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Incorporating *Nigella sativa* nanoemulsion into gelatin-guar gum films for enhanced healing of wound infections

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ABSTRACT

Aims: This study aims to investigate the impact of incorporating *Nigella sativa* essential oil nanoemulsion (NSNE) into gelatin (Ge) and guar gum (GG)-based films at various concentrations (0%, 2%, 4%, and 6%), to evaluate the antimicrobial properties of the resulting films against common bacterial strains associated with wound infections, as well as to assess their effects on the physical and chemical properties of the films, and to create a biomaterial with the potential to be utilized as wound dressing, possessing optimal properties capable of accelerating wound infection healing.

Methods: The nanoemulsion (NE) was obtained through ultrasonic irradiation. Polydispersity index (PDI), zeta potential, and particle size of NE were measured. For film preparation, gelatin (Ge) and guar gum (GG) were used, incorporating NSNE at concentrations of 0%, 2%, 4%, and 6%. Mechanical properties were evaluated using a universal testing machine, film thickness with a micrometer, and crystalline structure through X-ray diffraction (XRD) analysis. Scanning electron microscopy (SEM) was utilized for microstructure examination, and hydrophobicity was assessed by contact angle measurements. Antimicrobial activity was determined via the disk diffusion method against bacteria relevant to wound infections. Statistical analysis employed one-way ANOVA and Tukey post hoc tests with a significance level set at 5%.

Results: The particle size, PDI, and zeta potential of the NE were measured as 296±4.85 nm, 0.569±0.2, and -35.2±07 mV, respectively. The incorporation of NSNE into GE-GG-based films demonstrated promising antimicrobial efficacy against common wound infection bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The films maintained mechanical integrity, with no significant alterations in tensile strength (TS) and elongation at break (EAB) (p0.05). However, higher NSNE concentrations led to decreased hydrophobicity ($p<0.05$) and structural changes, as evidenced by increased pores and cracks observed in SEM images.

Conclusion: This study highlights the potential of NSNE-loaded films to assist in healing wound infections, combining antimicrobial properties with a biocompatible film matrix.

Keywords: Biomedical engineering, wound infection, nanoemulsion, *Nigella sativa*

INTRODUCTION

Wound care is a critical aspect of medical practice, constantly evolving with advances in medicine.[1] The process of wound healing involves the repair of the skin and underlying tissues, and it has been the subject of extensive research, including the use of medicinal plants and alternative therapies.[2] The bacteria responsible for wound infections encompass a range of pathogens, including multidrug-resistant Gram-negative bacteria such as *Pseudomonas aeruginosa (P. aeruginosa)*, *Escherichia coli (E. coli)*, and *Acinetobacter* species (spp.).[3] Additionally, *Staphylococcus aureus (S. aureus)*, *Enterobacteriaceae* family members, and *Enterococcus* spp. have been identified as common culprits in wound infections.[4,5] Furthermore, the prevalence of biofilms, where microorganisms adhere to surfaces and form a community within a self-produced matrix particularly those formed by *P. aeruginosa*, has been highlighted as a significant factor in wound infections, emphasizing the complex nature of microbial communities within infected wounds.[5,6] Wound infection is a critical issue due to its significant impact on wound healing and the overall well-being of patients. It has been noted that wound infections play a crucial role in the development of chronic wounds, leading to delays in the healing process and the current approaches to wound care are encumbered by several limitations, posing significant challenges in the treatment of chronic wounds. These limitations are multifaceted and encompass various aspects of wound care, including pathophysiological understanding, management modalities, and economic, clinical, and social impacts.

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The traditional wound dressings have several disadvantages, including the potential to cause further injury, limited antibacterial effects, and inadequate promotion of wound healing.[7] These limitations of traditional wound dressings have led to the development of novel dressings that aim to overcome these challenges.

Ge-based hydrogels have been characterized and investigated for their potential as wound dressings, showing high water uptake capacity and cell-interactive properties, which are essential for wound healing.[8,9] Besides, GG has shown potential for wound dressing applications due to its ability to form hydrogen bonding with water molecules, which is essential for maintaining a moist environment conducive to wound healing.[10]

Studies have shown that essential oils (EOs), such as those from St. John's Wort and Salvia cacaliifolia Benth., promote fibroblast migration and tissue repair, indicating their role in the wound healing process.[11,12] Furthermore, EOs have been reported to inhibit tissue remodeling-related proteins, suggesting promising wound healing properties.[13] The EO and seeds of *Nigella sativa (N. sativa)* have exhibited antimicrobial activity, making them potential candidates for combating bacterial and fungal infections.[14,15] Thymoquinone, an active compound in *N. sativa*, has been identified as having antimicrobial effects against both Gram-negative and Gram-positive bacteria, further supporting its potential for antimicrobial applications.[16]

NE form of EOs that have much smaller droplet sizes than coarse emulsions offer several advantages over coarse emulsions in microbiological applications. Their small droplet sizes provide improved emulsion homogeneity, stability against oxidation, coalescence, and creaming, enhanced solubility, and controlled release of volatile organic compounds.[17] The small droplet size of NEs expands their application options and presents a greater surface area, providing advantages over conventional macroemulsions.[18] Overall, the use of NEs in microbiological applications offers enhanced stability, improved bioavailability, and increased functionality of active compounds, making them a promising system for drug delivery and other microbiological applications.

This study introduces a novel approach by incorporating NSNE into GE/GG-based films, thereby aiming to enhance their antimicrobial efficacy for wound infection treatment. Additionally, the study evaluates the impact of NSNE incorporation on the physical and chemical properties of the films, contributing to the development of advanced wound dressing biomaterials with optimized healing properties.

