. In conclusion, SRA-DM demonstrated superior

sensitivity (84%) and specificity (100%) compared to EndNote's default de-duplication process, resulting in a 42.86% increase in the number of duplicate records detected. The paper acknowledged that no software can currently detect all duplicate records, and there are limitations to the SRA-DM, such as undetected duplicates due to discrepancies in data and extraneous information inserted into the title field.

In 2016, an international research team led by Erasmus MC-Erasmus University Medical Centre in Rotterdam developed a de-duplication method (colloquially referred to as the Bramer method) for de-duplicating database search results in EndNote, a popular reference manager, which is used by information professionals conducting exhaustive searches for systematic reviews[19]. The authors highlight the limitations of relying on unique identifiers like DOIs and PMIDs for identifying duplicates and propose using pagination as an alternative. They discuss the variations in page number formats used in different databases and provide a method for adapting the page number format of references to facilitate de-duplication. The paper addresses the challenges of existing de-duplication methods, which are time-consuming or impractical, and compares different software programs. The authors provide detailed instructions for customizing EndNote settings, creating export files with expanded page numbers, and installing filters for importing modified files. Overall, the paper contributes a practical and efficient method for de-duplicating database search results in EndNote, addressing the limitations and challenges of existing methods. This method is still very popular even though it was introduced in 2016.

In 2019, two researchers from the university library at the Vrije University in Amsterdam created AMSTERDAM EFFICIENT DE-DUPLICATION (AED) METHOD. The paper describes the authors' method of de-duplication, which provides a systematic approach to de-duplicating articles and claims to be 100% reliable[20]. The AED method explains per database/host what steps are needed to successfully de-duplicate data sets. This multi-step approach for efficient de-duplication includes collecting accession numbers during the initial search which is useful for an update search and then followed by manual assessment. If the data set is large authors advise following up with the Bramer method.

In 2021, Canadian researchers evaluated the accuracy and efficiency of commonly used electronic methods for flagging and removing duplicate references in systematic reviews[21]. Testing included the default settings (using the default algorithm of each program) in Ovid multifile search, EndNote desktop, Mendeley, Zotero, Covidence, and Rayyan. A benchmark set of unique, de-duplicated references was created through manual abstraction, and the performance of different de-duplication methods was compared against this benchmark set. The study identifies Ovid, Covidence, and Rayyan as the most accurate methods for identifying duplicate references, with Ovid and Covidence having high specificity and Rayyan demonstrating high sensitivity. The paper highlights the strengths and weaknesses of commonly used de-duplication methods and provides strategies for improving their performance to avoid unintentionally removing eligible studies and introducing bias into systematic reviews. The limitation of this paper is the fact that it does not provide specific details about the number of false-negative and false-positive duplicate references for each method or the overall accuracy, sensitivity, and specificity values. Still, the findings of the study are important for researchers in selecting database platforms and supporting software programs for conducting systematic reviews, highlighting those factors such as availability, ease of use, functionality, and capability must be taken into consideration.

In 2022, researchers were introduced to the most advanced de-duplication to date. The Swiss research team from the University of Bren has developed an automated, artificial intelligence-based algorithm named "Deduklick" which combines natural language processing algorithms with a set of rules created by expert information specialists[22]. This automated de-duplication uses a multistep algorithm of data normalization, calculates a similarity score, and identifies unique and duplicate references based on metadata fields, such as title, authors, journal, DOI, year, issue, volume, and page number range. Authors claim that the algorithm significantly reduced the time spent on analysis, simplifying the systematic review process. The performance was comparable to expert information specialists while preserving high metadata quality. The algorithm's transparent and explainable decision process, along with its reproducibility and adherence to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards[23], makes it a reliable tool for duplicate removal. Although this sounds groundbreaking, this paper is not free from limitations *e.g.*, it does not address potential biases or limitations in the algorithm's decision-making process, such as the impact of different metadata fields on duplicate detection or does not discuss potential limitations or challenges in implementing Deduklick in real-world systematic review processes. Also, the evaluation of the performance was limited to eight existing datasets, and it is unclear if these datasets represent the full range of systematic review scenarios.

In 2023, research team from the University of Edinburgh developed Automated Systematic Search Deduplicator (ASySD) an automated tool to conduct the de-duplication of systematic searches in biomedical databases[24]. In this paper, the authors compared ASySD with other existing tools, such as EndNote's default de-duplication option and the SRA-DM. As a result, ASySD outperformed the alternative methods, correctly removing > 95% of duplicate citations across five biomedical datasets, while removing a few citations incorrectly (specificity > 0.999). The paper's limitations are - the tool was only developed using preclinical systematic review datasets and its performance in other review areas has not been fully evaluated. The accuracy of ASySD depends on the quantity and quality of citation information, and it may not work well with older searches or citations lacking certain bibliographic information. ASySD may mistakenly remove some citations, which is a concern for smaller reviews where each relevant paper is important. Additionally, the memory requirements for larger datasets exceed what is possible on shinyapps.io, so users may need to run the Shiny application locally in R Studio, which can be challenging for non-R-proficient individuals.

IMPORTANCE OF DE-DUPLICATION IN EVIDENCE SYNTHESIS

De-duplication serves as an important step in evidence-based literature reviews hence research teams must be aware of

the two types of de-duplication in medical scientific writing[25]. As mentioned earlier the first type exists on a database level and is a result of the event when a single manuscript was concurrently entered into two or more databases. Usually detected *via* automatic de-duplication. The second type exists between various journals when a single manuscript/study was released in several publications/issues/editions. This category is often referred to as study de-duplication "as it aims to identify two distinct reports of the same study. Study deduplication, although the rarer of the two is only usually detected after data have been extracted from both papers, after the authors have been contacted, or sometimes not at all" [26]. Usually detected *via* screening in full text. Removing duplicate records is essential in maintaining data accuracy and integrity, as they can introduce inaccuracies and redundant data extraction and analysis efforts. De-duplication streamlines the process by focusing on unique evidence, minimizing confusion and misinterpretation in systematic reviews. It eliminates bias and overestimation as duplicates can bias the results of evidence synthesis by inflating the apparent number of studies available for analysis. "Using a primary study results multiple times in the same analysis overstates its sample size and number of events, falsely leading to greater precision in the analysis"[27]. It enhances the quality of synthesis as evidence synthesis aims to provide a comprehensive and accurate overview of the available evidence. "If the same study has more than one report - possibly with different author lists, different titles, and in different journals - both papers should often be cited, but they should only be included in the meta-analysis as one trial" [26,28]. De-duplication ensures that the synthesis is based only on a unique and high-quality set of studies. Reduce workload and improve efficiency as removing duplicates reduces the workload for reviewers and analysts, allowing them to focus on analysing unique studies. This enhances the efficiency of the evidence synthesis process. Provide transparent and reproducible research as de-duplication is an integral part of transparent and reproducible research. Documenting the process ensures that others can replicate the de-duplication and validation steps, enhancing the credibility of the review. Align with publication standards and guidelines as many publication guidelines, including the PRISMA, emphasize the importance of de-duplication to maintain the quality and credibility of systematic reviews. Most importantly enhances trust in findings as de-duplication increases the trustworthiness of the systematic review findings by demonstrating a rigorous and transparent approach to handling data.

WHAT ARE THE CHALLENGES AND LIMITATIONS OF DE-DUPLICATION IN SYSTEMATIC REVIEWS?

De-duplication in systematic reviews, while necessary, is not without challenges and limitations which were touched on earlier. It is important to be aware of these potential issues to effectively address them and ensure the accuracy and reliability of the review process. Conducting manual reviews to confirm duplicates is time-consuming, especially when titles and abstracts do not provide enough information for differentiation. Managing and processing large complex data sets manually can be time-consuming, and error-prone, automated de-duplication seems to be the best option however deciding on either manual or automated de-duplication requires careful consideration.

Variability in data contributes to the issue - variations in study titles, author names, and other bibliographic elements can complicate the de-duplication process as it makes it difficult to accurately identify duplicates requiring careful consideration of matching criteria.

Differences in database indexing - different databases use varied indexing and citation formats which leads to inconsistent or incomplete data *e.g.*, page numbers, which can affect how duplicates are identified, making it harder to determine if two records are indeed duplicates. Furthermore, as a result of those discrepancies automated de-duplication tools may produce "false negatives (duplicate citations that should have been deleted but were not) and false positives (duplicate citations that were deleted but should not have been)"[17]. Hence researchers need to account for these variations during the de-duplication process and often manual review is essential to confirm results.

Cross-language studies - dealing with studies published in different languages introduces challenges due to variations in titles, authors, and other bibliographic details. Non-journal sources - systematic reviews may include various types of sources beyond journal articles *e.g.*, reports, theses, and conference papers. These sources may have different indexing and citation formats, making de-duplication more complex.

Multiple versions of the same studies also contribute to the problem. Studies may be published in different versions (*e.g.*, conference abstracts, and full-text articles) or have been published in multiple journals. Deciding whether these are duplicates or unique records requires careful assessment as "there is currently no standard methodological approach to deal with overlap in primary studies across reviews"[27].

Risk of exclusion - overly aggressive de-duplication can lead to the inadvertent exclusion of potentially relevant studies, affecting the comprehensiveness of the review. Technological limitations - automated tools may not be able to handle certain complexities, such as very similar studies with nuanced differences *e.g.*, differences in journal names "and" instead of "&" or author information or order of authors names[17].

Resource constraints - limited access to paid-for automated tools, lack of personnel, or time can impact the thoroughness of the de-duplication process.

Striking a balance between efficiency and accuracy is essential to overcome these limitations. Navigating these challenges requires a combination of methodological rigor, technological tools, collaboration among the review team, and transparent reporting of the de-duplication process and outcomes.

THE ROLE OF PROSPECTIVE REGISTRATION OF SYSTEMATIC REVIEWS AND META-ANALYSIS AND HOW THIS HELPS IN DE-DUPLICATION?

Prospective registration involves registering systematic reviews and meta-analyses in publicly accessible databases before starting the research process. This practice has gained prominence in recent years, primarily due to its significant impact on de-duplication efforts. In a 2022 paper by a German team from Brandenburg Medical School (Theodor Fontane)[29], the authors stated that prospective registration of systematic reviews aims to reduce bias in research conduct and reporting, increase transparency, and prevent unintended duplication, thereby reducing research waste. There are several options available for prospective registration, including PROSPERO, the Registry of Systematic Reviews/Meta-Analyses in Research Registry, INPLASY, the Open Science Framework Registries, and protocols.io. These registries provide search functions to help authors avoid duplicate reviews.

Prospective registration discourages the submission of the same systematic review or meta-analysis to multiple journals, as researchers and publishers can easily identify prior registrations. Hence reduces the chances of duplicate publications, a common issue in medical literature, which can subsequently lead to de-duplication problems. Registered systematic reviews and meta-analyses are required to provide a detailed protocol outlining their research objectives, methodologies, and inclusion criteria. This transparency helps researchers identify potentially duplicate records, even before data collection begins. Prospective registration fosters collaboration by allowing other researchers to see that other reviews are ongoing or coming up in relation to their own field. But also fosters group work and discourages the chances of having redundant reviews at the same time.

THE ROLE OF REFERENCE CITATION ANALYSIS FOR PROPER CITATION AND DE-DUPLICATION

Another important tool to improve de-duplication in medical databases is reference citation analysis and this goes hand in hand with prospective registration. "Use of the unique registration number may be useful in helping track subsequent use or citation of the review to monitor its impact"[30]. It involves a meticulous examination of the references cited in articles, and it plays a critical role as *via* reference citation analysis, researchers can identify secondary publications that stem from the same primary research, such as conference abstracts, journal articles, and systematic reviews. This is crucial for de-duplication, as it helps consolidate related information into a single reference. Citation analysis also aids in ensuring that the primary sources are correctly attributed and cited in systematic reviews and meta-analyses. But also, can reveal citation errors, discrepancies, or inconsistencies in systematic reviews and meta-analyses. Identifying and rectifying these issues contribute to the overall quality of the research synthesis. This helps maintain accuracy and integrity in the research synthesis process.

BEST PRACTICES FOR DE-DUPLICATION IN LITERATURE REVIEWS

De-duplicating search results and studies effectively during the systematic review process is essential. A comprehensive understanding of the data set's characteristics and proper validation of de-duplication outcomes are also critical. Transparent documentation of de-duplication procedures and reproducibility of results are important. Yet there are no standardized guidelines for all aspects of de-duplication, leading to variations in practices and interpretations as shown in this opinion literature review.

Following best practices helps maintain the quality of the review and the credibility of its findings. The practice proposed in this opinion review reflects the personal approach of the librarian but can be applied broadly to all researchers working on a systematic review to achieve reliable and reproducible results.

Document the process - clearly document your de-duplication process in the review protocol. This documentation should include the criteria for identifying duplicates, the tools/software used, and any decisions made during the process of de-duplication so it could be replicated by others. Transparency in the methodology enhances the credibility of the review.

Utilize reference management software (*e.g.*, EndNote, Zotero, Mendeley) to manage and organize search results. These tools include automated (default) de-duplication features that help identify at least the exact matches and reduce obvious duplicates. *e.g.*, in EndNote the default settings are author, year, and title. These tools can also further help identify duplicates based on predefined criteria *e.g.*, volume, issue, and pages which require deciding on a method that is best for that project *e.g.*, the Bramer method for EndNote.

Manual review - conduct a manual review of potential duplicates identified by some of the automated tools. Establish criteria for matching - define explicit criteria for matching *e.g.*, titles, authors, publication dates, and other bibliographic information to confirm whether records are indeed duplicates. Decide on a threshold for matching to avoid excluding potentially relevant studies.

Handle multiple versions of the same paper - pay attention to different versions *e.g.*, conference abstracts, and full-text articles. Decide whether to treat them as separate records from the start or duplicates based on their content (usually screening in full text will solve this problem).

Address non-journal sources - be prepared to de-duplicate various types of sources beyond journal articles, such as conference proceedings, reports, and theses. Consider their unique indexing and citation formats.

Handle updates and overlapping searches of the existing systematic review strategy. If your review involves multiple search rounds, use the de-duplication process to identify studies already included in previous rounds to avoid the common assumption that updating the search strategy is as easy as taking it from where you left off.

Resolve discrepancies - in case of discrepancies or uncertainties, consult with your review team to make informed decisions about the status of potentially duplicated records. Document decisions - document all decisions made during the de-duplication process, including the rationale for excluding or retaining records. Transparency in decision-making enhances the review's reproducibility.

Remember that while automated tools can expedite the de-duplication process, often manual review is unavoidable and still crucial for accurate identification of duplicates, especially when titles and abstracts are not sufficient for differentiation. Consistency, thoroughness, and transparency are key principles when de-duplicating studies in systematic reviews.

ETHICAL CONSIDERATIONS IN DE-DUPLICATION

While de-duplication is essential for data integrity and research quality, it's important to approach the task in a manner that respects the rights of authors and researchers, maintains data privacy, and adheres to ethical standards *e.g.*, the European Code of Conduct of the Research Integrity published by All European Academies[31]. Ethical considerations in de-duplication align with principles of reliability, honesty, respect, and accountability in responsible research and data management outlined by the above code.

With that in heart, this opinion review proposes the following considerations, transparency and documentation: (1) Systematic review protocol should provide transparent information about the de-duplication process, including the criteria used, methods applied, and decisions made. Transparent documentation helps ensure accountability and reproducibility, allowing others to understand and verify the process; (2) Preservation of data integrity: While removing duplicates is necessary, ensure that the process does not alter or compromise the integrity of the original data. Keep the original data as a reference for any future inquiries; (3) Conflict resolution: In cases of disagreements or uncertainties about the status of a record, aim for consensus within the review team to resolve conflicts ethically and responsibly; and (4) Maintaining original records: Keep a copy of the original duplicated records, even if they are removed from the final dataset. This preserves a historical record of the research process.

FUTURE DIRECTIONS AND CHALLENGES

The future of de-duplication holds exciting possibilities as technology continues to evolve. Continued advancements in machine learning, deep learning, and natural language processing will enable more accurate de-duplication. Deduklick is already on that path as its first de-duplication tool to ease the de-duplication burden. Standardization efforts to harmonize data formats, identifiers, and metadata across different sources would also simplify de-duplication processes. Collaboration across disciplines, ongoing research, and innovative solutions will even further shape the future of de-duplication.

CONCLUSION

Accurate and reliable de-duplication stands as a cornerstone for quality in evidence-based literature reviews. By addressing issues of duplicate records and data redundancies, de-duplication plays a critical role in upholding the scientific rigor, transparency, and overall quality of systematic reviews, making them more trustworthy and impactful resources for evidence-based decision-making. Although this functionality is available *via* many tools not all of them keep up with current advancements in the field of computer science and continue to see de-duplication only as one of many functions' tools were designed to perform. With Deduklick and AsySD the future of de-duplication holds promise for more accurate, efficient methods that can handle increasingly complex and diverse datasets.

FOOTNOTES

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REVIEW

Crohn's disease and clinical management today: How it does?

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Abstract

Crohn's Disease (CD) is an Inflammatory Bowel Disease and is characterized by an immune-mediated nature. Its etiology results from the interaction between genetic, enviromental and microbial factors. Regarding pathophysiology, it involves high levels of interleukin (IL)-12, IL-17, and Th1 profile, along with loss of tolerance mechanisms, an increase in pro-inflammatory interleukins, beyond the possibility to affect any part of the gastrointestinal tract. Its symptoms include abdominal pain, chronic diarrhea, weight loss, anorexia, and fatigue, as well as blood in the stool or rectum. Additionally, conditions comprising musculo-skeletal, cutaneous, ocular, hepatic, and hematological alterations may be associated with this scenario and extra-intestinal presentation, such as erythema nodosum, anterior uveitis, osteoporosis, and arthritis can also occur. Today, clinical history, exams as fecal calprotectin, ileocolonocopy, and capsule endoscopy can be performed in the diagnosis investigation, along with treatments to induce and maintain remission. In this sense, anti-inflammatory drugs, such as corticosteroids, immunomodulators, and biological agents, as well as surgery and non-pharmacological interventions plays a role in its therapy. The aim of this review is to bring more current evidence to clinical management of CD, as well as to briefly discuss aspects of its pathophysiology, surveillance, and associated disorders.

Key Words: Crohn's disease; Inflammatory bowel diseases; Diagnosis; Treatment; Immunomodulation; Biological agents

Core Tip: Today, the clinical management of Crohn's disease (CD) involves both non-pharmacological and pharmacological therapies with the primary objective of inducing and maintaining remission. In this context, anti-inflammatory drugs, including corticosteroids, immunomodulators, and biological agents, can be employed either as monotherapy or in combination. Surgical treatment, while considered palliative, is not curative. Therefore, this review aims to provide an overview of current evidence regarding interventions for CD.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic condition characterized by inflammation in the gastrointestinal tract (GI) [1-3]. It encompasses a variety of diseases, with Crohn's disease (CD) and ulcerative colitis (UC) being the primary recognized types[2,3]. These diseases are persistent, debilitating, recurrent, and immune-mediated, affecting the digestive system[4,5]. The etiology of CD is multifactorial and results from the interaction between genetic and environmental factors and microbial exposure[6]. Several genes have been associated with CD, with the most evident link being related to the NOD2/CARD15 gene, which is associated with an earlier onset of the disease and a family history of CD[7]. Environmental risk factors include smoking, infections (especially *Clostridium difficile*, linked to disease relapses), use of medications (for example, antibiotics, mainly in the first year of life, aspirin, non-steroidal anti-inflammatory drugs, and oral contraceptives), a low-fiber diet, and stress[8]. Currently, the incidence of IBD is notably increasing, especially in developing countries and recently industrialized nations[9]. The disease, previously considered predominantly an affliction of young adults, is now diagnosed across all age groups, with about 25% of patients identified before the age of 20. The peak occurrence in childhood is during adolescence, but approximately 20% of children develop it before the age of 10, and about 5% before the age of 5[10]. Epidemiological studies in Japan also indicate an increase in IBD incidence, especially in males[11]. In Brazil, it is considered to have low rates of IBD. However, there are indications of an increase in its occurrence, even in the absence of detailed information on new cases[12]. Regarding age, research indicates a notable prevalence in individuals between 20 and 50 years old. Age groups between 20 and 60 years exhibited the highest rates of disease, with women registering a higher incidence in both CD and UC[13]. In the context of IBD, inflammation of the intestinal mucosa triggers symptoms such as abdominal pain, diarrhea, presence of blood in stools, weight loss, and the infiltration of immune cells like neutrophils and macrophages that release inflammatory substances, enzymes, and free radicals, contributing to lesions and ulcerations[2]. CD causes segmental inflammation that can affect any part of the digestive system, from the mouth to the anus. It often results in deeper ulcers that traverse all intestinal layers, potentially leading to the formation of fistulas and associated complications[5]. Although it most commonly affects the gut, this inflammatory condition can impact multiple organs[14,15]. The frequency of extra-intestinal manifestations ranges from 6% to 47%[16] and includes joint, mucocutaneous, hepatopancreatobiliary, and ocular manifestations[17,18]. The impact of IBD on patients' quality of life is significant, affecting physical and mental health as well as work performance. Moreover, it imposes a substantial burden on healthcare systems due to its chronic and recurrent nature. If inflammation is not properly controlled, serious complications can arise, such as abdominal abscesses, strictures, and intestinal obstructions, increasing the risk of developing tumors in the GI tract[5]. Therefore, this review aims to describe the current clinical management of CD to assist healthcare professionals in providing adequate care for these patients.

METHODOLOGY

For this review, the authors surveyed relevant articles in the United States National Library of Medicine (PubMed). The descriptors used, along with Boolean descriptors AND/OR, were: CD; pathophysiology; immunology; diagnosis; clinical management; pharmacological; non-pharmacological; aminosalicylates; corticosteroids; immunomodulators; infliximab; adalimumab; vedolizumab; ustekinumab; surgery. The eligibility criteria for this review were based on the discussion of clinical management, covering topics from the diagnosis of CD to the exploration of new therapies. Articles published within the last 10 years and available in English, Portuguese, or Spanish were considered. A total of 24638 articles were initially identified in the database, of which 124 met the inclusion criteria. Articles that did not address the topics mentioned in the title/abstract or upon full-text examination were excluded. Additionally, a manual search of the references in the included articles was conducted, leading to the inclusion of 18 more articles. In total, 142 articles were included in this review. The summary of the articles selection process is shown in Figure 1.

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Figure 1 Summary of the articles selection process.

PATHOPHYSIOLOGY AND CROHN'S DISEASE IMMUNOLOGY

Both immunological mechanisms of innate and adaptive immunity are interconnected on pathophysiology of CD. There is a dysregulation of the inflammatory and anti-inflammatory processes, as well as excessive release of cytokines[19] .

Histologically, the mucosa of the GI tract comprises three types of cells: Goblet cells, Paneth cells, and immune system cells[20]. These cells are crucial for maintaining the structural integrity and defense of the intestines and play an active role in homeostasis[21,22], causing any intestinal injury to potentially disrupt physiological functioning mechanisms, resulting in harmful responses to the body concerning food absorption and digestion[23]. Additionally, when there is tissue damage in the intestine, alterations in the microbiota can lead to mucosal inflammation, recruiting natural killer (NK) cells and monocytes to combat the injury or pathogen[23].

Studies in mice infected with microorganisms have demonstrated the crucial role of innate lymphoid cells (ILC3) in maintaining the integrity of intestinal mucosal tissues. The absence of these cells, along with IL-22, has been linked to the development of IBD[24]. In addition, Th2 profile response is observed in patients with UC while a Th1 profile response in patients with CD[24]. The high levels of interleukin (IL)-12 in the serum stimulate the maturation of immature T lymphocytes to Th1 profile[25]. Subsequently, cytokines released into the intestinal lumen contribute to the chemotaxis of Th1 cells to the mucosa of this organ. Lymphocytes synthesize chemokines like Interferon (IFN)-γ, IL-2, and tumor necrosis factor-alpha (TNF-α), which are related to the migration of neutrophils and macrophages to the site of inflammation, causing further damage to the mucosal epithelium[19,26].

Within this context, other cytokines such as IL-1, IL-6, IL-23, and transforming growth factor-beta (TGF-β) contribute to the differentiation of a Th17 profile during the antigen presentation process among a subset of immature lymphocytes [27]. These cells are responsible for synthesizing IL-17, which stimulates the migration and activity of neutrophils, thereby promoting a more inflammatory environment, along with T regulatory (T-reg) cells[27,28]. Furthermore, the high levels of IL-17 present in the tissues affected by CD can act on the intestinal epithelium, triggering the release of chemokines that further enhance the chemotaxis of inflammatory agents[28,29]. Macrophages and antigen-presenting cells (APC) also contribute to the synthesis of IL-12, IL-6, TGF-β, and IL-23. Therefore, the inflammatory process is capable of creating a feedback loop, continually stimulating the expression of substrates with Th1 and Th17 profiles[26].

Physiologically, the microbiota assist in the immune tolerance process, which occurs when the body's own antigens are captured by APCs, presented to naïve T lymphocytes, and, due to the heightened expression of immunomodulators such as IL-10 and retinoic acid, the lymphocytes are activated into T-reg cells. Studies have demonstrated that in individuals with CD, there is a loss of this tolerance mechanism and an increase in proinflammatory interleukins[30]. Supporting the theory of immune dysregulation between pro and anti-inflammatory processes, studies have shown that even in the presence of high levels of TGF-β and IL-10, which act as regulators of the immune response, local inflammation persists [27]. Furthermore, a diminished action of IL-10, responsible for regulating IL-23 levels, allows for the elevation of the latter, contributing to the stimulation of the Th17 profile[31].

The continuous and exaggerated inflammation of the intestinal mucosa, along with interactions between substrates and cytokines such as IL-13, IL-17, and TGF-β, can stimulate increased synthesis and deposition of the extracellular matrix, potentially leading to the strictures of the inflamed tissue. Furthermore, the inflammatory process could induce

hyperplasia and hypertrophy of the intestinal muscle, contributing to a stenosis process in the affected region[32].

CD is regarded as a systemic illness due to its potential for complications and the presence of various extraintestinal symptoms[33]. Accordingly, the most widespread disorders associated with this condition comprise musculoskeletal, cutaneous, ocular, hepatic and hematological alterations[34]. Firstly, CD-related arthropathy occurs, apparently, due to genetic predisposition and immune system dysregulation[14]. In this sense, the human leukocyte antigen gene (HLA)-B27 is considered a major factor to the development of arthropathies, regarding genetic susceptibility[35-37]. On the other hand, increasing evidence suggests that it could also be linked to an immune-driven inflammatory reaction, especially related to IL-23, which supports IL-17 production and, consequently, neutrophil recruitment and maintenance of the inflammatory status[38,39].

Regarding cutaneous manifestations, erythema nodosum (ED) and pyoderma gangrenosum (PG) are the most prevalent disorders associated with CD[40]. Apparently, both ED and PG are more frequent in women and patients with other extraintestinal manifestations[41]. Also, a study suggests that the susceptibility to cutaneous manifestations is associated with the TRAF3IP2 gene, which plays a role in IL-17-mediated cellular immune responses[42]. The third most prevalent extraintestinal manifestation of CD involves the eye, *e.g.*, episcleritis, scleritis, and uveitis, with approximately 3%-4% of CD patients affected[43-45]. Overall, the onset and maintenance of the inflammatory process in these structures, as mentioned above, seems to be also related to Th17 cells[46]. As for disease susceptibility, several studies suggests a relationship between HLA-DRB1*0103 and extraintestinal manifestations, including uveitis[47].

In terms of the hepatopancreatobiliary system, Primary Sclerosing Cholangitis (PSC) is considered the most common CD-related disorder[48]. While the pathogenesis of PSC is not yet fully understood, there is growing interest in the role of genetic factors, including certain HLA alleles, in its development. To date, HLA-B8, HLA-DRB1*0301, HLA-DRB3*0101 and HLA-DRB1*0401 are associated with PSC susceptibility[48-50].

CLINICAL MANAGEMENT OF THE CROHN'S DISEASE TODAY: HOW IT DOES?

The diagnosis of CD is complex and is made on the basis of symptomatic findings, physical examination, laboratory and imaging tests.

The role of clinical history and physical examination in CD

In terms of clinical aspects, CD presents diverse manifestations that are related to the intensity of transmural inflammation and its location, which, although it has a higher prevalence in the ileocolic segments, can affect any part of the gastrointestinal tract[51]. Symptoms are usually insidious, but can also develop acutely[52]. In most cases, they occur in young patients and include abdominal pain in the right iliac fossa, chronic diarrhea, weight loss, anorexia and fatigue. In cases that colonic inflammation is present, blood may appear in the stool or rectum[52,53]. In this sense, continuous blood loss and reduced absorption of iron, vitamin B12 and folic acid lead to anemia in 6.2%-73.7% of CD patients. Among these, iron deficiency anemia is the most prevalent[54,55]. It is also important to note the occurrence of extra-intestinal symptoms such as erythema nodosum, anterior uveitis, episcleritis, sclerosing cholangitis, osteoporosis, cholelithiasis, venous thromboembolism, nephrolithiasis, as well as arthritis of large joints or axial arthropathies[52-56]. These manifestations are shown in Figure 2.

When investigating the patient's clinical history, risk factors such as prior familiar cases of inflammatory bowel disease, diet low in fruit fiber, appendectomy, as well as a lifestyle with poor sleep quality, high stress and little physical activity should be taken into account[53,56]. Among the drugs with potential involvement are antibiotics, oral contraceptives and non-steroidal anti-inflammatory drugs[52,56]. Genetics also seems to be related to CD, among which it can be mentioned that the NOD2 gene in homozygosity increases the risk between 20 and 40 times[52]. In addition, one study suggests that exposure to cigarette smoke, together with chronic obstructive pulmonary disease, leads to systemic and intestinal ischemia, with epithelial dysfunction occurring in the latter and a greater risk of developing more severe forms of CD[57,58]. The physical examination should include an assessment of the hemodynamic state and look for signs of toxemia, malnutrition, dehydration and anemia. The abdomen may show distension or masses. When examining the pelvic and perianal region, lesions on skin or in the anal canal, as well as fistulas with or without abscesses should be investigated [53,57].

How laboratory tests can assist in CD diagnosis

Laboratory tests should be guided by a search for anemia, thrombocytosis, folate and 25-hydroxyvitamin D deficiency and increased acute phase proteins. Fecal Calprotectin (FC), a neutrophil-derived factor for CD in adults with sensitivity 83%-100% and specificity 60%-100%, can be useful for assessing disease activity and monitoring after diagnosis[53,57]. Other potential biomarkers include serum IgA antibodies against *Saccharomyces cerevisiae*[53], antineutrophil cytoplasmic antibodies, antibodies directed against CBir1 and OmpC, elafin for predicting intestinal stenosis in CD[59], as well as microRNA expression screening for intestinal dysbiosis[60,61].

Endoscopic procedures and other imaging exams in CD

Endoscopic procedures are the first line of examination following an assessment of the clinical and laboratory aspects of patients with a non-toxic presentation[52]. Ileocolonoscopy is considered the preferred examination for assessing luminal disease[62], while esophagogastroduodenoscopy is the choice for cases suspected of upper gastrointestinal tract involvement[57,62]. Typical findings may include friability, erosion, segmental inflammation, or aphthoid, longitudinal and serpiginous ulceration. In more advanced cases, it is possible to find fistulas, stenosis, mucosal cobblestoning and

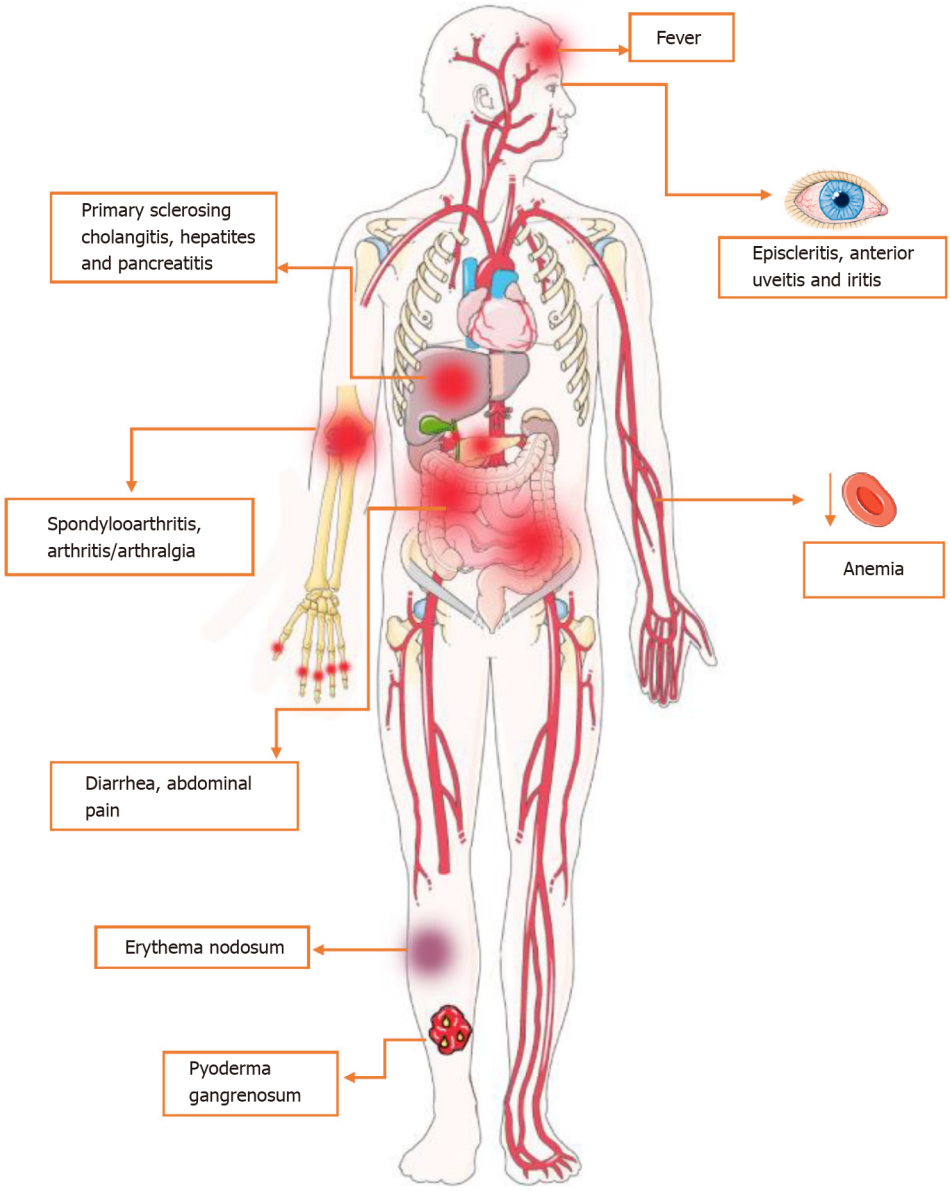

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Figure 2 Clinical presentation that can occur in Crohn's disease patients. The symptoms may be fever, abdominal pain, diarrhea, as well as extra-intestinal symptoms, such as episclerits, anterior uveitis, cholagintis, arthritis, erythema nodosum, and pyoderma gangrenosum.

wall stiffness[56,63]. Additionally, this technique allows for the collection of biopsy samples, characterized by epithelioid granulomas, preservation of globose cells, and transmural inflammatory infiltrate[63]. It is worth noting that endoscopy is also useful for determining the prognosis of CD, as well as screening for cancer associated with colitis and its characteristic lesions[64,65].

One of the limitations of traditional endoscopic methods is the difficulty of accessing the small bowel[66]. Thus, in cases of negative endoscopy, but significant symptomatology and suspicion of small bowel involvement, small bowel capsule endoscopy can be performed, which has a high negative predictive value[53]. The advantages of this method include no radiation exposure, no need for sedation and no pain. However, it is not possible to obtain a sample for biopsy or perform therapeutic interventions[57].

Radiographic techniques are considered for CD affecting the small intestine[53]. Even plain abdominal X-rays can be useful in visualizing dilation, obstruction, perforation or thickening of the intestinal wall. However, they are gradually being replaced by computed tomography (CT), especially CT-enterography, which, although it requires a high volume of intravenous contrast, has become the preferred exam for investigating the wall thickness and the relationships between the intestinal loops[52,56].

Although Magnetic Resonance Imaging (MRI) is a less available and more expensive exam, MR-enterography is an alternative to CT that allows the assessment of mesenteric vascularization and the presence of penetrating disease without the risk of exposure to ionizing radiation[62]. Another important point is that pelvic MRI is the preferred examination for investigating perianal fistulas or adjacent abscesses[53].

Another possibility is ultrasound (US), which, despite being operator-dependent, is highly available, well tolerated and has a sensitivity and specificity close to that of CT and MRI. Also, the accuracy and quality of US for visualizing the intestine can be increased with the use of oral or intravenous contrast[62]. However, it is of limited use in cases where gas is present, present less sensitive for colonic segments, and unable to assess the retroperitoneum and some areas of the GI tract[62,67].

Therefore, it is possible to classify the CD phenotype according to Montreal classification, based on age to diagnosis, localization of inflammatory lesions, and clinical behavior of the disease[65].This classification is shown in Figure 3.

That said, and despite all the complexity of diagnosing CD, professionals should also be aware of differential diagnoses that include ulcerative colitis, intestinal tuberculosis, eosinophilic gastroenteritis, diverticulitis, inflammation of Meckel's diverticulum, intestinal ischemia, chemotherapy-induced enteritis, the presence of a foreign body, neoplasms and others [66,67].

Medical management of CD: How to induce and maintain disease remission

Although IBD has no known cure to date, early medical intervention in the diagnosis of CD can improve clinical response to treatment, reduce inflammatory biomarkers and increase endoscopic remission rates[68]. In addition, patients treated in the early stages of the disease have fewer complications and need for hospitalizations[69]. The goal of conservative clinical management is to induce and maintain remission in patients with active disease, undergoing non-pharmacological interventions and medications, such as aminosalicylates, corticosteroids, immunomodulators, and immunobiologicals[70,71]. Surgical treatment depends on the presentation of the disease[5].

How non-pharmacological interventions can be used in CD treatment

The management of psychological comorbidities, such as anxiety and depression, can improve the disease status, treatment adherence and the need for high-cost care. Cognitive Behavioral Therapy can reduce rates of these comorbidities and improve the quality of life, both individually and in groups, in adults and adolescent patients[72]. A study showed reduction from 35.7% and 25.0% at baseline to 10.4% and 4.2% after therapy in rates of anxiety and depression, respectively[73]. Meditation and relaxation techniques can improve quality of life and possibly decrease inflammatory activity in IBD[74].

Dietetic interventions can be a viable option due to their low cost, availability, and few adverse effects. Diet can influence the immunological system and inflammatory response, although its interaction with intestinal mucosal defense and inflammatory cells is complex[75]. This approach is particularly considered for patients to avoid the use of steroid-based medications, especially children, in whom steroids may impact growth trajectory[76]. Data has indicated an elevated risk for IBD development in individuals with diets high in fat and meat, in contrast to a lower risk associated with high-fiber foods, fruits, and vegetables. Among the dietary options, Exclusive Enteral Nutrition has demonstrated efficacy in inducing CD remission and is considered the first-line therapy, especially in the pediatric population. However, adherence to this therapy can be a challenge in adults[23,77]. Additionally, the use of probiotics showed no significant effect on the induction or maintenance of CD remission[74].

Exercise has a positive impact on various clinical aspects of IBD, including disease activity, the immune system, quality of life, fatigue, and psychological factors[78]. The anti-inflammatory effects of physical exercise are based on myokines, exercise-specific cytokines that are released by myocytes during muscle contractions[79]. Resistance training is a viable and safe option; however, special attention should be given to patients with active disease, as exercise capacity may be limited during this period[78]. Smoking cessation should be strongly considered for smokers with CD, as it assists in modifying the course of the disease, reducing exacerbations, the need for surgical procedures, and improving the response to immunomodulatory therapy[80,81].

Aminosalicylates, corticosteroids, and immunomodulators: pharmacological options?

Anti-inflammatory medications are often the first choice in the treatment of IBD, such as corticosteroids and aminosalicylates[82]. Mesalazine, Balsalazide, and Olsalazine are medications derived from 5-aminosalicylic acid (5-ASA). Most guidelines do not recommend 5-ASA for the induction or maintenance period in CD[83,84]. However, 5-ASA is still used by many patients, possibly due to its safety profile, especially in the elderly[85].

Corticosteroids are the first-line treatment at the time of diagnosis and may be indicated regardless of the localization of the inflammatory lesion in moderate or severe disease. Systemic drugs such as Prednisone, Methylprednisolone, Hydrocortisone, and Budesonide can be used, with Budesonide being limited to mild or moderate disease in the ileocecal region[65]. Corticosteroid treatment should be administered for up to 4 wk, followed by a gradual dose reduction until complete cessation of use, typically concluding within 12 wk[86]. According to the British Society of Gastroenterology consensus guidelines, systemic steroids are effective for remission induction but are not suitable for the maintenance phase due to their toxicity and lack of efficacy[87]. For cases resistant to these drugs, immunomodulators or biological therapy may be considered[65].

CD relapse is common after discontinuation of corticosteroid therapy, and immunomodulators such as Azathioprine, Mercaptopurine, or Methotrexate are effective in maintaining remission. Early initiation of these medications is recommended in several cases[87,88]. Methotrexate is not recommended as monotherapy for induction, but it can be used for patients refractory to corticosteroids, with a preference for subcutaneous administration[89].