METHODS

This study does not require an ethics committee approval. approval. All procedures were carried out in accordance with the ethical rules and the principles.

Preparation of *Nigella sativa* oil Nanoemulsion and Characterization

A two-step methodology was employed to generate NSNE. In the initial phase, the oil phase was formulated by blending 5% (v/v) *N. sativa* oil, 3% (v/v) ethanol, and 3% (v/v) Tween 80, followed by an incubation period at 85 °C for 1 hour. Subsequently, distilled water was added dropwise into the solution while consistently stirring at 25°C, during the second phase. The resulting blend, featuring a final concentration of 5% oil (v/v), underwent homogenization at 13,000 rpm for 5 minutes using a Wiggenhauser homogenizer (D-130, Germany). To transform the coarse emulsion into NE, the prepared mixture underwent ultrasonic irradiation in a Bandelin Electronic RK 255H ultrasonic bath (Germany) at 20°C, with a power of 160 W and a frequency of 35 kHz, for a duration of 15 minutes. The resultant NE was then preserved in a dark bottle at +4°C.

The PDI, zeta potential, and particle size values of NSNE were measured by the Malvern Zetasizer Nano ZSP instrument (Malvern Instruments Ltd., Malvern, UK). The analyses were run in triplicate.

Preparation of *Nigella sativa* oil Nanoemulsion-loaded Films

In the initial phase, 2% (w/v) Ge was hydrated in distilled water at room temperature for 30 minutes and subsequently heated to 60°C using a magnetic stirrer. After cooling to room temperature, GG was introduced to the Ge solution at a concentration of 50% (w/w, Ge/GG) and stirred at 45°C for 3 hours. Glycerol, serving as a plasticizer, was then added (20% of the weight of Ge) and stirred for an additional 15 minutes. Following this, NSNE was incorporated into the solution at concentrations of 0%, 2%, 4%, and 6% (v/v), resulting in films named Ge/GG, Ge/GG-NSNE 2%, Ge/GG-NSNE 4%, and Ge/GG-NSNE 6%, respectively.

To eliminate bubbles, the film solutions underwent stirring at a reduced speed using a magnetic stirrer for an additional 30 minutes. Approximately 20 g of each film solution was poured into petri dishes (with a diameter of 9 cm) and allowed to dry at room temperature for 24 hours. After drying, the films were detached from the petri dishes and transferred to a desiccator with a controlled environment of 25°C and a relative humidity of 50±3%, saturated with magnesium nitrate, for a duration of 48 hours.

Characterization of Films

Mechanical properties of films: The thickness of the films was determined using a micrometer (Loyka, 5203, Ankara, Turkiye) with measurements taken at five random locations on films and subsequent calculation of mean values. The TS and EAB values of the films were determined employing a universal testing machine (Testform/AS1, Ankara, Turkiye). Film samples were prepared in strips measuring 6×1 cm and then subjected to testing. The initial grip separation was set at 40 mm/min, and the crosshead speed was 50 mm/min. Each film was tested three times.

X-ray diffraction analysis: To examine the crystalline structure of the films, XRD patterns were acquired utilizing a Bruker AXS D8 Advance X-ray diffractometer (Madison, WI, USA) operating at 42 kV, 30 mA, and 1.540 A° with CuKa radiation. The spectra were recorded over a range of 2θ angles from 5 to 60°C at room temperature.

Microstructure of films: The surface morphology of the film samples was investigated using a SEM (Carl Zeiss Gemini 300, Germany) after gold coating. The samples were examined under low vacuum conditions at a voltage of 15 kV.

Hydrophobicity of films: The contact angles were assessed employing a Theta Attension optical tensiometer (Biolin Scientific, Gothenburg, Sweden). Approximately 5 µL of ultrapure water was delicately dispensed onto the film surface using a micro-syringe. Measurements were taken on both sides of the water droplet at room temperature, and the results were reported as the average of three determinations.

Antimicrobial Activity

To investigate the antimicrobial properties of the produced films, the disk diffusion method was used, selecting certain bacteria responsible for wound infections. The pathogens were cultured in Mueller Hinton Agar at 37 °C for 18 hours. Subsequently, they were transferred into sterile saline, and the bacterial suspension's turbidity was adjusted ~$1.5x10^6$–10^7 CFU/mL. Sterile swabs were utilized to evenly spread the suspension onto Mueller-Hinton agar plates. Films with a diameter of 12 mm were sterilized using ultraviolet irradiation for 3 minutes. Following sterilization, the films were positioned on the plates and then incubated for 24 hours at 37°C. The diameter of the inhibition zones (mm) was measured as the diameter of the film+the zone, after three repeated measurements, and recorded as the mean±standard deviation (SD).

Statistical Analysis

To assess variations in mean values, a one-way analysis of variance (ANOVA) followed by Tukey post hoc tests was conducted using SPSS software (version 22, Chicago, IL), with a predetermined significance level set at 5%.

RESULTS

Mechanical Properties of Films

Thickness and mechanical properties (TS and EAB) of films are shown Table 1. When compared to the control film (Ge/GG) the thickness of films incorporated with 2% NSNE remained unchanged, while an increase in thickness was observed in films incorporated with 4% NSNE and 6% NSNE. ($p<0.05$). The increase in film thickness can be attributed to the rising oil content in the film. The films incorporated with NSNE at different concentrations retained their mechanical integrity, exhibiting no notable changes in TS and EAB values ($p>0.05$).