Biological agents: The role of immunobiologicals in an immune-mediated disease

Immunobiologicals have revolutionized the management of IBD by targeting the inflammatory processes associated with the disease, either by reducing pro-inflammatory cytokines or increasing regulatory cytokines[90,91]. British Society of

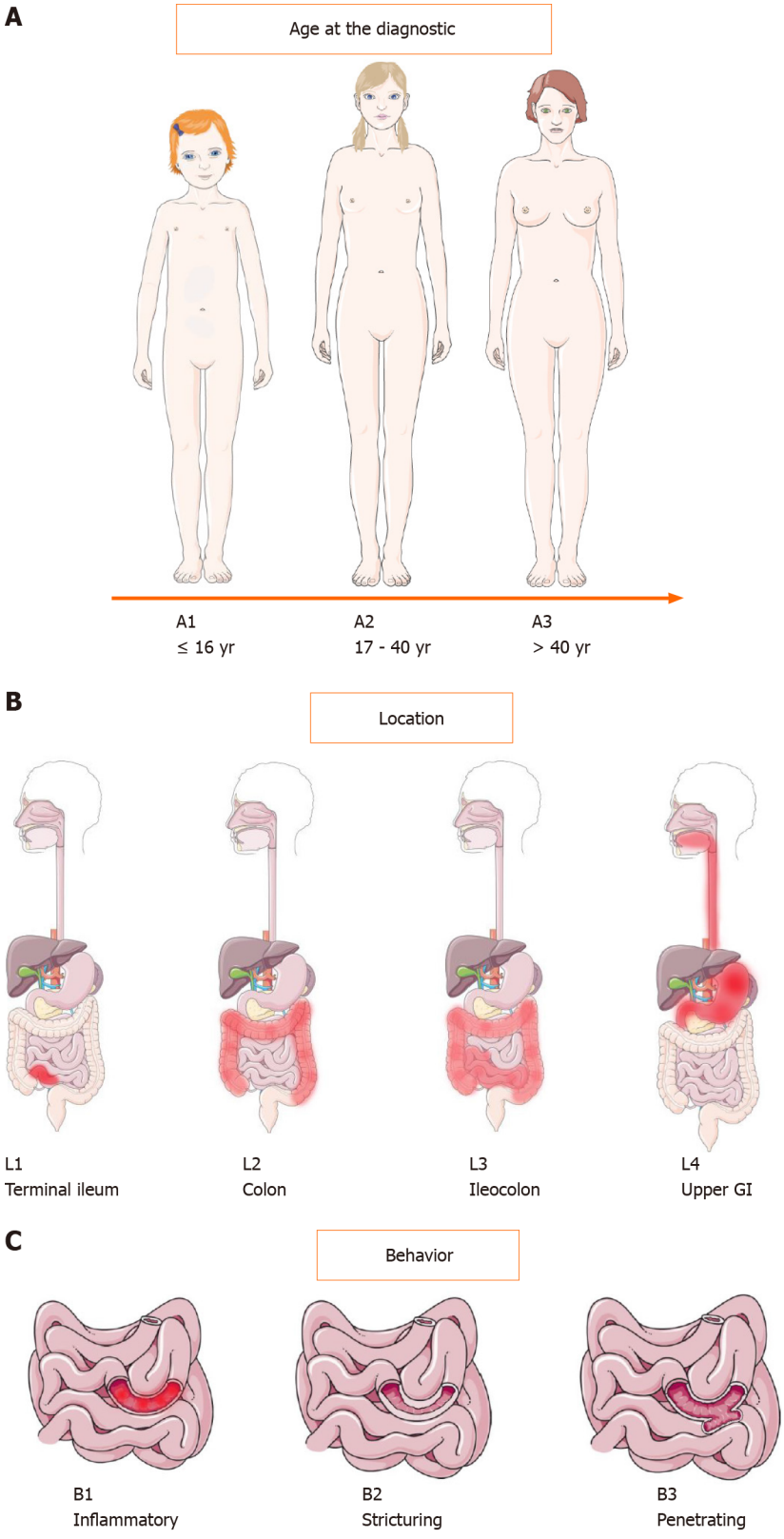

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Figure 3 Montreal classification of Crohn's disease. A: It involves age at the diagnostic; B: Inflammation locality; C: Behavior of the disease. For A, ages of ≤ 16 years, 17-40 years and > 40 years are classified to A1, A2 or A3, respectively. Depending on location (B), this disorder can be classified in L1 to terminal ileum, L2 for predominant disease in colon, L3 if ileocolonaccometiment, and L4 for illness in upper gastrointestinal tract. Finally, according to behavior, Crohn's disease can present as inflammatory, stricturing or penetrating, being classified in B1, B2 or B3, respectivelly. GI: Gastrointestinal tract.

Gastroenterology consensus guidelines recommend choosing a biological agent based on clinical factors, cost, safety, availability for use, as well as patient adherence and preference[92]. These drugs include monoclonal antibodies (mAbs) targeting TNFα, such as Infliximab (IFX), Adalimumab (ADA), and Golimumab; mAbs targeting integrins α4β7, such as Vedolizumab; and mAbs targeting the p40 subunit of IL-12 and IL-23, such as Ustekinumab (UST)[82,91].

TNF-α is a critical pro-inflammatory cytokine in intestinal inflammation[92]. Clinical evidence has shown that anti-TNF therapy, such as IFX, leads to better clinical success, early remission, higher rates of mucosal healing, and improved quality of life in approximately 60% of IBD patients[93]. Adalimumab, administered subcutaneously, has also demonstrated efficacy in treating active CD and in patients with a loss of response to or intolerance of IFX[94,95]. However, approximately 40% of patients do not respond to anti-TNF treatment[93].The concomitant use of immunomodulators like Azathioprine or Methotrexate may prevent immunogenicity, reducing the development of anti-drug antibodies and systemic inflammatory status[96].

Various methods are employed to evaluate the response and therapeutic efficacy of these therapies, including biomarkers like FC, C-reactive protein (CRP), serum levels of the anti-TNF agent, and anti-drug antibodies[93]. Reduced levels of FC may precede disease remission and mucosal healing and are correlated with endoscopic scores[97,98]. Also, elevated levels of FC at the onset of treatment may indicate a probable non-response to anti-TNF therapy and lower rates of clinical remission[99,100], and elevated CRP rates are associated with low response in severe CD[101]. Regarding serum levels of mAbs anti-TNF, higher chances of remission and mucosal healing are associated with levels above >2 μg/mL, a minimal concentration for IFX, for example, as well as for ADA[102,103]. Anti-drug antibodies are associated with remission when their levels are below 3.15 U/mL in IFX treatment, while elevated levels may neutralize clinical efficacy, lead to a loss of response, and negatively impact quality of life and disease complications[104,105]. Novel biomarkers, such as proteomic profiles and microRNAs (miRNAs), are still under investigation for clinical practice[93]. Thus, despite the wide availability of biomarkers, most of them are not specific to CD and reflect the body's inflammatory status, necessitating further clinical studies for IBD specifically.

Anti-integrin agents modulate inflammation by targeting integrins, preventing the migration of lymphocytes to the gastrointestinal mucosa. This serves as an alternative to anti-TNF therapy and may be used in patients with a loss of response, inadequate response, and/or anti-TNF intolerance[106]. Vedolizumab, an IgG1 humanized mAbs targeting integrin α4β7 without affecting α4β1, has demonstrated efficacy in inducing remission in CD, with a better safety profile than Natalizumab, another mAbs targeting integrin α4, which is associated with the development of Progressive Multifocal Leukoencephalopathy[106,107].

Ustekinumab targets the p40 subunit of IL-12 and IL-23. Studies have shown its efficacy and safety in the treatment of CD, with approximately half of the patients achieving long-term maintenance of response without loss of response, surgery, or intolerance[108,109]. However, some individuals may experience a loss of response to UST and shorter maintenance times due to factors such as infection, elevated inflammation levels, and inadequate medication concentrations[110,111]. In such cases, optimization strategies can be employed, such as shortening the treatment interval and intravenous reinduction. UNITI studies concluded that the treatment for 12 and 8 wk safely maintained clinical response and remission in patients with CD[112,113].

Surgery in CD: Can be palliative but is not curative

Approximately 15% to 47% of patients undergoing treatment may require surgical intervention[114]. While most patients initially present with the inflammatory phenotype, about 10% exhibit the stenosing phenotype at the time of diagnosis. According to the Montreal classification, within 10 years, the disease progresses to stenosing CD in approximately 15% of cases. An anti-TNF strategy may be considered in cases of strictures without complications, but surgical therapy is indicated for refractory disease[115,116]. In cases of acute small intestinal obstruction, hospitalization and immediate evaluation are necessary to rule out complications such as perforation, abscess, fistulizing disease, and signs of underlying malignancy[115]. Surgical options include segmental resection and stenoplasty[117].

Surgery can provide palliative relief but is not curative. Many patients may experience postoperative recurrence, with risk factors such as active smoking, age younger than 30 years, and previous surgeries for penetrating disease being associated with recurrence[118]. High-risk patients may receive anti-TNF or thiopurine prophylaxis for 8 wk post-surgery, with routine endoscopy, while low-risk patients may undergo endoscopic surveillance 6-12 mo after surgery [119,120]. On the other hand, early resection in CD with ileocecal stricture is associated with prolonged clinical remission and reduced exposure to corticosteroids and biological therapies[121]. Therefore, the decision to undergo surgery should be made on a case-by-case basis, considering disease phenotype and available treatment options.

New therapeutic strategies and patients vigilance

Toll-like receptor 4 (TLR4) is overexpressed in both CD and UC, and the binding between the receptor-ligand pattern recognition of DAMPs or PAMPs triggers a cascade of signaling and recruitment of inflammatory cells and cytokines, such as IL-6 and TNFα[122]. Evidence suggests a potential role for TLR4 antagonists in inflammation treatment, making it a promising alternative for future innovative treatments for IBD[123]. Therefore, more studies are necessary. Additionally, biosimilars are drugs that are similar to immunobiologicals without significant clinical differences in safety and efficacy[82]. The advent of this class of drugs promises to encourage a reduction in the prices of biologics and increase patient access to this form of treatment. Finally, new anti-integrin agents, such as Etrolizumab (humanized mAbs targeting the subunit β7 of the α4β7 and αEβ7 integrins), AJM300 (an orally administered humanized antagonist of α4 integrin), and mAbs IgG2 targeting MAdCAM-1 are in development[106], and more investigations are necessary to establish their efficacy and encourage gastroenterologists to consider them, potentially as first-line therapy in the future.

The vaccination of individuals with IBD varies according to the recommendations of each country, as well as by the clinical judgment of the patient's healthcare provider[124]. It is recommended that these patients be vaccinated before starting immunosuppressive therapy, and if they have already started, vaccines with live viruses should be avoided[125, 126]. Some authors recommend mandatory immunizations for these patients, including vaccines against influenza, chickenpox, hepatitis B, and triple viral. Other authors add to this list the vaccines for hepatitis A, tetanus, diphtheria, whooping cough, herpes zoster, and human papillomavirus (HPV)[127,128]. For hepatitis A and B vaccinations, it is recommended to perform serological tests to evaluate the presence of antibodies and immunity. If the test results are negative or show very low antibody levels, these patients should restart the vaccination schedule with three doses. Additionally, individuals over 50 years old should be immunized against the herpes zoster virus, and persons aged 9 to 45 years should be vaccinated for HPV, preferably covering the four main strains (6, 11, 16, and 18)[126-128]. The pneumococcal vaccine should be administered to all patients, following the recommendations of national immunization programs for chronic diseases[125]. Influenza immunization should be done annually[126]. Furthermore, in the current context, coronavirus disease 2019 (COVID-19) vaccination is essential. Although some studies have demonstrated that seroconversion rates in individuals with CD are similar to those without the disease, others have shown that the level of these antibodies and the duration of this immunity are shorter[129,130]. Therefore, some authors recommend administering one additional dose of the vaccine as a booster approximately 6 mo after completing the vaccination schedule[131].

In general, CD can predispose individuals to various intestinal neoplasms, including colorectal, small intestinal, and anal cancers. Colorectal cancer is the most strongly associated with CD, affecting mainly individuals between 40 and 50 years old who have a history of early onset symptoms[132]. The chronic inflammatory process in the rectal and colonic mucosa, excessive production of free radicals contributing to deoxyribonucleic acid damage, inactivation of tumor suppressor genes, and individual factors are all related to dysplastic mutations and the development of colorectal carcinoma[133]. Additionally, the ongoing inflammation can create an environment favorable to colonization by pathogens like Enterotoxigenic *Bacteroides fragilis*, which has been linked to the development of colorectal neoplasms [134]. Even the use of some immunosuppressive medications, such as TNF inhibitors, has been associated with an increased risk of cancer[135]. Similarly, the inflammatory process in the small intestine, coupled with mutations in genes like TP53 (a tumor suppressor) and IDH1 (which assists in the control of oxidative processes), has also been linked to a higher risk of small bowel adenocarcinoma[136]. Although rare, the inflammatory process of an anal fistula, a complication of CD, can increase the risk of anal cancer[137].

Recommendations for screening for colorectal cancer vary depending on the guidelines of different scientific organizations and local guidelines. Generally, it is recommended to undergo at least one colonoscopy within 8 years of the onset of symptoms to evaluate the extent and degree of mucosal damage[52,138]. TThe American Gastroenterological Association recommends annual screening for high-risk patients, evaluation every 1 to 2 years for individuals with extensive colitis or inflammation located on the left side of the colon, or every 1 to 3 years if two consecutive exams are negative. The management can be individualized based on medical criteria and the extent of mucosal damage[138]. On the other hand, the European Crohn's disease and Colitis Organization recommends annual screening for high-risk patients, evaluation every 2 to 3 years for individuals with moderate-risk factors, and, for low-risk patients, colonoscopy every 5 years[52].

Beyond the considerable physical impact of CD, the disease process can affect various aspects of mental well-being and interpersonal relationships[139]. This disease directly interferes with family planning for couples because, while the pathogenesis of CD is not related to infertility, studies have shown that women with this disease often choose not to become mothers due to conditions such as excessive pain, dyspareunia, anemia, depression, low libido, and malnutrition [140]. Additionally, concerns about the use of medications during pregnancy and their potential side effects, as well as the possibility of active disease during labor, which increases the risk of complications, contribute to reducing discussions about family planning[141]. Other complications such as miscarriages, placental abruption, eclampsia, and pre-eclampsia can be associated with pregnancy and CD[142]. Finally, men using medications or experiencing clinical conditions associated with pain have also been related to voluntary avoidance of having children[140,141].

CONCLUSION

This review provides an overview of the immunologic aspects of CD and discusses the clinical management of these patients. Recognizing gastrointestinal symptoms such as diarrhea, abdominal pain, anorexia, and fatigue can assisti in diagnosis. These symptoms may also be accompanied by extraintestinal manifestations and associated disorders, such as arthropathy, erythema nodosum, episcleritis, and anterior uveitis. Additionally, laboratory tests, such as Fecal Calprotectin, and imaging exams play a crucial role in the evaluation process, with endoscopic procedures being the first-line approach. Both ileocolonoscopy and capsule endoscopy are important tools in this diagnostic scenario. Non-pharmacological and pharmacological treatments form the cornerstone of disease management, with surgical therapy being considered in some cases. This includes the use of anti-inflammatory medications, such as corticosteroids and immunomodulators, as well as biological agents. Therefore, psychological interventions should be more widely prescribed, as they have the potential to improve treatment responses and, consequently, disease outcomes. Finally, therapies targeting the immune system are increasingly being studied, and more research is needed to elucidate the best approach to patients with CD, taking into account their specific phenotypes. Our work summarizes the current evidence available to date.

FOOTNOTES

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Using national census data to facilitate healthcare research

Michael Colwill, Andrew Poullis

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Abstract

National censuses are conducted at varying intervals across both the developed and developing world and collect detailed data on a wide range of societal, economic and health questions. This immense volume of data has many potential uses in the field of healthcare research and can be utilised either in isolation or in conjunction with other information sources such as hospital records. At a governmental level census data can be used for healthcare service planning by providing accurate population density information but also, through the use of more detailed data collection, by helping to identify high-risk populations that may require increased resource allocation. It can also be a key tool in addressing and improving healthcare inequality and deprivation by both identifying those populations with poorer healthcare outcomes and through helping researchers to better understand the causes of this inequality. Similarly, it has utility when studying the complex causes of disease and assessing the success of strategies designed to tackle these aetiologies. However, the maximum benefit from these various uses can only be realised if the data collection and analysis processes utilised are robust and this requires that census bureaus regularly review and modify their methods in a transparent and thorough way.

Key Words: Census data; Methodology; Epidemiology

Core Tip: National census data is collected widely across the world. Recently, more detailed data on a wide range of societal, economic and health questions has begun to be collected and this vast volume of data has enormous potential in healthcare research. Examples of potential utility are in assisting with healthcare service planning, analysing healthcare workforces, identifying healthcare inequality and it's causes and understanding the causes of disease. However, census data's utility is dependent upon robust and scientific data collection and analysis and this requires regular methodological review and improvement by national census bureaus.

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INTRODUCTION

National censuses are performed in the majority of countries in the developed world and a growing number of developing countries (see Figure 1). The breadth of the data collected has increased and diversified significantly in recent decades with many countries now collecting data on socioeconomic status and health conditions as well as basic population demographics[1]. Whilst the idea of using census data for healthcare research is not new[2], this vast collection of data remains underutilised. This article will discuss areas in which this data has utility and some of the pitfalls associated with it.

Healthcare service planning

The basic demographic data that is provided by a national census is crucial for all elements of government planning including healthcare provision. At a very basic level, providing accurate data on population density can be used to decide the location and provision of healthcare facilities[3]. An example of this was demonstrated by a Tanzanian study focusing on maternal outcomes in obstetric care based upon proximity to healthcare centres and found that a greater distance to healthcare facilities was associated with worse maternal outcomes[4]. This data has since been used to justify the construction of new healthcare centres in appropriate under-served regions in order to address this disparity.

Some countries go further than just basic population data, such as in the United States where the American Community Survey (ACS) is performed along with the decennial census. The ACS tracks social determinants of health such as income, housing and national origins as well as insurance coverage, fertility and disability. This allows the department of health and human services (HHS) to more precisely target resources to match the anticipated needs of each region and is a key part of HHS' long-term strategy.

Census data have also been used to analyse the healthcare services themselves as well as the populations they serve. A study from Japan in 2018 used several decades of census data and cross-referenced it with physician surveys and municipality borders to investigate concerns of disparity between the number of physicians in urban and rural practice [5]. The study identified an uneven distribution, which had been worsening over time, with a lack of physicians in rural settings and prompted government departments to start to develop strategies to mitigate this. Workforce analysis was performed in the United Kingdom using census data to review the make-up of the healthcare workforce and identified a heavy dependence upon foreign-trained workers indicating that domestic training programmes needed reforming[6].

Similarly, work by Gupta *et al*[7] used national census data from three different countries to analyse and compare inter-country healthcare worker provision. Their study gave a detailed snapshot of differences between these countries and the various challenges they faced which provided a mandate for the international community, including non-governmental organisations and charities, to direct and focus their resources demonstrating the utility of this data in a transnational, as well as national, setting. It should however also be noted that they found significant variability in the quality of data provided and this imposed limitations on the conclusions they were able to make and this will be discussed later in this article.

Addressing healthcare inequality

Studies have repeatedly shown that healthcare inequality, both at national and international levels, can impact upon mortality and morbidity[8,9] and contribute to deprivation. A recent example was data collected during the coronavirus pandemic which identified differences in outcome with those from deprived health systems having significantly worse survival[10] and thrust the issue of health inequality into the spotlight. Addressing this inequality is a complex political, societal and public health conundrum but census data can be a key element to identify inequalities and guide reform.

In order to allow politicians to appropriately allocate the resources required to address healthcare inequality, the location and nature of the inequality needs to be clearly identified and this is where census data has a role. In the United Kingdom, the 2019 NHS long term plan made addressing healthcare inequality a priority specifically targeting the most deprived 10% of the United Kingdom population. This plan, along with the Core20Plus5 initiative, combined the use of national census data, general practitioner records and hospital records to identify, at a local level, those groups who suffer from the highest levels of healthcare inequality[11]. Some examples of 'at-risk groups' were those from an ethnic minority or those with a disability.

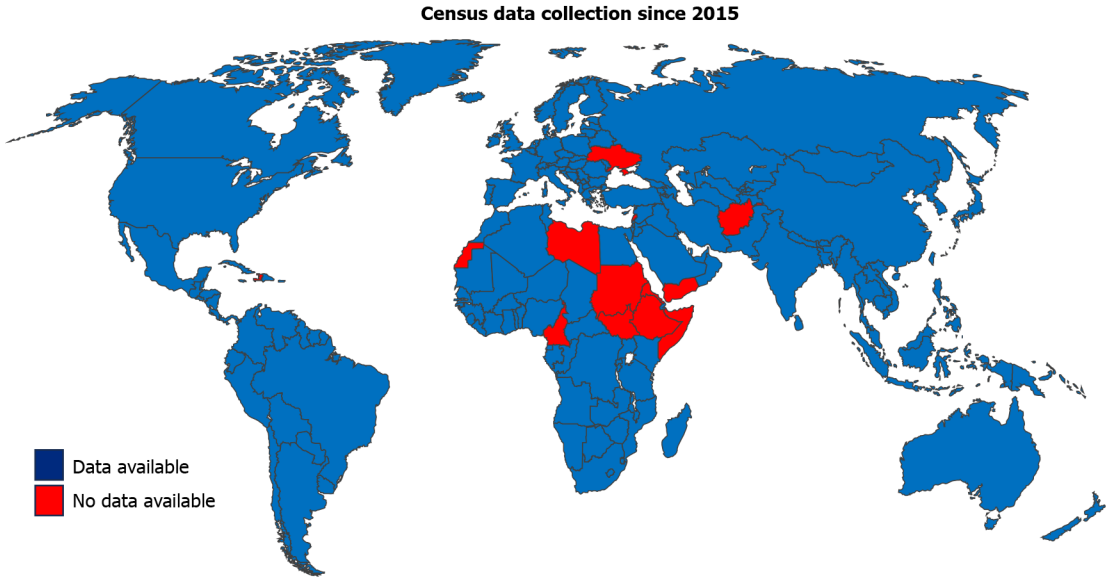

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Figure 1 World map showing which countries and territories have produced national census data since 2015. The figure is created from Powerpoint insert map tool.

Similar use of census data to identify populations with higher risk of deprivation have been used in the United States [12] with some interesting epidemiological findings. One example was the so called 'Hispanic paradox' where historically this population was believed to have better healthcare outcomes despite their high deprivation scores and risk profiles. However, recent and more detailed census data analysis has found that this may not be the case[13] demonstrating the importance of high quality data and statistical analysis.

Census data is also important when monitoring the progress, or lack of, with regards to tackling healthcare inequality. A large study in the United States entitled 'The Public Health Disparities Geocoding Project' used a five step data analysis process to determine, through census and health surveillance data, a picture of health inequalities over time. It identified both areas where improvements had been made but also areas where the problem persisted or had worsened and has been used to inform public policy[14].

Other work has focused on using census data to identify the causes of healthcare inequality. A study in the United States analysed this data and identified a significant association between the presence of greater numbers of liquor stores and the risk of health-related social problems in low income neighbourhoods[15]. Whilst there is clearly not a single cause for poorer health outcomes, this interesting analysis sheds light on possible environmental factors that will be an important part of reducing healthcare inequality.

Understanding the causes of disease

As previously mentioned, census data also have a further role in addressing healthcare inequality by assisting researchers to understand the causes of disease. Canadian researchers, through combining primary care records and census data, demonstrated a link between socioeconomic status and obesity[16] whilst a study in Spain used a similar methodology to demonstrate a link between deprivation and common cancers in order to better target screening programmes[17]. These studies show the utility of census data in assessing health disparities and environmental factors associated with chronic disease.

There are also examples of more detailed and complex use of census data for similar purposes. Moceri *et al*[18] used, in a case-control study, census data and birth certificates to reconstruct the early-life socioeconomic environment of elderly Alzheimer's patients and, through examining variables such as paternal occupation, parental age and birth order amongst others, found higher odds-ratios for developing Alzheimer's for certain characteristics. They also then combined this with genetic analysis of these patients to study the interaction between apolipoprotein ε4 allele and these socioeconomic risk factors.

As well as identifying risk factors for disease, census data have also been used to demonstrate effective interventions in improving public health. Patterson *et al*[19] used census data in England and Wales to demonstrate that active commuting, such as cycling or walking, was associated with lower cardiovascular risk. This is, in theory, a relatively easily achieved public health initiative and there are an increasing number of programmes attempting to increase this method of commuting with the aim of improving public health and reducing the risk of a wide variety of diseases.

Pitfalls

Whilst census data have multiple potential uses there are caveats that need to be addressed and recognised. Firstly, it's utility is dependent upon having robust and accurate data and there have been instances where poor or incorrect collection has had profound social impact. In the United States, the 1840 census incorrectly identified higher levels of insanity amongst the 'coloured' population, an argument then used by slave-owners to suggest that African-American

populations were not able to live as free people[20]. There has also been historically inaccurate data about native American populations and rates of disease leading to worsening healthcare inequality fuelled by the misappropriation of federal funding. A more recent example showed persisting inequalities when it comes to accurate population and health data collection in the Maori population in New Zealand[21] meaning that they receive less resource allocation from government funding. Moreover, there have been documented examples where census data has been deliberately falsified in order to obtain greater funding and support for specific regions. This was discussed and reviewed in detail by Adele with regards to the national census in Nigeria and identified chronic and deliberate falsification of data to obtain benefits from the government[22]. Given the implications for strategic planning and resource allocation, an inaccurate census can have profound impacts for communities and citizens and this is also true when the data is used for healthcare research. These examples underpin the need for a robust scientific process enabling accurate data collection and interpretation in order to have maximum benefit for those who need it the most.

Secondly, there has also been debate around the ethical considerations of using census data for healthcare research. Whilst the data is often anonymised, the nature of census data involves categorisation of respondents and there can be discontent with even simply the names of these categories. A recent example is the controversy surrounding the inclusion of a question asking for respondent's gender identification in England and Wales census for the first time[23]. Similarly, there have been concerns about racial categorisation in association with health labels and stigma[24] although interestingly a public panel consultation in 2018 found that members of the public were in support of census data collection and it's use in healthcare research[25].

Thirdly, there is also a concern that there is inherent bias[26] within the census process itself. This can take the form of non-respondent bias, as described by the United States census bureau[27], which has been shown to skew data significantly such as during the 'poll tax' era in the United Kingdom when non-respondent rates increased. There is also a concern about accurate female representation with this group being historically under-represented[26]. These pitfalls demonstrate the need for regular review of the methodological and analytical practices employed by census bureaus and appropriate improvements if indicated.

CONCLUSION

Internationally, census data collection is becoming more widespread, detailed and robust particularly in the developing world. This data, provided it is accurate to avoid defects, is an immensely rich resource which has utility in research to help with healthcare planning, reducing healthcare inequality and understanding more about the causes of disease. Provided that accurate data is collected in-line with good scientific practice and remains widely and freely available to researchers, it has the ability to be an invaluable resource in healthcare research.

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MINIREVIEWS

Machine learning and deep neural network-based learning in osteoarthritis knee

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Abstract

Osteoarthritis (OA) of the knee joint is considered the commonest musculoskeletal condition leading to marked disability for patients residing in various regions around the globe. Application of machine learning (ML) in doing research regarding OA has brought about various clinical advances viz, OA being diagnosed at preliminary stages, prediction of chances of development of OA among the population, discovering various phenotypes of OA, calculating the severity in OA structure and also discovering people with slow and fast progression of disease pathology, *etc.* Various publications are available regarding machine

learning methods for the early detection of osteoarthritis. The key features are detected by morphology, molecular architecture, and electrical and mechanical functions. In addition, this particular technique was utilized to assess non-interfering, non-ionizing, and in-vivo techniques using magnetic resonance imaging. ML is being utilized in OA, chiefly with the formulation of large cohorts viz, the OA Initiative, a cohort observational study, the Multi-centre Osteoarthritis Study, an observational, prospective longitudinal study and the Cohort Hip & Cohort Knee, an observational cohort prospective study of both hip and knee OA. Though ML has various contributions and enhancing applications, it remains an imminent field with high potential, also with its limitations. Many more studies are to be carried out to find more about the link between machine learning and knee osteoarthritis, which would help in the improvement of making decisions clinically, and expedite the necessary interventions.

Key Words: Osteoarthritis; Knee; Artificial intelligence; Machine learning; Deep neural network

Core Tip: Application of machine learning in research has various clinical advances viz, osteoarthritis (OA) knee being diagnosed at preliminary stages, prediction of development of OA, discovering various phenotypes. Large cohorts have been formulated viz, the OA Initiative, the Multi-centre Osteoarthritis Study and the Cohort Hip & Cohort Knee. Many studies are awaited to find about the link between ML and knee OA, which would improve making decisions clinically, and expedite the necessary interventions.

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INTRODUCTION

Osteoarthritis (OA) of the knee joint is considered the commonest musculoskeletal condition leading to marked disability for patients residing in various regions around the globe[1,2]. OA is a degenerative disease, still, there is no clear knowledge regarding the factors that are responsible for the progression of the disease pathology. In the late stages of the disease, the only option for treatment is total knee arthroplasty, which is not affordable by many, is very much invasive, and highly degrades the individuals' quality of living.

A breakthrough was brought about by artificial intelligence (AI), specifically by machine learning (ML) in the field of medical care, mainly in subspecialties like rheumatology, mainly OA[3-6]. Machine learning includes Supervised, Semi-supervised, Unsupervised, and Reinforcement learning[7]. Deep learning is a subtype of ML that depends on various layers of a network consisting of a neuron architecture, that allows a model to improvise itself and train on its own, which in turn will lead to great accuracy levels by extracting greater level features from the given data[8-10].

Application of ML in doing research regarding OA has brought about various clinical advances viz, OA being diagnosed at preliminary stages, prediction of chances of development of OA among the population, discovering various phenotypes of OA, calculating the severity in OA structure and also discovering people with slow and fast progression of disease pathology, *etc*. The present models for the prediction of the progression of OA have been based on the collaboration of texture descriptors separated from the trabecular bone, Kellgren Lawrence (KL) grade, and clinical and anthropometric data[11-14]. Methods of Machine learning are divided into supervised and unsupervised (Figure 1). In supervised analysis, the results are established and the data are tagged. Whereas, in unsupervised analysis, the results are not established and the data are not tagged. In addition to these, two new categories were added viz, semi-supervised & reinforcement learning, in which the outcomes are only partially established[7].

Models of semi-supervised learning comprise a mixture of data that are tagged & untagged and are predicated on weak monitoring, with limited tagged data that are used to gain information and for the monitoring of untagged data. On the other hand, reinforcement learning is an ML prototype in which learning happens collectively by a sequence of hit-and-miss trials to increase the result achieved after every hit and thereby increase the learning behavior. Amongst the above-mentioned methods, supervised ML methods are most frequently utilized in the health care systems and medical field[15].

ML is being utilized in OA, chiefly with the formulation of large cohorts viz, the OA Initiative (OAI)[16], a cohort observational study, the Multi-centre Osteoarthritis Study[17], an observational, prospective longitudinal study, and the Cohort Hip & Cohort Knee[18], an observational cohort prospective study of both hip and knee OA. Though ML has various contributions and enhancing applications, it remains an imminent field with high potential, also with its limitations.

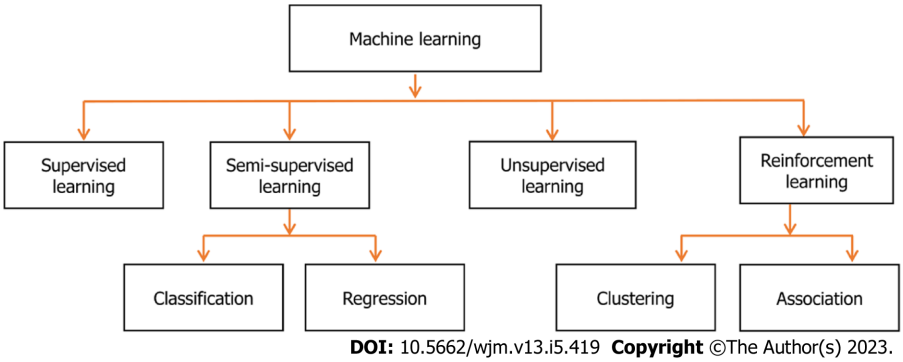

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Figure 1 Various algorithms used in machine learning.

LITERATURE REVIEW

Various publications are available regarding machine learning methods for the early detection of osteoarthritis. The detection of osteoarthritis of the knee in its early stages and the exact & persistent assessment needed to detect changes in cartilage were discussed by Mohd Hani *et al*[19]. The key features are detected by morphology, molecular architecture, and electrical and mechanical functions. In addition, this particular technique was utilized to assess non-interfering, non-ionizing, and in-vivo techniques using magnetic resonance imaging (MRI).

Nelson *et al*[20] elaborated on the various causative factors of Osteoarthritis of the knee viz age, mass, and problems to the joint because of the flexing and kneeling activities. The data was attained by the authors from the OAI to assess the advancement of the knee. Dual Echo Steady State MRI was utilized to scrutinize the various images that are available and to choose the part of comparison and later on, perform a computerized differentiation from the data that is available. The modus operandi and design utilized is the Active Appearance Model. The CDI values that are acquired, are fed into machine learning methods like support vector machines (SVM), artificial neural network (ANN) & Random Forest to achieve precise results.

In a study conducted by Kwon *et al*[21], the radiographic maps were used & applied the model of SVM grouping was to authorize the deep learning model. For the multi-classification system, the deep learning network that was used was the Inceptio-ResNet-v2. Calculations of various parameters viz sensitivity, precision, gait analysis, X-ray findings, and F1-values were separated and skilled with property extraction and depth learning features extraction respectively and the training & conditioning of the SVM model was done by utilizing the training set and the compiled data was utilized in the diagnosis of OA. They found an ambiguous learning technique based on radiographic findings, blue-print-dependent scrutiny of data & analysis of functions. Following the inferences of this study improved the accuracy of the diagnosis of OA of the knee.

Wahyuningrum *et al*[22] utilized Long Short-Term Memory (LSTM) for extraction of the features to inquire about the pre-processing through a convolution neural network (CNN). Their main concerns were on the process of cross-validation, of which 2/3 and 1/3 were taken as training and testing data respectively. They performed the analysis by cropping the data of knee images into 400 × 100 pixels which were then arranged as piled augmented images and in turn were introduced in the manner of sequences, as input into the CNN, because LSTM should be performed sequentially. Three convolution filters followed the stride of Max pooling, and 1000 softmax channels into the LSTM processed the entire data from CNN. They have proposed that the LSTM technique is based on the KL grade and is more precise than deep learning.

An analysis of the cases was performed by Galván-Tejada *et al*[23] on the grounds of the data available from the OAI analysis. In this study they utilized two kinds of radiological grades, the first one being a quantitative analysis score and the second being a semi-quantitative analysis score, with the results of radiological images analyzed by two different sets of radiologists. To measure the variables related to pain in the future, A single variable logistic regression test was conducted. They found that the formation of bone osteophytes acts as the predictor of joint pain in the early stages and there's no correlation between pain in the subsequent period and a decrease in joint space. By the findings of the study, they have found that by using plain radiographs the reasons for potential OA knee can be predicted early. They have demonstrated the correlation between the discriminant X-ray of retrogression and the knee pain that developed.

Due to the difficulties faced in diagnosing knee Osteoarthritis, Christodoulou *et al*[24] brought about the concept of deep neural networks for the categorization of issues faced in diagnosis. In this study, to solve the problems in diagnosing osteoarthritis, taking into consideration the variable health factors that cause osteoarthritis, they have introduced a deep learning network to solve the problems faced in classifying knee OA, as a new and efficient machine learning method. The efficiency of the new method was proven by making discrete subgroups of the treated patients, from the data available and by designing a category for the diagnosis of OA knee. Deep neural networks serve as a successful technique in tackling the several problems of machine learning technique viz classification of images, predilection and disease prognosis, *etc*.

Magnetic resonance imaging was utilized by Du *et al*[25] to find the biomedical information concealed in it to diagnose osteoarthritis. Four techniques of machine learning were utilized to assess the osteoarthritis progression and to figure out the transformation in grades according to Kellgren and Lawrence's (KL) classification system, narrowing of joint space in

both medial or lateral compartments. The 36-dimensional elements were divided into two separate 18-dimensional elements with each 18-dimensional element to be individually examined by a four-graded technique for analyzing the various changes of both lateral and medial compartments. In an attempt to narrow down the regions and increase the performance, the analysis by PCA was utilized. By this technique, the machine learning methods for analysis were used to project the increase in KL grades, and medial and lateral compartment joint space reduction, and helped in delineating the pathological progression of knee OA. For the analysis purpose of PCA, the CDI information was compiled from one of the 36-dimensional elements on the 3D knee model of the MR tibiofemoral compartment.

In another study conducted by Kawathekar *et al*[26], they used geometrical parameters from the radiographs of the knee (anteroposterior view), by calculating the distance between the femur and tibia in active form models. Other approaches like local binary arrangement, which is being utilized in most of the pattern recognition tools are also used in the diagnosis of OA knee.

MACHINE LEARNING TECHNIQUES

The typical programming consists of an Algorithm, that includes a couple of rules that require pre-processing data (input) that is utilized to calculate a form of processed data (output) (Figure 2).

The algorithms of machine learning (Figure 3) can learn from the available data and are utilized for the automatic calculation of the set of rules. To perform such approaches, three components[27,28] are essential viz, pre-processed input data, Output data that the algorithm is supposed to predict, and A comparative tool to verify the predicted output performance.

The working mechanism includes entering both the data into the pipeline, which in turn will learn on its own to transform one data into the other data form. Machine learning techniques can be utilized effectively when there is no feasibility or possibility for defining an algorithm by manual techniques and when there's adequate data that applies to the training method.

Various subtypes of machine learning are available like supervised and unsupervised methods. Supervised methods can be utilized when there is a definite idea about the output data and Unsupervised methods can be utilized to discover anonymous data patterns. For instance, Supervised-learning methods encompass linear array regression, gradient boosting and ANN, *etc*.

ARTIFICIAL NEURAL NETWORKS

In the recent past, AI has been used graciously in the field of medicine. AI is nothing but a division of software engineering that includes algorithm training to reflect the human learning process. The design of the human brain has been the basis of various machine learning methods like the ANN. The ANN consists of a congruence of neurons that are interrelated. The ground structure of the neurons, can transform their inner structure or initiate a process, based on the information that is being fed to them and in turn results in a yield that depends upon both the input provides and the present execution.

CONVOLUTION NEURAL NETWORKS (CNN)

CNN is utilized widely in various modalities like an assertion of images and videos, recommendation systems, and handling of basic languages. Convolutions are vastly used in processing images[29,30], this being one of the reasons behind its introduction to deep learning methods to perform visual tasks. CNN facilitates the advantage of knowing about the local patterns that are concealed in the data given unlike the other systems, which treat the input data variables globally. CNN belong to a special subgroup of ANN, which uses a system known as a convolution in a minimum of at most one of their steps. Yann LeCunn introduced CNN first in 1990 though it was not that popular at that time[31].

Convolutions involve the mathematical assessment of two different functions that are assertions of real values. In imaging modalities, various terminologies are used to describe the input and kernel, which indicates the first and second functions respectively. The output data that is attained is termed the feature map. CNN models date back to one among the historical deep neural network models, which have various layers that are hidden between the general ones, and this, in turn, helps in knowing more information regarding the features that are concealed in the images that are provided as the input. A second type of layer that is often utilized in CNN models conducts pooling undertaking. Due to this pooling, there is a reduction in spatial resolution and the most appropriate features are only retained, which is solely necessary for maintaining a feasible network size.

OBJECT CLASS DETECTION

In today's treatment modalities, technologies of machine vision are utilized mostly for the identification of surgical materials among the available objects. The features of medical image inquiry have helped doctors in various ways viz

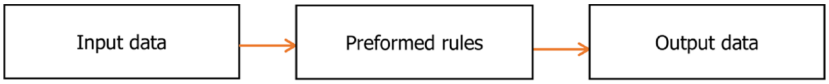

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Figure 2 Typical programming algorithm.

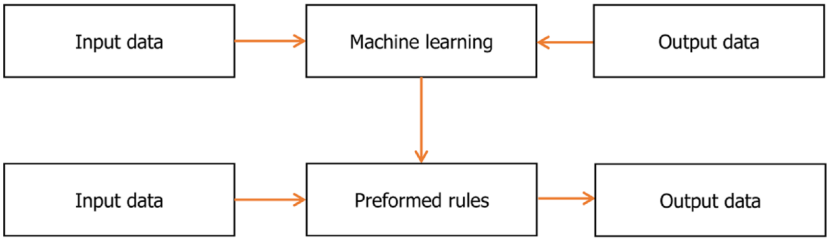

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Figure 3 Machine learning algorithm.

enhancing the diagnostic capabilities of pediatricians, outcomes which are object-guided, treatment-oriented, and extensive evaluation of various body organs like evaluation of clinical images of those in a state of vulnerability. The availability of advanced technological systems enhances the necessity for identifying and diagnosing various diseases like osteoarthritis, but there are various hurdles to the analysis of its probable location.

Nowadays various algorithms are available which are being used in health care to solve these hurdles. Numerous numbers of various technological advancements are happening in the day-to-day period, which focuses on the diagnosis and recognition of many other disease conditions. The traditional object analyzing methods can be utilized in a broad spectrum of imaging methods viz Radiography, Ultrasonography, Computed Tomographic images, and MRI.

There is no standard method of ML which is acceptable in order to apply them for real-time application[32]. Future research should concentrate on making algorithms that can detect OA in the stages of less severity. The lacunae in the real-time application can be fulfilled by utilizing data in larger numbers and multimodality data to make the ML models more accurate and increase the credibility simultaneously[9].

CONCLUSION

The future of medical science relies upon developing new methods of detecting osteoarthritis in its early stages, which would help in providing joint-preserving treatment modalities to patients. Machine learning techniques would develop a new revolution of augmented radiological methods. Machine learning can explore a wide range of designs, to identify the interaction and multiresolution models, which can easily predict the dynamics of the system to find the predisposing factors. Many more studies are to be carried out to find more about the link between machine learning and knee osteoarthritis, which would help in the improvement of making decisions clinically, and expedite the necessary interventions.

FOOTNOTES

Author contributions: Jeyaraman M contributed to conceptualization; Ratna HVK, Jeyaraman N, and Nallakumarasamy A contributed to initial draft; Ratna HVK and Jeyaraman M contributed to final draft; Sharma S, Khanna M, and Gupta A contributed to supervision; All authors accepted to publish the current version of the manuscript.

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MINIREVIEWS

Synoptic review on existing and potential sources for bias in dental research methodology with methods on their prevention and remedies

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Abstract

The results of years of dental study serve as the foundation for the practise of medicine and, for that matter, dentistry. Doctors may have their own preferences for techniques and materials, but whether directly or indirectly, their decisions are influenced by systematic reviews and meta-analyses. However, due to poorly conducted or presented research, this very basic foundation may not be reliable. Bias in research is one of several factors that might make study results or research itself unreliable. Bias can be introduced into research at many stages, deliberately or unknowingly. Bias can appear at any point during the research process, even before the study itself begins. There are many biases in research, but some of them are more relevant to dentistry research than others. Because it is said that "eyes

see what the mind knows", it is essential to have a complete understanding of the different types of bias, how and when they get entrenched, and what steps may be taken to prevent or lessen them if they do occur. This comprehensive summary of bias in dentistry research is provided by this synoptic review. The goal is to identify gaps and measures that have been taken-or that should have been taken-by providing both descriptive and evaluative summaries, as well as examples from the literature, when needed.

Key Words: Dental research; Bias (epidemiology); Research methodology; Research design; Epidemiologic methods

Core Tip: Be it clinical or *in-vitro*, bias may arise at any point in the course of research. Always make efforts to minimise, if not completely eradicate, any potential bias that could show up in a study. However, how can a researcher take preventative or remedial actions if they are oblivious that bias is being introduced into their study? This article lists and summarises every potential bias that could arise during a study so that the researcher is aware of the possibilities and can take the necessary precautions to contribute reliable scientific data to the literature.

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INTRODUCTION

The practice of medicine, or for that matter dentistry, is primarily based on outcomes generated from years of relevant research. Although doctors do have their own preference with regard to procedures and materials, the choice, either directly or indirectly, is based on systematic reviews and meta-analysis. However, this very fundamental base may not be reliable owing to improperly executed or improperly presented research. Of the various reasons that make the research outcome or the research itself unreliable, bias is an important aspect that is either knowingly or unknowingly introduced at various steps.