Table 1. Thickness and mechanical properties of films

Sample	Thickness (mm)	TS (MPa)	EAB (%)
Ge/GG	0.256±0.05[a]	3.0011.146[a]	78.192±12.286[a]
Ge/GG-NSNE 2%	0.258±0.11[a]	3.2940.619[a]	65.788±5.333[a]
Ge/GG-NSNE 4%	0.262±0.03[b]	3.1030.800[a]	73.036±6.918[a]
Ge/GG-NSNE 6%	0.265±0.06[b]	2.9100.264[a]	70.918±3.665[a]

Data are mean±SD. Different letters in the same column indicate significant differences ($p<0.05$)

X-Ray Diffraction Analysis

The main peaks of Ge in XRD graphs are typically observed at 2θ of 7° and 20°, representing the triple helix structure and single left-handed helix chain of Ge. The main peaks of GG in XRD graphs are typically observed at 2θ of 15°, 17°, 18°, and 23°, indicating the characteristic crystalline structure of GG. As seen in Figure 1 the sharpness of the 2θ of 17° peaks was observed to diminish in films incorporated with NSNE at concentrations of 4% and 6%. The XRD graph illustrates the typical semi-crystalline structure of polymers, and the addition of EOs has further reduced sharp peaks.

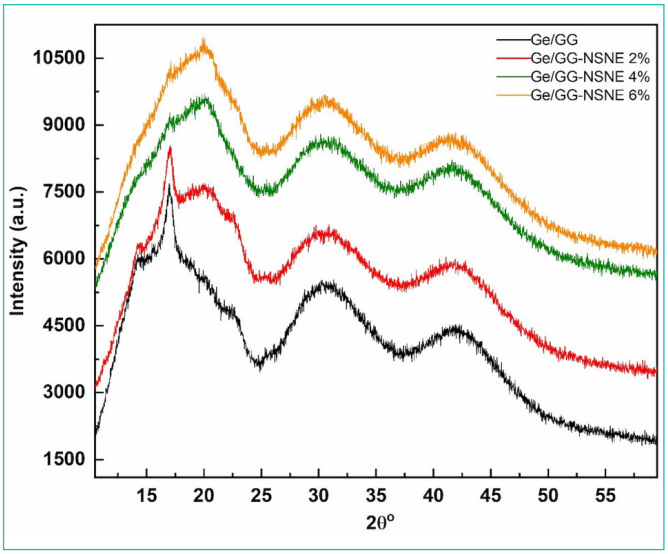

Figure 1. XRD diagram of film without NSNE and films loaded with 2% (w/w) NSNE, 4% (w/w) NSNE and 6% (w/w) NSNE

Microstructure of Films

The incorporation of NSNE has been shown to alter the microstructural characteristics of polymer films. As seen in Figure 2, the number of pores and the cracks in the films has increased proportionally with the increasing concentration of NSNE.

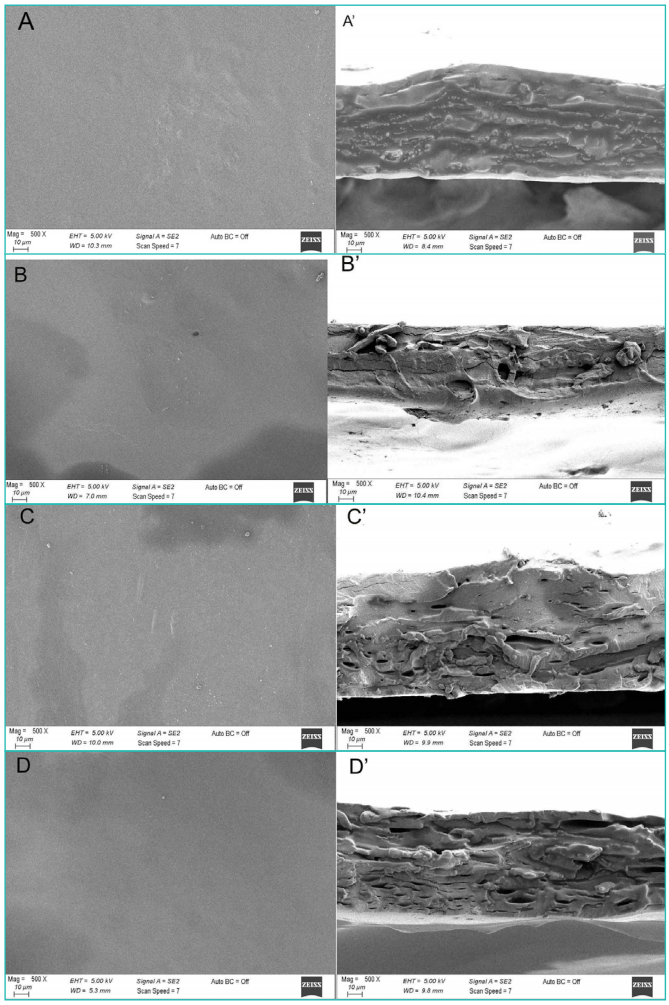

Figure 2. SEM images of surface (A, B, C, and D) and cross-section (A', B', C', and D') of the control film and films containing 2%, 4% and 6% of NSNE, respectively

Hydrophobicity of Films

Contact angles of films are given in Table 2. While it is expected that the nature of the oil would increase hydrophobicity, it was observed that the hydrophobicity remained unchanged in films incorporated with 2% and 4% NSNE and decreased in the film incorporated with 6% NSNE ($p<0.05$).