By definition, bias is any tendency that prevents unprejudiced consideration of a question[1]. Bias can occur at any phase of research, even before starting the study itself. Bias is not just a yes or no variable, and its interpretation cannot be restricted to whether bias is present. Instead, a critical reader should consider the efforts taken in a study to decrease the degree of bias and also critically evaluate the influence of the bias on the conclusion[2]. There is a long list of biases in research, but some of them are more applicable to dental research. The "eyes see what the mind knows"; hence, a thorough knowledge of types of biases, how and at which stage they are introduced, and the ways to avoid or reduce them are crucial for justifying the results of the research. Awareness of these biases is imperative to promote rigorous and unbiased dental research. Researchers, peer reviewers, journal editors, and funding agencies should collaborate to establish robust methodologies, transparent reporting standards, and effective peer-review processes to minimize the impact of bias and enhance the reliability and validity of dental research. Additionally, promoting open access to research findings and fostering a culture of transparency and reproducibility can ensure a comprehensive and unbiased body of dental literature.

This synoptic review provides a concise but accurate overview of all materials related to bias in dental research. The aim is to provide both descriptive and evaluative summary along with examples from literature to identify gaps and measures taken or should have been taken. In dental research, like any other field, various types of biases may affect the validity and reliability of the results. A detailed and well-categorized flowchart listing all possible biases that can occur in clinical and *in vitro* research is depicted in Figure 1. A special emphasis is given to certain categories of biases, such as those related to split-mouth study, inter-examiner studies, and blinded studies. Finally, a brief description of various biases that are difficult to categorize under a single category are mentioned in detail under the special bias category. The following detailed discussion about various biases adheres to the headings and subheadings with reference to Figure 1 for ease of understanding and clarification.

BASIC TYPES OF BIAS[3]

Random error/random variability

It refers to the unavoidable fluctuation in data that results from chance. There is no way to completely prevent or control this type of inaccuracy. Measurement precision and accuracy, as well as the outcomes of statistical analysis, can be impacted by random error. To lessen the effects of random bias, researchers often use statistical techniques and large sample sizes. In addition, if investigators are themselves involved in sample collection, it would further reduce the

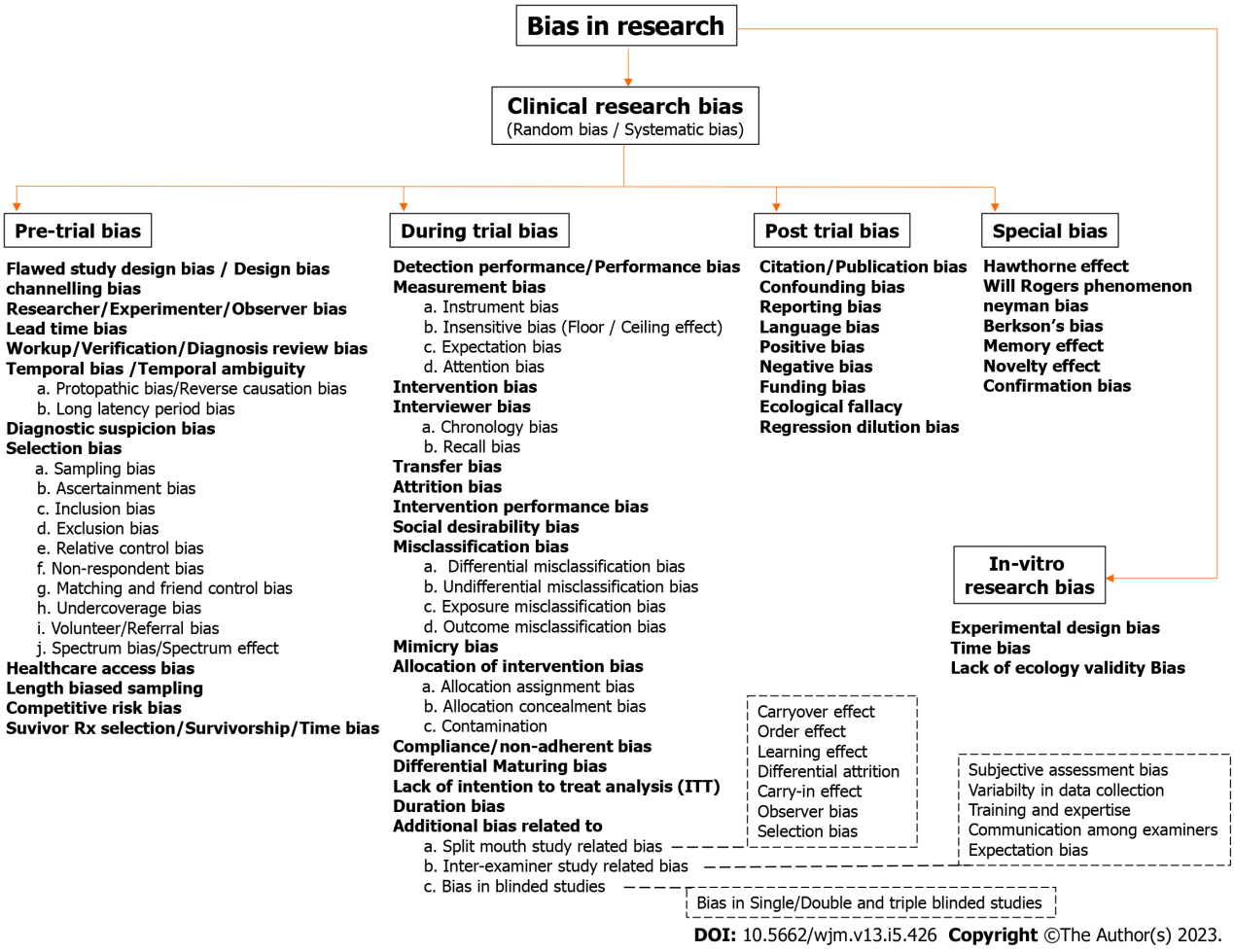

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Figure 1 An exhaustive list of possible biases that can occur in clinical and *in-vitro* research.

chances of random bias.

Systemic bias (or systematic bias)

It is a type of prejudice that is present throughout the planning, execution, or analysis of research investigations. This bias is systematic or persistent, which means that it does not result from chance but rather from certain defects or problems in the study process. Systematic bias has an enormous effect on the reliability and validity of research findings. Typical examples of systemic bias in research include selection bias, sampling bias, measurement bias, and funding bias. The various types of systemic bias have been discussed under different subheadings along with the measures to be taken to avoid or at least reduce them, wherever relevant.

CLINICAL RESEARCH-RELATED BIAS

The pretrial stage ranges from selecting a topic for the research to literature search, preparing a research proposal, and designing a study and even screening tests and patient recruitment. Bias at the stage of study design and patient recruitment can cause fatal flaws in the resultant data, which are almost impossible to compensate during analysis. The following is the list of possible biases that can occur in the pretrial phase of a research.

Flawed study design bias (design bias)

It refers to a type of systematic bias that arises from errors or inadequacies in the planning, structure, or methodology of a research study. This bias can significantly impact the validity and reliability of the findings as it introduces systematic errors that are not due to random chance. Almost none of the studies with this bias get published in reputed journals as they get rejected either at the editor or reviewer level.

Channeling bias

As the name suggests, channeling bias occurs at the stage when patients are channeled to a specific group intentionally. This bias occurs in nonrandomized trials wherein patients' prognostic factors and degree of illness determine the study cohort in which they are placed[4].

Researcher bias/experimenter bias/observer bias

It occurs when researchers' personal beliefs, expectations, or preferences influence the study design, data collection, analysis, or interpretation. This bias can introduce systematic errors and affect the objectivity and integrity of dental research.

Lead time bias

It occurs when a new diagnostic test or screening method is introduced, which leads to the early detection of a disease without improving the patient's overall health or survival. As the new diagnostic/screening test is more sensitive, the disease is detected earlier. The patients appear to have a longer survival time from the time of diagnosis compared with those diagnosed later using the older methods. This time difference creates the illusion that the new test or method improves patient outcomes.

Work-up bias (verification bias/diagnostic review bias)

It is a potential source of bias in diagnostic studies. Study participants may be subjected to different levels of diagnostic work-up based on their initial test results or other factors. For example, individuals with positive initial test results may receive additional more definitive tests or clinical evaluations, whereas those with negative results may not undergo further assessment. Those selected for verification may not be representative of the entire study population, and this differential verification process can lead to an overestimation or underestimation of the test accuracy. These issues can result in biased estimates of the test sensitivity, specificity, positive predictive value, and negative predictive value.

Temporal ambiguity

It refers to a situation wherein it is unclear or ambiguous whether an exposure (such as a treatment or risk factor) occurred before or after the outcome of interest (such as a disease or health event). This ambiguity can introduce a bias and make it challenging to establish a causal relationship between the exposure and the outcome. In retrospective studies, if the timings of the exposure and outcome are not clearly established, it can lead to temporal ambiguity. Even participants or medical records may not provide accurate or detailed information about the exposure and outcome timings, thus making it difficult to determine which occurred first.

Protopathic bias: Also known as reverse causation bias, it occurs in certain epidemiological studies when the symptoms or early manifestations of a disease/condition may lead individuals to modify their behavior or seek medical attention. This behavioral change or medical intervention may include exposure to a particular treatment, medication, or lifestyle modification. Researchers may mistakenly attribute the exposure (*e.g.*, a specific treatment or medication) to be the cause of the outcome (*e.g.*, the development of the disease) when, in fact, the exposure occurred after the initial symptoms or early signs of the disease developed. For example, in a hypothetical study, an association is sought between amoxicillin 500 mg administered to patients experiencing fever (cause) and gastric upset (outcome as a side effect). Some patients having fever may also have undiagnosed or yet-to-manifest gastric upset. After taking amoxicillin for fever, if gastric upset occurs, although it was already present subclinically and only manifested later on, it would be assumed that the drug caused the side effect.

Long latency period bias: Some diseases or health events have long latency periods, meaning there is a substantial delay between the exposure to a risk factor and the development of the outcome. During this extended period, other factors or interventions may come into play, adding complexity to the temporal relationship.

Diagnostic suspicion bias

Healthcare providers or researchers might have preconceived beliefs or suspicions about the likelihood of a particular diagnosis based on the patient's symptoms, medical history, or other factors. These prior beliefs can influence the decisions made during the diagnostic process. Healthcare providers may be more likely to order specific tests related to the suspected diagnosis and may interpret the results with a bias toward confirming that diagnosis.

Selection bias

It occurs during the identification of a study population wherein participants or subjects are not randomly selected, resulting in a nonrepresentative sample. For instance, if certain demographic groups are overrepresented or underrepresented in the study, it can affect the generalizability of the findings. Selection bias arises when participants or study subjects are not adequately representative of the target population. This bias can occur because of inappropriate sampling methods or exclusion criteria, which may limit the generalizability of the study findings to the broader dental patient population. This type of bias is commonly observed in case–control and retrospective cohort studies wherein exposure and outcome would have already occurred at the time of selection[5]. This bias can be reduced by incorporating strict randomization and blinding with best possible efforts. Allocation concealment should be carefully designed and also reported at the time of publishing the findings.

Sampling bias: It is a type of selection bias wherein the sample is not representative of the target population. For example, if the study only includes patients from a specific dental clinic and not from other clinics in the area, the findings may not reflect the characteristics of the broader population.

Ascertainment bias: This bias arises as a result of disproportionate statement of the eligible population. Here, the category of patients examined does not correspond to the incidents in the population.

Inclusion bias: Inclusion bias occurs when study participants are selected to represent the research population, and groups with different experiences are ignored. This bias is especially common in quantitative research. For example, an online oral health survey is supposed to be conducted using available internet sources. One may choose a study group based on possible confounding factors but might skip the fact that some people have access to the internet while others do not.

Exclusion bias: Exclusion bias occurs when some subgroups are intentionally excluded from the sample population before randomizing them into groups. For example, in a periodontal surgery in which patients should be followed up for 4-6 mo, intentionally excluding those patients who stay very far from the research site to avoid later dropouts can result in exclusion bias.

Relative control bias: This type of bias occurs when patients' relatives are included in the study as a control group. This inclusion may or may not have an effect on the actual outcome of the study depending on the parameters to be checked. If it is a placebo-controlled trial, patients and their relatives can easily discuss the intervention received by them and might use each other's medicines.

Nonrespondent bias: This type of bias usually occurs in a large-scale survey, prospective study, or longitudinal study. Many participants may not respond to a survey; however, their actual opinion might be significantly different and would have affected the results of the study if all of them had responded. Maintaining a patient record (email ID/phone number/address) might help in reaching out to non-responders and reducing this bias.

Matching and friend control bias: Friend controls are useful because researchers can quickly locate them *via* the instances. Friends are expected to be more motivated and have higher response rates than general population controls who are unaware of the case. Introverted people will appear on fewer or even no friend rosters, and sociable people will appear on more of those rosters. Extroverts are therefore more prone than introverts to develop into friend controllers. Based on the degree to which the exposures of interest are linked to sociability or personality, selection bias will alter the results[6].

Under-coverage bias: Under-coverage bias arises when a representative sample is drawn from a small proportion of the target population. Online surveys are especially vulnerable to under-coverage bias. For instance, if a survey on oral precancerous lesions is planned and the researcher includes patients visiting a dental hospital as the study population, the vast majority of people who may be at a greater risk but not motivated enough to visit a hospital will be left out. The results of such research cannot be reliably applied to the wider population.

Volunteer/referral bias: Part of the study population who either volunteered for the study or were referred for the specific disease would logically be more motivated for the treatment and follow-ups than non-volunteers. The results of such a study would have very low external validity. For instance, in a study on the prevalence of oral precancerous lesions in young adults, if the study population comprises patients visiting/referred to a dental hospital for consultation, the results would not apply to the general population.

Spectrum bias: Also known as spectrum effect or spectrum of disease bias, it is a type of bias in clinical research and diagnostic testing. This bias occurs when the characteristics of the study population do not accurately represent the broader population of individuals who may be affected by the condition of interest. In other words, the study sample does not adequately capture the entire range or spectrum of disease severity or characteristics seen in the real-world population.

Healthcare access bias

It describes a situation in which the accessibility and availability of healthcare services and resources might have an impact on the demographic characteristics or results of the study. This bias can appear when specific groups of people have unequal access to healthcare services, thereby resulting in the unequal representation of those groups in research studies or in divergent health outcomes.

Lenth-biased sampling

It occurs when the selection of study participants is more likely to include individuals who have experienced a longer duration of disease or exposure. This bias can occur because individuals with longer durations of disease/exposure are more likely to be detected or referred for testing. This bias can result in the overestimation of the sensitivity of a diagnostic test because it is more likely to identify cases in later stages of the disease, which are easier to detect. Conversely, it may result in the underestimation of specificity because individuals with shorter disease durations or milder conditions may not be included.

Competitive risk bias

Competitive risk may cause bias in epidemiology and survival studies. This bias occurs when researchers study time-to-event data without properly considering competing hazards. These are circumstances that could prevent the relevant event from occurring or alter the likelihood of its occurrence. To prevent skewed findings and incorrect conclusions, competitive risk should be considered in the study. Other occurrences that could precede or compete with the main event are referred to as competitive risks. For instance, while evaluating cancer-specific mortality in a cancer study, death from causes unrelated to the disease is regarded as a competitive risk.

Survivor treatment selection bias

A type of bias that can occur in observational studies, particularly in those assessing the efficacy of therapies or interventions, is the survivorship bias or immortal time bias. The bias in survivor treatment selection occurs when individuals who suffer the relevant event (such as death) soon after the start date of the study or before they are eligible to receive the therapy are not included in the analysis. Short-term non-survivors are not included in the study because only people who have survived long enough to receive the treatment are included, which can give the impression that the treatment is more beneficial than it actually is. This bias may cause the advantages of the treatment to be overestimated. The phrase "immortal time" describes the timeframe during which people are regarded as "immortal" because they have not yet gone through the event (such as death) or have not yet received the therapy of interest.

DURING-TRIAL BIAS

Detection performance bias

This type of bias occurs when the measurement or assessment of outcomes is influenced by the researcher's knowledge of the participants' exposure status. This bias happens when there are differences in the care or treatment provided to study groups owing to the knowledge or expectations of healthcare providers or participants. For example, in a clinical trial assessing the effectiveness of a new drug, if the healthcare providers are aware of the treatment assignment (*e.g.*, *via* differences in appearance or side effects), they may provide varying levels of care to participants in the treatment and control groups, thereby leading to performance bias.

Measurement bias

Instrument bias: In many research investigations, data are gathered with the aid of measurements tools, such as questionnaires, scales, sensors, lab equipment, and diagnostic testing. These tools measure or evaluate specific variables of interest. When an instrument consistently overestimates or underestimates the true value of a variable being measured, bias is introduced, which produces unreliable results. The proper selection of a good-quality instrument can reduce this bias.

Insensitive measure bias: Also known as "floor effect" or "ceiling effect", it is a type of measurement bias that can occur in research when a measurement instrument or scale is not sensitive enough to capture the complete range of values or responses within a sample. This bias can lead to inaccurate or incomplete data, thereby affecting the validity and reliability of the study findings. "Floor effect" occurs when a measurement instrument is unable to detect or distinguish values at the lower end of the range. Hence, a substantial portion of participants score at the lowest possible value, creating a "floor" for the data. Conversely, the "ceiling effect" happens when the instrument cannot differentiate values at the upper end of the range. Several participants achieve the highest possible scores, creating a "ceiling" for the data.

Expectation bias: Also known as observer-expectancy bias or experimenter-expectancy effect, it is a type of cognitive bias that can impact the outcomes of studies, particularly experimental and observational research. This bias occurs when researchers, either knowingly or unknowingly, have certain expectations about the results of the study, which can influence their observations and potentially affect participant behavior or data collection. In a study by Nevins *et al*[7], the questionnaire given to the test group was forwarding or positive-answer provoking, which could have obvious answers.

Attention bias: It is type of cognitive bias arising from selectively focusing on certain stimuli or information while ignoring or downplaying others. Individual cognitive processes and experiences may contribute to this bias. Attention bias can influence how participants perceive and react to intervention or stimuli in clinical research. For instance, owing to ingrained beliefs, concerns, or expectations, participants may pay more attention to specific symptoms, treatments, or results. Participants' reports of their experiences may be impacted by attention bias, which could result in inaccurate self-reporting.

Intervention bias

Interviewer bias: It refers to the systematic difference in the manner in which information is solicited, recorded, and interpreted[5,8]. This bias can be minimized or eliminated if interviewers are blinded to the outcome of interest or if the outcome of interest has not yet occurred, as in prospective studies.

Chronology bias: Comparing the intervention with historic findings creates chronology bias. Historic controls are past data rather than present data. Hence, comparing current interventions with such data may affect the results because the two data are collected in different timeframes. Over the years, the methods of diagnosis, investigations, and treatment change, which might improve the results and decrease the rate of complications. For example, a study to identify the prevalence of the MB2 canal (mesio-buccal canal) in upper first molar using cone beam computed tomography (CBCT) and comparing the results with data published decades earlier when CBCT was not available will yield biased results. Such bias can be minimized by conducting prospective studies, or if retrospective, the data should be compared only with findings in the recent past.

Recall bias: In studies that rely on participants' memory or self-reporting, there is a risk of recall bias. Participants may not accurately recall past dental experience or behaviors, thus leading to inaccurate data. This bias occurs when participants in a study inaccurately remember or report past events or experiences. In dental research, this bias can affect

studies that rely on self-reported data, such as surveys or questionnaires, leading to distorted associations between exposures and outcomes. The simplest way to reduce this bias is to frame questions in such a way that it is easy for participants to recall and/or questions from the recent past.

Attrition bias: Also known as dropout bias or nonresponsive bias, it is a type of bias than can occur in research studies when there is a differential loss of participants or data during the course of the study. This bias arises when the characteristics of individuals who dropout or are lost to follow-up are different from those who remain in the study. This can distort the findings and compromise the validity of the results. Attrition bias can manifest in various types of research, including longitudinal studies, clinical trials, surveys, and observational studies. Some common reasons for attrition include participant withdrawal, loss to follow-up, nonresponse to surveys, and incomplete data collection.

Intervention performance bias: It arises when there are differences in the care or treatments provided to different study groups, which can affect the outcomes. For example, if one group receives better dental care than the other, it could influence the results. Similarly, if a particular treatment in a study, such as periodontal surgery or disimpaction, is performed by two or three different doctors, it can result in bias. The variations in the performance of different clinicians might affect the outcomes.

Social desirability bias: Social desirability bias is a form of response bias that is related to systematic errors in the way participants respond to survey questions, interviews, or assessments. To win others' praise or to escape criticism from others, people may deliberately or unconsciously alter their responses to conform to cultural standards, values, or expectations. The responses may not accurately reflect the participants' actual experiences or behaviors owing to social desirability bias. This bias might have an impact on survey results, patient-reported outcomes, self-reported data, *etc.* Aggarwal *et al*[9], in their study on an oral questionnaire about tobacco smoking status, validated the social desirability bias by identifying that a whopping 30% of the participants underreported their smoking status.

Misclassification bias

Differential misclassification bias: Misclassification bias occurs when the exposure or outcome status of the study participants is incorrectly classified. This can happen for various reasons, including measurement errors, inaccurate data collection methods, or imperfect diagnostic tests. Differential misclassification occurs when the probability of misclassification differs between comparison groups in a systematic manner. In this scenario, the bias introduced can lead to incorrect associations that favor one group over another. This type of bias can either exaggerate or attenuate the true effect.

Nondifferential misclassification bias: This bias occurs when misclassification occurs randomly or with equal probability in all study groups. In such instances, the bias introduced may be toward the null (*i.e.*, it tends to make associations appear weaker than they truly are), but it does not systematically favor one group over another.

Exposure misclassification bias: It occurs if the exposure itself is poorly defined or if proxies of exposure are utilized.

Outcome misclassification bias: Here, misclassification of the outcome can occur if nonobjective measures are used to define the outcome. For example, using change in color of the gingival margin to determine the presence of gingivitis instead of a more objective method, such as gingival index or sulcular bleeding index.

Mimicry bias

It occurs during trial when the investigator is examining how exposures are related to a disease. It is important to ensure that the outcome being investigated is the true disease and not a condition mimicking the disease, which could lead to false conclusions about the causes of the disease of interest.

Allocation intervention bias

It typically refers to a type of bias that can occur during the process of assigning the study participants to different intervention groups in experimental research settings. This bias, if not properly managed, can undermine the validity of the study findings and often arises in randomized controlled trials (RCTs) and other experimental designs.

Allocation assignment bias: This occurs when the investigator either consciously or unconsciously assigns certain participants to a specific intervention group based on their characteristics or other factors. For example, if participants who are more likely to benefit from the intervention are placed in the treatment group and those less likely to benefit in the control group, it introduces selection bias.

Allocation concealment bias: This type of bias occurs when the process of randomization itself is not adequately concealed from the researchers or study staff who are responsible for assigning participants to groups. If researchers are aware of upcoming assignments, they may either inadvertently or intentionally influence the allocation process.

Contamination bias: This bias occurs when participants in different intervention groups interact or share information with each other, leading to the exchange of elements of the interventions. This exchange can blur the distinction between groups and undermine the study's ability to determine the true effects of the interventions.

Compliance bias

The degree to which study participants follow the recommended therapy or intervention protocols is referred to as compliance. For instance, participants in clinical trials might be told to take a certain drug, adhere to a certain diet, or make a certain behavioral change. When the participants do not adhere to these guidelines diligently, noncompliance happens. When there are systematically different compliance levels across treatment groups (such as intervention groups) and control groups (such as placebo groups) or between subgroups within the same study group, compliance bias is present. Such discrepancies in compliance may skew the study findings.

Differential maturing bias

This bias is observed in group randomized trials and reflects uneven secular (long-term) trends among the groups in the trial, favoring one condition over another[10].

Lack of intention to treat analysis

Clinical trials and other intervention studies sometimes employ intention to treat (ITT) analysis, wherein all participants are evaluated according to their initial treatment assignment regardless of what transpired later, such as noncompliance, dropouts, or protocol violations. ITT analysis ensures that the groups being compared remain comparable even if some participants did not adhere to their assigned therapy, thus maintaining the advantages of randomization. In the absence of ITT analysis, the research may be systematically different from those who discontinue or do not fully adhere to the treatment, thereby resulting in exaggerated or underexaggerated treatment effects that could mislead clinical or policy decisions.

Duration bias

The length of the study or follow-up period is a crucial factor in clinical research. This period should be selected in accordance with the study question and the desired results. Long-term effects or late-occurring events may not be captured if the study duration is too short. On the contrary, very protracted follow-up times can be expensive and may result in participant attrition, making it difficult to sustain the validity of the study. These issues cause duration bias in either situation. Additional bias related to: Split-mouth study-related bias, inter-examiner-related study bias, bias in blinded studies, questionnaire/survey-related bias.

BIAS ASSOCIATED WITH THE SPLIT-MOUTH STUDY DESIGN

The split-mouth study design is a type of within-subject design often used in clinical research, especially in dentistry and some medical fields. In this design, each subject serves as their control, with one side of the mouth (*e.g.*, left side) receiving one treatment or intervention and the other side (*e.g.*, right side) receiving a different treatment or intervention. Some potential biases related to the split-mouth study design are as follows: (1) Carryover effect: There may be a carryover effect from one treatment to the other, particularly if there is a washout period between interventions. This effect could impact the results of the study as the treatments may not be entirely independent of each other; (2) Order effect: The order in which the treatments are administered can introduce bias. For example, if one treatment is more effective, it might influence the response to the subsequent treatment on the other side of the mouth; (3) Learning effect: Participants may become more accustomed to the study procedures or interventions over time, resulting in different responses for later treatments compared with earlier ones; (4) Differential attrition: There might be differences in dropout rates between the two sides of the mouth, which could introduce bias in the analysis; (5) Carry-in effect: The baseline characteristics of the two sides of the mouth may not be entirely equal, leading to potential confounding; (6) Observer bias: If the outcome measures are assessed subjectively or by different observers, biases may arise because of variations in interpretation or assessment; and (7) Selection bias: The choice of the split-mouth design could introduce bias if certain participants are selected for the study based on specific criteria or characteristics.

To mitigate these biases, researchers employing a split-mouth study design should carefully plan the study, randomize the order of treatments, and ensure standardized and blinded assessments of outcomes. Furthermore, it is essential to consider the potential for bias when interpreting the results of such studies and to be cautious while making generalizations beyond the study population.

INTER-EXAMINER-RELATED STUDY BIAS

Also known as inter-rater or inter-observer bias, it refers to a type of bias that can occur in research studies when different examiners or observers assess the same subjects or data points differently. This bias can influence the results and conclusions of the study, potentially leading to inaccurate or misleading findings. Understanding and addressing this bias is important to ensure the validity and reliability of research outcomes. Some common factors contributing to inter-examiner bias are as follows: (1) Subjectivity in assessments: When examiners employ subjective criteria for evaluating subjects or data, their interpretations and judgments may vary. For instance, in dental research, different doctors may diagnose the same condition differently based on their experiences and personal biases; (2) Variability in data collection: Different examiners may use slightly different methods or instruments to collect data, leading to inconsistencies in the collected data. This variation can affect the reliability of the results; (3) Training and expertise: The level of training and

expertise may differ among examiners, leading to differences in their ability to interpret and analyze the data accurately; (4) Communication among examiners: If there is a lack of standardized communication or guidelines for examiners, they may inadvertently influence each other's judgments, leading to biased outcomes; and (5) Expectation bias: Examiners might be influenced by their expectations or prior beliefs about the study outcomes, consciously or unconsciously affecting their observations and assessments.

Several strategies can be employed to mitigate inter-examiner bias and enhance the reliability of the findings. Implementing clear and standardized protocols for data collection and assessment can reduce variability between examiners. Inter-rater reliability can be calculated to assess the level of agreement among examiners, which can help identify potential bias and guide improvements in the assessment process. Moreover, consistent training can be provided to all examiners to ensure that they have a shared understanding of the assessment criteria and methodologies. In some cases, blinding the examiners to certain aspects of the study (*e.g.*, treatment groups) can help prevent their expectations from influencing the results. In addition, having multiple independent examiners evaluate the same subjects or data can help identify discrepancies and potential biases. A review process can be implemented in which an experienced and impartial researcher checks and validates the assessments of different examiners. By recognizing the potential for inter-examiner bias and taking steps to minimize its impact, researchers can enhance the credibility and accuracy of their findings. One of the best examples of measures taken to reduce inter-examiner bias has been presented in our previous study (Agrawal[11], 2011) on the reliability and reproducibility of a sign grading system for plaque and calculus.

BIAS IN BLINDED STUDIES

Bias in research can occur in various forms, and single, double, or triple-blind studies are not entirely immune to these biases. Although these study designs are adopted to reduce certain types of biases, they may still be affected by other sources of bias. The ways in which each study design can be impacted have been discussed below: (1) Single-blind studies: In a single-blind study, the participants are unaware of their group assignment (*e.g.*, treatment or control), but the researchers are aware of the groups. Bias can still arise in this design if the researchers inadvertently influence the participants' behavior or the study outcome. For example, unintentional cues or communication from researchers to participants may subtly affect the way in which participants respond or behave during the study; (2) Double-blind studies: In a double-blind study, both the participants and the researchers directly involved in the study are unaware of the group assignment. This design helps reduce potential bias caused by participants' expectations or researchers' conscious or unconscious influence. However, bias can still occur if those administering the treatments or interventions (*e.g.*, nurses or doctors) are aware of the group assignment and unconsciously treat participants differently based on that knowledge; and (3) Triple-blind studies: Triple-blind studies go one step further by also keeping the data analysts or statisticians blinded to the group assignment. This design aims to minimize bias during data analysis. However, researchers might still inadvertently introduce bias during the conduct of the study even if the analysts remain blinded to the groups.

POST-TRIAL BIAS

Bias in research after the conclusion of the trial could occur during data analysis or publication or interpretation of the results. Some possible biases that can occur in the post-trail stage are discussed below.

Publication bias/citation bias

This bias occurs when studies with positive or significant results are more likely to be published and those with null or negative findings are less likely to be published. In dental journals, 82% of the published articles report positive results [12]. This bias can create an overrepresentation of certain results and lead to an inaccurate overall picture. It occurs when research findings are selectively published based on their statistical significance or favoring positive outcomes. This bias can lead to an overrepresentation of studies with favorable results, potentially distorting the overall understanding of the effectiveness or safety of dental interventions. Despite the measures adopted by the International Committee of Medical Journal Editors[13,14], citation bias continues to exist. Although centralized documentation of all trials provides information about unpublished trials, the results of such studies can only be speculated. Locating unpublished studies *via* trial registers, search engines, and other databases can reduce this bias.

Confounding bias

It occurs when the observed association between variables are attributed to three possible causes: The exposure itself, the outcome of interest, and an independent factor[5]. One such example is the association between obesity and dental caries (confounded by frequent eating or a high-sugar diet). After the completion of the study, the identified confounders can be controlled by analyzing the association only in those cohorts that are similar in terms of the identified confounding factors. Stratified analysis or multivariable regression analysis can be used to control the identified confounders; however, the role of unidentified confounders cannot be controlled. Unknown confounders can, however, be controlled with randomization.

Reporting bias

It involves the selective reporting of outcomes within a study. Researchers may emphasize certain findings while downplaying or omitting others, which can skew the overall conclusions.

Language bias

This type of bias may occur when research studies published in a particular language are favored over those published in other languages, resulting in an incomplete representation of the available evidence.

Positive bias

The value, effect, or data observed are greater than the actual or casual data, which is away from null. Here, the perceived value is closer to 1.0 than the actual value.

Negative bias

In this case, the value, effect, or data observed are less than the actual/casual data and can be termed null. The perceived value is lower to 1.0 than the actual value.

Funding bias

If the research is funded by a specific dental product manufacturer or entity with vested interests, there might be a tendency to favorably report outcomes related to their products. The bias refers to the potential influence of financial or nonfinancial conflicts of interest on the study outcomes. Dental research studies funded by industry sources might have a higher likelihood of reporting results favorable to the sponsor's products or interests, which can compromise the impartiality and independence of the research.

Ecological fallacy

Data are collected and analyzed at the population or group level in some investigations. For instance, researchers may examine health results, risk factor exposure, or other aspects for entire populations or geographical areas. When researchers base their conclusions or judgements about individuals within such groups only on the observed group-level data, they are committing an ecological fallacy. Problems can arise as individual-level traits or connections might be different from those observed in groups.

Regression dilution bias

When the values of the independent variable (exposure) are measured incorrectly, measurement error arises. This inaccuracy may be the result of inaccurate data acquisition techniques, tools, or participant self-reporting. When performing regression analysis, measurement error in the exposure variable tends to weaken the relationship between the exposure and the result. Thus, the expected impact of exposure on the result is less significant than the actual impact. Regression dilution bias can cause researchers to draw wrong conclusions about how strong and important a relationship between two variables is. They might overestimate the genuine effect of an exposure on the result, which affect policy, clinical practice, or public health.

SPECIAL BIAS

The Hawthorne effect

The Hawthorne effect is a psychological phenomenon that can significantly influence research outcomes. This effect occurs when participants in a study modify their behavior or performance simply because they are aware of being observed or receiving special attention. In an RCT evaluating a novel manual toothbrush design for orthodontic patients, the methodology necessitated that patients brush in front of the investigator[15]. This could have led to the Hawthorne effect as the performance of patients in home brushing may not be same as that in brushing in front of the dentist.

The Hawthorne effect can significantly influence research outcomes in various fields. For instance: (1) Bias in participant responses: Participants may alter their responses so that these are aligned with what they believe the researchers desire rather than providing genuine or accurate information; (2) Enhanced performance: Participants might perform better than they typically would because of the extra attention received during the study, leading to inflated results; (3) Social desirability bias: Participants may alter their behavior to appear more socially acceptable or desirable, resulting in responses that do not accurately reflect their usual behavior; and (4) Temporary changes: The effect is often short-lived, and once the observation or special treatment ends, participants may revert to their typical behavior.

Of these, the naturalistic observation strategy might be useful in mitigating the Hawthorne effect. It means that researchers can conduct observations unobtrusively without the participants being aware of it, thereby enhancing the authenticity of the results.

Will Rogers phenomenon

In clinical research and epidemiology, a shift in the staging or classification of people when a new diagnostic test or set of criteria is introduced is referred to as the "Will Rogers phenomenon", after the American comedian Will Rogers. This modification may appear to improve the outcomes or survival rates for some populations, but there may be no real

change in the severity or prognosis of the disease. In essence, this phenomenon illustrates the notion that "moving people across groups can improve the image of both groups". An interesting study in this regard was published by Oshman *et al* [16] in which similar patients were presented at two different timepoints to a group of specialists. When the patients' age was added at one timepoint, the agreement between the diagnosis changed significantly.

Neyman bias

Neyman bias, often referred to as "incidence–prevalence bias" or "Neyman's bias", is a statistical phenomenon that can affect how epidemiological or clinical research findings are interpreted, especially in cross-sectional and observational studies. This bias, which bears Jerzy Neyman's name, occurs when the incidence rate of a condition or disease is incorrectly estimated based on its prevalence in the studied population.

This bias develops when researchers estimate the incidence rate of a disorder based on its prevalence, particularly when the length of the condition differs among people. This approach can lead to problems because prevalence is affected by the frequency of new cases as well as the length of the illness in those patients in whom the illness is already present.

Individuals with a prolonged disease duration are more likely to be counted in the prevalent cases, which results in an overestimation of the incidence rate. In contrast, individuals with a shorter disease duration are less likely to be included, which causes the incidence rate to be underestimated.

Berkson's bias

A statistical phenomenon known as Berkson's bias, often referred to as Berkson's paradox or Berkson's fallacy, can affect how clinical research findings are interpreted, particularly in studies conducted in hospitals or using data from hospitals. When the study subjects are selected from a hospital or clinical context, Berkson's bias occurs. Owing to the fact that they are seeking medical attention or treatment, participants in these studies often have a higher risk of suffering from one or more health issues. Therefore, the correlation between risk factors and diseases may be inflated. In other words, simply because people who have the condition are more likely to be in a hospital or clinical setting, certain risk variables may seem to be more strongly connected with the disease. In a retrospective study by Jain[17], the author concluded that mandibular molars were the most frequently treated posterior teeth in both sexes and that women constituted the majority of those receiving posterior root canal therapy. However, the data were obtained from patients who reported to their college, and hence, the conclusion may not be applicable to the general population. In the discussion section, the authors compared their findings with a previous study that also had Berkson's bias. The study by Al-Negrish[18] aimed to determine the incidence and distribution of root canal treatment in a dentition of Jordanian population, but the study sample was from the department of dentistry of a hospital in Jordan.

Memory effect

Also known as recall bias or retrospective bias, it is a prevalent form of bias in research studies that can significantly impact the validity and reliability of the findings. This bias occurs when participants' memories of past events are flawed or selectively recalled, leading to inaccurate or distorted data.

In research, the memory effect can arise in various scenarios: (1) Retrospective studies: Research designs that rely on participants' recollection of past experiences, habits, or exposures are particularly vulnerable to the memory effect. Participants may inadvertently misremember or exaggerate certain events or behaviors, which can skew the results; (2) Surveys and questionnaires: When individuals are asked to report past events *via* surveys or questionnaires, their ability to accurately recall specific details can be influenced by various factors. These factors include the emotional impact of an event, the passage of time, or even external influences, such as media coverage or personal beliefs; (3) Longitudinal studies: The memory effect can occur even in longitudinal studies that track participants over an extended period. Participants might remember events differently or become influenced by subsequent experiences, leading to alterations in their responses; (4) Case–control studies: In case–control studies in which researchers compare individuals with a specific condition to those without it, recall bias can be especially problematic. Patients may be more likely to recall specific exposures or events owing to their current condition, leading to an overestimation of the association between the condition and the exposure; and (5) Self-reported data: Any study relying heavily on self-reported data is susceptible to the memory effect. People may unintentionally misrepresent their experiences or provide socially desirable responses, leading to biased conclusions.

NOVELTY BIAS

Novelty-related bias refers to the tendency of individuals, organizations, and societies to place greater emphasis and value on novel information, ideas, products, or experiences than on established or conventional ones. This bias can manifest in various aspects of human behaviors, decision-making processes, and cultural preferences. While embracing novelty can pave the way for progress and positive change, an excessive bias toward it has certain drawbacks. In academia and scientific research, there can be a pressure to publish novel findings, which might lead to a focus on flashy but potentially less rigorous or significant studies. As for the actual clinical research studies, evaluation of a novel item (*e.g.*, a fancy toothbrush design or a noncommercially available mouthwash) can also introduce a bias in that the participants use the novel product more seriously and without skipping the recommended dose or duration or both. Over the weeks, as the novelty effect fades off and opinions of clinicians might. In a recent study, comparing the manual toothbrush with a novel sonic toothbrush, the authors unknowingly introduced a potential novelty bias[7]. Had the investigators asked the patients to use the sonic toothbrush for a few weeks before starting the trial, the results might

have been different. On the contrary, some individuals may develop a bias against novelty, preferring familiar and established practices owing to a fear of the unknown or a desire for stability. This resistance to change can hinder progress and stifle innovation.

CONFIRMATION BIAS

Confirmation bias has been included under special bias category as it exerts its effect at every stage of clinical research right from study design to conducting the trial and publishing the results and even in clinical case scenarios. Researchers might design studies in a way that unconsciously favors the hypothesis they believe to be true, leading to biased data collection or experimental design. During data collection and analysis, researchers might unconsciously focus on aspects of the data that support their hypothesis while overlooking or downplaying those that are against it. After the completion of the research, there may be a tendency to submit and publish studies with positive or confirmatory results, whereas those with negative or contradictory findings might remain unpublished or receive less attention. Furthermore, confirmation bias can affect the peer review process because reviewers might be more critical of studies that challenge established beliefs and more lenient toward those that confirm prevailing notions. Clinicians involved in research or medical practice may be influenced by confirmation bias when interpreting patient data or deciding on treatments. They might give more weightage to data that support their initial diagnosis or treatment plan.

BIAS IN *IN VITRO* STUDIES

In vitro studies are experiments conducted in a controlled laboratory environment using isolated cells, tissues, or organs outside of their natural environment. While these studies are valuable for investigating specific biological mechanisms and testing hypotheses, they are not free from potential biases. Some possible biases in *in vitro* studies include the following: (1) Publication bias: *In vitro* studies that yield positive or significant results are more likely to be published than those with negative or inconclusive findings. This bias can lead to an overrepresentation of positive outcomes in the literature; (2) Selection bias: The choice of cell lines or experimental models may not entirely represent the complexity of human biology. Researchers might select cells that are easier to work with or those that support their hypothesis, potentially overlooking relevant but less convenient models; (3) Sampling bias: In *in vitro* studies, researchers often use specific cell lines or samples obtained from a specific source, such as a commercial supplier. These samples may not accurately represent the target population or disease being studied; (4) Measurement bias: Errors such as inaccurate calibration of instruments or subjective interpretation of results can occur during the measurement process. These errors can introduce discrepancies between the observed and actual outcomes; (5) Contamination or cross-contamination: *In vitro* experiments are susceptible to contamination by bacteria, fungi, or other cell lines. If not appropriately controlled, such contamination can affect the validity of the study; (6) Experimental design bias: The design of the *in vitro* study may introduce a bias. One such example is using higher concentrations of a substance to achieve more significant effects even if these concentrations are not physiologically relevant; (7) Time bias: *In vitro* studies may be conducted over a relatively short period, whereas biological processes often occur over longer timeframes. This discrepancy can lead to an incomplete understanding of the dynamics involved; and (8) Lack of ecological validity: Findings from *in vitro* studies might not translate accurately to the complexities of the human body and its interactions in the natural environment.

Hence, *in vitro* study results must be interpreted cautiously, and their limitations should be considered. To gain a more comprehensive understanding, results from such studies should be complemented with data from other research approaches, such as animal studies or clinical trials.

CONCLUSION

Researchers should be aware of these biases and adopt measures to minimize their impact to ensure the validity and reliability of their findings. Transparent reporting of study methods and potential sources of bias is crucial for the dental research community to assess the quality of the research.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Assessing the readability of online information about jones fracture

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Abstract

BACKGROUND

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look toward the internet as their primary source of information to learn about their respective medical conditions. The American Medical Association and National Institute of Health strongly recommend that online medical information be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds.

AIM

To assess the readability of online information regarding Jones fracture. Our hypothesis is that the reading level of medical information published on websites far exceeds the recommended reading level of 6th-8th grade as proposed by the American Medical Associate and National Institute of Health. The result of this study can help us formulate improved recommendations for publishing more comprehensible material and, thus, eventually improve patient compliance and clinical outcomes.

METHODS

The exact phrase "Jones fracture" was queried on the three most common search engines, Google, Yahoo!, and Bing, on December 28, 2022. As of December 2022, Google held 84%, Bing held 9%, and Yahoo! held 2% of the worldwide search engine market share. Web pages uniform resource locator from the first three pages of search results were recorded from each search engine. These web pages were classified according to academic, physician-sponsored, governmental and non-government organizations (NGO), commercial, and unspecified as per

formally defined categories. Websites associated with an educational institution or medical organization were classified as academic. Websites with products for sale, corporate sponsorship, or advertisements were classified as commercial. Governmental websites or NGOs comprised those that received government subsidies or grants. Webpages that were independently owned by physicians or physician groups were respectively classed as physician sponsored. The remainder of websites that did not fall under the above categories were classified as unspecified.

RESULTS

A total of 93 websites were analyzed for reading assessment. A whopping 44% of websites were commercial, followed by 22% of physician-sponsored websites. Third place belonged to non-government organization websites holding a 15% share. The academic website held a meager 9% portion, while unspecified sites were 3%. The table illustrates mean readability scores, along with average cumulative grade level. The average grade level was 10.95 ± 2.28 for all websites, with a range of 6.18 to 18.90. Since *P* values were more than 0.05, there was not a significant statistical difference between the first page results and the results of all pages. Thus, we can rationalize that readability scores are consistent throughout all pages of a website.

CONCLUSION

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look towards the internet as the primary source of information to learn about their respective medical conditions. Our study demonstrates that current online medical information regarding Jones fracture is written at an extraordinarily high-grade level, with an average grade level of all websites at 10.95, nearly an 10th-grade educational level. The American Medical Association and National Institute of Health strongly recommend that online medical information should be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds. On the contrary, most of the medical information evaluated was at an 10th-grade level, which far exceeds recommendations by AMA and NIH. This is particularly relevant because readability scores are directly proportional to the level of comprehension attained by readers, thus directly impacting patient outcomes. In conclusion, we suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

Key Words: Jones fracture; Jones fracture treatment; Jones fracture management; Jones fracture prevention; Jones fracture types; Jones fracture location

Core Tip: With technological advancements, many patients look toward the internet as their primary source of information to learn about their respective medical conditions. The American Medical Association and National Institute of Health strongly recommend that online medical information be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds. Readability measures how easy a piece of text is to read. This, in turn, affects how much information people can understand and retain. Our study aims to assess the readability of online information regarding Jones fracture. A total of 93 websites were analyzed for reading assessment. The overall mean average grade level of all websites in the study was 10.95230 ± 2.27862, corresponding to a 10th-grade reading level. In Conclusion, most of the medical information evaluated was at an 11th-grade level, far exceeding AMA and NIH recommendations.

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INTRODUCTION

Sir Robert Jones originally described the Jones fracture in 1902 as a self-named fracture occurring within 3/4th of an inch from the base of the 5th metatarsal. Generally, the Jones fracture is defined as all fractures of the proximal 5th metatarsal distal to the tuberosity within 1.5 cm of this area[1]. Interestingly, the most common fracture of the foot is the Jones fracture, *i.e.*, fracture of the 5th metatarsal. Many classification systems exist describing the Jones fracture; however, the most widely used is the anatomical classification, dividing the proximal part of the 5th metatarsal into three zones[2].

Table 1 Unpaired *t*-test for overall pages *vs* first page of websites

Readability metric	Overall mean ± SD (range)	First page mean ± SD (range)	Percentage mean difference	*P* value
Flesch reading ease	55.3970 ± 12.7618 (23.2-86.1)	55.3290 ± 15.9812 (9.9-87.4)	2.32	0.553
Flesch-kincaid	10.1450 ± 2.7082 (5.0-20.1)	10.3840 ± 3.2056 (4.9-18.9)	0.47	0.915
Gunning fog	12.7830 ± 2.8099 (8.2-23.5)	12.4210 ± 3.1772 (7.6-20.7)	3.86	0.287
SMOG	9.3530 ± 1.9114 (5.6-15.8)	9.3000 ± 2.3224 (5.1-16.3)	2.27	0.523
Coleman-liau index	12.2460 ± 1.4778 (7.5-16.1)	11.9970 ± 2.2096 (8-19)	4.64	0.41
Automated readability	10.2340 ± 3.0205 (4.6-22.0)	10.5680 ± 3.6182 (3.8-19.2)	1.05	0.824
Average grade level	10.95230 ± 2.27862 (6.18-18.88)	10.93420 ± 2.73413 (6.22-18.48)	0.09	0.888

Zone 1 is the most proximal area, comprising the 5th metatarsal tuberosity and the metatarsocuboid joint. Injuries in Zone 1 are usually avulsion fractures, which are non-operatively managed to result in adequate clinical outcomes. Zone 2 consists of an area bordering the 4th and 5th intermetatarsal junction, stretching to the metaphyseal-diaphyseal junction and distal to Zone 1. A fracture in Zone 2 is classified as an actual Jones fracture. Finally, we have Zone 3, which contains the proximal 1.5 cm of the metatarsal diaphysis. Fractures in Zone 2 and Zone 3 are known to have high non-union rates with non-operative management, hence, promoting operative management as the preferred treatment[2].

With the turn of the century, the internet has become a valuable source of medical information for patients[3]. As per the 2022 Health Information National Trends Survey, approximately 74.7% of people initially browsed the internet to gain insight into their medical problems[4]. With increased online dissemination of medical information, the public has turned to the internet as their first source of information regarding medical illnesses. In fact, online resources have been shown to increase compliance with treatment goals and self-governed lifestyle changes[5].

Readability is the measure of how easy a piece of text is to read[6]. This, in turn, affects how much information people are able to understand and retain. Given that health literacy actively corresponds to positive patient outcomes, the readability of a text comes into play in how effectively a piece of information can be comprehended by patients[7]. The American Medical Association and the National Institute of Health recommend that patient education materials should be written between a 6th and 8th-grade level[8,9].

Our study aims to assess the readability of online information regarding Jones fracture. Our hypothesis is that the reading level of medical information published on websites far exceeds the recommended reading level of 6th-8th grade as proposed by the American Medical Associate and National Institute of Health. The result of this study can help us formulate improved recommendations for publishing more comprehensible material and, thus, eventually improve patient compliance and clinical outcomes.

MATERIALS AND METHODS

The exact phrase "Jones fracture" was queried on the three most common search engines, Google, Yahoo!, and Bing, on December 28, 2022. As of December 2022, Google held 84%, Bing held 9%, and Yahoo! held 2% of the worldwide search engine market share[10]. Web pages uniform resource locator (URLs) from the first three pages of search results were recorded from each search engine. These web pages were classified according to academic, physician-sponsored, governmental and non-government organizations (NGO), commercial, and unspecified as per formally defined categories[11]. Websites associated with an educational institution or medical organization were classified as academic. Websites with products for sale, corporate sponsorship, or advertisements were classified as commercial. Governmental websites or NGOs comprised those that received government subsidies or grants. Webpages that were independently owned by physicians or physician groups were respectively classed as physician sponsored. The remainder of websites that did not fall under the above categories were classified as unspecified[12-14] (Tables 1-3).

RESULTS

A total of 101 website results were obtained, 49 from Google, 28 from Yahoo!, and 24 from Bing. Only eight websites were excluded, making a total of 93 pages manually analyzed (49 from Google, 21 from Yahoo!, and 23 from Bing; Figures 1 and 2). Exclusions were made due to duplication results. All the text from the images was included as part of the analysis. On pages where there was information about multiple subjects, only information relevant to Jones fracture was selected for analysis.

Table 2 Percentage of websites and their grade level

Classification	*n*	Percentage	Average grade level	St. Dev.	Maximum	Minimum
Academic	8	0.0860	11.36	± 3.33	18.88	8.54
Physician sponsored	21	0.2258	10.93	± 1.71	13.42	8.74
Governmental & NGO	21	0.2258	11.51	± 2.56	15.68	6.18
Commercial	40	0.4301	10.84	± 2.06	18.02	6.46
Unspecified	3	0.0323	7.61	± 1.24	8.32	6.18

NGO: Non-government organizations.

Table 3 Unpaired *t*-test for government non-government organizations *vs* academic websites

Readability metric	Government & NGO mean ± SD (range)	Academic mean ± SD (range)	Percentage mean difference	*P* value
Flesch reading ease	49.0330 ± 17.4883 (28.0-86.1)	53.5880 ± 14.3224 (23.2-66.6)	4.438	0.276
Flesch-Kincaid	10.7860 ± 2.9066 (5.0-15.6)	10.825 ± 4.083 (7.5-20.1)	0.362	0.554
Gunning fog	13.529 ± 2.869 (8.2-18.5)	13.1500 ± 4.5056 (9.6-23.5)	2.838	0.475
SMOG	9.8860 ± 2.1763 (5.6-13.4)	9.7880 ± 2.8478 (7.2-15.8)	0.998	0.453
Coleman-liau index	13.1670 ± 2.2265 (7.5-16.1)	12.0500 ± 0.7051 (11.2-13.0)	8.857	0.086
Automated readability	10.200 ± 2.8660 (4.6-16.0)	11.0000 ± 4.7413 (7.2-22.0)	7.547	0.290
Average grade level	11.51330 ± 2.55634 (6.18-15.68)	11.36250 ± 3.33421 (8.54-18.88)	1.319	0.665
Complex words	130.81 ± 117.662 (44-459)	122.380 ± 51.578 (70-210)	6.663	0.304
Percentage of complex words	0.1746810 ± 0.0552751 (0.0538-0.2356)	0.1414500 ± 0.0334975 (0.1063-0.1860)	21.024	0.187
Average words per sentence	16.90710 ± 3.23038 (14.03-25.71)	20.32130 ± 8.64815 (14.12-40.74)	18.341	0.090
Average syllables per word	1.67520 ± 0.19457 (1.21-1.85)	1.56750 ± 0.07459 (1.49-1.68)	6.645	0.041

NGO: Non-government organizations.

DISCUSSION

With advances in technology, operative and conservative management options for Jones continue to grow. Vast amounts of information are available on the internet. In fact, in today's age, patients tend to utilize the internet as their primary source of information before actually seeing a medical practitioner. Therefore, it is of utmost significance how medical information is disseminated to patients.

Our study aimed to assess the readability of online information regarding Jones fracture. A vast majority, 77%, of websites were written above an eighth-grade level, far surpassing recommendations set by AMA and NIH[15-17]. The overall mean average grade level of all websites in the study was 10.9523 ± 2.27862, corresponding to a 10th-grade reading level. Strikingly, governmental and NGO websites were found to have the highest average grade level of 11.51 ± 2.56, representing an 11th-grade level of comprehension. Even though previous studies[18] have shown Academic websites to have the high readability score, in our case, academic websites ranked 2nd with an average grade level of 11.36 ± 3.33, which was still an eleventh-grade reading level. Considering these results, full comprehension of online medical information would require the completion of at least a secondary education.

CONCLUSION

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look towards the internet as the primary source of information to learn about their respective medical conditions. Our study demonstrates that current online medical information regarding Jones fracture is written at an ex-traordinarily high-grade level, with an average grade level of all websites at 10.95, nearly an 10th-grade educational level. The American Medical Association and National Institute of Health strongly recommend that online medical information should be written at the 6th to 8th-

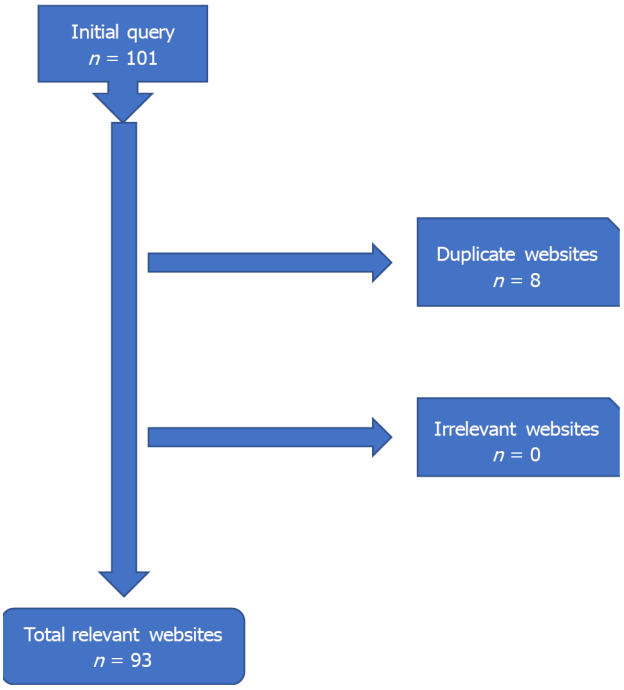

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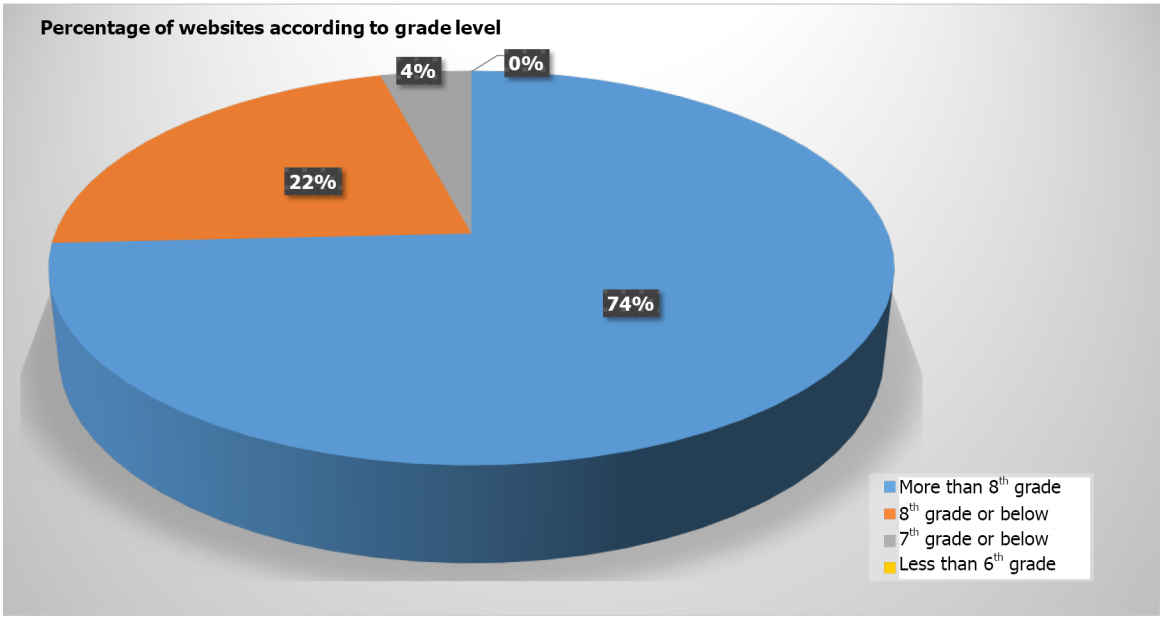
Figure 1 Flow diagram of inclusion and exclusion criteria.



DOI: 10.5662/wjm.v13.i5.439

Figure 2 Percentage of websites and their grade level.

grade level to aid comprehension by patients of all literacy backgrounds. On the contrary, most of the medical information evaluated was at an 10th-grade level, which far exceeds recommendations by AMA and NIH. This is particularly relevant because readability scores are direct-ly proportional to the level of comprehension attained by readers, thus directly impacting patient outcomes. In conclusion, we suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

ARTICLE HIGHLIGHTS

Research background

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look toward the internet as their primary source of information to learn about their respective medical conditions. The American Medical Association and National Institute of Health strongly recommend that online medical information be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds.

Research motivation

With advances in technology, operative and conservative management options for Jones continue to grow. Vast amounts of information are available on the internet. In fact, in today's age, patients tend to utilize the internet as their primary source of information before actually seeing a medical practitioner. Therefore, it is of utmost significance how medical information is disseminated to patients.

Research objectives

To assess the readability of online information regarding Jones fracture. Our hypothesis is that the reading level of medical information published on websites far exceeds the recommended reading level of 6th-8th grade as proposed by the American Medical Associate and National Institute of Health. The result of this study can help us formulate improved recommendations for publishing more comprehensible material and, thus, eventually improve patient compliance and clinical outcomes.

Research methods

The exact phrase "Jones fracture" was queried on the three most common search engines, Google, Yahoo!, and Bing, on December 28, 2022. As of December 2022, Google held 84%, Bing held 9%, and Yahoo! held 2% of the worldwide search engine market share. Web pages uniform resource locator from the first three pages of search results were recorded from each search engine. These web pages were classified according to academic, physician-sponsored, governmental and non-government organizations (NGO), commercial, and unspecified as per formally defined categories. Websites associated with an educational institution or medical organization were classified as academic. Websites with products for sale, corporate sponsorship, or advertisements were classified as commercial. Governmental websites or NGOs comprised those that received government subsidies or grants. Webpages that were independently owned by physicians or physician groups were respectively classed as physician sponsored. The remainder of websites that did not fall under the above categories were classified as unspecified.

Research results

A total of 101 website results were obtained, 49 from Google, 28 from Yahoo!, and 24 from Bing. Only eight websites were excluded, making a total of 93 pages manually analyzed (49 from Google, 21 from Yahoo!, and 23 from Bing). Exclusions were made due to duplication results. All the text from the images was included as part of the analysis. On pages where there was information about multiple subjects, only information relevant to Jones fracture was selected for analysis.

Research conclusions

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look towards the internet as the primary source of information to learn about their respective medical conditions. Our study demonstrates that current online medical information regarding Jones fracture is written at an extraordinarily high-grade level, with an average grade level of all websites at 10.95, nearly an 10th-grade educational level. The American Medical Association and National Institute of Health strongly recommend that online medical information should be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds. On the contrary, most of the medical information evaluated was at an 10th-grade level, which far exceeds recommendations by AMA and NIH. This is particularly relevant because readability scores are directly proportional to the level of comprehension attained by readers, thus directly impacting patient outcomes. In conclusion, we suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

Research perspectives

We suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

FOOTNOTES

Author contributions: Al-Kharouf KFK conceived the methodology for the manuscript, performed the literature search and analysis for the study, and wrote the manuscript; Khan FI performed the literature search and analysis for the study and wrote the manuscript; Robertson GA advised on the study, and reviewed and edited the manuscript.

Institutional review board statement: Not required, no human or animal involved in our study.

Informed consent statement: The dataset consisted of anonymized synthesize evidence from published studies. Thus, no informed consent for data sharing was required.

Conflict-of-interest statement: Khaled Farid Khaled Al-Kharouf, Faisal Idrees Khan, and Greg Robertson have no conflicts of interest to declare. None have received fees for serving as a speaker or a consultant for commercial organizations. None have received research funding from commercial organizations. All are employees of the UK National Health Service, though not of any commercial organizations. None own stocks or shares in related commercial organizations. None own patent related to the topic of this study.

Data sharing statement: Technical appendix and datasets are available from the corresponding author at kfk990@gmail.com.

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ORIGINAL ARTICLE

Retrospective Study

Impact of COVID-19 lockdown on hospital admissions for epistaxis in Germany

Adrian Hoenle, Martin Wagner, Stephan Lorenz, Helmut Steinhart

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Abstract

BACKGROUND

Reports of a decrease in hospital admissions during the coronavirus disease 2019 (COVID-19) lockdown period have raised concerns about delayed or missed diagnoses and treatments for non-COVID-19-related illnesses.

AIM

To investigate the impact of the COVID-19 pandemic-induced lockdown and its end on hospital admissions of patients with epistaxis in Germany.

METHODS

A retrospective analysis based on the national database of the Hospital Remuneration System was used to compare hospital admissions during defined time periods between 2019 and 2022 with the lockdown period as the reference period. This was done on a weekly basis before, during, and after the lockdown. An Interrupted Time Series was used as the analysis method.

RESULTS

In our analysis, we included 26183 patients. The implementation of the lockdown led to a substantial reduction in the overall occurrence of epistaxis among patients ($P < 0.05$). This effect was most pronounced in the age group of 0-39 years, where the decrease was highly significant ($P < 0.001$). However, there was no change observed in patients aged 80 years and older (not significant). With the end of the lockdown period, the overall number of patients, especially in the youngest age group, increased abruptly and significantly ($P < 0.01$).

CONCLUSION

During the lockdown period, there was a decrease in hospital admissions for

younger patients with epistaxis, possibly due to the fear of COVID-19 exposure. We also conclude that the severity of epistaxis was not underestimated in the elderly during the pandemic.

Key Words: COVID-19; Epistaxis; Lockdown; Pandemic; Emergency medicine; Otolaryngology

Core Tip: The coronavirus disease 2019 (COVID-19) pandemic has had a significant impact on healthcare systems worldwide. In an effort to reduce the spread of the virus, many countries have implemented lockdown measures that restrict movement and social interaction. While these measures have been effective in reducing the transmission of COVID-19, they have also had unintended consequences on healthcare delivery and hospital admissions. Several studies have reported a decrease in hospital admissions during the COVID-19 lockdown period. We showed that the pandemic-induced lockdown resulted in a direct decrease in hospitalizations especially for young patients with epistaxis and an immediate increase in hospitalizations with its end. This might be caused by fear of exposure to COVID-19, unintended consequences of public health recommendations to minimize non-urgent healthcare, or stay at home orders. These findings match with results from previous studies. Conversely, these measures did not lead to any change in older patients, which suggests that at least in this age group, the symptoms of epistaxis should not be underestimated, even with regard to a possible exposure to the coronavirus.

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INTRODUCTION

Epistaxis

Epistaxis is a common emergency in the field of ear, nose, and throat, with varying degrees of severity. Roughly 60% of the population are expected to encounter it at least once during their lifetime[1]. The causes of epistaxis can range from idiopathic to cancerous lesions, with only about 6% of cases requiring medical or surgical attention[2,3]. Idiopathic or spontaneous factors are the primary cause of epistaxis, constituting the cause in at least 70% of cases. These occurrences are frequently associated with conditions such as hypertension, atherosclerotic disease, smoking, or the use of oral anticoagulation medications[4]. Age has also been shown to be a factor in the incidence of epistaxis, with the risk increasing as individuals get older[5].

The Little area, located along the anterior septum, is the origin of approximately 90% of epistaxis cases[6]. Blood supply to this region is provided by the Kiesselbach plexus, composed of second-order branches from both the external and internal carotid arteries[7]. Hemorrhage in this area is commonly referred to as anterior epistaxis and can often be managed using conservative approaches like nostril pressure, topical vasoconstrictors or hemostatic agents, cryotherapy, electrocautery, or anterior nasal packing[8].

In contrast, posterior epistaxis, accounting for only 5% to 10% of cases, originates from the more posterior regions of the nasal cavity[9]. Managing posterior-based nasal bleeding with anterior and posterior nasal packs is less successful, with success rates ranging from 48% to 83%[10-12]. In some cases, nasal hemorrhage persists despite packing or recurs upon pack removal. Posterior epistaxis can be effectively treated through endoscopic or open surgical approaches involving direct ligation or cauterization of the affected artery, with a reported success rate of 97%[13,14].

Endovascular embolization is another viable option to halt nasal bleeding, with reported success rates ranging from 71% to 100%. However, this approach may entail minor complications, such as septal perforation, sinusitis, headache, facial or jaw pain, and facial edema[2,13]. More serious complications, including stroke, facial nerve paresis, soft-tissue necrosis, and even blindness, can occur as a result of inadvertent embolization[14-16].

Recent research indicates that epistaxis may serve as an initial symptom of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. This virus can affect the nasal epithelium, potentially increasing the risk of developing epistaxis[17,18].

Hospital admissions during coronavirus disease 2019 pandemic

The global healthcare systems have been profoundly affected by the coronavirus disease 2019 (COVID-19) pandemic. To mitigate the virus's spread, numerous nations have enacted lockdown measures, curtailing mobility and social interactions. While these steps have effectively curbed COVID-19 transmission, they have also led to unintended repercussions for healthcare provision and hospital admissions. Several studies have reported a decrease in hospital admissions during the COVID-19 lockdown period[19,20]. This decrease has been attributed to several factors, including the cancellation of elective surgeries, reduced emergency department visits, and a decrease in the incidence of some

illnesses due to lifestyle changes[21-23].

While the decrease in hospital admissions during the COVID-19 lockdown period may seem like a positive outcome, it has raised concerns about delayed or missed diagnoses and treatments for non-COVID-19-related illnesses. This could potentially result in long-term health consequences for patients[24].

Aim of the study

The aim of this study was to examine whether there was a decline in hospital admissions for epistaxis in Germany during the COVID-19 lockdown. Given the potential impact of the COVID-19 pandemic on healthcare delivery and the need for timely treatment of epistaxis to prevent complications, it is important to investigate whether there has been a decline in hospital admissions for this condition during the lockdown period. By examining hospital admission rates for epistaxis during the COVID-19 lockdown period and comparing them to pre-lockdown rates, we can determine whether there was a significant decrease in hospital admissions for this condition.

Motivated by the pressing need to understand how the COVID-19 pandemic might have influenced the healthcare-seeking behavior of individuals, the authors of this study meticulously collected and analyzed nationwide data. The authors recognized that fear of potential coronavirus exposure could have deterred patients from seeking necessary medical attention, even for severe conditions such as epistaxis. This study not only highlights the authors' commitment to addressing a critical gap in medical research during a global crisis but also showcases their dedication to ensuring comprehensive and accurate data collection.

This approach is positioned in the temporal course prior to the outcome following the inpatient admission and can therefore be considered as a complement to other recently published studies that have dealt with patient outcomes after coronavirus infection. In this domain, models using machine learning approaches have been introduced to, for example, estimate the mortality risk of patients with pre-existing diabetes based on various input parameters or to perform early classification of COVID-19 patients through deep learning techniques[25,26].

The findings of this study will provide valuable insights into the impact of the COVID-19 pandemic on the management of epistaxis and may inform the development of strategies to ensure timely access to care for patients with this condition. Furthermore, the authors' contributions extend beyond the immediate scope of the research by shedding light on the broader challenges of maintaining regular healthcare services during times of crisis. Through this study, the authors aim to support healthcare systems in adapting to unforeseen disruptions and guaranteeing that patients receive the essential care that they require, irrespective of external circumstances.

MATERIALS AND METHODS

Patient population

This epidemiological retrospective observational study was performed by using quasi-anonymous open-access population data from the Institute for the Hospital Remuneration System in Germany[27]. This database was used to access the weekly number of hospital admissions for patients with epistaxis (ICD R04.0), with the ICD-10 diagnosis not differentiating between the cause and type of epistaxis (for example anterior or posterior). All patients of all ages and gender who were admitted within Germany with the diagnosis of epistaxis during the specified period were included.

The study period extended from February 1st to June 8th of the years 2019 to 2022. This period was divided into 6-wk blocks, with the government-initiated coronavirus lockdown from March 15, 2020 to April 26, 2020 forming a separate group (lockdown). This resulted in a uniform study period of 18 wk per year to rule out the seasonal incidence of epistaxis[28-30]. Cases before March 15, 2020 (2020 wk 12) were classified into the pre-lockdown group, and cases after April 26, 2020 (2020 wk 18) were classified into the post-lockdown group. These respective time periods of inpatient admission were compared to investigate whether there have been time-dependent changes in patient numbers as the outcome of interest.

Statistical analysis

The average number of hospital admissions for epistaxis per week was calculated according to time period, gender, and age. Depending on age, patients were grouped into 0-39 years, 40-79 years, or 80 years and older. To evaluate the data, weekly case numbers were presented using an interrupted time series (ITS) analysis. This is a quasi-experimental design suitable for measuring the population-level impact of healthcare interventions[31,32]. The ITS was presented in tabular form. An AutoRegressive Integrated Moving Average (ARIMA) forecast model without seasonal effects was used as a counterfactual in order to provide a more accurate estimate of what would have happened in the absence of the intervention than linear regression[33]. The counterfactual was calculated from the pre-lockdown group as well as from the lockdown group. The results are presented as a percentage deviation from the predicted value, with the respective time boundaries of the Interrupted Time Series corresponding to the start and end of the COVID-19 lockdown.

Values are reported as absolute numbers (*n*), mean, standard deviation (SD), and 95% confidence interval (95%CI). Due to the metric scaling, analysis of variance was performed to test for mean differences. A *t*-test for independent samples was used as a *post-hoc* test with Bonferroni correction to avoid alpha error accumulation[34].

A P value of $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS, version 29.0.0.0 (IBM Corporation, Armonk, NY, United States) and Microsoft Excel, version 2019 (Microsoft Corporation, Redmond, WA, United States).

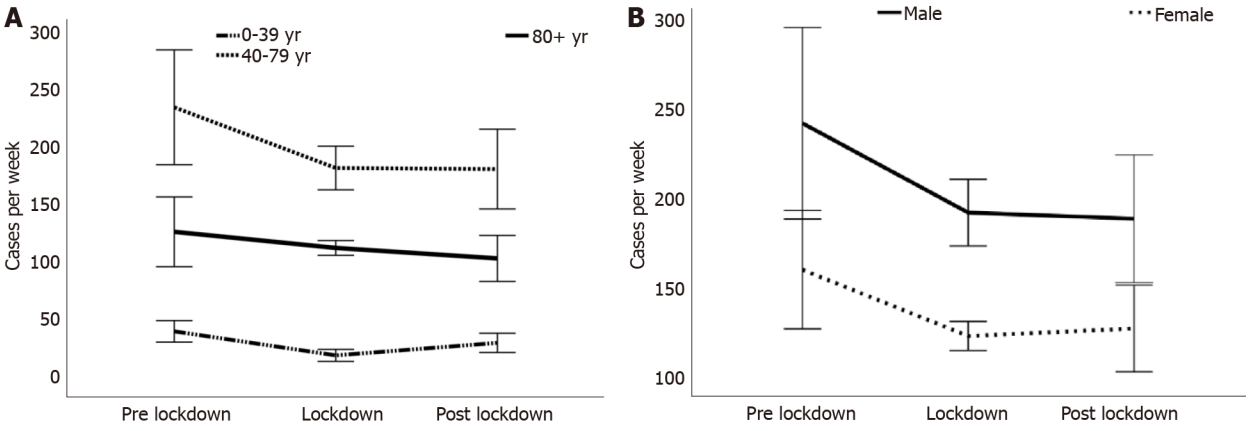

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Figure 1 Cases per week grouped by time period presented as the mean and standard deviation. A: Age-dependent; B: Male and female.

The recommendations for good practice in secondary data analysis established by the German Working Group on the Collection and Use of Secondary Data were taken into full account. According to the Professional Code of Conduct of the Regional Medical Association, the study did not require ethical approval since it did not involve the use of identifiable patient data.

RESULTS

Overall, 26183 inpatient cases were included in the analysis with a male-to-female ratio of 1.51. The largest age group was the 40-79-year-old group (n = 15145; 58%), followed by those over 80 years of age (n = 8526; 33%). The smallest group was made up of patients aged up to 39 years old (n = 2512; 9%).

It was found that regardless of age and gender, most weekly admissions occurred during the 2019 study period. In contrast, 2020 marked the lowest weekly case numbers in the group up to 39 years compared to the other study periods (P < 0.001). In the age group of 40-79 years, the highest case numbers were also observed in 2019 (P < 0.001), with a statistically constant value in the following years. Only in the group of those over 80 years old, a statistically constant weekly case number was observed for each study year.

Fragmented into 12 equally sized time periods (Table 1), the weekly caseloads for epistaxis were significantly different compared to the restriction period (P < 0.001).

Cases had decreased by 22% from 401.5 (SD 84.2) in the pre-lockdown period to 314.2 (SD 20.6) during the lockdown period, irrespective of age and gender (P < 0.05). Subsequently, there was a marginal, non-significant increase of 0.3% to 315.0 (SD 56.2). Notably, Figure 1 illustrates that the majority of patients were middle-aged and male.

When considering gender, a notable decrease of 29% (P < 0.01) was observed during the lockdown period for female patients, while male patients experienced a decrease of 21%. In the post-lockdown period, cases increased by 11% among females, whereas male patients showed a slight additional decrease of 2% (Table 2, Figure 1B).

The male-to-female ratio remained approximately constant during the individual study periods. During the lockdown period, there was an increase of 3.4%, and during the subsequent post-lockdown period, there was a decrease of 4.8% (not significant; Table 2).

The lockdown period led to a significant decrease in epistaxis cases, especially among young people (0-39 years of age), with a reduction of 51% (P < 0.001). After the end of the restriction period, there was a clear and continuing statistically significant, increase in patient numbers by 50% (P < 0.01). In the age group of 40-79 years, there was also a significant decrease in the number of cases by 22% (P < 0.01) with the start of the lockdown period, which remained constant thereafter. Only in the group of the oldest patients (80+ years of age), a statistically constant weekly number of cases was observed. This number decreased by 11% at the beginning of the lockdown period and then by an additional 8% (Table 2, Figure 1A).

These observations are also consistent with the results of the ITS. Compared to the actual case numbers, the counterfactual for the lockdown resulted in a significantly higher number, which was also most pronounced in the youngest age group. With the end of the lockdown period, as described above, there was again an increase in patient numbers. Based on the estimated value from the counterfactual calculated from the lockdown period, there were also significant deviations from the actual case numbers during the post-lockdown period. Here too, the effect was strongest in the youngest age group (Table 3, Figure 2).

DISCUSSION

This study aimed to investigate the influence of the nationwide Corona lockdown on hospital admissions pertaining to

Table 1 Total cases of patients with epistaxis in Germany

Category		All	Gender		Age group (yr)		
			Female	Male	0-39	40-79	80+
February 1, 2019- March 14, 2019	*N*	2620	1075	1545	249	1580	791
	Cases per week		179.3 (36.8)	257.5 (70.0)	41.5 (8.2)	263.3 (62.9)	131.8 (37.8)
March 15, 2019-April 26, 2019	*N*	3082	1201	1881	301	1768	1013
	Cases per week		171.4 (16.3)	268.7 (20.6)	43.0 (8.6)	252.6 (17.6)	144.7 (8.1)
April 27, 2019-June 8, 2019	*N*	2210	920	1290	233	1297	680
	Cases per week		153.3 (14.6)	215.0 (24.5)	38.8 (8.7)	216.2 (24.4)	113.3 (13.9)
February 1, 2020-March 14, 2020	*N*	2526	955	1571	269	1449	808
	Cases per week		136.4 (42.3)	224.4 (69.7)	38.4 (12.4)	207.0 (61.2)	115.4 (40.6)
March 15, 2020-April 26, 2020 (lockdown)	*N*	1885	735	1150	118	1091	676
	Cases per week		122.5 (8.1)	191.7 (18.7)	19.7 (5.2)	181.8 (18.8)	112.7 (6.4)
April 27, 2020-June 8, 2020	*N*	1790	718	1072	123	2090	587
	Cases per week		119.7 (20.8)	178.7 (15.4)	20.5 (6.0)	180.0 (19.7)	97.8 (12.2)
February 1, 2021-March 14, 2021	*N*	2042	853	1189	166	1185	691
	Cases per week		142.2 (9.2)	198.2 (24.2)	27.7 (6.4)	197.5 (13.9)	115.2 (8.5)
March 15, 2021-April 26, 2021	*N*	2127	889	1238	218	1226	683
	Cases per week		148.2 (12.2)	206.3 (7.6)	36.3 (8.6)	204.3 (11.6)	113.8 (14.2)
April 27, 2021-June 8, 2021	*N*	1998	808	1190	217	1086	695
	Cases per week		115.4 (32.5)	170.0 (54.3)	31.0 (8.6)	155.1 (48.8)	99.3 (30.4)
February 1, 2022-March 14, 2022	*N*	2046	812	1234	184	1193	669
	Cases per week		135.3 (19.5)	205.7 (35.0)	30.7 (5.7)	198.8 (34.4)	111.5 (15.5)
March 15, 2022-April 26, 2022	*N*	1953	753	1200	213	1096	644
	Cases per week		125.5 (11.7)	200.0 (22.3)	35.5 (7.5)	182.7 (15.3)	107.3 (6.0)
April 27, 2022-June 8, 2022	*N*	1904	743	1161	221	1094	589
	Cases per week		106.2 (27.0)	165.9 (46.3)	31.6 (10.1)	156.3 (42.0)	84.1 (22.9)

Cases per week: Mean, standard deviation (SD).

epistaxis. Therefore, in addition to the 6-wk lockdown, hospital admissions from 2019 to 2022 were also investigated nationwide. This was done over a total period of 18 wk per year to compensate for seasonal differences in the incidence of epistaxis. From our point of view, epistaxis is a clear and serious symptomatology, while other diseases with ambiguous symptoms such as stroke could be underestimated in terms of possible exposure to the coronavirus[35-38]. The decrease in patients with various diagnoses during the Corona lockdown has already been reported in numerous studies[39-44].

The majority of patients in this study were between 40 and 79 years old and male. This is consistent with previous publications that have associated older age and male gender with a higher incidence of epistaxis[4,45-47].

It was shown that the introduction of the COVID-19 lockdown was associated with a decrease in nationwide hospital admissions for epistaxis. This was not dependent on gender. However, a strongly significant decrease was observed in the youngest age group of 0-39 years. In the oldest age group of 79 years and older, there was no significant decrease in the number of patients. With the end of the COVID-19 lockdown 6 wk later, the numbers significantly increased again in the age group up to 39 years, while no significant changes were observed in patients aged 40 years and older.

Using ITS analysis, a clear association could be demonstrated both with the start and the end of the COVID-19 lockdown. The difference to the counterfactual was most pronounced in the youngest patient group, in line with the significance described above. It can be inferred from this that both the start and end of the COVID-19 lockdown had a direct influence on the number of hospital admissions.

The significant decrease in patients with epistaxis in the youngest age group could be due, on the one hand, to a greater fear of exposure to the coronavirus in this group. Another reason could be the less severe symptomatology on average in younger patients[48-50]. This is supported by the subsequent significant increase in patient numbers in this age group after the lockdown, which was also shown in the ITS and was associated with it.

Table 2 Total cases of patients in regard to the coronavirus disease 2019 lockdown in Germany

Category	Pre-lockdown			Lockdown		Post-lockdown		
	Cases per week	95%CI	*P* value	Cases per week	95%CI	Cases per week	95%CI	*P* value
Total	401.5 (84.2)	367.5-435.5	0.05	314.2 (20.6)	292.5-335.8	315.0 (56.2)	297.9-332.1	NS
Female	159.7 (33.1)	146.3-173.0	0.01	112.5 (8.1)	113.9-131.0	126.7 (24.3)	119.3-134.1	NS
Male	241.8 (53.7)	220.1-263.5	NS	191.7 (18.7)	172.1-211.2	188.3 (35.8)	117.4-199.2	[a]
MFR	1.52 (0.18)	1.45-1.59	NS	1.57 (0.18)	1.38-1.76	1.50 (0.22)	1.43-1.56	NS
0-39	40.4 (9.3)	36.7-44.2	[a]	19.7 (5.2)	14.2-25.1	30.5 (8.3)	27.7-33.0	0.01
40-79	234.4 (49.7)	214.3-254.5	0.01	181.8 (18.8)	162.0-201.6	180.9 (34.5)	170.4-191.4	NS
80+	126.6 (30.2)	114.4-138.8	NS	112.7 (6.4)	105.9-119.4	103.6 (19.9)	97.5-109.6	NS

[a]$P < 0.001$.

Cases per week: Mean, standard deviation (SD); Reference period: Lockdown group, MFR: Male-to-female ratio; NS: Not significant.

Table 3 Interrupted time series analysis

Category	Lockdown			Post-lockdown		
	Cases per week	95%CI	$\Delta_{pred-rep}$ (%)[a]	Cases per week	95%CI	$\Delta_{pred-rep}$ (%)[b]
Total						
Reported	314.2 (20.6)	292.5-335.8		315.0 (56.2)	297.9-332.1	
Predicted	364.7	152.8-576.9	+13.9	253.3	214.9-291.7	-24.5
Female						
Reported	112.5 (8.1)	113.9-131.0		126.7 (24.3)	119.3-134.1	
Predicted	126.3	67.5-185.1	+12.3	123.0	102.0-143.0	-3.0
Male						
Reported	191.7 (18.7)	172.1-211.2		188.3 (35.8)	117.4-199.2	
Predicted	242.0	131.0-352.0	+26.3	139.1	100.0-178.1	-35.4
0-39						
Reported	19.7 (5.2)	14.2-25.1		30.5 (8.3)	27.7-33.0	
Predicted	41.3	23.9-59.3	+52.3	20.0	6.0-33.0	-52.5
40-79						
Reported	181.8 (18.8)	162.0-201.6		180.9 (34.5)	170.4-191.4	
Predicted	212.1	92.3-332.5	+14.3	127.4	89.4-165.1	-42.0
80+						
Reported	112.7 (6.4)	105.9-119.4		103.6 (19.9)	97.5-109.6	
Predicted	127.0	64.0-189.0	+11.3	113.0	86.0-129.0	+8.3

[a]Based on the counterfactual calculated from the pre-lockdown period.

[b]Based on the counterfactual calculated from the lockdown period. Cases per week: Mean, standard deviation (SD).

Limitations

First, the hospital data analyzed represent a comprehensive inquiry into inpatient admissions for epistaxis in Germany. However, no data on outpatient visits to emergency departments are available in the dataset. Second, adjustments for comorbidities, socio-economic factors, or place of residence were not feasible due to the absence of these patient-level data. Third, while coding issues might have arisen for different diagnoses, we determined that these were unlikely to be a significant confounding factor, given the size of the population.

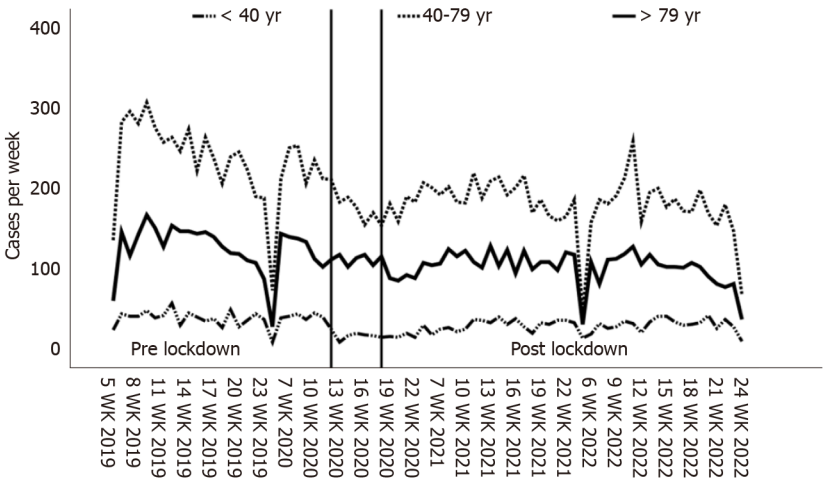

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Figure 2 Hospital admissions by age. Vertical lines indicate the beginning and end of the coronavirus disease 2019 lockdown.

CONCLUSION

Our study demonstrated that, based on actual case numbers and simulated calculations using ARIMA forecast along with ITS analysis, the pandemic-induced lockdown led to a direct reduction in hospitalizations, particularly among young patients with epistaxis, and an immediate surge in hospitalizations upon its termination. This could be attributed to concerns about COVID-19 exposure, unanticipated outcomes of public health advice to reduce non-urgent healthcare visits, or adherence to stay-at-home orders, aligning with findings from prior research[39-44].

Conversely, these measures did not lead to any change in older patients, which implies that at least in this age group, the symptoms of epistaxis should not be underestimated, even with regard to a possible exposure to the coronavirus.

These findings highlight how a nationwide intervention, such as the COVID-19 lockdown in this instance, can directly influence hospital admissions within specific segments of the population.