Table 2. Contact angle of films

Sample	Contact Angel (°)	Droplet photographs
Ge/GG	108.27±12.28[a]	
Ge/GG-NSNE 2%	36.47±1.68[a]	
Ge/GG-NSNE 4%	41.61±1.30[a]	
Ge/GG-NSNE 6%	25.78±1.25[b]	

Data are mean±SD. Different letters in the same column indicate significant differences ($p<0.05$)

Antimicrobial Activity of Films

The antimicrobial effects of the films against *E. coli*, *P. aeruginosa*, *Enterococcus faecalis (E. faecalis)*, *S. aureus*, and *Klebsiella pneumoniae (K. pneumoniae)* were determined. As seen in Table 3 it was observed that the effectiveness against the other bacteria, except for *S. aureus*, was not dependent on the incorporated oil ratio. While the effectiveness in films other than the control film showed a slight increase with the dose, this increase was not statistically significant ($p>0.05$). For *S. aureus*, films with 2% and 4% added oil were found to be statistically similar, but those with 6% incorporated oil exhibited a significantly higher efficacy ($p<0.05$).

DISCUSSION

NEs are a category of emulsions characterized by their transparency or translucency, with droplet sizes falling within the range of 20 to 500 nm.[19] These droplets are smaller than those found in conventional emulsions, contributing to the kinetic stability, and improved functional

Table 3. Inhibition zones of films

Strains	Zone of inhibition (mm)			
	Ge/GG	Ge/GG-NSNE 2%	Ge/GG-NSNE 4%	Ge/GG-NSNE 6%
E. coli	12.0[b]	14.36±0.37[a]	14.66±0.57[a]	14.78±0.54[a]
P. aeruginosa	12.0[c]	16,51±0.44[b]	16.08±0.80[b]	16.06±1.10[b]
E. faecalis	12.0[d]	17.23±0.25[c]	17.11±0.59[c]	17.16±0.14[c]
S. aureus	12.0[f]	13.0[e]	13.0[e]	15.23±0.25[d]
K. pneumoniae	12.0g	15.58±0.14[f]	15.50±0.43[f]	15.92±0.14[f]

Data are mean±SD. Different letters in the same row indicate significant differences ($p<0.05$)

performance of NEs.[20] Studies have shown that the droplet size of NEs can be influenced by various factors such as the type of oil used and the emulsification process.[21,22] The NE exhibited a mean particle size of 296 nm, a PDI of 0.569, and a zeta potential of -35.2 mV, indicating a moderately sized and well-stabilized colloidal system with a relatively uniform size distribution, thus holding promise for various applications in wound dressing.

NEs may disrupt the structural integrity of the films, leading to a reduction in TS.[23] However, in this study, the incorporation of NSNE did not cause any change in TS and EAB values ($p>0.05$). The mechanical properties of films with the addition of oil NE may remain unaltered due to the specific properties of the oil and its interaction with the film matrix. For instance, similar to present study, castor oil has been found not to plasticize the film matrix and may produce in situ blend compatibilizers due to the presence of certain functional groups.[24,25] Similarly, the incorporation of oils such as palm oil and essential oils has been observed to increase the elasticity and thickness of film packages without significantly affecting the TS.[26]

The addition of oils into Ge-based films has been found to have several effects on the properties of the resulting films. Incorporating oils into polymer films can indeed alter the sharp XRD peaks and reduce the crystallinity of the films. Similar to present study, the addition of limonene to polylactic acid resulted in a decrease in the degree of crystallinity due to enhanced polymer chain mobility and the plasticization effect.[27] Similarly, the incorporation of essential oils into carrageenan-based films was found to influence the mechanical properties and decrease the crystallinity of the films.[28]

The formation of pores in the film matrix can be attributed to various factors, including the reduction in intermolecular forces between the polymer chains due to the incorporation of NEs.[29] Furthermore, the incorporation of lipid compounds into hydrocolloid-based films has been reported to decrease water vapor permeability, potentially leading to the formation of pores in the film matrix.[30] The addition of EOs to film formulations has been reported to weaken the film by decreasing cohesion forces within the structure, potentially leading to the formation of pores.[31] These factors collectively contribute to the formation of pores in the film matrix following the incorporation of oil NEs.

Hydrophobicity is an important feature in film samples intended for wound dressing applications. The hydrophobic nature of the film can prevent the ingress of water and microorganisms, while maintaining a moist environment at the wound interface, which is conducive to wound healing. Additionally, hydrophobic films can effectively act as a barrier to microorganisms and remove excess exudates from the wound surface, promoting an optimal environment for wound healing.[32] The decrease in hydrophobicity when oil NE is incorporated into the film matrix can be attributed to several factors. The addition of NEs can lead to an increase in the molecular spaces between the protein, resulting in decreased hydrophobicity.[33] Furthermore, the reduction in intermolecular forces between the polymer chains due to the incorporation of NEs can contribute to the decrease in hydrophobicity. These factors collectively contribute to the observed decrease in hydrophobicity when oil NE is incorporated into the film matrix. Consistent with the present study, films developed by Acharya[34] and Mutlu[35] addition NEs to the film matrix decreases surface hydrophobicity.

The antibacterial effect of *N. sativa* has been extensively studied. Thymoquinone, an active principle of *N. sativa*, has been found to possess strong antibacterial properties against Gram-positive bacteria such as *S. aureus* and *Streptococcus* mutants.[36] Thymoquinone has been shown to have potency in preventing bacterial biofilm formation.[37] Topical gel formulations prepared using ethylacetate extract of *N. sativa* have demonstrated antibacterial properties against acne-causing microorganisms, indicating its potential as an alternative remedy for dermal acne.[38] These findings highlight the potential of *N. sativa* and its active components in combating bacterial infections and supporting skin health.