ARTICLE HIGHLIGHTS

Research background

The COVID-19 pandemic has had a profound impact on global healthcare systems, leading many nations to enforce lockdowns that restrict movement and social interactions in an effort to curb virus transmission. Although effective against COVID-19, these measures have inadvertently affected healthcare delivery and hospital admissions. Numerous studies have noted a decline in hospital admissions during the lockdown, attributed to factors such as postponed elective surgeries, decreased Emergency Room visits, and lifestyle-related illness reductions. Despite the apparent benefits of reduced admissions, apprehensions arise over potential long-term health implications due to delayed diagnoses and treatments for non-COVID-19 conditions.

Research motivation

Concerns have arisen due to reports of reduced hospital admissions during the COVID-19 lockdown, suggesting potential delays or omissions in diagnosing and treating non-COVID-19-related illnesses. The decrease in hospital visits during this period has sparked worries about the impact on timely medical interventions. The lockdown's effect on hospital admissions has prompted discussions regarding possible disruptions to the identification and management of non-COVID-19-related medical conditions.

Research objectives

To examine how the COVID-19 pandemic-induced lockdown and its conclusion affected hospital admissions among patients with epistaxis in Germany.

Research methods

Utilizing quasi-anonymous open-access data from Germany's Institute for the Hospital Remuneration System, this retrospective observational study analyzed hospital admissions for epistaxis, considering patient age and gender. The study covered February 1 to June 8 from 2019 to 2022, segmented into six-week periods, with a distinct lockdown group from March 15 to April 26, 2020. Statistical analysis employed Interrupted Time Series and AutoRegressive Integrated Moving Average models, presenting deviations from predicted values. Ethical approval was not necessary due to the absence of identifiable patient data, aligned with ethical guidelines.

Research results

In total, 26183 inpatient cases were analyzed, with a male-to-female ratio of 1.51. The 40-79-year-old group (n = 15145; 58%) had the most cases, followed by those over 80 years (n = 8526; 33%), and the smallest group was aged up to 39 years (n = 2512; 9%). Weekly admissions peaked in 2019 across age and gender groups, while the 2020 lockdown period saw the lowest weekly case numbers for those under 39 years ($P < 0.001$). In the age group of 40-79 years, 2019 had the highest case numbers ($P < 0.001$), remaining constant in subsequent years; those over 80 years showed consistent weekly case numbers. Fragmented into 12 time periods, weekly epistaxis cases significantly differed during the restriction ($P < 0.001$). There was a 22% decrease in cases during the lockdown ($P < 0.05$), followed by a slight increase of 0.3%, particularly affecting middle-aged males. Lockdown caused a notable 29% decrease in female cases ($P < 0.01$) and 21% in males, with an 11% post-lockdown increase in females and 2% decrease in males. The male-to-female ratio remained stable. Lockdown led to a significant 51% decrease in young patients (0-39 years, $P < 0.001$) and a subsequent 50% increase ($P < 0.01$), while the 40-79 age group had a 22% decrease ($P < 0.01$) and the oldest group remained constant. These trends were consistent with ITS results, showcasing the impact on different age groups.

Research conclusions

Our study demonstrated that the pandemic-induced lockdown led to a direct decrease in hospitalizations, particularly among young patients with epistaxis. This was followed by a rapid increase after the lockdown was ended. Possible factors contributing to this trend include COVID-19-related fears, unintended consequences of healthcare recommendations, or stay-at-home orders – findings that align with previous research. Notably, older patients were not similarly affected, highlighting the importance of addressing epistaxis symptoms, even in the context of potential COVID-19 exposure. These results emphasize the significant impact of a nationwide intervention like the COVID-19 lockdown on hospital admissions in specific demographic groups.

Research perspectives

Further and more comprehensive research based on larger datasets is necessary to obtain insights into lockdown-induced changes in hospital admissions for other diagnoses as well.

FOOTNOTES

Author contributions: Hoenle A designed the study, collected and analyzed the data, and wrote and edited the manuscript; Wagner M and Lorenz S collected data for the manuscript; Steinhart H assisted in study design and edited the manuscript.

Institutional review board statement: The study was reviewed and approved by the Ethikkommission Marienhospital Stuttgart Institutional Review Board.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous secondary clinical data which is publicly available.

Conflict-of-interest statement: All the authors report no conflicts of interest for this article.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at adrian.hoenle@gmx.net. Participants gave informed consent for data sharing.

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Retrospective Study

Effect of vaccination status on CORADS and computed tomography severity score in hospitalized COVID-19 patients: A retrospective study

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Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic is continuing. The disease most commonly affects the lungs. Since the beginning of the pandemic thorax computed tomography (CT) has been an indispensable imaging method for diagnosis and follow-up. The disease is tried to be controlled with vaccines. Vaccination reduces the possibility of a severe course of the disease.

AIM

The aim of this study is to investigate whether the vaccination status of patients hospitalized due to COVID-19 has an effect on the CT severity score (CT-SS) and CORADS score obtained during hospitalization.

METHODS

The files of patients hospitalized between April 1, 2021 and April 1, 2022 due to COVID-19 were retrospectively reviewed. A total of 224 patients who were older than 18 years of age, whose vaccination status was accessible, whose severe acute respiratory syndrome coronavirus 2 polymerase chain reaction result was positive, and who had a Thorax CT scan during hospitalization were included in the study.

RESULTS
Among the patients included in the study, 52.2% were female and the mean age was 61.85 years. The patients applied to the hospital on the average 7th day of their complaints. While 63 patients were unvaccinated (Group 1), 20 were vaccinated with a single dose of CoronaVac (Group 2), 24 with a single dose of BioNTech (Group 3), 38 with 2 doses of CoronaVac (Group 4), 40 with 2 doses of BioNTech (Group 5), and 39 with 3 doses of vaccine (2 doses of CoronaVac followed by a single dose of BioNTech, Group 6). CT-SS ranged from 5 to 23, with a mean of 12.17.

RESULTS
CT-SS mean of the groups were determined as 14.17, 13.35, 11.58, 10.87, 11.28, 10.85, respectively. Accordingly, as a result of the comparisons between the groups, the CT-SS levels of the unvaccinated patients found to be significantly higher than the other groups. As the vaccination rates increased, the rate of typical COVID-19 findings on CT was found to be significantly lower.

CONCLUSION
Increased vaccination rates in COVID-19 patients reduce the probability of typical COVID-19 symptoms in the lungs. It also reduces the risk of severe disease and decreases CT Severity Scores. This may lead to a loss of importance of Thorax CT in the diagnosis of COVID-19 pneumonia as the end of the pandemic approaches.

Key Words: COVID-19; CORADS; Computed tomography severity score; Thorax computed tomography; SARS-CoV-2; Vaccination

Core Tip: This is a retrospective study to evaluate the effect of vaccination status on CORADS and computed tomography (CT) severity score in hospitalized coronavirus disease 2019 (COVID-19) patients. Accordingly, as a result of the comparisons between the groups, the CT severity score levels of the unvaccinated patients were significantly higher than the other groups. As the vaccination rates increased, the rate of typical COVID-19 findings on CT was found to be significantly lower.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic still continues, although the number of cases has decreased[1]. The disease most commonly affects the lungs, and since the beginning of the pandemic, thorax computed tomography (CT) has been an indispensable imaging method for diagnosis and follow-up[2]. The disease is tried to be controlled with the introduction of vaccines and the increase in vaccination rates. Vaccination reduces the probability of severe course of the disease and the CORADS score, which is an indicator of lung involvement[3-6].

CoronaVac vaccine (Sinovac Life Sciences, Beijing, China), which is an inactive vaccine, has been started to use from the elderly population and healthcare workers in our country as of January 2021[7]. As of April 2021, the BNT-162b2 (BioNTech/Pfizer) vaccine, which is an mRNA vaccine, has begun to be used[8]. Reminder doses are also applied as the pandemic continues.

The aim of this study was to investigate whether the vaccination status of patients hospitalized due to COVID-19 had any effect on the CT severity score (CT-SS) and CORADS scoring assessed during hospitalization.

MATERIALS AND METHODS

Study design

This study was planned as a retrospective, cross-sectional study and was conducted with the approval of Erzincan Binali Yildirim University Clinical Research Ethics Committee (Date: 27.10.2022 / Decision No: 04/16).

The files of patients admitted to the COVID-19 inpatient clinic between April 1, 2021 and April 1, 2022 were retrospectively reviewed.

Inclusion criteria: (1) Being over 18 years of age; (2) Having a positive polymerase chain reaction (PCR) test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and receiving inpatient treatment in one of the COVID-19 inpatient clinics; (3) Having a Thorax CT scan on the seventh day of complaints during hospitalization; (4) Having access to COVID-19 vaccination information during hospitalization; (5) Among the patients for whom vaccination information was available, it was defined as 14 days or more from the last vaccine dose for patients who received 2 or more vaccines, and 14 days or more from the date of vaccination for patients who received a single dose of vaccine.

Accordingly, 224 patients out of a total of 2000 patients were included in the study. In our study, patients who met the inclusion criteria were divided into 6 groups: Unvaccinated (Group 1), single dose CoronaVac vaccine (Group 2), single dose BioNTech vaccine (Group 3), 2 doses CoronaVac vaccine (Group 4), 2 doses BioNTech vaccine (Group 5) and 2 doses CoronaVac + 1 dose BioNTech vaccine (Group 6). Patients were evaluated according to the severity of involvement in thorax CT and whether there is typical involvement or not. At the same time, it was investigated whether the acute phase parameters of the patients were affected by the vaccination status.

Laboratory tests

The SARS-CoV-2 PCR test was studied with DS CORONEX COVID-19 Multiplex Real Time qPCR (DS Nano and Biotechnology). The hemogram test of the patients was performed using the Sysmex XN-1000 Hematology System (Sysmex Corporation, Kobe, Japan); and biochemical tests were studied with AU 5800 (Beckman Coulter, California, United States) and coagulation tests with Ceveron® alpha (Diapharma Group, Ohio, United States).

Thorax CT

All patients had a CT scan without intravenous contrast material on the day they were admitted to the hospital (Siemens SOMATOM Sensation 16, Forchheim, Germany). All patients were scanned in the supine position using an adult CT protocol; reconstruction images of the 1.5 mm lung window were obtained using tube voltage = 130kV, effective mAs = 70, slice thickness = 5 mm, collimation = 16 × 1.2, pitch = 0.8. In children, reconstruction images of the lung window of 1.5 mm were obtained with protocol tube voltage = 110kV, effective mAs = 60, slice thickness = 8 mm, collimation = 16 × 1.2, pitch = 0.8 (14 years and younger). CT-SS and CORADS score were evaluated by 3 independent experienced radiologists.

CT severity score

The result of the CT examination on admission to hospital was used to define the CT severity value. CT severity of the patients was defined for each lung segment, and the sum of the severity value of each lobe was used to arrive at a final severity score. CT severity scores were calculated using the method described by Pan *et al*[9] (Table 1). The mean value of two measurements was recorded as the final value.

CORADS score

The CORADS system was used to determine the probability of disease based on the severity of lung involvement in CT examinations performed during hospitalization. CORADS 0 indicates that the examination is not of sufficient quality to be evaluated. CORADS 1 indicates either a normal thorax scan or the presence of a non-infectious disease. CORADS 2 identifies findings that are unusual for COVID-19 but are common in other infectious diseases such as bronchitis and bronchopneumonia. CORADS 3 describes findings that could be related to COVID 19 as well as other viral pneumonias or non-infectious diseases. CORADS 4 identifies findings that are typical for COVID 19 but may also be relevant for other viral pneumonias. Its difference from CORADS 5 is its atypical involvement. CORADS 5 category implies a very high level of suspicion for pulmonary involvement by COVID-19. Patients who are positive according to the reverse transcription-PCR test result are defined as CORADS 6[10].

Statistical analysis

NCSS (Number Cruncher Statistical System) 2020 (Kaysville, Utah, United States) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used while evaluating the study data. The suitability of the quantitative data to the normal distribution was tested by the Shapiro-Wilk test and graphical analyses.

Oneway ANOVA test was used for the comparison of normally distributed quantitative variables between groups, and Bonferroni test was used for post hoc evaluations. The Kruskal-Wallis test was used for the comparison of quantitative variables that did not show normal distribution, and the Dunn-Bonferroni test was used for post hoc evaluations.

Pearson Chi-Square test was used to compare qualitative data. Statistical significance was accepted as $P < 0.05$.

RESULTS

The study was conducted in a university hospital from the eastern of Turkey between April 1, 2021 and April 1, 2022, with a total of 224 cases, of which 52.2% (n = 117) were female and 47.8% (n = 107) were male. The ages of the cases ranged from 22 to 97, and the mean age was 61.85±15.36 years.

A statistically significant difference was found between the age distributions of the groups ($P < 0.001$); when the significances are analyzed; the mean age of those who were not vaccinated was found to be significantly lower than the single dose CoronaVac, two doses CoronaVac, two doses BioNTech and three doses vaccine groups ($P = 0.021$; $P = 0.001$; $P = 0.011$; $P = 0.001$; $P < 0.05$, respectively).

Table 1 Computed tomography severity scoring

CT severity score	Extent of lesions for each lung lobe, %
0	0
1	< 5
2	5-25
3	26-50
4	51-75
5	> 75

CT: Computed tomography.

The mean age of those who received a single dose of CoronaVac was higher than those who received a single dose of BioNTech ($P = 0.049$), and significantly lower than the two-dose CoronaVac and three-dose vaccine groups ($P = 0.002$; $P = 0.001$; $P < 0.01$, respectively).

The mean age of those who received a single dose of BioNTech was also found to be significantly lower than the two-dose CoronaVac and three-dose vaccine groups ($P = 0.001$; $P = 0.001$; $P < 0.01$, respectively).

The ages of those who received two doses of CoronaVac were also significantly higher than those who received two doses of BioNTech ($P = 0.001$; $P < 0.01$). The age of those who received two doses of BioNTech was also significantly lower than the group that received three doses of vaccine ($P = 0.001$; $P < 0.01$).

No statistically significant difference was found between the distribution of the groups according to gender ($P > 0.05$) (Table 2).

A statistically significant difference was found between the CT severity scores according to the groups ($P < 0.01$). When it was analyzed from which groups the significance originated, no significant difference was found between the severity scores of those who were not vaccinated and those who received a single dose of CoronaVac and a single dose of BioNTech ($P > 0.05$). The severity score was significantly higher than those who received two doses of CoronaVac, two doses of BioNTech, and three doses of vaccination ($P < 0.05$) (Table 3, Figure 1).

A statistically significant difference was found between the distribution of the groups according to the CORADS classification according to their typicality ($P = 0.001$; $P < 0.01$). When the significances were analyzed, the typical incidence rate in those who were not vaccinated and those who received a single dose of CoronaVac vaccine was significantly higher than in all other groups. Typical rates of those who received a single dose of BioNTech and those who received two doses of CoronaVac vaccine were also found to be higher than those who received two doses of BioNTech and three doses of vaccine (Table 4).

A statistically significant difference was found between the C-reactive protein (CRP) levels of the groups ($P < 0.01$). When it was analyzed from which groups the significance originated, CRP levels of those who received a single dose of BioNTech vaccine were found to be statistically significantly lower than those who received two doses of CoronaVac, two doses of BioNTech, and three doses of vaccine ($P = 0.012$; $P = 0.014$; $P = 0.008$; $P < 0.05$). No significant difference was found between the CRP levels of the other groups ($P > 0.05$) (Table 5, Figure 2).

A statistically significant difference was found between the lactate dehydrogenase (LDH) levels of the groups ($P < 0.01$). When it was analyzed from which groups the significance originated, LDH levels of those who received three doses of vaccine, two doses of CoronaVac vaccine, one dose of BioNTech, and those who were not vaccinated were statistically significantly lower ($P = 0.010$; $P = 0.038$; $P = 0.001$; $P < 0.05$). The LDH levels of those who received two doses of BioNTech were significantly lower than those who were not vaccinated ($P = 0.040$; $P < 0.05$). No significant difference was found between the LDH levels of the other groups ($P > 0.05$) (Table 5, Figure 3).

There was no statistically significant difference between the lymphocyte levels of the groups ($P > 0.05$) (Table 5).

A statistically significant difference was found between the procalcitonin levels of the groups ($P < 0.01$). It was analyzed from which groups the significance originated, the procalcitonin levels of those who received a single dose of BioNTech were found to be statistically significantly lower than those who received a single dose of CoronaVac vaccine, two doses of CoronaVac and two doses of BioNTech ($P = 0.001$; $P = 0.001$; $P = 0.042$; $P = 0.037$; $P <0.05$). No significant difference was found between the Procalcitonin levels of the other groups ($P > 0.05$) (Table 5, Figure 4).

There was no statistically significant difference between D-Dimer levels according to the groups ($P > 0.05$) (Table 5).

DISCUSSION

In our country, COVID-19 vaccination was started with the CoronaVac vaccine, which is an inactive vaccine. The vaccine was first started to be administered to healthcare workers and elderly individuals, and vaccination continued from older individuals to younger individuals gradually. While the pandemic was continuing, the BNT-162b2 (BioNTech) vaccine, which is an mRNA vaccine, has also been started to be used in our country[7,8]. This explains why the average age of those who received two doses of CoronaVac and three doses of vaccine in our study was higher.

Table 2 Evaluations of the groups by age and gender

	n	Age	Gender	
		mean ± SD	Male	Female
[1]Unvaccinated	63	50.95 ± 13.54	27 (42.9)	36 (57.1)
[2]Single dose CoronaVac	20	60.75 ± 8.82	9 (45.0)	11 (55.0)
[3]Single dose BioNTech	24	50.1 ± 11.015	7 (29.2)	17 (70.8)
[4]Two doses CoronaVac	38	73.55 ± 9.34	21 (55.3)	17 (44.4)
[5]Two doses BioNTech	40	59.13 ± 13.88	24 (60)	16 (40)
[6]Three doses	39	74.23 ± 10.29	19 (48.7)	20 (51.3)
Total	224	61.09 ± 15.36	107 (47.8)	117 (52.0)
P value		0.001[a,c]	0.204[b]	
Post hoc		1 < 2, 4, 5, 6; 2 > 3; 2 < 4, 6; 3 < 4, 6; 4 > 5; 5 < 6	-	

[a]Oneway Anova test.
[b]Pearson chi-square test.
[c]$P < 0.01$.

Table 3 Evaluation of computed tomography severity scores of the groups

Group	*n*	CT severity scores				*P* value
		Mean	SD	Median	Min-Max	
[1]Unvaccinated	63	14.17	4.56	15	5-23	0.002[b]
[2]Single dose CoronaVac	20	13.35	4.12	13.5	5-21	
[3]Single dose BioNTech	24	11.58	5.29	11.5	5-20	1 > 4; $P = 0.015$[a]
[4]Two doses CoronaVac	38	10.87	4.42	10.5	5-19	1 > 5; $P = 0.021$[a]
[5]Two doses BioNTech	40	11.28	5.25	12.0	5-21	1 > 6; $P = 0.045$[a]
[6]Three doses	39	10.85	5.01	9	5-22	
Total	224	12.17	4.950	12	5-23	

Kruskal Wallis test & post hoc Dunn test.
[a]$P < 0.05$.
[b]$P < 0.01$. CT: Computed tomography.

Thorax CT has been used since the beginning of the pandemic to diagnose COVID-19 suspected patients, especially those with negative SARS-CoV-2 PCR tests. With the CORADS scoring system, patients are evaluated for COVID-19 with Thorax CT. At the same time, Thorax CT is used for disease severity scoring[11,12]. The pandemic is tried to be controlled with the introduction of COVID-19 vaccines. In the study of Haas *et al*[13], it was shown that 2 doses of BioNTech vaccine reduced the risk of severe disease and death. Hu *et al*[14] also found that 2 doses of inactive vaccine were highly effective against the Delta variant and reduced the risk of severe disease. In the study of Sagiraju *et al*., it was shown that full dose vaccination reduces the risk of severe disease and mortality, regardless of the vaccine type[15]. In the study of Mahajan *et al*[16], where they evaluated vaccinated and unvaccinated patients with a positive SARS-CoV-2 PCR test, they found that the CT-SS of vaccinated people was mild-moderate, while unvaccinated individuals were moderate-severe. Again, in the studies conducted by Russo *et al*[4], Ravindra *et al*[5], Fatima *et al*[6], and Yavuz *et al*[17], when the CT SS of vaccinated and unvaccinated patients were compared, it was shown that vaccinated patients had milder pulmonary involvement. In our study, it was shown that unvaccinated individuals had moderate-severe lung involvement, and patients who received two doses of CoronaVac or two doses of BioNTech or three doses of vaccine had moderate lung involvement. Also, it was shown that incomplete vaccination was not effective in reducing CT SS. This finding supports the literature. At the same time, it is an indication that incomplete vaccination will not have any effect on severe disease.

In our study, unlike other studies, the group that had a reminder dose with mRNA vaccine after 2 inactivated vaccines was also evaluated. Accordingly, the CT SS of the patients who received 3 doses of vaccine was found to be lower than those who received 2 doses of vaccine, although it was not statistically significant. This is an evidence of the necessity of reminder doses.

Table 4 Evaluation of groups according to CORADS classification

Group	*n*	CORADS		*P* value
		Typical	Not typical	
[1]Unvaccinated	63	57 (90.5)	6 (9.5)	0.001[a]
[2]Single dose CoronaVac	20	18 (90)	2 (10.3)	
[3]Single dose BioNTech	24	14 (58.3)	10 (41.7)	
[4]Two doses CoronaVac	38	26 (68.4)	12 (31.6)	
[5]Two doses BioNTech	40	15 (37.5)	25 (62.5)	
[6]Three doses	39	17 (43.6)	22 (56.4)	
Total	224	147 (65.6)	77 (34.4)	

[a]$P < 0.01$. Pearson chi-square test.

Table 5 Distribution of Acute Phase Variables by Groups

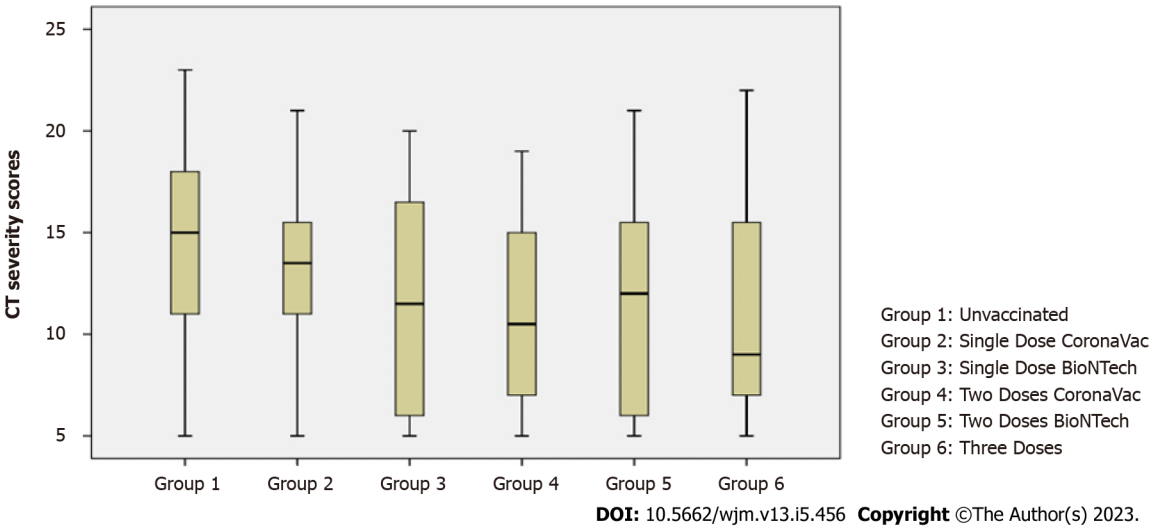
		[1]Unvaccinated	[2]Single dose CoronoVac	[3]Single dose BioNTech	[4]Two doses CoronaVac	[5]Two doses BioNTech	[6]Three doses	*P* value[c]	Post Hoc
CRP	mean ± SD	52.95 ± 40.49	49.75 ± 31.26	34.0 ± 32.3	66.58 ± 52.75	84.35 ± 72.99	81 ± 62.88	0.006[a]	3 < 4, 5, 6
	Median (Min-Max)	46 (3-173)	39.5 (12-124)	22 (2-106)	59 (4-204)	66 (2-341)	54 (2-286)		
LDH	mean ± SD	359.17 ± 164.41	315.2 ± 58.2	331.83 ± 116.61	401.5 ± 305.74	294.05 ± 89.57	276.36 ± 94.2	0.001[a]	6 < 5, 3, 1; 5 < 1
	Median (Min-Max)	359 (164-1213)	311 (195-461)	328 (207-692)	368 (156-1943)	290 (152-640)	273 (131-538)		
Lymphocyte count	mean ± SD	1335.4 ± 512.04	1396.5 ± 470.93	1502.92 ± 724.42	1637.11 ± 1896.86	1628.75 ± 86.02	1477.95 ± 867.82	0.660	
	Median (Min-Max)	1290 (390-2790)	1375 (510-2050)	1340 (420-3450)	1200 (340-12360)	1575 (550-5200)	1320 (260-4640)		
Procalcitonin	mean ± SD	0.52 ± 0.94	0.28 ± 0.29	0.13 ± 0.13	0.77 ± 2.39	0.25 ± 0.2	0.27 ± 0.31	0.001[a]	3 < 1, 2, 4, 5
	Median (Min-Max)	0.3 (0.1-6)	0.3 (0.1-1.3)	0.1 (0.1-0.7)	0.2 (0.1-15)	0.1 (0.1-0.7)	0.1 (0.1-1.2)		
D-DIMER	mean ± SD	1041.08 ± 1559.79	955.95 ± 1156.16	1396.71 ± 1608.97	1561.63 ± 2118.83	1462.2 ± 2934.47	1388.41 ± 1922.45	0.106	
	Median (Min-Max)	596 (24-11805)	549.5 (133-4661)	714.5 (140-6193)	942.5 (251-13077)	622 (166-17459)	820 (194-9440)		

[a]$P < 0.01$.

[c]Kruskal Wallis test & post hoc Dunn test.

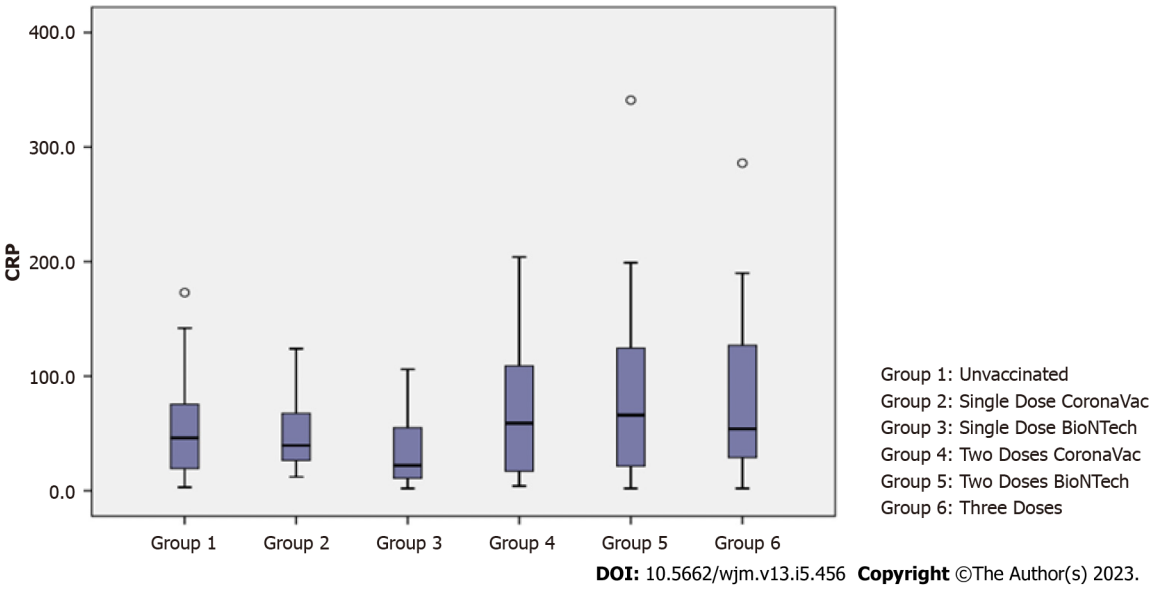
Since the beginning of the pandemic, Thorax CT has had an important place in the diagnosis of suspected COVID-19 patients, especially those with negative PCR tests. Patients with negative SARS-CoV-2 PCR test were not included in our study, and patients were admitted to the hospital on the 7th day of their symptoms on average. When the CORADS scores of the diagnosed cases were evaluated, it was found that the typical appearance was significantly less as the number of vaccinations increased. This suggests that while the pandemic continues, the role of CT in the diagnosis of COVID-19 may remain in the background.

In a study evaluating the effectiveness of the CoronaVac vaccine and hospitalizing 292 patients diagnosed with COVID-19, no significant difference was found between the acute phase reactants of vaccinated and unvaccinated patients[17]. In another study conducted in Poland, no significant difference was found between acute phase reactants [18]. In our study, different acute phase reactants (CRP and procalcitonin) responses were observed depending on the number and type of vaccination, and it was thought that it could not be interpreted clearly since only the acute phase reactants of the patients at the time of admission to the hospital were examined. Otherwise, LDH level is associated with the prognosis of the disease[19]. In our study, the higher LDH levels of unvaccinated or incompletely vaccinated patients showed that vaccination would reduce the probability of a severe course of the disease but, only the measurements of the



DOI: 10.5662/wjm.v13.i5.456

Figure 1 Distribution of computed tomography severity scores of the groups. CT: Computed tomography.



DOI: 10.5662/wjm.v13.i5.456

Figure 2 Distribution of C-reactive protein levels of the groups. CRP: C-reactive protein.

patients at the time of admission to the hospital were evaluated in our study.

Strength of the study

The strength of our study is that, unlike other studies, we evaluated the group that received a booster dose with mRNA vaccine after 2 doses of inactive vaccine.

Limitations of the study

The acute phase reactants in the follow-up of the patients were not evaluated. This is one of the limitations of the study. Again, not evaluating the comorbid diseases and mortality rates of the patients are other limitations of our study.

CONCLUSION

In conclusion, our study showed that being vaccinated, regardless of the type of vaccine, leads to a decrease in CT severity scores. Again, our study suggests that with the effect of vaccination, we may no longer see the CT images that we are accustomed to seeing in COVID-19 patients, and CT may remain in the background in diagnosis.

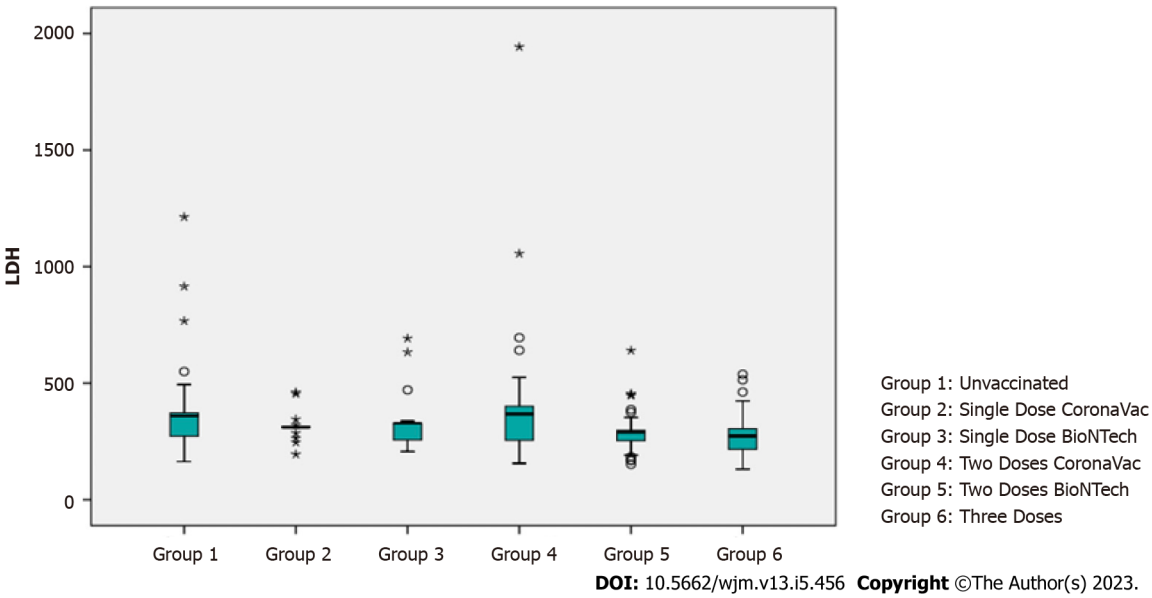


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Figure 3 Distribution of lactate dehydrogenase levels of the groups. LDH: Lactate dehydrogenase.

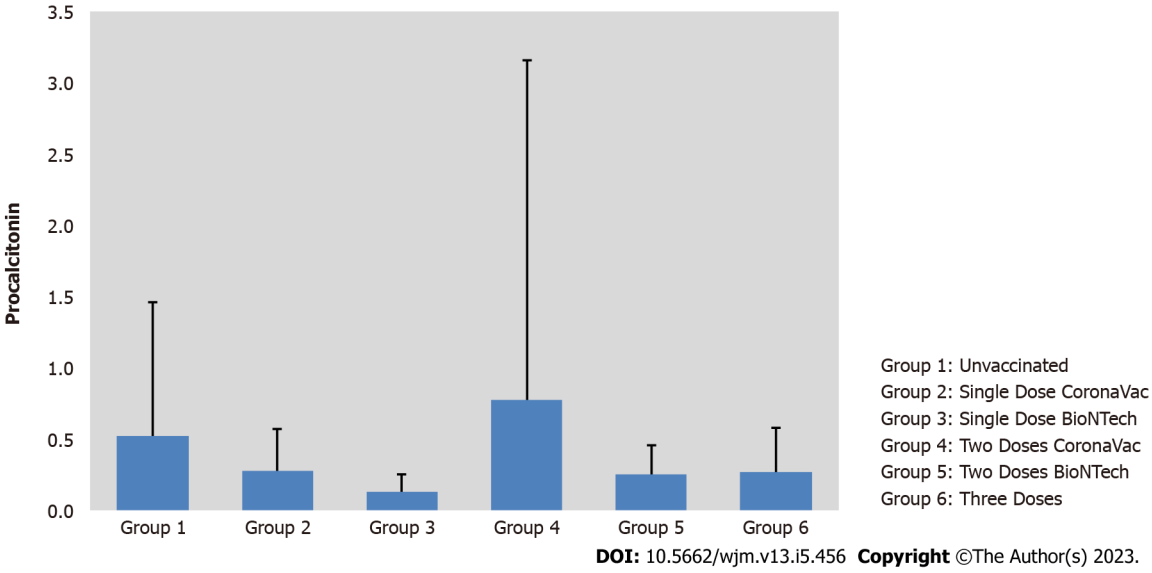

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Figure 4 Distribution of procalcitonin levels of the groups.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease 2019 (COVID-19) pandemic is still continuing. Vaccination has an important place in preventing the disease and vaccination reduces the possibility of a severe course of the disease.

Research motivation

It is important to investigate whether vaccination has any effect on the computed tomography (CT) severity score (CT-SS) and CORADS score of COVID-19 patients.

Research objectives

We aim to investigate whether the vaccination status of inpatient treatment for COVID-19 has any effect on the CT-SS and CORADS score taken during hospitalization.

Research methods

This single-center retrospective study was conducted between April 1, 2021 and April 1, 2022 with a total of 224 patients older than 18 years of age, whose vaccination status was accessible, who had positive severe acute respiratory syndrome

coronavirus 2 polymerase chain reaction results, and who had a thorax CT taken during hospitalization.

Research results

Among the patients included in the study, 52.2% were female and the mean age was 61.85 years. The patients applied to the hospital on the average 7th day of their complaints. While 63 patients were unvaccinated (Group 1), 20 were vaccinated with a single dose of CoronaVac (Group 2), 24 with a single dose of BioNTech (Group 3), 38 with 2 doses of CoronaVac (Group 4), 40 with 2 doses of BioNTech (Group 5), and 39 with 3 doses of vaccine (2 doses of CoronaVac followed by a single dose of BioNTech, Group 6). CT-SS ranged from 5 to 23, with a mean of 12.17. CT-SS mean of the groups were determined as 14.17, 13.35, 11.58, 10.87, 11.28, 10.85, respectively. Accordingly, as a result of the comparisons between the groups, the CT-SS levels of the unvaccinated patients found to be significantly higher than the other groups. As the vaccination rates increased, the rate of typical COVID-19 findings on CT was found to be significantly lower.

Research conclusions

The increase in vaccination rates in COVID-19 patients reduces the CT Severity Score and the CORADS score.

Research perspectives

It is important that COVID-19 vaccinations continue. With the effect of vaccination, the possibility of severe course of the disease will decrease. There will be a need for studies in which more patient data are analyzed and data obtained from patients with different vaccines are evaluated.

FOOTNOTES

Author contributions: Binay UD designed and performed the research and wrote the manuscript; Karavas E designed the research and supervised the report; Karakeçili F designed the research and contributed to the analysis; Barkay O, Aydin S, Senbil DC provided clinical advice; Barkay O, Aydin S, Senbil DC supervised the report. All authors have read and approved the final manuscript.

Institutional review board statement: This study was reviewed and approved by the Erzincan Binali Yildirim University Ethics Committee (Approval No: 04/16).

Informed consent statement: Since it is a retrospective study, patient consent was not obtained.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

Data sharing statement: Data will be shared upon request.

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ORIGINAL ARTICLE

Observational Study

Study on good clinical practices among researchers in a tertiary healthcare institute in India

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Abstract

BACKGROUND

Good clinical practice (GCP) is put in place to protect human participants in clinical trials as well as to ensure the quality of research. Non-adherence to these guidelines can produce research that may not meet the standards set by the scientific community. Therefore, it must be ensured that researchers are well-versed in the GCP. But not much is known about the knowledge and practices of the GCP in the medical colleges of North India.

AIM

To assess the knowledge and practices of researchers about GCP and analyze these with respect to the demographics of participants.

METHODS

This is a cross-sectional study. A self-structured questionnaire about GCP, after expert validations, was circulated among researchers, at a tertiary healthcare institute, All India Institute of Medical Sciences (AIIMS), Rishikesh. A total of 59 individuals, who were selected by universal sampling, participated in the study. All healthcare workers who have been investigators of Institutional Ethics Committee-approved research projects, except residents and faculty, and are still a part of the institute have been included in the study. The study was approved by the Institutional Ethics Committee of AIIMS, Rishikesh. We used descriptive analysis and the Chi-squared test to analyze data. P value < 0.05 was considered significant.

RESULTS

Out of 59 participants, only 11 (18.6%) were certified for GCP. Most of the participants (64.4%) had "Average" knowledge, 33.9% had "Good" knowledge and 1.7% had "Poor" knowledge. Only 49% of participants had satisfactory practices related to GCP. There was a significant difference in the knowledge based on the current academic position for the items assessing knowledge of institutional review board (P = 0.010), confidentiality & privacy (P = 0.011), and participant safety & adverse events (P < 0.001). There was also a significant difference in knowledge of research misconduct (P = 0.024) and participant safety & adverse events (P = 0.011) based on certification of GCP. There was a notable difference in the practices related to recruitment & retention on the basis of current academic position (P < 0.001) and certification of GCP (P = 0.023). We also observed a considerable difference between the knowledge and practices of GCP among the participants (P = 0.013).

CONCLUSION

Participants have basic knowledge of GCP but show a lack thereof in certain domains of GCP. This can be addressed by holding training sessions focusing on these particular domains.

Key Words: Clinical trial; Ethics; Good clinical practice; Knowledge; Research; Research subjects

Core Tip: There is a lack of knowledge about the good clinical practices (GCP) in the researchers of medical colleges. In order to improve the quality of research, as well as, make research a better experience for the participants of research, we must work on improving awareness of the GCP among researchers. This can be done by organising training sessions or workshops which throw light on the principles of GCP.

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INTRODUCTION

With the increase in research studies involving human subjects, there arises a need to have certain guidelines in place to protect human subjects. Additionally, the review of research by the research ethics committee (REC) has been mandated by international standards[1,2]. A lack of guidelines can lead to misuse of participants as well as other resources. Good clinical practice (GCP) provided by the International Conference on Harmonization (ICH) sets ethical and scientific standards and guidelines for conducting research involving human participants[3]. The two important principles of these guidelines include protecting the rights of human participants and the credibility of the data generated[4]. Such guidelines are required for the welfare of individuals partaking in a trial.

Knowledge of GCP before taking on a research project would lead to increased safety and efficacy of the project. A study conducted in Saudi Arabia, in which 85% of respondents had been trained for GCP, estimated that 97% of respondents believed that GCP guidelines were followed in trials and they improved the quality of the trial. While 59% of the respondents were cynical towards the institutional review board (IRB) approval process or the monitoring of the clinical trials[5]. Another study, conducted among dental faculties in India, established that more than 93% of the respondents wanted research ethics education for post-graduates, principal investigators as well as members of the REC, while less than 20% thought that the REC was not needed because of the presence of scientific committee[6].

The objective of our study was to estimate the knowledge and practices of researchers in a tertiary care institute in Northen-India. We also want to determine the difference in knowledge and practices based on research experience, GCP certification, and current academic position in the institute. Our null hypothesis stated that there is no knowledge/practice gap of GCP among researchers in a tertiary care research institution.

MATERIALS AND METHODS

Study Design

We conducted a cross-sectional study, which used a self-administered questionnaire at the All India Institute of Medical Sciences, Rishikesh between February and April 2023.

Study Population

We recruited all the researchers who had Institutional Ethics Committee-approved research projects. Here, the individuals, other than faculty and residents, who have conducted research in the institute are referred to as researchers.

Questionnaire

The questionnaire was self-structured and based on the GCP guidelines[7]. The study tool included 4 sections. The first section contained a summary of the study and informed consent for participation in the study. This was followed by section 2, which had questions that characterized the demographics of the participants. This included age, sex, experience in research, current academic position, number of publications, and certification for GCP. The third section consisted of 13 questions that assessed the knowledge of participants regarding GCP guidelines. Three of these questions were evaluated by a Likert scale (1- strongly agree, 2- agree, 3- neutral, 4- disagree, 5- strongly disagree), 9 were True/False questions and 1 was a Yes/No question. The last section assessed the application of GCP principles by the participants in practice through 10 case scenarios. In these, 4 options were given out of which one had to be chosen by the participants. Questions were based on the course of GCP by National Institute on Drug Abuse (NIDA) Clinical Trials Network and references were taken from previous studies[5,7].

All 23 items assessing the Knowledge and practices of participants were categorized into 1 of the 12 modules of NIDA Clinical Trials Network's course of GCP: Introduction (n = 2), IRB (n = 2), informed consent (n = 2), confidentiality & privacy (n = 4), participant safety & adverse events (n = 3), quality assurance (n = 2), the research protocol (n = 1), documentation & record-keeping (n = 0), research misconduct (n = 4), roles & responsibilities (n = 0), recruitment & retention (n = 2), investigational new drugs (n = 1)[7].

The questionnaire was subjected to content validation by researchers well versed in the GCP guidelines and modifications were made according to their suggestions.

The questionnaire was sent to the participants as Google forms and it was ensured that the participants fully understand the items before responding to them. To ensure quality, the responses were verified and screened for non-response/invalid responses.