These findings collectively suggest that the developed films, particularly those supplemented with 2% and 4% NSNE, hold promise as wound dressing materials for wound healing applications. Further research could explore optimization strategies to fine-tune the material properties, considering the observed changes in hydrophobicity and microstructure. Additionally, in vivo studies would be crucial to validate the efficacy and safety of these films in practical wound care scenarios. Overall, this study contributes valuable insights into the development of functionalized films for potential biomedical applications.

CONCLUSION

The incorporation of NSNE into Ge/GG-based films demonstrated minimal impact on mechanical properties, underscoring their potential for wound healing applications. Notably, the films maintained their structural integrity, with no significant alterations in TS and EAB values, while exhibiting enhanced antimicrobial efficacy. These findings emphasize the clinical relevance of the developed films, especially those enriched with 2% and 4% NSNE, as promising wound dressings with both structural resilience and heightened antimicrobial properties for effective biomedical applications.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study does not require an ethics committee approval due to its design.

Informed Consent

This study does not require an informed consent.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Investigation of polypharmacy and potential drug-drug interactions in a group of hospitalized pediatric patients: a single-center study

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ABSTRACT

Aims: Polypharmacy involves the use of multiple medications to manage one or more clinical conditions. This study aimed to determine the prevalence of polypharmacy and potential drug-drug interactions during hospitalizations in childhood and to investigate the nature of common interactions.

Methods: Data for this retrospective cross-sectional observational study were obtained from the hospital database records of pediatric patients admitted to the pediatric department of a university hospital during the first six months of 2020. A total of 601 pediatric prescriptions from 877 hospitalizations involving 2620 medications were examined for drug-drug interactions using the drugs.com/interaction checker tool.

Results: Of the evaluated 601 patients, 48.1% were female and 51.9% were male children. The mean age of the hospitalized patients was 4.78±5.2 years, ranging from 0 to 18 years, with a median age of 2 years. The mean length of the hospital stay was 5.5 (min 1-max 56) days. The mean number of prescribed medications per child was 4.38±2.4 (min-max 1-16). Potential interactions were identified in 49.1% of the prescriptions. The prescription rate of antimicrobial treatment for hospitalized patients was 86%, and this group had a high occurrence of major drug-drug interactions ($p<0.05$). Patients taking multiple medications had significantly longer hospital stays ($p<0.05$). Clarithromycin and ceftriaxone are among the most commonly interacting drugs.

Conclusion: The use of multiple drugs is common among hospitalized pediatric patients. There is a high risk of interaction during multiple antimicrobial treatments, especially in tertiary care hospitals. The increased risk of interactions associated with specific drug groups should prompt clinicians to make informed decisions when prescribing drugs.

Keywords: Polypharmacy, pediatrics, hospitalized patients, drug-drug interactions

INTRODUCTION

Polypharmacy, referred to as the use of multiple medications, is a concern that needs to be consistently kept in mind in pediatric practice because of its life-threatening consequences in childhood. The most significant consequence of polypharmacy is an increased risk of drug-drug interactions (DDIs), which can result in toxicity or treatment inefficacy. Using multiple medications is known to lead to medication administration errors, an increased need for emergency interventions, and extended hospital stays.[1] Therefore, it is necessary to assess the interaction risks of medications as a routine aspect of treatments that require a combination of medications. However, uncertainties related to this subject are multifaceted. The source of information regarding potential DDIs in pediatrics largely consists of data from the adult population owing to ethical, financial, and methodological limitations that restrict studies on the consequences of polypharmacy in the pediatric population.[2] While the pediatric age group is not regarded as young adults, the neonatology, infancy, childhood, and adolescence stages are distinct from each other in terms of physiological, psychological, and pharmacological aspects. Growth and development alone significantly influence pharmacology and affect every phase of drug disposition. Absorption, distribution, metabolism, elimination, pharmacodynamic components, and expected effects were not uniform across the age groups.[3]

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In addition to the limitations of clinical drug studies in pediatric populations, the lack of standardization in definitions and guidelines for pediatric polypharmacy and drug interactions necessitates further research on this subject. Data from such studies are valuable in guiding therapeutic interventions. This study aimed to investigate the prevalence of polypharmacy and evaluate drug-drug interactions in children admitted to a university hospital to illustrate the current situation and highlight the issues related to this subject.

METHODS

Ethics

This study was initiated after the decision of the Afyonkarahisar Health Sciences University Clinical Researches Local Ethics Committee (Date: 02.10.2020, Decision No: 2020/442). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This was a descriptive, cross-sectional study. The data for this study were obtained through retrospective file analysis from a single center. The study was outlined following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist, which is used for reporting observational studies in Health Sciences.

Collecting Data

The study included all pediatric patients aged 0-17 years who received inpatient treatment and follow-up within the Pediatric Health and Diseases Department of Afyonkarahisar Health Sciences University Faculty of Medicine during the first six months of 2020. The sampling period of the study was determined by considering that the frequency of polypharmacy including antibiotherapy would be higher in the first 6 months of the year. Patient data were accessed by examining the hospital's information system database. Patient names were anonymized from the hospital database. The demographic information and length of stay of 877 pediatric patients hospitalized between January 1, 2020, and June 31, 2020, were analyzed, along with all medications used during their hospitalization. The investigated prescriptions were selected from the day the patients received the highest number of medications during their hospital stay.

Criteria for Inclusion and Exclusion

Daycase admissions, healthy newborn follow-ups, and patients who received volume support solely for maintaining fluid and electrolyte balance without prescription (276 patients in total) were excluded from the study. The remaining 601 patients were included and evaluated in this study. The study design is illustrated in Figure 1. Nutritional support treatments, drugs administered for radiological diagnosis, blood products used for intravenous expansion, fluids, insulin, and drugs used in topical applications were excluded from the analysis. The drugs.com database, also used in various studies, was used to evaluate drug-drug interactions.[4] A total of 601 prescriptions were evaluated using the Drugs. com/Interaction Checker tool. According to this guideline, the drug interactions of the prescriptions were classified into three categories. Drug combinations with clinically significant interactions, where avoidance of co-administration is recommended because the potential interaction risk outweighs the benefits, are classified as major interactions. Drug combinations with a moderate degree of clinical significance to be used only in specific cases are categorized as moderate interactions. Drugs with minimal clinical significance in terms of interactions, where an alternative drug or monitoring plan is recommended, are classified as drugs with minor interactions.

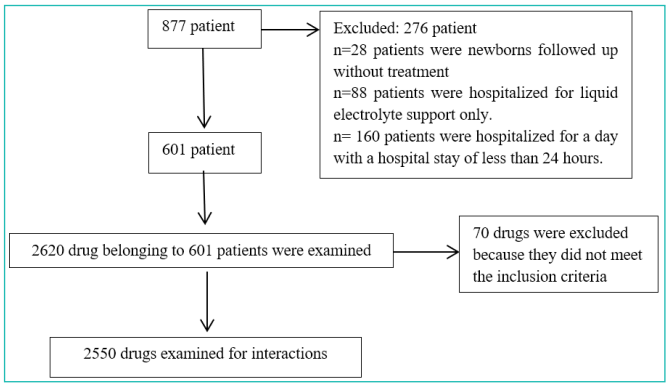

Figure 1. Inclusion and exclusion criteria for patients and prescriptions

Statistical Analysis

Statistical Package for the Social Sciences (SPSS, version 20.0; IBM Corp. 2019 IBM SPSS Armonk, NY.) was used for data analysis. The data and results of potential drug-drug interaction assessments were determined using descriptive statistics, such as mean, standard deviation, and percentage distribution. The normality distribution of the data was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The Mann-Whitney U test was used because parametric conditions could not be met for differences in the number of medications and drug-drug interactions between children receiving antibiotics and those who did not. The Chi-Square test was used to compare the percentage distributions of categorical data between the groups, while the Spearman test was used to evaluate the correlation between two continuous variables. A significance level of $p<0.05$ was adopted.

RESULTS

A total of 601 patients were evaluated, comprising 48.1% females and 51.9% males. The mean age of the hospitalized patients was 4.78±5.2 years, with an age range from 0 to 17 years and a median age of 2 years. Among the hospitalized patients, 68.9% stayed for one day, 13.8% stayed for two days, and the remaining 17.3% stayed for three or more days. As age decreased, length of hospital stay increased (p<0.05). No significant relationship was found between age and number of prescribed medications (p>0.05). A total of 2.3% of patients were admitted to the hospital seven times within six months. The mean length of the hospital stay was 5.5 days. The mean number of prescribed medications during hospitalization was 4.38±2.4 medications per child. years (Table 1). In terms of the number of medications, 97% of the patients were prescribed at least two medications. The percentage of patients who received four or more medications was 56.4%. The distribution of the number of medications used is shown in Figure 2. Of the 2,620 medications from the 601 evaluated prescriptions, 2,550 met the criteria and were evaluated for potential drug-drug interactions.

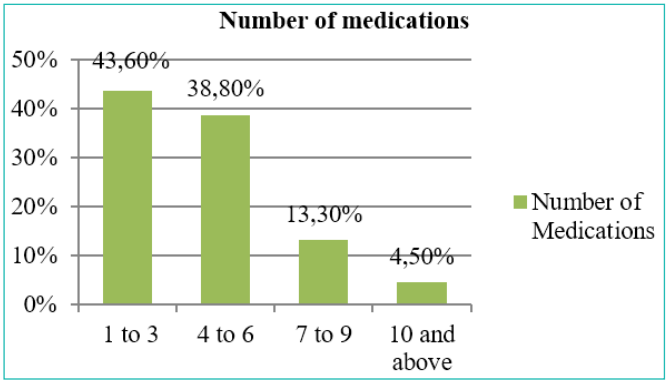

Figure 2. The distribution of the number of medications

Of all the prescriptions given to hospitalized patients, 85.5% contained at least one antibiotic. When evaluating all medications administered to hospitalized children, 34% consisted of antibiotics. Third-generation cephalosporins are the most commonly prescribed antibiotics. Specifically, cefotaxime (23.5%), ceftriaxone (23.1%), clarithromycin (16.7%), metronidazole (7.9%), amikacin (7.2%), meropenem (6.3%), vancomycin (4.1%), and other antibiotics (11.2%) were prescribed in the descending order. Within the scope of this study, the most frequently prescribed non-antibiotic drugs were paracetamol, ibuprofen, methylprednisolone, midazolam, phenobarbital, esomeprazole, levetiracetam, salbutamol, and budesonide.

A total of 49.1% of the prescriptions exhibited drug-drug interactions. Among prescriptions, 20.5% had major interactions, 43.6% had moderate interactions, and 29.8% had minor interactions. Among the 601 prescriptions, the total number of potential drug-drug interactions per patient demonstrated a significant relationship with the length of hospital stay and presence of antibiotics within the prescription (p<0.05). During hospitalization, the maximum interaction was found in a patient with 39 potential DDIs, and the highest number of active ingredients administered to a single patient in one day was 14 (min-max=0-39). The average number of DDIs was 2.1±3.1. A significantly higher potential interaction rate was found for prescriptions containing antibiotics (p<0.05). As age decreased, the number of potential drug-drug interactions increased (p<0.05). Strong positive (r=0.716) and significant (p<0.05) relationships were found between the number of drugs and risk of interaction (Table 2).