Statistics

Data analysis was done using Jamovi software. We report the positive responses for the items in mean scores.

For True/False and Yes/No items, a score of 1 was given to the correct response and 0 to the incorrect response. For the Likert scale, a score of 0 was given to the most negative response and a score of 4 to the most positive response. The composite score for knowledge was calculated by adding the scores of all 13 items, the maximum possible score was 22. As for the practice-based questions, a score of 1 was given to the correct answer and a score of 0 was given if the participant chose any other options. The total score was calculated by simple summation of the scores of all 10 items (maximum score = 10). The knowledge was classified as "good" (score > 16), "average" (score = 11-16), and "poor" (score < 11). Similarly, the practices of the participants were classified as "satisfactory" (score ≥ 7) and "unsatisfactory" (score < 7).

Demographic data were described using descriptive statistics. Chi-squared test in the bi-variate analysis was used to determine the relationship between knowledge and demographic details like certification of GCP, current academic position, and experience in research. The same was done to determine the association between practices of GCP and independent variables of demographics. To determine the association of knowledge and practices of GCP, a chi-square test was used. $P < 0.05$ was considered significant.

Ethical Consideration

The approval to conduct this research was taken from the Institutional Ethics Committee of All India Institute of Medical Sciences, Rishikesh, India.

Informed consent, which was subjected to the approval of the Institutional Ethics Committee, was taken from all the individuals before enrolling them in the study.

In order to maintain privacy, any data which may have identified the individual participants of the study was not disclosed.

RESULTS

A total of 135 individuals were contacted, out of which 67 consented to fill out the questionnaire and 59 responses were valid. Table 1 shows the demographic details of the participants. The age of the respondents ranged from 19 years to 38 years, with a mean age of 23 years and a median of 22 years. Most of the participants were Bachelor of Medicine, Bachelor of Surgery students (66.1%). Only 10.2% of participants had more than 3 years of research experience while a majority (57.6%) had less than 1 year of experience in conducting a research. Eighteen individuals (30.5%) had research publications. Out of the respondents, 25.4% have been resource persons for academic classes on research methodologies.

The relationship between research experience and certification of GCP has been shown in Figure 1.

The participants' knowledge was assessed by 13 items, of which 10 were "Yes/No" or "True/False" items and 3 used the Likert scale. Knowledge scores in our sample ranged from a minimum of 10 (45% correct) to a maximum of 18 (82% correct). The mean score was 15.4 (out of 22) with a standard deviation of 1.95. Knowledge was deemed "Good" if the score was more than equal to 17, "Average" if the score was from 11 to 16, and "Poor" if the score was less than 11.

Table 1 Demographics of participants

Variables		Number	Percent (%)
Sex	Female	25	42.4
	Male	34	57.6
Age	< 25 years	47	79.7
	≥ 25 years	12	20.3
Academic qualification	BSc	8	13.6
	MBBS	39	66.1
	MPH	3	5.1
	MSc	6	10.2
	PhD	3	5.1
Total research experience	< 1 year	34	57.6
	1-3 years	19	32.2
	> 3 years	6	10.2
Number of publications	Not yet published	41	69.5
	1-3	15	25.4
	> 3	3	5.1
Have been a resource person for academic classes on research methodologies	Yes	15	25.4
	No	44	74.6
Certified for GCP course	Yes	11	18.6
	No	48	81.4

BSc: Bachelor of science; MBBS: Bachelor of medicine, Bachelor of Surgery; MPH: Master of public health; MSc: Master of science; PhD: Doctor of philosophy; GCP: Good clinical practice.

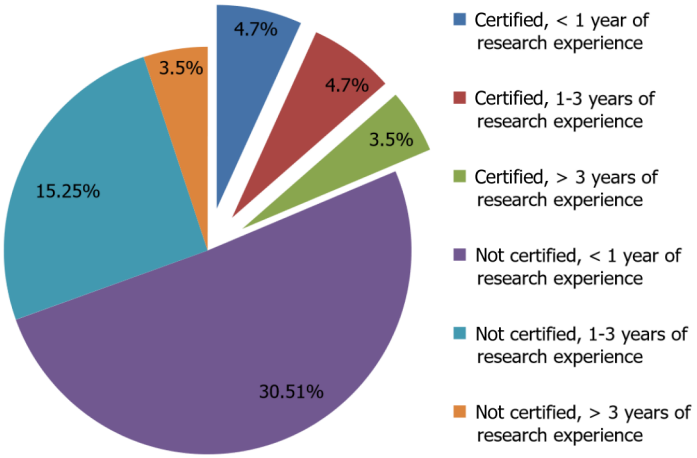

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Figure 1 Research experience with respect to certification of good clinical practice.

As seen in Figure 2, the maximum number of correct answers were given for the statement "A consent document must be submitted to the Institutional Review Board for its approval before enrolling participants in the study." (Item No. 2) with 96.6% correct responses. The statement with the maximum number of incorrect answers was "Review by the ethics committee is time consuming and makes it difficult to conduct research." (Item No. 13) which had 25.4% correct answers.

There was a significant difference in the response of the participants on the basis of current academic position for the statements "A consent document must be submitted to the IRB for their approval before enrolling participants in the study." (P = 0.010), "Participant's information can be disclosed if he/she makes a credible threat to harm another person." (P = 0.011), "The severity of an adverse event is same as its seriousness." (P < 0.001). We also found a considerable

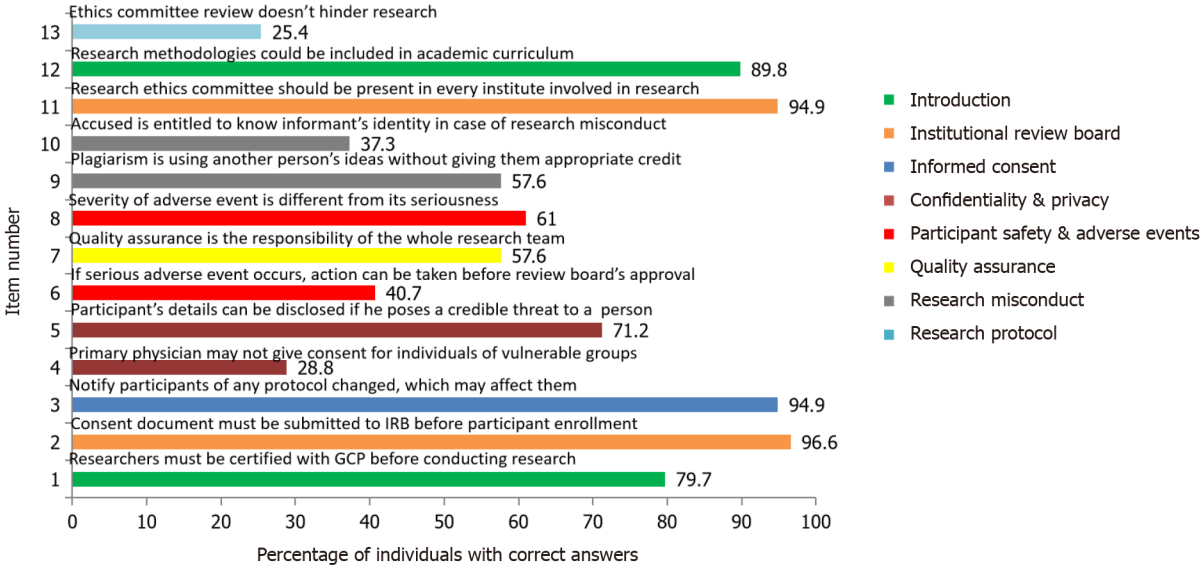

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Figure 2 Module-wise arrangement of questions assessing knowledge. GCP: Good clinical practice.

difference on the basis of certification of GCP for the following statements: "The severity of an adverse event is same as its seriousness." (P = 0.011), and "Research misconduct consists of- fabrication, falsification and plagiarism. Plagiarism is using another person's ideas after giving appropriate credit." (P = 0.024). This is shown in the Supplementary Table 1.

Most of the respondents (64.4%) had "Average" knowledge and 33.9% had "Good" knowledge. No noteworthy differences in the total knowledge scores were found on the basis of duration of research experience, certification of GCP, and current academic position. However, there was a significant relationship between the practices of the participants and their academic positions in the institute. This is shown in Table 2.

Practices

The practice of the participants in accordance with GCP was assessed with the help of 10 multiple-choice questions, with a score of 1 for each correct response. Out of a total score of 10 for practice-based questions, scores more than equal to 7 were considered "Satisfactory" and scores less than 7 were considered "Unsatisfactory".

Item numbers 1, 2, and 5 which assess recruitment & retention, informed consent, and research misconduct respectively, had the maximum number of correct responses as shown in Figure 3.

As shown in Supplementary Table 2, a significant difference was found in the responses for item 3, which assesses the domain of Recruitment & Retention, based on the current academic position (P < 0.001) and certification of GCP (P = 0.023).

Only 29 participants (49.2%) had "Satisfactory" practices of GCP. Table 2 shows a significant difference in the overall practices of participants on the basis of their current academic position (P = 0.008).

Knowledge and Practices

Table 3 shows the results of a chi-squared test for independent samples, which showed a significant relationship between the knowledge and practice scores of the study participants; χ^2 (2, n = 59) = 8.62, P = 0.013.

DISCUSSION

This questionnaire-based study is the first report in North India specifically targeting knowledge and practices of GCP among researchers (excluding faculty and residents) in a medical college[5,6,8,9].

In our study, most of the researchers were not certified for GCP, which is comparable to a study by Goel *et al*[8] stating a lack of formal training for GCP in health care providers[8]. As the research experience increased, more proportion of individuals were certified with GCP. This suggests that individuals with more research experience are more likely to pursue GCP certification. Most of the respondents believed that one must be GCP certified before conducting research, despite this the scores of most of the participants in knowledge and practices were "Average". This goes on to show that individuals are aware of the GCP on the surface but do not fully understand the principles of GCP. There is a gap in knowledge and practices among participants concerning GCP. The mean score for knowledge of the participants was 70% of the maximum possible score, indicating most of the individuals had some knowledge of GCP. This differs from a study conducted in Japan which showed that ≤ 50% of nurses had knowledge about GCP[10]. Another study conducted among doctors in medical colleges in India stated a lack of knowledge of GCP[11].

Table 2 Associations between demographics, and total knowledge and practices scores

Demographics		Knowledge of good clinical practice					Practice of good clinical practice			
		Good	Average	Poor	χ^2	*P* value	Satisfactory	Unsatisfactory	χ^2	*P* value
Research experience (in years)	< 1	13	20	1	1.67	0.796	17	17	0.0357	0.982
	1-3	5	14	0			9	10		
	> 3	2	4	0			3	3		
Certified for GCP	Yes	3	7	1	4.54	0.103	3	8	2.59	0.108
	No	17	31	0			26	22		
Academic position in the institute	BSc	2	6	0	6.67	0.573	3	5	13.7	[a]0.008
	MBBS	17	21	1			23	16		
	MPH	0	3	0			3	0		
	MSc	1	5	0			0	6		
	PhD	0	3	0			0	3		

[a]$P < 0.01$.

GCP: Good clinical practice; BSc: Bachelor of science; MBBS: Bachelor of medicine, Bachelor of Surgery; MPH: Master of public health; MSc: Master of science; PhD: Doctor of philosophy.

Table 3 Association between knowledge and practices of good clinical practice

Practices of GCP	Knowledge of GCP		
	Good	Average	Poor
Satisfactory	15	14	0
Unsatisfactory	5	24	1
χ^2	8.62		
P value	[b]0.013		

[b]$P < 0.05$.

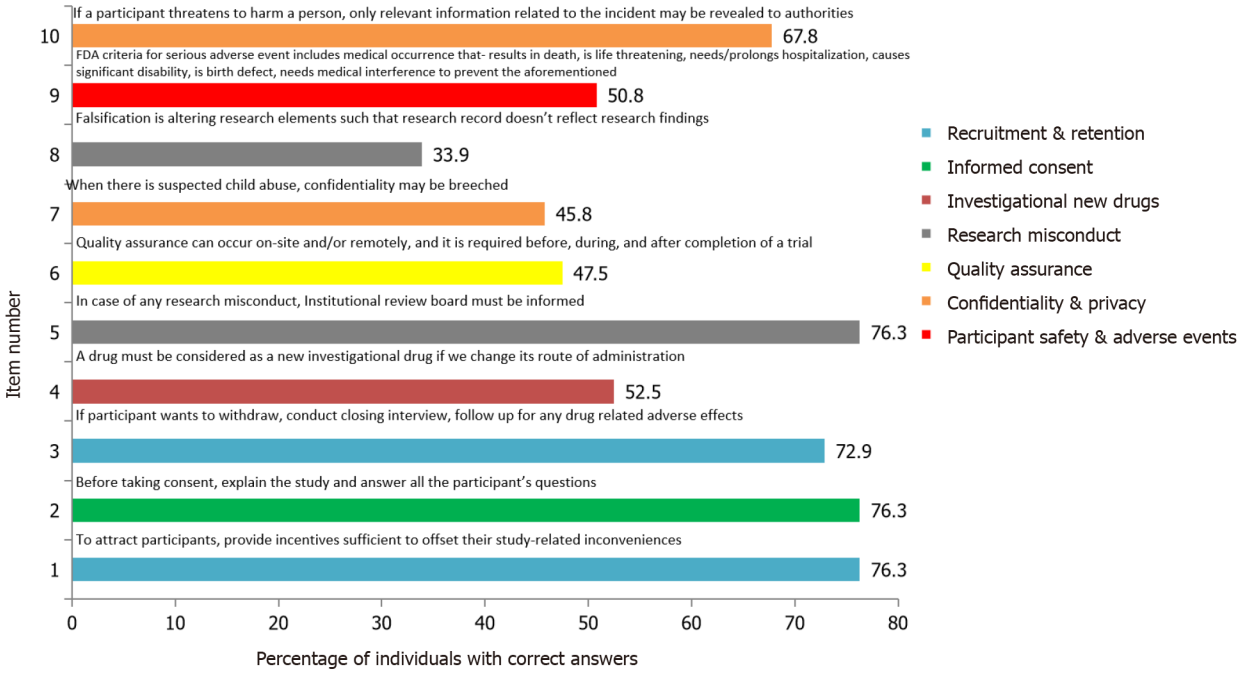
GCP: Good clinical practice.

The scores for the knowledge questions related to the topics of IRBs, and informed consent were high, indicating good knowledge of these domains. Participants obtained lower scores for questions related to confidentiality & privacy, research protocol, quality assurance, participant safety and adverse events, and research misconduct, which signifies that participants are less certain or have mixed opinions about these topics. In our study, about 74.6% of the participants believe that the review by the ethics committee is time-consuming or are unsure about this. This is similar to the finding of El-Dessouky *et al*[12], Than *et al*[9]. This delay could be perceived due to the participants' lack of understanding of the ethics committee, meticulous evaluation by the ethics committee, or due to the increased workload of the committee.

For the practice-based questions, we observed that statements related to recruitment & retention, and informed consent had a higher mean score in comparison to those related to confidentiality & privacy, quality assurance, participant safety and adverse events, research misconduct, and investigational new drugs.

We also found a significant difference between the knowledge and practices of the study participants for GCP. We also found statistical difference in the knowledge of researchers with respect to certification of GCP, or positions within the institute for items which assessed IRB, confidentiality & privacy, participant safety & adverse events, research misconduct. A noteworthy difference was found in the overall practices of GCP based on the academic positions in the institute, along with a difference in practices related to recruitment & retention for various academic positions, and certification of GCP.

At present there is no formal education about GCP in undergraduate or paramedical courses in the institute. In order to build a proper foundation of knowledge of clinical research, one must be familiar with the principles of GCP. We suggest the addition of a course on GCP in the curriculum of undergraduates and post-graduates to familiarize individuals with clinical research. Interactive training sessions can also be held, which have been shown to be effective by some studies[8,13,14].



DOI: 10.5662/wjm.v13.i5.466

Figure 3 Module-wise arrangement of questions assessing practices. FDA: Food and drug administration.

Limitations

This study was not able to include the residents as well as faculty of the tertiary care institute as per the directions of the IRB. These categories include most of the researchers of the institute, thus their exclusion leading to reduced sample size as well as discrepancies between the study population and the target population. We conducted the research only in a tertiary care institute, which may limit the generalization of our results. Due to fewer participants in the study, there could be a lack in the credibility of subgroup analysis.

CONCLUSION

Our study concluded that most of the researchers (except faculty and residents) in the medical college are not certified for GCP. Individuals with more research experience are more likely to pursue GCP certification. There was a gap in the knowledge and practices of GCP among the researchers. The understanding of IRB, informed consent, and recruitment & retention was good compared to that of confidentiality & privacy, quality assurance, participant safety and adverse events, and research misconduct. There is a significant difference in knowledge or practices of individuals on the basis of GCP certification or current academic position for some domains of GCP. there is also a significant difference between the knowledge and practices of GCP. This study recommends the incorporation of GCP courses into the academic curriculum or planned training sessions for GCP for the researchers of the institute, which focus on the domains where lack of knowledge was found.

ARTICLE HIGHLIGHTS

Research background

With the increase in research studies involving human subjects, there arises a need to have certain guidelines in place to protect human subjects. Additionally, the review of research by the research ethics committee has been mandated by international standards. A lack of guidelines can lead to misuse of participants as well as other resources. Good clinical practice (GCP) provided by the international conference on harmonization sets ethical and scientific standards and guidelines for conducting research involving human participants. The two important principles of these guidelines include protecting the rights of human participants and the credibility of the data generated. The level of understanding of these GCPs among researchers is the real question to assess.

Research motivation

GCP is an essential part of research life, and assessing this will have both awareness and accountability among researchers. One method of implementation is to assess first.

Research objectives
The objective of our study was to estimate the knowledge and practices of GCP among researchers in a tertiary care institute in India.

Research methods
A self-structured questionnaire about GCP, after expert validations, was circulated among researchers, at a tertiary healthcare institute. A total of 59 individuals, who were selected by universal sampling, participated in the study. All healthcare workers who have been investigators of Institutional Ethics Committee-approved research projects, except residents and faculty, and are still a part of the institute have been included in the study. The study was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences, Rishikesh. We used descriptive analysis and the Chi-squared test to analyze data. P value < 0.05 was considered significant.

Research results
Out of 59 participants, only 18.6% were certified for GCP. Sixty-four-point-four percent had "Average" knowledge, 33.9% had "Good" knowledge and 1.7% had "Poor" knowledge. Only 49% of participants had satisfactory practices related to GCP. There was a significant difference in the knowledge based on the current academic position for the items assessing knowledge of institutional review board (IRB) (P = 0.010), confidentiality & privacy (P = 0.011), and participant safety & adverse events (P < 0.001). There was also a significant difference in knowledge of research misconduct (P = 0.024) and participant safety & adverse events (P = 0.011) based on certification of GCP. There was a notable difference in the practices related to recruitment & retention on the basis of current academic position (P < 0.001) and certification of GCP (P = 0.023).

Research conclusions
This study concluded that most of the researchers (except faculty and residents) in the medical college are not certified for GCP. Individuals with more research experience are more likely to pursue GCP certification. There was a gap in the knowledge and practices of GCP among the researchers. The understanding of IRB, informed consent, and recruitment & retention was good compared to that of confidentiality & privacy, quality assurance, participant safety and adverse events, and research misconduct.

Research perspectives
There is a lack of knowledge about the GCP in the researchers of medical colleges. In order to improve the quality of research, as well as, make research a better experience for the participants of research, we must work on improving awareness of the GCP among researchers through organising training sessions or workshops which throw light on the principles of GCP.

FOOTNOTES

Author contributions: Harshita H designed, collected data, analyzed, wrote, reviewed, approved the manuscript; Panda PK gave concept, designed, analyzed, critically reviewed, and approved the manuscript.

Institutional review board statement: The study was reviewed and approved by Institutional Ethics Committee of All India Institute of Medical Sciences, Rishikesh, India.

Informed consent statement: All study participants provided informed written consent before enrolling in the study.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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ORIGINAL ARTICLE

Observational Study

Inflammatory bowel disease among first generation immigrants in Israel: A nationwide epi-Israeli Inflammatory Bowel Disease Research Nucleus study

Mira Stulman, Gili Focht, Yiska Loewenberg Weisband, Shira Greenfeld, Amir Ben Tov, Natan Ledderman, Eran Matz, Ora Paltiel, Shmuel Odes, Iris Dotan, Eric Ian Benchimol, Dan Turner

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Abstract

BACKGROUND

Israel has a high rate of Jewish immigration and a high prevalence of inflammatory bowel disease (IBD).

AIM

To compare IBD prevalence in first-generation immigrants *vs* Israel-born Jews.

METHODS

Patients with a diagnosis of IBD as of June 2020 were included from the validated epi-IIRN (Israeli IBD Research Nucleus) cohort that includes 98% of the Israeli population. We stratified the immigration cohort by IBD risk according to country of origin, time period of immigration, and age group as of June 2020.

RESULTS

A total of 33544 patients were ascertained, of whom 18524 (55%) had Crohn's disease (CD) and 15020 (45%) had ulcerative colitis (UC); 28394 (85%) were Israel-born and 5150 (15%) were immigrants. UC was more prevalent in immigrants (2717; 53%) than in non-immigrants (12303, 43%, $P < 0.001$), especially in the < 1990 immigration period. After adjusting for age, longer duration in Israel was associated with a higher point prevalence rate in June 2020 (high-risk origin: Immigration < 1990: 645.9/100000, ≥ 1990: 613.2/100000, $P = 0.043$; intermediate/low-risk origin: < 1990: 540.5/100000, ≥ 1990: 192.0/100000, $P < 0.001$). The prevalence was higher in patients immigrating from countries with high risk for IBD (561.4/100000) than those originating from intermediate-/low-risk countries (514.3/100000; $P < 0.001$); non-immigrant prevalence was 528.9/100000.

CONCLUSION

Lending support to the environmental effect on IBD etiology, we found that among immigrants to Israel, the prevalence of IBD increased with longer time since immigration, and was related to the risk of IBD in the country of origin. The UC rate was higher than that of CD only in those immigrating in earlier time periods.

Key Words: Epidemiology; Inflammatory bowel disease; Immigration; Environment

Core Tip: In this nationwide study, we compared inflammatory bowel disease (IBD) rates between first-generation immigrants originating from countries of varying IBD risk *vs* Israel-born residents. Our focus on the Jewish population was aimed at narrowing the genetic variation of IBD that is usually present in immigration cohorts. We found that the prevalence rate was lower among patients from intermediate- and low-risk regions compared to patients from high-risk regions but in both, the prevalence increased in association with duration in Israel after immigration. This finding, especially among immigrants from intermediate- and low-risk countries, lends support toward the role of environmental factors in IBD pathogenesis in Israel.

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INTRODUCTION

The incidence of inflammatory bowel disease (IBD) is rising sharply in low socio-demographic index regions such as Africa, Asia, and South America[1-3]. Increasing rates have also been observed among immigrants from low-resource countries to developed countries[4-6], and to a lesser extent, also *vice versa* (*e.g.*, from the Faroe Islands to Denmark, where the excess ulcerative colitis (UC) risk in Faroese immigrants was no longer seen after immigration to the new country)[7], implying probable environmental triggers for developing IBD. However, these nationwide studies have included immigrants from various ethnicities[5,8,9] with different genetic backgrounds compared to the host country, hampering attention to the environmental effect on IBD risk. In contrast, in Israel, which is defined as a high prevalence country for IBD, reaching 0.59% of the total population in 2021, and 0.67% among the Jewish population[10,11], the vast majority of immigrants are Jewish or first-degree relatives of Jewish residents who share a similar genetic background[12]. This poses a unique opportunity to study the impact of environmental factors in a high-risk westernized country among immigrants and Israel-born residents with relatively similar genetic predisposition to IBD[13,14], originating from countries with varying degrees of IBD risk.

Israel is a melting pot of various ethnicities and cultures, characterized by waves of immigration from all over the world. As a country with a population of 9.2 million (as of June 2020), Israel has absorbed 1.5 million Jewish immigrants since 1990 with little population drift[15]. Israel has a universal healthcare system where all residents are insured by one of four Health Maintenance Organizations (HMOs), making administrative studies relatively accurate. However, previous Israeli studies comparing IBD rates among Israel-born residents *vs* immigrants were conducted in small regional cohorts[16-23].

We aimed to compare the rate of IBD in first-generation immigrants *vs* Israel-born residents using a nationwide cohort of patients with IBD. We also aimed to determine whether the duration of residence in Israel affects the rate of IBD in these immigrants. Finally, we aimed to examine whether the rate of IBD in immigrants is related to the IBD risk in their country of origin.

MATERIALS AND METHODS

We utilized the Israeli IBD Research Nucleus (epi-IIRN), a validated nationwide IBD cohort including 98% of the patients with IBD in Israel [50503 patients with IBD as of June 2020: 27490 with Crohn's disease (CD) and 23013 with UC]. Case-ascertainment algorithms were validated for each of the four HMOs in Israel [89% sensitivity, 99% specificity, 92% positive predictive value (PPV), 99% negative predictive value (NPV)]. Further algorithms classified disease type as CD or UC (92% sensitivity, 97% specificity, 97% PPV, 92% NPV)[10,24].

We constructed a prevalence cohort including all Jewish patients with IBD in the epi-IIRN database who had an active diagnosis by June 2020, stratified by Israel-born and first-generation immigrants. Our focus on the Jewish population was based on the Israeli Law of Return, which almost exclusively gives Jews the right to receive citizenship in Israel. Indeed, 99.4% of immigrants in the epi-IIRN cohort immigrated under this definition.

Country of birth and date of immigration were retrieved from the HMOs' electronic medical records for all patients with IBD. We categorized the countries of origin by degree of risk for IBD based on published systematic reviews[3,25] and according to Agrawal *et al*[5] as: Low risk [*i.e.,* incidence of CD, < 1.95/100000 person-years (PY) and UC, < 3.10/100,000 PY], intermediate risk (*i.e.,* CD, 1.95-6.38/100000 PY or UC, 3.10-7.71/100000 PY), and high risk (*i.e.,* CD, > 6.38/100000 PY or UC, > 7.71/100000 PY). Each risk category was further stratified into age groups: 0-34, 35-65, and 65+ years as of June 2020. Duration of residence in Israel was inferred by immigration time periods, divided as the years < 1990, 1990-2001, and 2002-2020, based on immigration reports from the Israeli Central Bureau of Statistics[12]. When further stratifying by risk in country of origin and age groups, time periods were divided as the years < 1990 and 1990 onwards (*i.e.,* at least 30 years of residence in Israel, or less than 30 years), again based on the national data published by the Israel Central Bureau of Statistics, with these time periods available for each risk stratum[15].

In order to minimize the possibility that IBD was diagnosed prior to immigration and was only new to the Israeli medical system, we performed a sensitivity analysis, which only included immigrants with at least a five-year lookback period from the date of immigration to the date of the first IBD code, as previously validated in the epi-IIRN cohort[24].

Patients who originated from countries with no risk data available or with missing data such as date of birth, country of origin, or immigration date were excluded. The date of death was obtained by linking each patient's unique identifying code by deterministic approach to the Israeli Ministry of Health national mortality database. Socioeconomic status scores were based on a scale of 1-10 and grouped into three categories: 1-3, low; 4-6, intermediate; and 7-10, high[26], and current residence in Israel was divided into rural and urban based on address categorization by the Israeli Central Bureau of Statistics.

Statistical analysis

Factors were compared between the cohorts using ANOVA and Kruskal-Wallis tests, corrected for multiple comparisons by the Bonferroni method, as well as chi-square tests where appropriate. Point prevalence rates were calculated by dividing the annual number of living patients by the average population as of 2020, reported per 100000 with 95% confidence intervals (CI) and age-standardized by the direct method to the Israeli population for comparison between the groups. Proportions were compared *via* chi-squared contingency tables. SPSS version 28 (IBM Corporation, Armonk, NY, United States) and R for statistical computing[27] were used for statistical analyses. The Institutional Review Board of Shaare Zedek Medical Center approved the study.

RESULTS

A total of 33544 Jewish patients were included in the study, of whom 18524 (55%) had CD and 15020 (45%) had UC; 85% were Israel-born (n = 28394) and 15% (n = 5150) were immigrants. Of the latter, 25 % (n = 1293) originated from high-risk countries, 70% (n = 3615) from intermediate-risk countries, and 5% (n = 242) from low-risk countries. Due to the small sample size in the low-risk group, immigrants from low- and intermediate-risk countries were grouped together and compared with those from high-risk countries. Data regarding the number of immigrants and the risk category assigned to each country are detailed in Supplementary Table 1. Immigrants from Poland (n = 191) and Switzerland (n = 12) were excluded from this analysis, because the last available IBD data were from 1951-1960 and 1960-1969, respectively. The majority of immigrants were from former USSR (Union of Soviet Socialist Republics) countries (56%) and Europe (21%), followed by North America (11%), the Middle East (6%), South America (4.4%), Africa (1.5%), and Asia (0.1%). Additional

Table 1 Basic characteristics of the inflammatory bowel disease cohort by June 2020 [*n* (%), mean ± SD and median (interquartile range) are displayed as appropriate][1]

	Origin from high-risk countries (*n* = 1293)	Origin from intermediate- and low-risk countries (*n* = 3857)	Israel-born (*n* = 28394)
Prevalence-crude rate[2]	561.4	514.3	528.9
Sex (Female)	651 (50)	1953 (51)	14234 (50)
Age at immigration	25 ± 18	25 ± 17	N/A
IBD phenotype (Crohn's disease)	685 (53)	1748 (45)	16091 (57)
Age at diagnosis (yr)	41 ± 18	46 ± 17	32 ± 15
Age at June 2020	43 ± 17	60 ± 17	54 ± 19
Immigration periods			
< 1990	501 (39)	1358 (35)	N/A
1990-2001	229 (19)	2199 (57)	
2002-2020	543 (42)	300 (8)	
Socioeconomic status			
Low	65 (5)	247 (7)	1348 (5)
Intermediate	550 (52.5)	2247 (58)	10680 (37)
High	646 (50)	1247 (32)	15589 (55)
Missing	32 (2.5)	116 (3)	777 (3)
Residential location			
Rural	111 (9)	155 (4)	3173 (11)
Urban	1182 (91)	3701 (96)	25221 (89)

[1]Proportions between groups were significantly different, $P < 0.001$.
[2]Per 100000.
IBD: Inflammatory bowel disease; N/A: Not applicable.

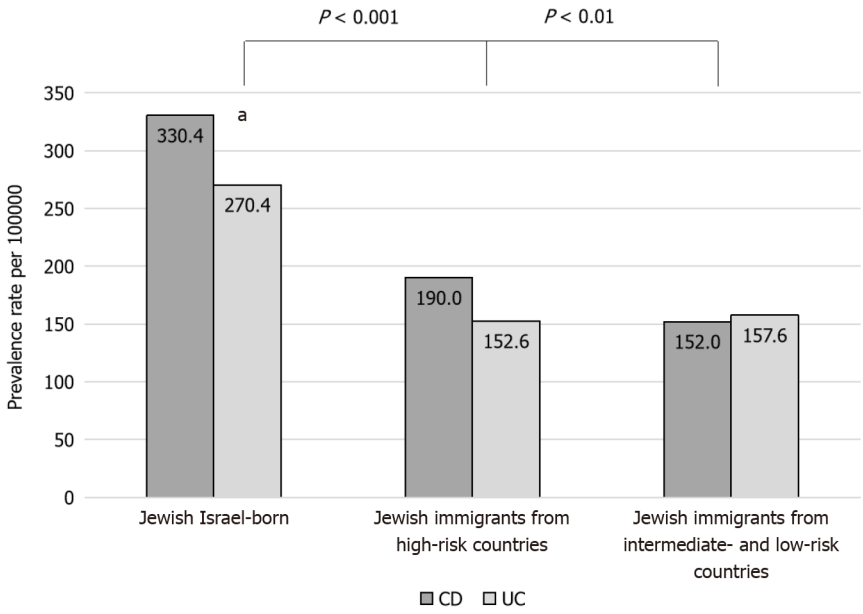
socio-demographic characteristics are provided in Table 1.

Among the Israel-born cohort, 16091 (57%) had CD and 12303 (43%) had UC, while in the immigration cohort, UC dominated: 2433 (47%) CD *vs* 2717 UC (53%) (odds ratio (OR) = 1.46 (95%CI: 1.38-1.55); $P < 0.001$]. UC dominated among immigrants from low- and intermediate-risk countries [1748 (45%) CD *vs* 2109 (55%) UC, $P < 0.001$], while immigrants from high-risk countries were more likely to be diagnosed with CD [685 (53%) *vs* 608 (47%), $P = 0.0023$)].

Crude IBD point prevalence rates in 2020 were highest among immigrants originating from high-risk countries [561.4 per 100000 (95%CI: 546.8-576.2)] followed by Israel-born residents [528.9 per 100000 (95%CI: 522.7-535.0)] and were lowest among immigrants from intermediate- and low-risk countries [514.3 per 100000 (95%CI: 493.2-535.9); $P < 0.001$]. In the immigration cohort, age-standardized point prevalence rates in 2020 were 342.6 per 100000 for immigrants originating from high-risk countries and 309.5 per 100000 for intermediate- and low-risk countries [OR = 1.1 (95%CI: 1.03-1.17); $P = 0.0041$]. For the Israel-born cohort, the standardized rate was twice as high (600.8 per 100000) as the entire immigration cohort (318.1 per 100000) [OR = 1.9 (1.65-2.17); $P < 0.001$]. When stratified by phenotype, CD prevalence rates were highest in the Israel-born cohort, followed by immigrants from high-risk countries, and were lowest among immigrants from intermediate- and low-risk countries (330.4 *vs* 190.0 *vs* 152.0 per 100000, respectively; $P < 0.001$; Figure 1).

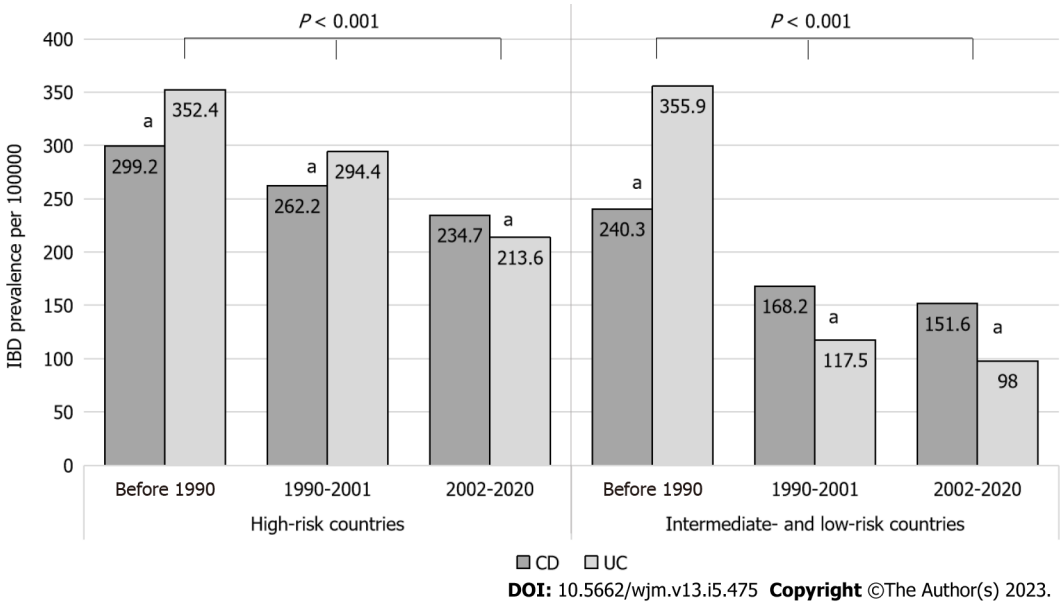
Among the immigrants, there was a significant association between longer duration of living in Israel and higher IBD prevalence rates, regardless of risk in the country of origin (Figure 2). CD became dominant among patients who immigrated after 1990 from intermediate- and low-risk countries, and from 2002 among patients who immigrated from high-risk countries. When stratifying by both IBD risk and age groups, prevalence for both CD and UC increased between time periods in each sub-group, with the exception of the group aged 35-64 years among immigrants from high-risk countries (Figure 3).

In the sensitivity analysis, there was a significant and positive association between longer duration in Israel and higher IBD prevalence rates for all countries of origin and among all age groups, including those aged 35-64 years (Supplementary Figure 1). For this analysis, 1057/5150 (21%) were excluded due to insufficient look-back period from the time of immigration; 1930 (47%) had CD and 2163 (53%) had UC; and 1115 (27%) originated from high-risk countries and 2978 (73%) from intermediate- and low-risk countries. UC was more common among individuals originating from intermediate- and low-risk countries [1571 (53%) UC *vs* 1407 (47%) CD, $P < 0.001$], as well as from high-risk countries [572



DOI: 10.5662/wjm.v13.i5.475

Figure 1 Inflammatory bowel disease prevalence among Jewish Israel-born and first-generation immigrants in June 2020. [a]*P* < 0.01. CD: Crohn's disease; UC: Ulcerative colitis.



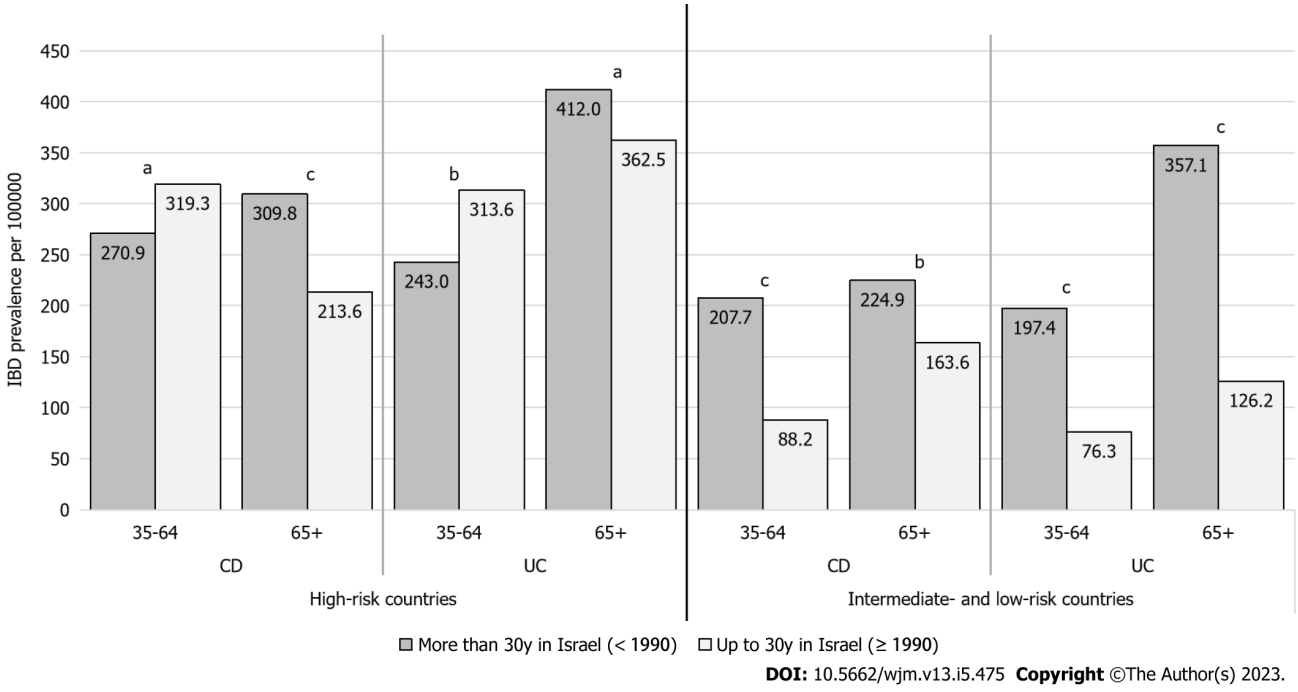
DOI: 10.5662/wjm.v13.i5.475

Figure 2 Inflammatory bowel disease prevalence among Jewish immigrants originating from varying risk countries in June 2020, stratified by duration of residence in Israel. [a]*P* < 0.01. CD: Crohn's disease; UC: Ulcerative colitis.

(51%) UC *vs* 543 (49%) CD], although in the latter, the difference was not statistically significant ($P = 0.34$).

DISCUSSION

In this nationwide study, we focused on the Jewish population to narrow the genetic variation of IBD that is usually present in immigration cohorts. We found that the IBD prevalence rate was lower among patients originating from intermediate- and low-risk regions compared to patients from high-risk regions but in both, the prevalence increased in association with duration of living in Israel after immigration. This finding, especially among immigrants from intermediate- and low-risk countries, lends support toward the role of environmental factors in IBD pathogenesis in Israel. In accordance with our findings, nationwide studies in Canada[8,28] and Sweden[9] have reported a lower risk of IBD among immigrants from low-risk countries compared to the non-immigrant population.



DOI: 10.5662/wjm.v13.i5.475

Figure 3 Inflammatory bowel disease prevalence among Jewish immigrants from varying risk countries in June 2020, stratified by age group and by duration of residence in Israel. [a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001. CD: Crohn's disease; UC: Ulcerative colitis.

Previous studies on immigration to Israel were small and regional such as from the Beer Sheva region (1961-1985 and 1979-1987)[16,17], Central Israel (1970-1980)[18,19], the Kinneret sub-district of Northern Israel (1965-1989)[20,21], and Israeli Kibbutz settlements (1987-2007)[22,23]. These studies mainly compared IBD prevalence and incidence between Israel-born *vs* European-American-born and Asian-African-born Jewish immigrants. Most of these studies showed higher prevalence rates in European-American-born immigrants or Israel-born individuals, similar to our study. Our analysis, however, included all immigrants in Israel, including a substantial number of patients from low- and intermediate-risk countries, such as former USSR countries, who initially demonstrated higher UC rates but shifted to the local higher rate of CD in association with longer duration in Israel.

Although UC was predominant among patients who immigrated before 1990, CD became more prominent during the period from 2002 onwards, around the time when the switch from UC to CD dominance occurred in Israel (in 2006, as demonstrated in our previous study)[10]. High UC rates have been previously demonstrated in immigrants from both low-risk[29,30] and high-risk countries[7]. UC dominance, at least at first, may be explained by the hypothesis that UC is predominantly driven by exposure to environmental risk factors that may occur at any point in life, whereas CD is predominantly driven by genetic-environmental interactions in early life at critical stages of immunologic development [31].

Environmental risk factors affecting immigrant populations worldwide may include changes in diet, water supply, hygiene, socioeconomic status, access to the health care system, use of antibiotics, urbanization, and possibly psychological stress[32-34]. Vangay *et al*[35] demonstrated 'westernization' of the gut microbiome among 281 Southeast Asian individuals who immigrated to the United States. That study demonstrated loss of native gut microbiome diversity and function, amplified by longer duration in the United States.

Our study had several strengths. This study was the first to describe and compare the immigrant *vs* Israel-born IBD population in Israel on a national scale, who likely have similar genetic backgrounds, highlighting the role of environmental factors in IBD development in Israel. We utilized the national epi-IIRN cohort, which is based on validated algorithms accurately distinguishing patients with IBD, IBD type, and incident cases[24], thus minimizing selection bias and loss to follow-up. In Israel, healthcare coverage is universal, minimizing healthcare access bias between the immigrant and Israel-born groups. We conducted a sensitivity analysis that excluded patients without sufficient look-back period (and who may have had the disease at immigration), in an effort to minimize lead-time bias.