Table 1. Descriptive statistics and chi-square / Mann-Whitney U-test and Spearman's rho results at hospitalized children

	Variables	Drug drug interaction n(%) Yes 295(49.1) No 306(50.9)	Number of potential interactions Mean (SD) 2.1(3.7) Median (IQR) 0 (3) Min, Max 0.39
Gender	Female n(%) 289 (48.1%) Male 312 (51.9%)	p>0.05*	p>0.05**
Age (year)	Mean (SD) 4.7(5.2) Median (IQR) 2(8) Min, Max 0.17	p<0.05**	p<0.05***
Hospital stay (day)	Mean (SD) 5.07(5.2) Median (IQR) 4(4) Min, Max 1-5	p<0.05**	p<0.05***
Number of drugs	Mean (SD) 4.48(2.4) Median (IQR) 4(3) Min, Max 1.16	p<0.05**	p<0.05***
Number of prescriptions for antibiotics	Yes n(%) 514(85.5) No 87 (14.5)	p<0.05*	p<0.05**
	Total 601		

*: p: chi-square ($\chi 2$) p value; **p : MannWhitney U test p value; p***:Spearman's rho p value

Table 2. Relationship between age, number of medication and potential drug-drug interaction

		Potantial drug drug interaction
Age	Spearman's rho	-.189*
	p	0.000
	n	601
Number of medication	Spearman's rho	.716*
	p	0.000
	N	601

Corelation is signifficant at the 0.01 level*

DISCUSSION

Hospitals are establishments that provide inpatient healthcare services and are characterized by the management of complex and challenging cases, often involving lengthy hospital stays. Therefore, the use of multiple medications is inevitable for many patients.[5,6] Polypharmacy was initially defined in the mid-20th century as the extensive use of several medications. While there is currently no universal consensus regarding a definitive cut-off number, the literature includes definitions such as using two or more medications, four or more medications, or five or more medications for a minimum of 240 days. Although polypharmacy is commonly encountered in the geriatric population, it is also prevalent among pediatric patients admitted to clinical settings.[7] In a study conducted in Turkiye that analyzed the active ingredients used by all hospitalized patients for a month, it was found that the average number of active ingredients per pediatric patient was 19.30±22.10.[8] There is no clear consensus regarding the definition of pediatric polypharmacy. In addition to pediatric studies that apply known polypharmacy criteria from the literature, there are also studies suggesting that the simultaneous use of two or more medications for at least one day, considering the lower burden of chronic diseases in children than in adults, could be considered as a criterion.[9] Due to variations in clinical conditions and definitions, the prevalence of polypharmacy ranges widely from 18% to 100%.[10] In this study, when the use of four or more medications was defined as a criterion, it was determined that the polypharmacy rate among hospitalized patients was approximately 56%. Numerous studies have also demonstrated that children with identified polypharmacy tend to have longer hospital stay. Polypharmacy has been explored as an independent risk factor for morbidity, mortality, and hospitalization, and its predictive value has been examined in several studies.[11,12] Determining the rates of polypharmacy among hospitalized children is significant for revealing preventable potential drug interactions and identifying the risks of side effects associated with multiple drug use.[13] While studies have suggested a higher prevalence of polypharmacy in early childhood, no such correlation was found in this study.[14]

Drug-drug interactions are defined as the occurrence of an increased or decreased effect that occurs outside the anticipated effect when at least two drugs are used concomitantly. This can complicate the clinical process and potentially lead to drug toxicity or reduced efficacy. Side effects tend to be more evident and dramatic as they present more noticeable manifestations. Studies have shown that the potential risk of side effects when using two drugs is approximately 6%, whereas this rate increases to 50% when using five drugs and nearly 100% when using eight or more drugs.[15]

This study observed that one of every two prescriptions demonstrated at least one potential risk associated with drug-drug interactions. The frequency of exposure to drugs that have a drug-drug interaction (DDI) and the frequency of related adverse effects are poorly understood. Different outcomes can also be observed in the literature.[16] This difference between studies on pediatric polypharmacy is due to many differences in sample size, age, patient groups, polypharmacy, and analysis criteria. There are also studies in the literature in which some modeling is proposed to minimize the variability related to study designs. In a methodological study, Zheng et al.[17] underlined the need for standardization to determine prevalence rates despite the existence of numerous studies investigating drug-drug interactions. Just as critical as these differences are, the potential risk of drug-drug interaction is greater than apparent due to physiological changes related to pediatric age and the potential confounding effects of the current disease and the drugs used.[18]

According to recent data, a retrospective data analysis study using the Pediatric Health Information System (PHIS) database, which included data from 498,956 hospitalized children from 43 different hospitals, found that 49% of hospitalizations in hospitalized pediatric patients were associated with ≥1 potential DDI.[19] Similar results were obtained in a study conducted by Daignault et al.[20] When we compare the results of our study, we can conclude that they are compatible with the intervals stated in the literature.