Limitations of our study include lack of access to national data necessary to calculate person-time and incidence rate ratios, as well as lack of data regarding lifestyle habits such as smoking and dietary intake among the immigration cohorts in the origin countries, as well as in Israel. Thus, we could not quantify the impact of specific environmental risk factors. We also excluded immigrants from countries without known IBD risk data, although the IBD immigration from these cohorts was negligible, such as some of the African countries (Supplementary Table 1).

It is almost impossible to fully disentangle periods of immigration and cohort effects explored in immigration studies in a meaningful way[36,37]. In our study, it is possible that there are periods of arrival cohort effects, but these cannot be distinguished from duration of residence effects. However, we did stratify the analysis by IBD risk in the country of origin, which may account for the cohort effect to a certain extent.

CONCLUSION

In conclusion, we found a high prevalence rate of IBD among immigrants to Israel and identified a positive association between duration of time after immigration and IBD prevalence, alluding to environmental risk factors in Israel. Future studies should explore associations between immigration with time to IBD onset, and should examine specific environmental factors among immigrants to further our understanding of the elusive IBD etiology.

ARTICLE HIGHLIGHTS

Research background

Israel has a high rate of Jewish immigration and a high prevalence of inflammatory bowel disease (IBD). The study of IBD among immigrants is of paramount importance for several compelling reasons. Immigration itself facilitates population growth and changes in demographics, thereby influencing prevalence trends. Moreover, immigration introduces individuals to new environments, dietary habits, hygiene practices, and lifestyle behaviors, which can significantly alter their risk of developing IBD as they assimilate into their host countries.

Research motivation

Investigating IBD among immigrants provides a unique opportunity to dissect the complex interplay between genetics, environment, and migration in disease development, especially if focusing on a specific ethnic group of immigrants with similar predisposition to IBD. In this study, we compared IBD rates between first-generation immigrants originating from countries of varying IBD risk *vs* Israel-born residents, focusing specifically on the Jewish population in an effort to narrow the genetic variation of IBD that is usually present in immigration cohorts, in an increasingly interconnected world.

Research objectives

We aimed to compare the rate of IBD in first-generation immigrants *vs* Israel-born residents using a nationwide cohort of patients with IBD. We also aimed to determine whether the duration of residence in Israel affects the rate of IBD in these immigrants. Finally, we aimed to examine whether the rate of IBD in immigrants is related to the IBD risk in their country of origin.

Research methods

Patients with a diagnosis of IBD as of June 2020 were included from the validated Israeli IBD Research Nucleus cohort that includes 98% of the Israeli population. We stratified the immigration cohort by IBD risk according to country of origin, time period of immigration, and age group as of June 2020.

Research results

Of the 33544 Jewish patients that were ascertained, 18524 (55%) had Crohn's disease and 15020 (45%) had ulcerative colitis (UC); 28394 (85%) were Israel-born and 5150 (15%) were immigrants. UC was more prevalent in immigrants (2717; 53%) than non-immigrants (12303, 43%, $P < 0.001$), especially in the < 1990 immigration period. The prevalence was higher in patients immigrating from countries with high risk for IBD (561.4/100000) than those originating from intermediate-/low-risk countries (514.3/100000; $P < 0.001$); non-immigrant prevalence was 528.9/100000. After adjusting for age, longer duration in Israel was associated with a higher point prevalence rate in June 2020 (high-risk origin: Immigration < 1990: 645.9/100000, ≥ 1990: 613.2/100000, $P = 0.043$; intermediate/low-risk origin: < 1990: 540.5/100000, ≥ 1990: 192.0/100000, $P < 0.001$).

Research conclusions

Our focus on the Jewish population was aimed at narrowing the genetic variation of IBD that is usually present in immigration cohorts. We found that the prevalence rate was lower among patients from intermediate-and low-risk regions compared to patients from high-risk regions but in both, the prevalence increased in association with duration in Israel after immigration. This finding, especially among immigrants from intermediate- and low-risk countries, lends support toward the role of environmental factors in IBD pathogenesis in Israel.

Research perspectives

Future studies should explore associations between immigration with time to IBD onset, and should examine specific environmental factors among immigrants to further our understanding of the elusive IBD etiology.

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FOOTNOTES

Author contributions: Stulman M designed and conceptualized the study, collected, analyzed, and interpreted the data, and drafted the manuscript; Focht G, Loewenberg Weisband Y, Greenfeld S, Ben Tov A, Ledderman N, and Matz E contributed to data acquisition; Paltiel O, Odes S, Dotan I, and Benchimol EI contributed in data analysis and interpretation; Turner D designed and conceptualized the study, contributed to data analysis and interpretation, and drafted the manuscript.

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Informed consent statement: The Shaare Zedek Medical Center IRB has waived the requirement to obtain informed consent.

Conflict-of-interest statement: All the authors declare that they have no conflicts of interest to disclose.

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

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ORIGINAL ARTICLE

Basic Study

Sequential extraction of RNA, DNA and protein from cultured cells of the same group

Ying-Yu Cui

Specialty type: Medical laboratory technology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

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Abstract

BACKGROUND

Efficient extraction of nucleic acids and proteins (ENAP) from cells is a prerequisite for precise annotation of gene function, and has become laboratory routine for revealing the mysteries of life. However, cell samples are often from different culture dishes, resulting in inevitable experimental errors and sometimes poor repeatability.

AIM

To explore a method to improve the efficiency of ENAP, minimizing errors in ENAP processes, enhancing the reliability and repeatability of subsequent experimental results.

METHODS

A protocol for the sequential isolation of RNA, DNA, and proteins from the same cultured HepG2 cells using RNAzol reagent is presented here. The first step involves culturing HepG2 cells to the exponential phase, followed by the sequential isolation of RNA, DNA, and proteins from the same cultured cells in the second step. The yield of nucleic acids and proteins is detected in the third step, and their purity and integrity are verified in the last step.

RESULTS

The procedure takes as few as 3-4 d from the start to quality verification and is highly efficient. In contrast to the existing kits and reagents, which are primarily based on independent isolation, this RNAzol reagent-based method is characterized by the sequential isolation of RNA, DNA, and proteins from the same cells, and therefore saves time, and has low cost and high efficiency.

CONCLUSION

The RNA, DNA, and proteins isolated using this method can be used for reverse transcription-polymerase chain reaction, polymerase chain reaction, and western blotting, respectively.

Key Words: Sequential extraction; Ribonucleic acid; Deoxyribonucleic acid; Protein; Cultured cells

Core Tip: Sequential extraction of nucleic acids and proteins from cultured cells of the same group. Life is a way of material (mainly protein and nucleic acid) movement, and health lies in movement. Cell is the most fundamental structural and functional unit of life. Therefore, the effective isolation of nucleic acids and proteins from cells is the foundation and prerequisite for revealing the mysteries of life. However, during laboratory routine for isolation of nucleic acids and proteins, cell samples are often from different culture dishes, usually leading to inevitable experimental errors and sometimes poor repeatability. The present research tries to explore the possibility to simultaneously isolate nucleic acids and proteins from the same sample, while reducing experimental errors and ensuring consistency during experimentation. The present study established a selective protocol for sequential isolation of RNA, DNA and proteins from the same cells with the characteristics of easy operation, rapid extraction and high efficiency. RNAzol reagent was used for the sequential isolation of RNA, DNA, and proteins from the same cultured HepG2 cells, resulting in a novel protocol containing four steps. A protocol for sequential isolation of RNA, DNA and proteins was established and the procedure takes as few as 3-4 d from the start to quality verification and is highly efficient. The quality of RNA, DNA and proteins isolated through sequential isolation protocol can be used for reverse transcription - polymerase chain reaction (PCR), PCR and western blot, respectively. The present procedure is not only easy, rapid and high efficient, but also economical and practical, especially for researchers in developing and underdeveloped countries.

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INTRODUCTION

The essence of life relies on molecular kinetics, which primarily involves the interactions of nucleic acids (DNA and RNA) and proteins. Therefore, the extraction of nucleic acids and proteins is crucial for deciphering the secrets of life. It is becoming increasingly important for researchers in the post-genomic era to efficiently isolate nucleic acids and proteins of high purity and integrity from cultured cells. Various commercial kits are available for the isolation of nucleic acids and proteins, using various methods[1]. Recently, methods for RNA[2], DNA[3] and protein[4,5] micro-isolation have been updated, respectively, and simultaneous isolation of RNA and DNA or protein has also been reported[6,7], but there has been no reported method for simultaneous isolation of RNA, DNA, and protein from cultured cells of the same group. Methods for the independent extraction of nucleic acids and proteins from different groups of cultured cells comprise several steps, are expensive, and time-consuming; these factors affect the productivity, purity, and integrity of the isolated samples. Most importantly, it is very difficult to ensure that the number of cells, cell growth status, and metabolic status of the cultured cells are consistent across the different groups, which inevitably increases the chances of experimental errors between earlier and later experiments. Although the complete elimination of errors is not achievable during experimentation, it is essential to minimize errors as far as possible. The quality of the RNA and proteins isolated from different groups of cultured cells could be a key factor responsible for the inconsistencies in gene expression data obtained by reverse transcription (RT)-polymerase chain reaction (PCR) and western blotting that are often observed. By referring to related literature[8-10] and repeated experimentation, the present study established a relatively rapid procedure for the sequential extraction of RNA, DNA, and proteins from the same group of cultured cells. The method described herein is not only easy and inexpensive, but also has high reproducibility, comparability, and credibility, and ensures consistency during experimentation.

MATERIALS AND METHODS

Reagents

The following reagents were used in this study for the successive extraction of nucleic acids and proteins: RPMI 1640 (HyClone), penicillin-streptomycin (10000 units/mL penicillin and 10000 μg/mL streptomycin; Invitrogen), HEPES (Sigma, United States), fetal bovine serum (FBS; Sijiqing, China), RNAzol (Cohen-Bio Corp., Beijing, China, lot no. NA6111), sodium dodecyl sulfate (SDS; Amresco), agarose (Fluka, Spain), diethyl pyrocarbonate (DEPC; Sigma), acrylamide (BBI, Canada), bis-acrylamide (BBI), tetramethylethylenediamine (TEMED; Sigma), ammonium persulfate

(Sigma,), guanidine hydrochloride (BBI, Canada), Tris (Sigma), glycine (Sigma), ethidium bromide (E.B; Sigma), MOPS (Serva, Sino-American Biotechnology Co.), PRO-STAIN™ protein marker II (SBS Genetech Co., Beijing, China), horseradish peroxidase-anti-glyceraldehyde-3-phosphate dehydrogenase (HRP-anti-GAPDH; Kangchen Bio-tech, Shanghai, China), and sediment type mono-ingredient TMB substrate solution (PA108-01, Tiangen Biotech Co., Beijing, China).

Primers

The following primers were used in this study: c-Myc: Forward: 5′-AGCAAACCTCCTCACAGC-3′, and reverse: 3′-GATGCCTTGAGAACACGC-5′ (GenBank accession number: NM_002467).

Equipment

The following equipment was used for the sequential extraction of nucleic acids and proteins: CO_2 water jacketed incubator (Thermo Forma MODEL 3111, series II, HEPA FILTER, Forma Scientific Inc.), inversion microscope (XDS-1B, COIC), an agarose gel electrophoresis apparatus (DYY-III-4, LiuYi Corp. Beijing), SDS-polyacrylamide gel electrophoresis (PAGE) apparatus (POWER-PAC200/300, Bio-Rad), a MultiMage™ Light Cabinet (Alpha Innotech Corporation), and a ultraviolet spectrometer (Ultrospec®2100 Pro, Amersham Pharmacia Biotech).

Cell culture

Duration: 1-3 d, depending on the cell line used.

The cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 mmol/L HEPES at pH 7.4, and maintained at 37 °C in a humidified incubator with 5% CO_2. When the cells reached a confluence of 75%, they were detached with 0.25% trypsin and subsequently seeded into 24-well microtiter plates at a density of 10^4 cells /mL with 2 mL complete culture media /well, and cultured in a 5% CO_2 incubator for 24, 48, 72, and 96 h. The cells were collected from three wells at each time point and counted with a hemocytometer. The mean number of cells calculated from the three wells represented the y-axis value, and the time point was represented on the x-axis of a cell growth curve, which was analyzed to identify the exponential growth phase of the cells.

RNA isolation

Duration: Approximately 1 h.

Homogenization: The cells were directly lysed in the exponential phase of growth in a culture dish by adding 1 mL RNAzol reagent to a dish of diameter 3.5 cm, and the cell lysate was pipetted several times for homogenization.

The homogenized samples were incubated for 5 min at room temperature (RT, 25 °C) to allow the complete dissociation of nucleoprotein complexes.

The samples were centrifuged at 11000 × *g* for 10 min at 4 °C, following which the clear supernatant solution was transferred to a fresh 1.5 mL Eppendorf tube.

A 0.2 mL aliquot of chloroform was added to the Eppendorf tube and the sample tubes were securely capped. The tubes were vigorously shaken by hand for 15 s and incubated at RT for 3 min.

The samples were centrifuged at 11000 × *g* for 15 min at 4 °C. Following centrifugation, the mixture separated into a lower, phenol-chloroform phase, an interphase, and an upper colorless aqueous phase. The RNA remained exclusively in the aqueous phase, the DNA remained in the interphase, and the proteins were retained in the lower organic phase. The volume of the aqueous phase was approximately 60% of the volume of RNAzol reagent used for homogenization. The aqueous, interphase, and organic phases were collected in fresh 1.5 mL Eppendorf tubes, and the tubes containing the interphase and organic phase were stored at 4 °C for the isolation of DNA and proteins.

A 0.5 mL aliquot of isopropyl alcohol was added to the tube containing the aqueous phase, mixed evenly, and allowed to stand at room temperature for 10 min.

The mixture was centrifuged at 11000 × *g* for 10 min at 4 °C and the supernatant was discarded. The RNA precipitate, often invisible before centrifugation, formed a gel-like pellet on the sides and bottom of the tube.

At least 1 mL of 75% ethanol was added for washing the RNA pellet, once.

The sample was mixed by vortexing and subsequently centrifuged at a speed < 7500 × *g* for 5 min at 4 °C. The supernatant obtained after centrifugation was discarded.

The RNA pellet was air-dried for 5-10 min. The RNA was dissolved in an appropriate volume of RNase-free water by pipetting the solution a few times and incubating for 10 min at 55-60 °C. A 2 μL aliquot of the RNA solution was diluted 200-fold for detection of absorbance (A) at 260 nm, and the remnant was stored at - 80 °C until further use.

DNA isolation

Duration: Approximately 1 h.

Any remaining aqueous phase was removed from the interphase layer and discarded, and 0.3 mL of 100% ethanol was added per milliliter of RNAzol reagent used for the initial homogenization of the interphase and phenol phases. The samples were mixed by inversion and allowed to stand at room temperature for 3 min.

The DNA was sedimented by centrifugation at a speed less than 2000 × *g* for 5 min at 4 °C. The phenol-ethanol supernatant was then transferred to a fresh tube and stored at 4 °C for protein isolation.

A 0.3 mL aliquot of 0.1 M sodium citrate-10% ethanol solution was added to wash the DNA pellet, at least twice. During each wash, the DNA pellet was kept in the washing solution for 30 min at room temperature (with periodic

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Figure 1 Determination of the integrity of the total RNA isolated from the cultured HepG2 cells. Electrophoresis of the total RNA on a formaldehyde-denaturing agarose gel and subsequent staining with 0.5 mg/mL ethidium bromide revealed discrete bands corresponding to 28S, 18S, and 5S RNA molecules.

mixing) and centrifuged at 2000 × *g* for 5 min at 4 °C.

The DNA pellet was suspended in 75% ethanol (1.5-2 mL of 75% ethanol per mL of RNAzol reagent), stored for 20 min at room temperature (with periodic mixing), and centrifuged at 2000 × *g* for 5 min at 4 °C.

The DNA was air-dried for 5-15 min in an open tube. The DNA isolated from 10^6 cells was dissolved by adding 20-60 µL of 8 mmol/L NaOH, such that the concentration of the isolated DNA reached 0.2-0.3 µg/µL.

Protein isolation

Duration: Approximately 1 h.

For protein isolation, 1.5 mL of isopropanol was added to the previously obtained phenol-ethanol supernatant (approximate volume: 0.8-1 mL of RNAzol reagent), and allowed to stand at room temperature for 10 min.

The protein precipitate was sedimented by centrifugation at 12000 × *g* for 10 min at 4 °C, and the supernatant was discarded.

A 2 mL solution of 0.3 M guanidine hydrochloride in 95% ethanol was added to wash the protein pellet, thrice. During each wash cycle, the protein pellet was kept in the wash solution for 20 min at room temperature (15-30 °C) and centrifuged at 7500 × *g* for 5 min at 4 °C. After the final wash, the protein pellet was vortexed in 2 mL ethanol, stored in ethanol for 20 min at room temperature, and finally centrifuged at 7500 × *g* for 5 min at 4 °C.

The protein pellet was air-dried for 5-10 min and dissolved in 50 µL of 1% SDS by pipetting. Notably, the complete dissolution of the protein pellet might require incubating the sample at 50 °C. Any insoluble material was sedimented by centrifugation at 10000 × *g* for 10 min at 4 °C, and the supernatant was transferred to a fresh tube. A 1 µL aliquot of the sample was subsequently used for detecting the concentration and purity of the isolated proteins, and 10 µL of the sample was used for western blotting. The remainder was stored at −20 °C for future use.

RESULTS

The expected yields of RNA, DNA, and proteins from 1 × 10^6 cultured HepG2 cells are 5-10 µg, 4-7 µg, and 10-12 µg, respectively. Nucleic acids and proteins have an $A_{280}/A_{260} \geq 1.8$ when diluted with Tris-EDTA buffer (TE; 10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0) and 0.1% SDS, respectively.

Electrophoresis of the isolated RNA on a formaldehyde-denaturing agarose gel and subsequent staining with ethidium bromide revealed discrete bands of high molecular weight, corresponding to RNA molecules of size 7-15 kb (mRNAs and hnRNAs), two predominant ribosomal RNA bands of size ~5 kb and ~2 kb (28S and 18S rRNAs, respectively), and a low molecular weight RNA molecule of 0.1-0.3 kb (tRNA, 5S) (Figure 1).

The total RNA obtained using this method is free from contamination with protein and DNA and can be used for molecular cloning (RT-PCR) (Figure 2).

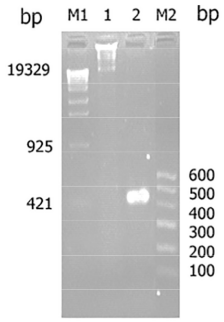
The DNA isolated using this method can be used for the detection of integrated foreign genetic materials by using PCR and restriction endonucleases.

The resulting protein isolated by this method can be analyzed for the presence of specific proteins by western blotting (Figure 3).

Notes

The cells should be lysed directly in a culture dish with appropriate RNAzol reagent only when they are in the exponential phase of growth, *i.e.* only cells in the exponential phase of growth should be used for isolation.

Disposable gloves should be worn always, and sterile, disposable plasticware and automatic pipettes should be reserved for RNA isolation. The glassware and plasticware should be kept RNase-free during RNA isolation to protect the RNA from contamination and degradation by RNases.



M1 λDNA/Eco 1301
1 Genomic DNA
2 c-Myc fragment
M2 DNA 100 bp marker

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Figure 2 Identification of genomic DNA and determination of the quality of the total RNA isolated from cultured HepG2 cells. Electrophoresis of the genomic DNA and products of reverse transcription (RT)-polymerase chain reaction (PCR) amplification of the c-Myc fragment on a 2.0% agarose gel and subsequent staining with 0.5 mg/mL ethidium bromide revealed the presence of 1.0-20 kb genomic DNA fragments (Lane 1), and a 500 bp c-Myc fragment that was amplified by RT-PCR using the total RNA as the template (Lane 2).

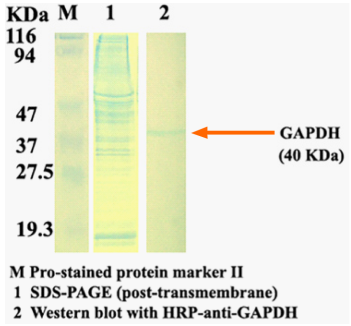

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Figure 3 Identification of the quality of the total proteins isolated from the same cultured HepG2 cells. The total proteins were separated on 12% gels by SDS-PAGE (Lane 1). Following transfer onto a polyvinylidene fluoride membrane, the 40-kDa GAPDH-specific band was blotted with HRP-anti-GAPDH and observed using sediment type mono-ingredient TMB substrate solution (Lane 2).

The isolated RNA and DNA samples should not be dried by centrifugation under vacuum.

DISCUSSION

Understanding the functions of genes in the postgenomic era is crucial for deciphering cellular mechanisms. Therefore, the development of effective methods of cell culture and novel techniques for the isolation of RNA, DNA, and proteins from cultured cells, especially the microextraction of nucleic acids and proteins from < 10^6 cultured cells, have become increasingly important. The protocol described herein offers such a method for isolating nucleic acids and proteins with relative rapidity and efficiency. The results demonstrate that the procedure allows the sequential isolation of RNA, DNA, and protein with high purity and integrity. The advantage of this method is that it allows the almost synchronous isolation of nucleic acids and proteins from the same cultured cells, which not only saves time, money, manpower, and material resources, but also preserves the identity of the isolated materials and enhances the reproducibility and reliability of the experimental results.

RNAzol is a ready-to-use reagent that is used for the isolation of total RNA from cells. The reagent consists of a monophasic solution of phenol and guanidine isothiocyanate, and the RNAzol-based technique of RNA isolation is superior to the single-step RNA isolation method developed by Chomczynski and Sacchi[8,9]. RNAzol maintains the integrity of the RNA during cell lysis and dissolution of cellular components. The addition of chloroform followed by centrifugation separates the solution into an aqueous phase and an organic phase, and the RNA remains exclusively in the aqueous phase. The RNA is recovered by precipitating the aqueous phase with isopropyl alcohol. The DNA in the interphase layer can then be isolated by precipitation with ethanol, and the proteins can be isolated from the organic phase by an additional precipitation step with isopropyl alcohol[4,10].

In the classical isolation methods, RNA, DNA, and proteins are extracted independently, which requires the preparation of a triplet group of cells as well as several reagents that need to be added separately during isolation. These protocols involve numerous steps and are time-consuming. In these conditions, RNA can easily be contaminated and

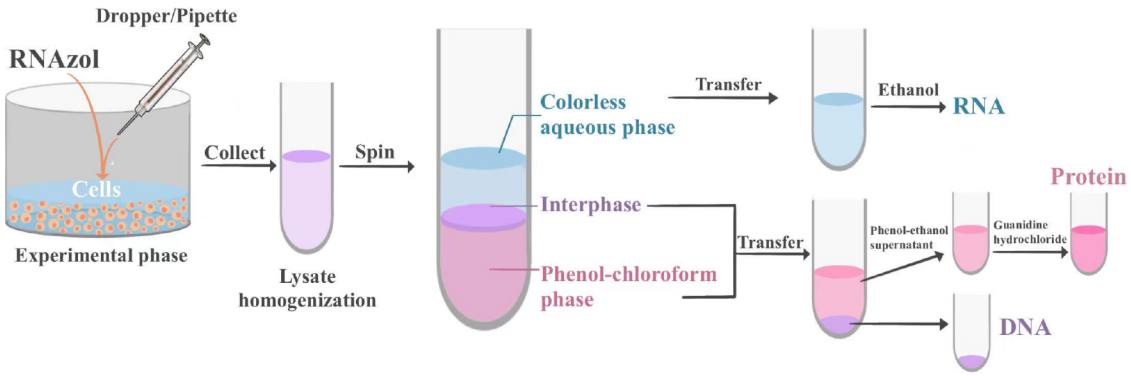

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Figure 4 Flow chart of ENAP protocol.

degraded by extraneous RNases. Therefore, the operational difficulty of the classical isolation methods is higher and the chances of successful isolation are decreased. Additionally, the various commercially available kits for the extraction of nucleic acids are expensive. RNAzol reagent facilitates the sequential isolation of RNA, DNA, and proteins from the same cultured cells, and preserves the identity of the isolated materials. The method developed herein has several advantages, including good comparability and reproducibility, a simple protocol, short duration of experimentation, improved work efficiency, reduced chance of RNA degradation, and it does not require the use of proteinase inhibitors for isolating single proteins. The quality of the isolated RNA, DNA, and proteins was validated by formaldehyde-denaturing agarose gel electrophoresis, RT-PCR, SDS-PAGE, and western blotting. And the findings reveal that the isolated nucleic acids and proteins can be used in molecular biology studies. Of note, this protocol is not suitable for lifelong cells without proliferative ability, *e.g.* neural cells and myocardial cells, or cells with relatively weak proliferative ability, *e.g.* stem cells with relatively small numbers.

CONCLUSION

Together, the present study describes a novel protocol for the sequential micro-extraction of RNA, DNA, and proteins from the same cells (Figure 4). The procedure has easy operation, allows rapid isolation, has high efficiency, and is economical and practical, especially for researchers in developing countries.

ARTICLE HIGHLIGHTS

Research background

Life is a way of material (mainly protein and nucleic acid) movement and health lies in movement. Cell is the most fundamental structural and functional unit of life, therefore, the effective isolation of nucleic acids and proteins from cells is the foundation and prerequisite for revealing the mysteries of life. However, during laboratory routine for isolation of nucleic acids and proteins, cell samples are often from different culture dishes, usually leading to inevitable experimental errors and sometimes poor repeatability.

Research motivation

The present research tries to explore the possibility to simultaneously isolate nucleic acids and proteins from the same sample, while reducing experimental errors and ensuring consistency during experimentation.

Research objectives

The present study established a selective protocol for sequential isolation of RNA, DNA and proteins from the same cells with the characteristics of easy operation, rapid extraction and high efficiency.

Research methods

RNAzol reagent was used for the sequential isolation of RNA, DNA, and proteins from the same cultured HepG2 cells, resulting in a novel protocol containing four steps.

Research results

A protocol for sequential isolation of RNA, DNA and proteins was established and the procedure takes as few as 3-4 d from the start to quality verification and is highly efficient.

Research conclusions

The quality of RNA, DNA and proteins isolated through sequential isolation protocol can be used for reverse transcription (RT) - polymerase chain reaction (PCR), PCR and western blot, respectively.

Research perspectives

The present procedure is not only easy, rapid and high efficient, but also economical and practical, especially for researchers in developing and underdeveloped countries.

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FOOTNOTES

Author contributions: Cui YY designed and performed the research, and analyzed the data and wrote the manuscript.

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ORIGINAL ARTICLE

Basic Study

Urine exosome mRNA-based test for monitoring kidney allograft rejection: Effects of sample transportation and storage, and interference substances

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Abstract

BACKGROUND

Exosomes are 30-150 nm nanovesicles with sophisticated nucleic acids cargo, actively secreted by all cells within human body, and found in abundance in all body fluids, including urine. These extracellular vesicles have tremendous potential for next generation diagnostics, theoretically enabling noninvasive assessment of organ and tissue function *via* liquid biopsy analysis.

AIM

Recently, feasibility of an exosomal molecular test was demonstrated for post-organ transplant monitoring: Analysis of urine-derived exosomal mRNA cargo allowed early detection of kidney allograft rejection. Here, we further studied urine-derived exosomes and their mRNA content as a highly promising diagnostic modality. This included stability studies of urine samples and exosomal mRNA upon transportation from the point of collection to a centralized testing facility, short-term storage of urine at different conditions upon receipt till the point molecular assay is performed, and effects of various potentially interfering substances on the downstream quantitative polymerase chain reaction (qPCR) assay.

METHODS

The urine specimens were stored at various conditions and pre-processed in different ways. Next, samples were passed through the columns to capture all extracellular vesicles, the vesicles were lysed to release their content and the exosomal RNA was purified on the mini-columns, reverse transcription was performed, next pre-amplification, followed by a qPCR analysis for a panel of

mRNA markers.

RESULTS

To ensure exosomal RNA integrity, the harvested urine specimens should be shipped refrigerated, by overnight delivery. Urine can next be stored at the test site for up to 1 wk at 4 °C, and long term should be frozen at -80 °C. Urine specimens must be centrifuge at low G-force to deplete cells and debris, to ensure consistent top results in downstream molecular assays. All commonly used medications (tacrolimus, cyclosporin A, mycophenolic acid, everolimus, sirolimus, ascomycin, teriflunomide) were tested and confirmed that they do not cause assay interference.

CONCLUSION

mRNA from urine-derived exosomes was shown to be stable across a broad range of conditions and produced accurate results when analyzed *via* qPCR assay for detection of kidney allograft rejection. We identified the most optimal conditions for every step of the process, ensuring pre-analytical sample integrity and robust qPCR results.

Key Words: Kidney allograft; Post-transplant monitoring; Liquid biopsy; Exosome; mRNA; Quantitative polymerase chain reaction

Core Tip: Recently, it was demonstrated that analysis of urine-derived exosomal mRNA allows early detection of kidney allograft rejection. We further studied exosomes and their mRNA content as a highly promising diagnostic modality. This included stability studies of urine samples and exo-mRNA upon transportation from the point of collection to centralized testing facility, short-term storage of urine at different conditions upon receipt till the point molecular assay is performed, and effects of various interfering substances on the quantitative polymerase chain reaction assay. mRNA from the urine-derived exosomes was proven to be stable across broad range of conditions and produce robust results in post-transplant monitoring assays.

Citation: McFaul M, Ventura C, Evans S, Dundar H, Rumpler MJ, McCloskey C, Lowe D, Vlassov AV. Urine exosome mRNA-based test for monitoring kidney allograft rejection: Effects of sample transportation and storage, and interference substances. *World J Methodol* 2023; 13(5): 492-501

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INTRODUCTION

There is an urgent need for advanced post-transplant monitoring molecular tests, allowing to rapidly determine allograft rejection at the earliest stage using non-invasively collected patients' samples. At the moment one of the most widely utilized tests is serum creatinine, which has a number of limitations[1,2]. The next gen molecular assay, now rather widely used in laboratories world-wide is based on cell free DNA[3,4]. This test analyses percentage of the donor-derived (dd) cfDNA in the blood of organ recipient, and if the fraction increases over time and crosses a predetermined threshold (typically 1% for kidney transplants)- this indicates organ rejection. Although this test was already commercialized a few years ago, its specificity is suboptimal, as other-than-allograft rejection events can also trigger increase in dd cfDNA percentage. This test does not work well for detection of the early stage organ rejection (TCMR1a). Also, this approach is minimally invasive, rather than non-invasive- as it requires the collection of a patient's blood (which is an inconvenience for immunosuppressed individuals). Consequently, clinicians still need sensitive and specific biomarkers that overcome the above listed limitations of cfDNA-based tests for post-transplant monitoring.

Exosomes are recently discovered nanovesicles secreted by all cells within human body and found in abundance in all body fluids. They contain very sophisticated cargo of nucleic acids and proteins, reflecting the content of parental cells[5-9]. Exosomes and other extracellular vesicles have tremendous potential as biomarkers for disease diagnosis, as they theoretically should allow access to the health state of all organs and tissues *via* liquid biopsy analysis, using not only blood, but also urine, saliva, and other non-invasively collected samples[10-13]. The value of urinary exosomes in post-transplant diagnostics was demonstrated in a recent study for early detection of kidney transplant rejection[14]. Notably, urine collections are better suited for active surveillance monitoring compared to frequent blood draws and urinary exosomes exhibit promising clinical utility for detecting early kidney allograft rejection and stratifying rejection etiology.

In this study, we further characterized the aforementioned assay to understand the preanalytical limitations for an eventual migration into the clinical diagnostic domain. An emphasis was placed on the viability of urine as a matrix to facilitate at home collections from post-kidney transplant patients and correlate expected outcomes for the advanced exosome assay. This included the stability of exosomal mRNA in urine upon transportation from the point of collection to a centralized testing facility, the storage of urine samples at different conditions upon receipt until the point molecular

assay is performed, and the effect of various potentially interfering substances on the downstream quantitative polymerase chain reaction (qPCR) assay. mRNA from the urine-derived exosomes was shown to be stable in a broad range of conditions and produced accurate results when analyzed *via* qPCR assay for detection of kidney allograft rejection. We identified the most optimal conditions for every step of the process, ensuring preanalytical sample integrity and robust qPCR results.

MATERIALS AND METHODS

Specimen transportation boxes

The following box was utilized in studies on transportation of urine specimens: Small box cat #56519 (Therapak); exterior dimensions: 11 × 9 × 11 in; inner dimensions: 8 × 6 × 8 in; wall thickness: 1.5 in. Gel packs (12 oz Gel Pack #PP12 (Sonoco)) were frozen overnight at -20 °C and placed in the boxes, to refrigerate urine specimens. Temperature monitor #40510 (DeltaTrak) was used to monitor temperature inside the box, up to 72 h.

Isolation of Exosomes from the human urine samples

Exosome isolation was performed according to the ExoLution protocol (Exosome Diagnostics, a Bio-Techne brand). In brief, 10 mL urine samples were combined with 2.5 mL of 5×BB buffer in 15 mL tubes. ISS-MHV positive extraction control was added to each sample and loaded onto ExoLution Columns in 50 mL tubes and centrifuged for 5 min at 5000 × *g*. Flow-through was discarded and columns were washed with 10 mL of wash buffer and re-centrifuged at 5000 × *g*. ExoLution Columns were then transferred to fresh 50 mL tubes and 550 µL of lysis mix was added directly onto the ExoLution Column membrane surface. Columns were then centrifuged for 5 min at 5000 × *g*. Flowthrough containing exosomal RNA was collected and transferred to the RNA purification step.

RNA purification

RNA was further purified according to the ExoLution protocol using silica spin columns. Briefly, after a chloroform separation, the aqueous phase was transferred to 1.5 mL reaction tubes containing ethanol and loaded onto silica spin columns. Spin columns were centrifuged for 30 sec at 11000 × *g*. The flow-through was discarded and the spin columns were re-assembled onto the next set of fresh 2 mL collection tubes. The previous steps were repeated until the entire sample was loaded onto each column.

Next, RNA Prep buffer was added to the silica spin columns, and columns were centrifuged for 30 sec at 11000 × *g*. The flow-through was discarded, and the spin columns were re-assembled on the next set of fresh 2 mL collection tubes. RNA Wash buffer was next added to the spin columns, and columns were centrifuged for 30 sec at 11000 × *g*. The flow-through was discarded, and the spin columns were re-assembled on the next set of fresh 2 mL collection tubes. The wash process was then repeated a second time.

The spin columns were centrifuged for 3 min at 16000 × *g* to dry the membranes. The 2 mL collection tubes were discarded, and the spin columns were placed in fresh 1.5 mL reaction tubes. 15 µL of TE buffer was added directly onto the silica membranes. The columns were incubated for 5 min. Purified exosomal RNA was finally eluted by column centrifugation for 1 min at 16000 × *g* and immediately carried forward to reverse transcription.

Reverse transcription

Reverse transcription (RT) reaction mix was prepared using SuperScript® VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific, cat# 11754-250): 10x SuperScript Enzyme Mix 2 µL, 5x VILO Reaction Mix 4 uL, exosomal RNA 14 µL. Samples were placed into thermocycler and were subject to the following cycling conditions: 25 °C: 10 min, 42 °C: 70 min, 85 °C: 5 min, hold: 4 °C.

Pre-amplification

Pre-Amplification was carried out as follows: TaqMan™ PreAmp Master mix (Thermo Fisher Scientific, 4488593): 12.5 uL, Primer mix 0.5 uL, RT reaction samples 12 uL. Samples were placed into thermal cycler and the following cycling parameters were utilized: Initial denaturation 95 °C for 10 min; 14 cycles: (95 °C for 15 sec, 60 °C for 4 min); hold: 4 °C.

qPCR

qPCR analysis was performed in triplicate, under the following conditions: TaqMan™ Fast Universal PCR Master Mix (Thermo Fisher Scientific, cat# 4367846): 10 µL, 20x assay mix: 1 µL[11], nuclease-free water: 7 µL, 5x diluted PreAmp sample: 2 µL. qPCR plates were analyzed on QuantStudio 5 (QS5) fast real-time PCR machine with 96-well 0.1 mL Block. Fast cycling conditions were utilized: initial denaturation 95 °C for 20 sec, 40 cycles: (95 °C for 1 sec, 60 °C for 20 sec).

Interference substances: Medications commonly prescribed to transplant patients

All pure drug compounds were acquired from Fisher Scientific: Teriflunomide (cat# AC467112500), Cyclosporin A (cat# AAJ6319106), Sirolimus (cat# AAJ62473MF), Everolimus (cat# AAJ60139MB), Mycophenolic acid (cat# AAJ6190509), Tacrolimus (cat# AAJ63571MF), Ascomycin (cat# AAJ66751MC), dissolved in DSMO to produce 5000× stocks, and tested for potential interference with assay at concentrations 50× exceeding expected urinary excretion level transplant patients. Expected levels: Tacrolimus: 0.53 µg/mL; Cyclosporin A: 10.5 µg/mL; Mycophenolic acid: 16.67 µg/mL; Everolimus: 0.03 µg/mL; Sirolimus: 0.10 µg/mL; Ascomycin: 0.47 µg/mL; Teriflunomide: 18.33 µg/mL.

RESULTS

Ideally, active surveillance testing for post-transplant monitoring should be facilitated through an at home non-invasive collection of the patients' specimens. Alternatively, the procedure could be performed with ease in a healthcare provider's office. Regardless of the point of collection, the specimen must be transported to a centralized laboratory, accessioned and temporarily stored prior to analysis.

The objective of this study was to explore stability of urinary exosomes and their diagnostic value with a previously described analytical method[14] for the detection and stratification of kidney allograft rejection. Briefly, in this assay the urine sample is first passed through the column to capture all extracellular vesicles, next the vesicles are lysed to release their content and the exosomal RNA is purified on the mini-column, reverse transcription is performed, next pre-amplification, followed by a qPCR analysis for a panel of mRNA markers. The output Ct values are analyzed by an algorithm that generates the scores that allow determination of rejection for kidney allograft, and type of rejection: TCMR *vs* ABMR. The entire assay takes less than 6 h to complete, which enables the laboratory to produce rapid "same day" results.

Taking into account the novelty of urine as a sample of choice for this molecular assay, and also exosomes, particularly their RNA cargo, as markers of organ rejection, it is of high importance to characterize every step in more detail, to ensure subsequent smooth clinical implementation of this test. Here we explored: (1) Stability of urine specimens and exosomal mRNA upon transportation from the point of collection to testing facilities, (2) storage of urine samples at different conditions upon receipt till the point molecular assay is performed, and (3) effect of various potentially interfering substances on the downstream qPCR assay.

Stability of exosomal mRNA upon urine transportation at different temperatures, and effects of various urine upfront processing techniques

Stability of exosomal mRNA upon preanalytical conditions mimicking urine transportation at different temperatures, and effects of various urine upfront processing techniques were studied in detail. Results are shown in Figure 1. Urine specimens were derived from 4 donors, and processed following the complete workflow, with qPCR assay performed for a panel of 9 mRNA targets. Preanalytical conditions mimicking specimen shipment and urine processing included: 2 d storage at +4 °C; 2 d at +20 °C; 2 d at +40 °C; whole urine frozen and thawed (mimicking transportation in frozen state); urine pre-processed to remove cells and debris by centrifugation for 20 min at 2000 × *g*, and supernatant subject to single or double freeze/thaw cycles.

Storage of urine samples at elevated temperature, +20 °C and especially +40 °C caused gradual degradation of mRNA, as indicated by Ct (threshold cycles) shift that varied among targets and donors (as expected). While for some donors and certain mRNA targets Ct shift was minimal (< 0.5 Ct), for other donors and certain mRNA targets effects were substantial (> 5 Ct). Importantly, variable increases in Ct values introduce unpredictable changes to the outputs of algorithms analyzing levels of multiple mRNA targets and as a consequence will decrease accuracy of calculated outcomes. Normalization to PGK1 or other RNA largely addresses this issue. However, overall it is recommended that urine specimens be transported refrigerated, to ensure temperatures are consistently maintained below +20 °C.

Freezing urine specimens on dry ice and -80 °C, followed by defrosting, caused mild increase in Ct values compared to unfrozen specimens, presumably due to partial mRNA degradation upon sample thawing; 2 freeze/thaw cycles did not have significant further effects on mRNA integrity.

Specimens which were frozen without initial centrifugation at low G-force to deplete cells and debris generated somewhat variable Ct values for some samples and mRNA targets. This indicates the need for preanalytical centrifugation of urine specimens to ensure removal of interfering cellular RNA fraction (including blood-derived: See discussion below), consistent qPCR results, and assay algorithmic output scores.

In summary, for exosome-based molecular assays, the preanalytical process plays an important role in downstream assay integrity. Urine specimens must be transported under refrigeration, and centrifugation of the samples ensures depletion of cells and debris, and consistent, reproducible results. Effects of urine freezing and prolonged storage on the accuracy and reproducibility of the assay will be further discussed below.

Next, temperature of the urine specimens was monitored during conditions mimicking transportation, to identify the optimal gel pack volume that would ensure sample refrigeration (Figure 2). One, two, three or four 12 oz gel packs were frozen overnight at -20 °C and placed in the 1.5-inch thick styrofoam box, that was stored at ambient temperature. Results are shown in Figure 2.

At 24 h, which is typical for overnight sample shipment, temperature inside the box with single gel pack (12 oz) was approximately 14 °C; two, three or four gel packs successfully maintained temperature below 4 °C. By 48 h, temperature inside the boxes with 1-3 gel packs (12 oz) was > 15 °C; only four gel packs secured temperature of approximately 8 °C. By 72h, temperature inside all boxes with 1-4 gel packs (12 oz) was > 18 °C.

Overall, for molecular tests, harvested urine specimens should be shipped by next day overnight delivery, in Styrofoam boxes ≥ 1.5 inch thick with gel packs totaling 24-48 oz, to ensure optimal refrigeration and specimen temperature for 24-48 h.

Stability of exosomal mRNA upon prolonged urine storage at different temperatures

Stability of exosomal mRNA was next studied upon prolonged urine storage at different temperatures. Results are shown in Figure 3. Urine samples were derived from 4 donors, and processed following the complete workflow, with qPCR assay performed for a panel of 9 mRNA targets. Conditions included: 2 d at +4 °C; 7 d at +4 °C; 14 d at +4 °C; 4 d at -80 °C; 30 d at -80 °C.

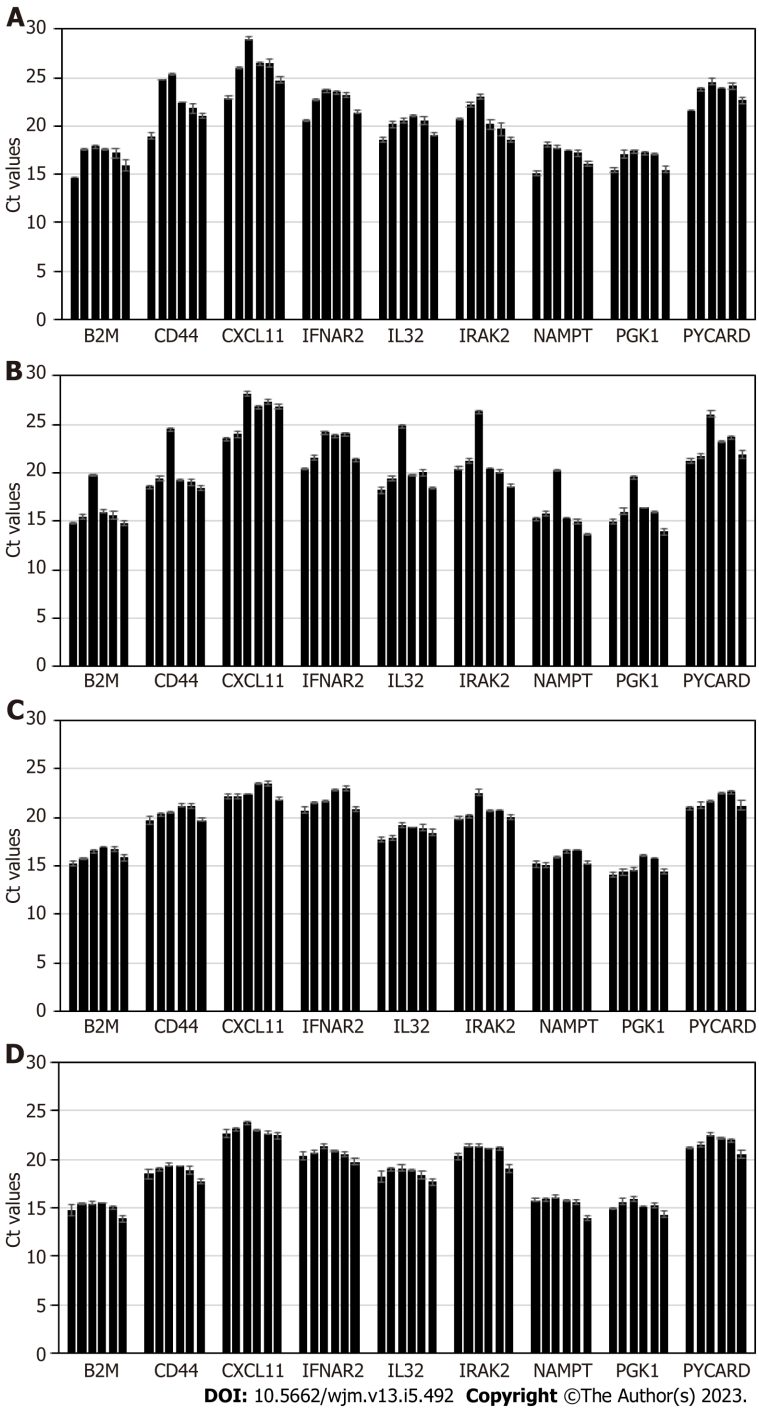

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Figure 1 Stability of exosomal mRNA upon urine transportation at different temperatures, and effects of various urine upfront processing techniques. A: Donor 1; B: Donor 2; C: Donor 3; D: Donor 4. For each donor, levels of 9 mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Conditions mimicking urine transportation and handling, left to right: 2 d at +4C; 2 d at +20C; 2 d at +40C; freeze/thaw; 2x freeze/thaw; urine freeze/thaw without pre-processing.

Storage of urine samples at +4 °C for up to one week did not affect mRNA integrity and assay outcome; at 2 wk of storage gradual degradation of mRNA is occurring, as indicated by Ct shift that varied for different targets and donor-to-donor (as expected). Thus, unfrozen urine specimens should ideally be tested within 7 d of specimen receipt and refrigerated storage.

Once samples were frozen at -80 °C, and then defrosted and tested in assay, Ct values typically shift up compared to fresh samples, and for some donors and certain mRNA targets the change is significant (> 2 Ct). Prolonged sample storage at -80 °C does not cause any further Ct increase. Thus, for the purpose of consistency and reproducibility, all urine samples should be either processed unfrozen (within a week, as stated above) or all subject to centrifugation at low G-force, frozen, then defrosted and tested at any time point. As mentioned above, normalization to PGK1 or other RNA, will ensure consistent assay performance.

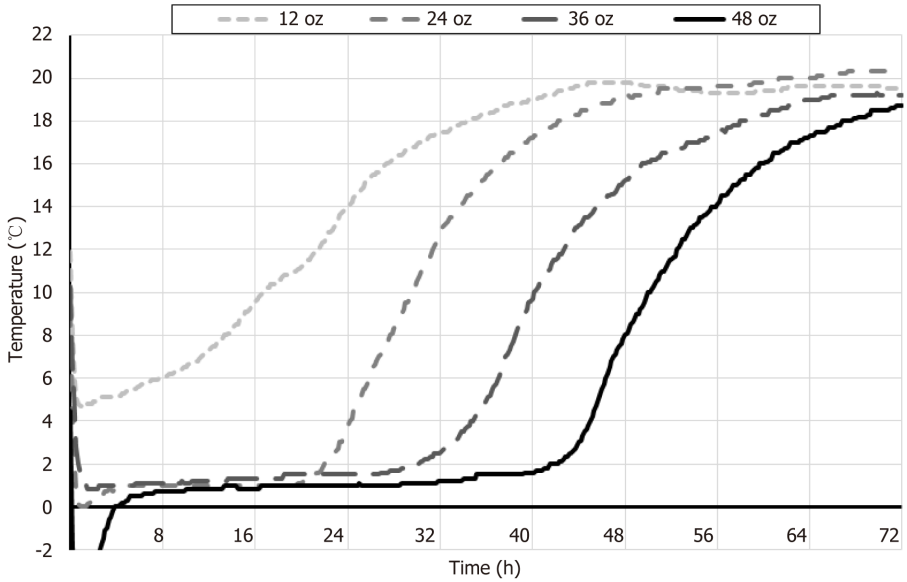

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Figure 2 Optimal refrigeration of urine specimens upon conditions mimicking shipment. One, two, three or four 12 oz gel packs were frozen overnight at -20C and placed in the 1.5 inch thick styrofoam box, to provide refrigeration for urine specimens. The graphs display temperature inside the box, over the range of 72 h.

Effect of interference substances on qPCR assay

First, medications commonly prescribed to transplant patients, were studied for potential interference with exosome molecular assay. Urine samples derived from 4 donors were utilized, and various parent drugs and metabolites were spiked in before exosome purification, at concentrations exceeding 50-fold the expected urinary excretion levels for transplant patients. Expected urine levels for immunosuppressants and other common medications were: Tacrolimus: 0.53 μg/mL; Cyclosporin A: 10.5 μg/mL; Mycophenolic acid: 16.67 μg/mL; Everolimus: 0.03 μg/mL; Sirolimus: 0.10 μg/mL; Ascomycin: 0.47 μg/mL; Teriflunomide: 18.33 μg/mL.

Samples were processed following the complete workflow, and qPCR assay was performed for a panel of 9 mRNA targets. Results are displayed in Figure 4. None of the drugs caused major interference with assay at the dose significantly exceeding the typical circulating levels in bodily fluids, as indicated by Ct values within +/- 1 compared to controls. This demonstrates that molecular assay is robust, and all common medications are depleted from the mRNA analytes during the exosome and RNA purification workflow, and the trace amounts remaining do not have negative impact on reverse transcription or real-time PCR.

Next, effect of trace amount of blood in urine was studied. Hematuria is a fairly common condition, and blood was expected to impact the performance of exosome molecular assay. Urine samples derived from 4 donors were utilized, and blood (0%-1%) was spiked into urine before exosome purification. Results are shown in Figure 5.

Blood in urine did not inhibit mRNA target detection but rather added its own "signal" (originating from blood-derived cellular mRNA) to that of the urinary exosomes. For all mRNA analytes, Ct values significantly decrease indicating several fold increase in mRNA levels due to blood-derived mRNA co-purifying with exosomal RNA. However, once the urine specimens are centrifuged (which is an obligatory part of the exosome assay, as was described above), the bulk of blood cells are successfully depleted and Ct values generated are very similar to normal urine specimens. Spiking in serum instead of blood confirmed the results - assay interference is coming from blood-derived components that can be successfully removed by centrifugation.

In case blood cells are lysed, the contents obviously cannot be easily spotted or removed from urine; however such RNA is rapidly degrading in urine that contains high levels of RNases, and thus interference with assays should be minimal – if any.

Overall, centrifugation of the urine samples ensures depletion of blood-derived cells and large debris, and consistent, reproducible results. As a precaution, urine samples with visible hematuria should be excluded for this molecular test.

DISCUSSION

Solid organ transplantation has made tremendous progress in the last decade- aided by imaging techniques, donor-recipient human leukocyte antigen matching, and immunosuppressive therapy. In the United States alone, more than 40000 organ transplants are performed annually, with kidney, liver, heart, and lung being among the most common.

Today, major challenges for transplantation are improving long-term graft viability and preserving patient quality of life. Allograft rejection represents the greatest risk of transplant failure among patients. Early identification of subclinical injury, and the subsequent differentiation of injury type, could enable earlier clinician intervention and provide

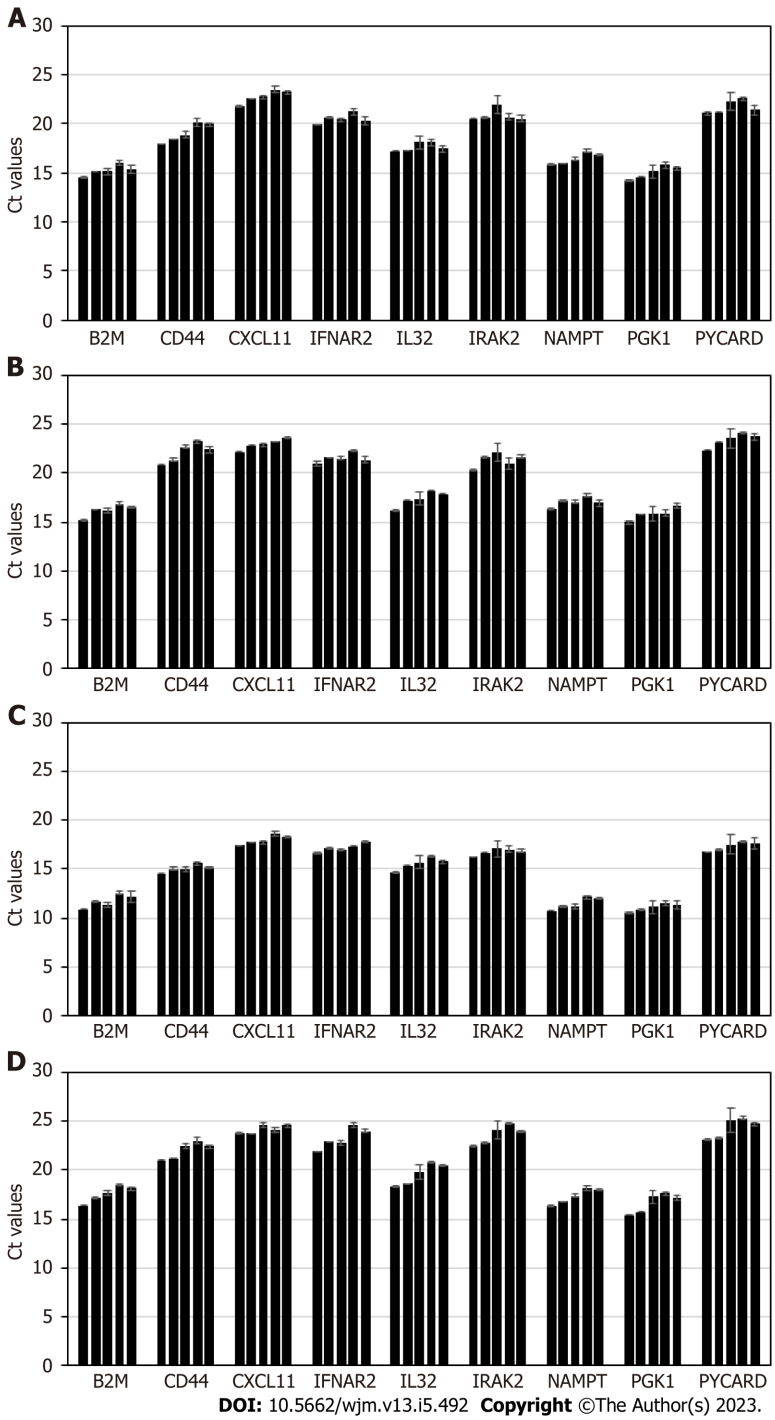

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Figure 3 Stability of exosomal mRNA upon prolonged urine storage at different temperatures. A: Donor 1; B: Donor 2; C: Donor 3; D: Donor 4. For each donor, levels of 9 mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Urine storage conditions, left to right: 2 d at +4C; 7 d at +4C; 14 d at +4C; 4 d frozen at -80C; 30 d frozen at -80C.

opportunities for personalized treatment. Further, minimally invasive methods of post-transplant monitoring could improve patients' quality of life while serving as a rejection screening tool and adjunct to histopathology.

One of the more promising discoveries in recent years has been the use of exosomal mRNA signatures to determine allograft health[14]. Urine has long been known as a valuable source of molecules serving as diagnostic markers for renal disease, and urinary exosomes have been shown to be effective screening tools for kidney allograft rejection. With herein demonstrated mRNA stability and overall robustness, urinary exosomal assays could represent a non-invasive and less burdensome approach to monitor graft health and also potentially open the door for at-home sample collections.

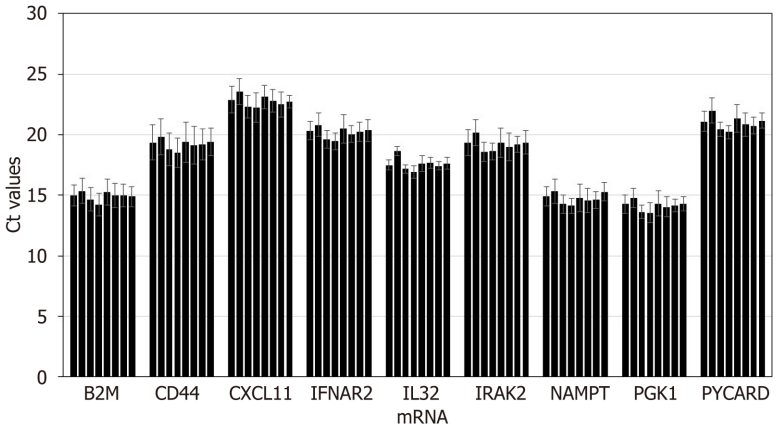

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Figure 4 Effect of common drugs on performance of the exosome molecular assay. Urine samples derived from 4 donors were utilized, and various drugs were spiked into urine before exosome purification, at levels exceeding the typical urinary concentrations by 50-fold. Nine mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Drugs, left to right: Control, Tacrolimus, Mycophenolate, Cyclosporin A, Sirolimus, Everolimus, Teriflunomide, Ascomycin.

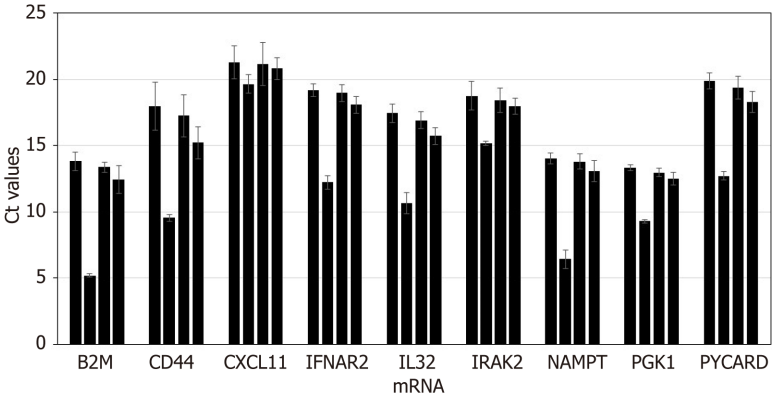

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Figure 5 Effect of blood on performance of the exosome molecular assay. Urine samples derived from 4 donors were utilized, and blood or serum was spiked into urine before exosome purification. Levels of 9 mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Conditions left to right: control urine specimens, processed following the standard protocol; urine specimens spiked with 1% blood and processed without additional centrifugation; urine specimens spiked with 1% blood and pre-processed following the standard protocol (centrifugation for 20 min at 2000 × *g*); urine specimens spiked with 1% serum and processed without additional centrifugation.

CONCLUSION

To conclude, we characterized molecular assay utilizing urine-derived exosomes. This included stability of urine samples upon transportation from the point of collection to a centralized testing facility, storage of urine at different conditions upon receipt till the point molecular assay is performed, upfront processing, and effect of various interference substances on the downstream qPCR assay. mRNA from urine-derived exosomes was shown to be stable across a broad range of conditions and produced accurate results when analyzed *via* qPCR assay for kidney allograft rejection. We identified the most optimal conditions for every step of the process, ensuring preanalytical sample integrity and robust qPCR results.

ARTICLE HIGHLIGHTS

Research background

Exosomes are nano-sized extracellular vesicles with nucleic acid and protein cargo, actively secreted by all cells within human body, and found in abundance in all body fluids, including urine. These extracellular vesicles have tremendous potential for next generation diagnostics, theoretically enabling noninvasive assessment of organ and tissue function *via* liquid biopsy analysis.

Research motivation

Recently, feasibility of an exosomal molecular test was demonstrated for post-organ transplant monitoring: analysis of urine-derived exosomal mRNA cargo allowed early detection of kidney allograft rejection. Taking into account the novelty of this approach, urine and in particular extracellular vesicles with their diverse RNA cargo have to be better characterized to ensure robustness of this molecular assay.

Research objectives

We further studied urine-derived exosomes and their mRNA content as a highly promising diagnostic modality. This included stability studies of urine samples and exosomal mRNA upon transportation from the point of collection to a centralized testing facility, short-term storage of urine at different conditions upon receipt till the point molecular assay is performed, and effects of various potentially interfering substances on the downstream quantitative polymerase chain reaction (qPCR) assay.

Research methods

The urine specimens were stored at various temperatures and conditions and pre-processed in different ways. Next, samples were passed through the columns to capture all extracellular vesicles, the vesicles were lysed to release their content and the exosomal RNA was purified on the mini-columns, reverse transcription was performed, next pre-amplification, followed by a qPCR analysis for a panel of mRNA markers.

Research results

To ensure exosomal RNA integrity, the harvested urine specimens should be shipped refrigerated, by overnight delivery. Urine can next be stored at the test site for up to 1 wk at 4 °C, and long term should be frozen at -80 °C. Urine specimens must be centrifuged at low G-force to deplete cells and debris, to ensure consistent top results in downstream molecular assays. All commonly used medications (tacrolimus, cyclosporin A, mycophenolic acid, everolimus, sirolimus, ascomycin, teriflunomide) were tested and confirmed that they do not cause assay interference.

Research conclusions

mRNA from the urine-derived exosomes was proven to be stable across a broad range of conditions and produce robust results in molecular post-transplant monitoring assays. We identified optimal conditions for every step of the workflow, ensuring pre-analytical sample integrity and robust downstream qPCR results.

Research perspectives

Exosomes and in particular their mRNA cargo have the potential to revolutionize post-transplant monitoring, and detect early rejection events for kidney as well as other allografts- based on molecular analysis of urine, saliva and other body fluids.

FOOTNOTES

Author contributions: McFaul M, Ventura C, Evans S, Dundar H performed the experiments; Rumpler MJ, McCloskey C, Lowe D, Vlassov AV planned the study and wrote the manuscript.

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CASE REPORT

Successful hip revision surgery following refracture of a modern femoral stem using a cortical window osteotomy technique: A case report and review of literature

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Abstract

BACKGROUND

The ExeterTM Universal cemented femoral component is widely used for total hip replacement surgery. Although there have been few reports of femoral component fracture, removal of a broken femoral stem can be a challenging procedure.

CASE SUMMARY

A 54-year-old man with a Dorr A femur sustained a refracture of a primary ExeterTM stem, two years after receiving a revision using a cement-within-cement technique (CWC) through an extended trochanteric osteotomy (ETO). The technical problems related to the CWC technique and the ETO played a major role in the stem fatigue refracture. We performed revision surgery and removed the distal cement using a cortical femoral window technique, followed by re-implantation with an uncemented, modular, distally-fixed uncemented stem. The patient experienced an uneventful postoperative recovery.

CONCLUSION

Re-fracture of a modern femoral ExeterTM stem is a rare event, but technical complications related to revision surgery can lead to this outcome. The cortical

window osteotomy technique can facilitate the removal of a broken stem and cement, allowing for prosthetic re-implantation under direct vision and avoiding ETO-related complications.

Key Words: Exeter stem; Femoral stem breakage; Femoral osteotomy; Total hip arthroplasty; Case seport

Core Tip: We analyzed the causes of failure in a patient with an Exeter stem refracture, and discussed how to resolve it using a known but little used technique. When removing a broken stem, a window osteotomy facilitates the extraction of the distal cement and allows for prosthetic reimplantation, thereby minimizing the complications of an extended osteotomy. Finally, this preoperative technique, if correctly planned, can be performed by using ordinary instruments and does not consume host bone. This technique should be an addition to the armamentarium of a revision hip surgeon when faced with the challenge of extracting a fracture cemented femoral stem.

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INTRODUCTION

Femoral stem fracture is a rare complication in total hip replacement (THR). Over the years, the stems have been redesigned and failure has been greatly minimized because of improved designs, the use of modern materials, and better cementation techniques[1]. The ExeterTM stem (Stryker, Newbury, United Kingdom) has shown excellent long-term survival[2,3]. Fractures are an extremely rare complication with a rate of around 0.2%[4,5]. Fracture of this particular design has already been reported, and was mainly associated with the original stem in the 70s[6-9]. However, as far as we are concerned, presented here is the first case of a re-fracture of an ExeterTM stem in the same patient. Removal of a broken femoral component is a technically demanding procedure and several techniques have been described to treat this complication[10-14] and to preserve as much bone as possible. In this case report, we described a patient with an ExeterTM stem re-fracture, analyzed the possible causes of this complication and surgically managed the case by using a simple technique that aimed to preserve bone and the abductor mechanism.

CASE PRESENTATION

Chief complaints

The patient's chief complaint involves post-operative hip pain, with an absence of concomitant traumatic antecedents.

History of present illness

A 54-year-old man [170 cm, 90 kg, body mass index (BMI) 31.1 kg/m^2] was admitted to another center 7 years ago for a one-stage bilateral THR due to osteoarthritis.

Five years later, he started to develop pain in his right thigh without previous trauma and was diagnosed with a fracture in the mid-proximal third of the femoral stem (Figure 1A and B). On reviewing his postoperative note, an ExeterTM V40TM/TridentTM X3TM (Stryker, Newbury, United Kingdom) hybrid right THR was implanted through a postero-lateral approach, using a 44 mm stem and a 50 mm cup was placed with a 32 mm ceramic (alumina) BioloxTM Forte (CeramTec, Plochingen, Germany) femoral head and a 10 highly cross-linked polyethylene (HXLPE). At revision surgery, the broken femoral stem was removed using an ETO, and a new ExeterTM V40TM stem was implanted by employing a CWC using the same stem size as the first intervention (Figure 1C). The patient evolved favorably without pain and returned to his functional status. Two years after revision the patient came to our institution, presenting pain in the operated limb, without reporting any associated trauma. Antero-posterior (AP) X-ray of both hips was taken, showing a new fracture of the right stem at the level of the mid-distal third, without apparent bone fracture and with the distal cement mantle undamaged (Figure 1D). The patient was scheduled for a femoral revision with a cement-less distal fixation stem. A minimally invasive window on the lateral cortex of the femur was made, to extract the cement mantle and the distal part of the fractured stem.

History of past illness

The information in question was not required for the case report.

Personal and family history
The information in question was not required for the case report.

Physical examination
Two years after revision the patient came to our institution, presenting pain in the operated limb, without reporting any associated trauma.

Laboratory examinations
The information in question was not required for the case report.

Imaging examinations
AP X-ray of both hips was taken, showing a new fracture of the right stem at the level of the mid-distal third, without apparent bone fracture and with the distal cement mantle undamaged (Figure 1D).

FINAL DIAGNOSIS
New fracture of the right stem at the level of the mid-distal third, without apparent bone fracture and with the distal cement mantle undamaged.

TREATMENT
Surgical technique
The following technique provides a method of "windowing" the femur, facilitating cement removal and firm fixation of the new prosthesis, in this case, an uncemented distally-fixed, RestorationTM Modular stem (Stryker, Newbury, United Kingdom). Preoperative radiographs must include an AP pelvic radiography covering both hips. Femoral radiographs should include the whole bone in AP as well as lateral (L) views. Calibration of the image is recommended in order to detect Dorr A narrow femoral canals like this one in which an uncemented, distally-fixed stem may not easily fit. In this case we used the known diameter of the failed femoral head, which was 32 mm, to calibrate the radiographs.

The patient was placed on the lateral decubitus position, with two anterior and one posterior supports in order to keep the pelvis stable and well-oriented during the whole surgery. A postero-lateral approach was used. The length was calculated with the previous preoperative planning on the radiography, measuring the distance from the tip of the greater trochanter to the end of the cement and the cement restrictor. The fascia lata and gluteal fascia were divided in line with skin incision. The fibers of the gluteus maximus were separated bluntly in line with skin incision. The sciatic nerve was then identified and protected.

When the patient reported a history of more than one revision, the fascia might have been scarred with the vastus externus, and dissection should be performed to separate these two different structures. Once the plane has been divided, a Charnley retractor was positioned, taking both sides of the fascia lata. The proximal cement and the proximal part of the fractured stem were removed with specially-designed chisels that had to be inserted between the cement and the bone, and then the cement was gently smashed with a light hammer (Figure 2A). All the proximal cement that was under direct vision was removed proximally, leaving the unseen polymethylmethacrylate and the cement restrictor to be resected through the cortical window. Direct vision of the removal prevented a cortical femoral perforation that could lead to an intraoperative femoral fracture. All necrotic tissues, pseudomembranes end cement were removed in order to maximize contact between the uncemented revision stem and the host bone. A cement chisel was introduced into the femoral canal, with the leg in the femoral position in order to measure the length of the cement where the tip of the stem was implanted. The vastus lateralis was detached and the cortical window was drawn with a surgical marker (Figure 2B). The cortical window could be made laterally or anterolaterally. The first option makes it easier for the surgeon to perform the windowing and to remove cement under direct vision. The second option has been advocated by some authors for its benefit of a lower incidence of postoperative femoral fracture[16]. But in all the cases this window is indicated, a longer stem bypassing at least 2 diaphyseal femoral diameters must be implanted.

The cortical window was made with a narrow saw in order to achieve maximal control of the cut (Figure 2B) and then it was detached with gentle chiseling (Figure 2C). The window was kept in a soaked swab until the femoral reconstruction was complete. Once the cement mantle was partially removed with a cement chisel, the fractured distal part of the cemented stem could be observed under direct vision. The next step was to impact the fractured stem from its tip in order to remove it retrogradely (Figure 2D). The window also allows for direct vision of the cement mantle as well as the cement restrictor. The distal part of the Exeter stem was removed. It is important to notice that this Exeter stem has been deficiently cemented proximally, and well-cemented distally, but the surgeon forgot to use the centralizer, which is of utmost importance for the proper functioning of this implant.

Femoral broaching was performed in order to achieve good contact between the host bone and the distal fixation stem. After this, the definitive conical distal stem was impacted into the femoral canal as preoperatively planned, under direct vision through the window, to achieve rotational stability and prevent subsidence (Figure 3A and B). Proximal cone reaming was then performed to prepare for the cone body placement. A BioloxTM Delta (CeramTec, Plochingen, Germany) 32 mm ceramic femoral head was implanted, retaining the highly cross-linked polyethylene, which was in

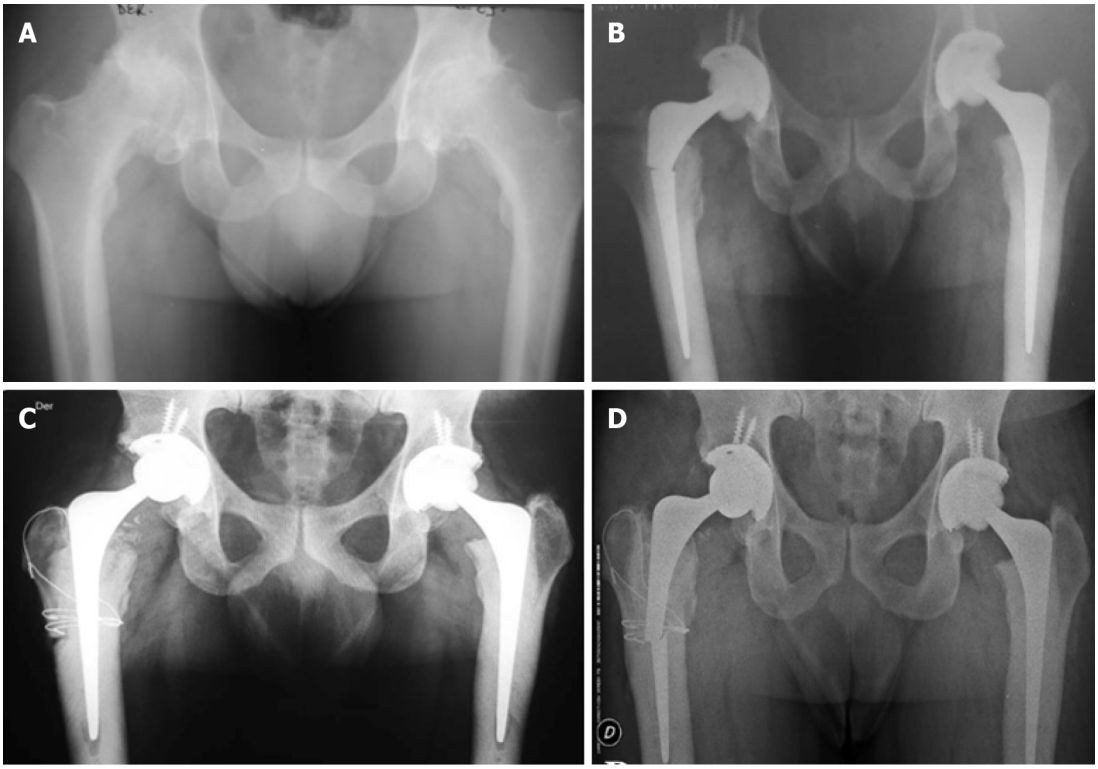

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Figure 1 X-ray. A: Antero-posterior preoperative X-ray of both hips where severe bilateral osteoarthritis is observed. A marked narrow endomedular canal is observed; B: Anteroposterior X-ray of both hips 5 years after operation, showing rupture at the proximal middle third level of the femoral stem; C: Immediate postoperative X-ray, showing femoral revision surgery. Trochanteric osteotomy and a poor proximal cement mantle are shown; D: Anteroposterior X-ray of both hips, 2 years after revision surgery, showing a further rupture of the Exeter® stem, at the level of prior femoral osteotomy.

good condition. Finally, reduction, stability test and limb length measurement were performed. Once procedure was finished, the cortical window was covered and fixed with wire cerclages (Figure 3C). Final postoperative AP X-rays exhibited excellent implant alignment, and the femoral windows was correctly bypassed with the new implant (Figure 3D).

OUTCOME AND FOLLOW-UP

Postoperative, routine exercises included isometric exercises of the lower limbs and ambulation with a walker was allowed as tolerated from the first postoperative day. 48 h later, the patient was discharged home, with good pain tolerance and without clinical complications. Six months after operation, the patient had no limitations in activities of daily living. The most recent radiographic control showed stable prosthetic implants in correct position, and complete healing of the window osteotomy (Figure 4A and B).

DISCUSSION

This case report describes two consecutive mechanical complications of a polished tapered high offset stem in a 54-year-old man having receiving operation in another center. The patient had a BMI > 30 and a femur with a Dorr A narrow medullary canal. Of note, a revision surgery was performed by employing a similar surgical technique, adding an ETO and using the CWC technique, and inserting an implant with the identical characteristics, that led to a refracture of the femoral stem. In our institution, the patient was revised to an uncemented distal fixation modular prosthesis, by removing the fractured stem through a femoral cortical window, allowing immediate full load as tolerated. The benefits of cortical windows are that this technique allows for easy removal of distal cement mantle or, as in this case, also the distal part of a broken cemented stem. Moreover, the osteotomized part can be easily fixed with some cerclage wires, it does not compromise the abductor mechanism, the patient has no restrictions to prevent a damage of this mechanism, and the stem can be fixed to an intact major trochanter, which is not possible when an ETO is performed. Furthermore, an ETO could have led to pseudoarthrosis, lack of proximal support and a new stem fracture.

In certain cases, as in Dorr A femurs, primary hip arthroplasty can be challenging. These femurs have strong thick cortices where uncemented fixation could be related to incomplete fitting of the stem because this one gets stuck in the proximal diaphysis before metaphyseal fixation occurs. Short partial neck preserving stems corresponding to the type 2B

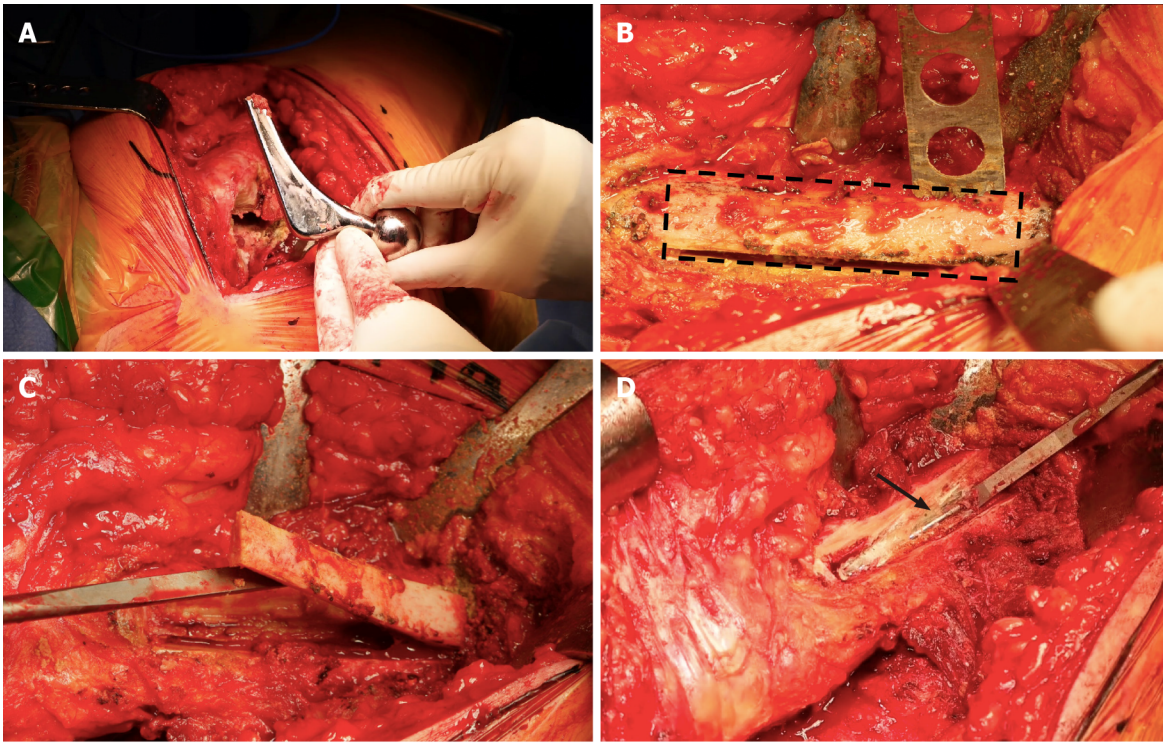

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Figure 2 Surgical operation. A: Intraoperative images of implant removal during re-revision surgery, showing the removal of the proximal part of the broken femoral stem; B: Planning and execution of lateral or window femoral osteotomy (black dotted line) with oscillating saw; C: Osteotomized segment of the window and exposure of the medullary canal; D: Removing the cement and distal segment of the stem (black arrow).

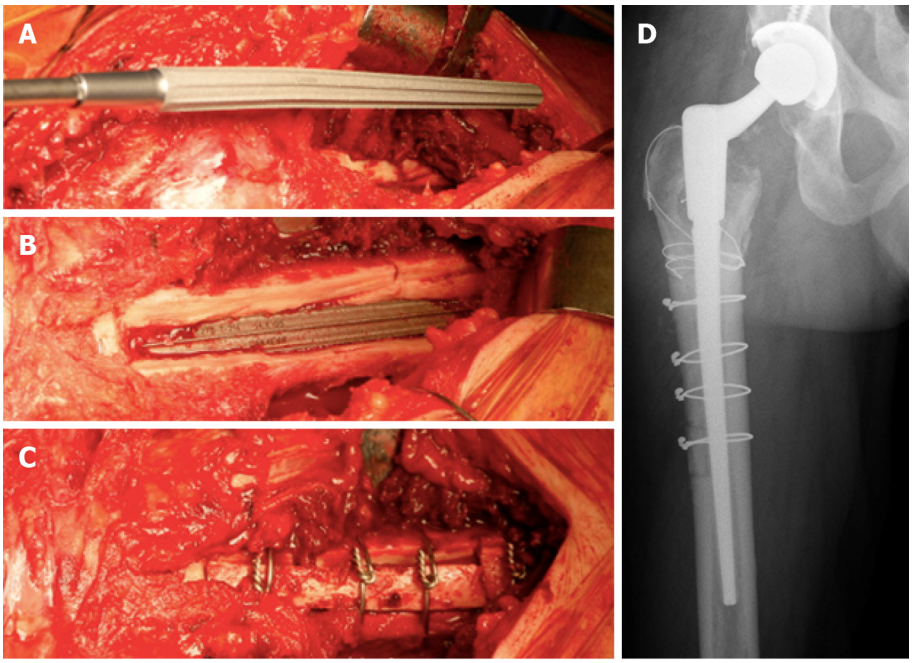

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Figure 3 Femoral broaching was performed in order to achieve good contact between the host bone and the distal fixation stem. A: Intraoperative images during re-revision surgery. Definitive cementless distal fixation modular stem; B: Direct view of the insertion of the new stem, through the femoral window; C: Osteosynthesis of the osteotomized segment with wires; D: A femur Anteroposterior X-ray, where the final immediate postoperative result is shown with an uncemented distal fixation femoral stem and osteosynthesis of the cortical window.

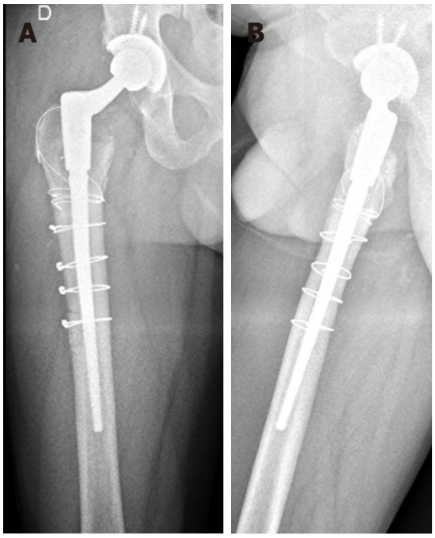

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Figure 4 Antero-posterior and lateral X-ray. A: Lateral X-ray; B: Antero-posterior X-ray. Antero-posterior and lateral X-ray 3 mo after re-revision surgery, in which the correct position of the femoral implant and consolidation of osteotomy are shown.

of the Khanuja *et al*[17] classification, could be an alternative in young patients with Dorr A femurs, since the fixation is more proximal and the femoral canal could be less invaded[18].

Since the introduction of the ExeterTM Universal Stem in 1988, there have been many reports on stem fracture, with the cause being multifactorial[19-25]. Factors such as poor medial support, insufficient proximal cement mantle, varus orientation of the stem, and increased patient weight (or a combination of these factors) have been considered. Fracture of the femoral component of the cemented total hip prosthesis is a rare but documented complication[26], with incidence ranging from 0.23% to 10.7%. In the current case, implantation of an under-sized stem, usually in a "champagne glass" femur, contributed to fracture in the body of the prosthesis. Although the fracture of a polished conical femoral stem is a rare event, a defect in the cementation technique can lead to a higher stress and suffices to increase the risk of fatigue rupture even in non-obese patients. Moreover, as reported by O'Neill *et al*[27], the cement-in-cement revision could also predispose stems to the breakage of a polished stem, probably because the the new stem must be proximally implanted, with less cement mantle, which would lead to poor metaphyseal fixation, thus generating a probable cantilever effect. For this reason, the 125 mm Exeter 44 mm offset stem was developed specifically for cement-in-cement revision, being 25 mm shorter than the standard stem and narrower in the distal and antero-posterior directions.

One of the main technical problems with a broken femoral stem is the removal of the distal part and its cement mantle. ETO is a popular and reproducible technique that can resolve most complications in a complicated THR, such as infection, aseptic loosening or a periprosthetic fracture. However, it is not without complications, and nonunion, subsidence, and trochanteric migration with subsequent Trendelenburg, at considerable rates, have been described[28-31]. The time to healing of the osteotomy and the restriction on immediate postoperative weight-bearing are considerable aspects when executing it. Several reports have described multiple methods to facilitate cement extraction and minimize complications during the procedure[32,33]. However, most of them require a protracted surgical time and may be associated with perforations of the femoral cortices or inadvertent intraoperative fractures[34].

Nelson *et al*[35] developed the original cortical window technique in 1980, creating a window on the lateral aspect of the femur. Klein *et al*[36], in a series of 21 THR revisions using a window made in the antero-lateral aspect of the femur to extract the distal cement, attained good results with an osteotomy consolidation rate of 17 wk, without thigh pain or loosening. The benefits of cortical windows are that this technique allows for easy removal of distal cement or, as in this case, also the distal part of a broken cemented stem. Moreover, the osteotomy can be easily fixed with two or three cerclage wires, it does not compromise the abductor mechanism, the patient has no restrictions to prevent the damage of this mechanism, and the stem can be fixed to an intact major trochanter, which is not possible when ETO is performed. Although preoperative planning of the window length is mandatory, it is possible to extend it if the surgeon needs to.

CONCLUSION

We analyzed the causes of failure in a patient with an Exeter stem refracture, and discussed how to resolve it using a known but little used technique. When removing a broken stem, a window osteotomy facilitates the extraction of the distal cement and allows for prosthetic reimplantation, thereby minimizing the complications of an extended osteotomy. Finally, this preoperative technique, if correctly planned, can be performed by using ordinary instruments and does not consume host bone. This technique should be an addition to the armamentarium of a revision hip surgeon when faced with the challenge of extracting a fracture cemented femoral stem.

FOOTNOTES

Author contributions: Garcia-Mansilla A collected the images and was a major contributor to the manuscript; Luco JB obtained consent from the patient and contributed to the editing of the manuscript; Buttaro M contributed to the editing of the manuscript and was the main supervisor of the project; All authors read and approved the final manuscript.

Informed consent statement: Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Conflict-of-interest statement: All the authors declare that they have no competing interests.

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