This study was based on a patient sample from a regional hospital unit with a high prevalence of infectious diseases and lower respiratory tract infections, particularly during months when complex cases are frequent, resulting in a high rate of antibiotic usage. Research conducted in Turkiye has indicated varying prevalence of antibiotic usage in pediatric hospitals, ranging from 30% to 80%.[21,22]

Upon examining frequently observed interactions in this study, certain antibiotics associated with major side effects were found to be responsible for this rate, especially clarithromycin, which strongly inhibits the CYP450 3A4 microsomal enzyme. This

inhibition can lead to increased blood concentrations of the co-administered drugs, resulting in toxicity.[23] During winter, when there is an increased incidence of lower respiratory tract infections, careful monitoring is recommended for signs and symptoms of hypercortisolism due to enhanced systemic absorption when using the inhaler budesonide with clarithromycin. Adrenal and immunosuppressive effects in children and adolescents, along with ocular manifestations, such as glaucoma and cataracts, as well as growth and developmental issues, should be closely observed. The literature reports cases of secondary Cushing's syndrome associated with this combination. In cases where combined use is unavoidable, dose intervals should be widened, and an alternative with lower lipophilicity and shorter half-life, such as beclomethasone, should be considered.[24,25]

The frequency of combinations involving clarithromycin with drugs such as methylprednisolone, digoxin, midazolam, and colchicine is noteworthy, considering the potential for specific toxic manifestations of the affected drug to emerge.[26]

Another major risk factor was an increase in nephrotoxicity risk during the concomitant or sequential use of cephalosporins, one of the most commonly used antibiotic therapies, with a second nephrotoxic agent. Monitoring renal function is important for the combined use of amikacin, vancomycin, acyclovir, and furosemide with cefotaxime and ceftriaxone.[27]

The co-administration of ceftriaxone and furosemide has a hypokalemic effect, and prolonged use of proton pump inhibitors with furosemide can lead to hypomagnesemia-related fluid and electrolyte imbalances, which should not be overlooked.[28]

A point-prevalence study conducted in a Turkish pediatric clinic to investigate the prevalence of inappropriate drug use reported that clarithromycin and ceftriaxone were the most commonly used antibiotics.[21] Although not examined in this study, considering the rates of potential interaction risks, further studies on the necessity of using these two drugs could be the subject of another research endeavor.

In cases of co-administration of aminoglycoside derivatives and nonsteroidal anti-inflammatory drugs (NSAIDs), especially during the treatment of patent ductus arteriosus using ibuprofen or indomethacin along with amikacin, renal damage and electrolyte balance should be monitored. A study examining the hospitalization data of 107 neonatal infants revealed that infants were exposed to a nephrotoxic agent every six days on average.[29] Another study that identified polypharmacy and potential interactions between antiepileptics and cardiovascular drugs emphasized the importance of avoiding therapeutic duplications.[30]

Limitations

The limitation of this study lies in the fact that knowledge about evidence-based pediatric drug interactions is largely derived from studies conducted on adults, and the effects of drugs in specific age groups of children are yet to be established through well-documented research. Therefore, there is a need for more pediatric pharmacokinetic studies, and more research is required to determine the clinical significance of theoretically identified interactions.[31,32]

Drugs with a narrow therapeutic index should be administered similar to those that result in harmful consequences. Small amount of dosage beyond this range. However, this may occasionally result in toxic effects. It is essential to recognize the increased chances of drug interactions when taking various medications. Renal function and serum drug concentrations should be monitored if patients being treated in hospitals are simultaneously taking medications, such as vancomycin, aminoglycosides, and phenytoin.[33]

Research conducted on clinicians' awareness of polypharmacy and potential interactions, as shown in Zapata et al.'s[34] extensive review of 34 studies covering a wide geographical area, indicated that alerts and reminders regarding drug interactions are often overlooked by clinicians, ranging from 58% to 98%.

It is necessary for potential interactions to have a level of evidence that prompts clinicians to take action, and for physicians managing polypharmacy to be well-versed in alternative preventive solutions before interactions occur. Increasing the visibility of drug-drug interaction pharmacovigilance data and developing programmes based on artificial intelligence technology in the clinic can be recommended to reduce risks. The use of technologies that support clinical decision-making and the determination of their contribution to the solution may be the subject of another study.[35]

CONCLUSION

Polypharmacy is common among hospitalized pediatric patients, particularly in tertiary care hospitals where the use of multiple antibiotics increases the risk of potential interactions. Elevated interaction risks within specific drug groups should prompt clinicians to make informed decisions when prescribing these drugs. Pediatric hospitalized patients should have their complete therapeutic regimen optimized by considering potential drug-drug interactions and using antibiotics more effectively.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Researches Local Ethics Committee (Date: 02.10.2020, Decision No: 2020/442).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Examining the relationship between object relations, relationship attachment and separation anxiety in adults with depressive tendencies

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ABSTRACT

Aims: This study aims to examine the relationship between object relations and relationship dependence and separation anxiety in depressive-prone adults.

Methods: The sample of the study consisted of 404 people between the ages of 18-60. Out of 404 people, 113 people with moderate to severe depressive tendencies were included in the study. Data were collected from the participants through Beck Depression Inventory, Bell Object Relationships and Reality Testing Inventory (BORRTI), Spann-Fischer Relationship Dependency Scale (SFIDS), Adult Separation Anxiety Questionnaire and Socio-demographic Information Form prepared by the researcher.

Results: 113 out of 404 individuals showed moderate to severe depressive tendencies. A statistically positive and significant relationship was found between adult separation anxiety and Object Relations and BORTTI (alienation, insecure attachment, egocentrism, distortion of reality, uncertainty of perceptions, delusion hallucination) sub-dimensions. A positive and significant relationship was found between Relationship Dependency and Object Relationships and BORTTI (insecure attachment, egocentrism, social inadequacy) sub-dimensions.

Conclusion: A positive and significant relationship was found between object relations sub-dimensions and adult separation anxiety in depressive adults. A positive and significant relationship was found between object relations and relationship dependence in depressive adults.

Keywords: Depression, object relations, relationship attachment, separation anxiety

INTRODUCTION

Depression has been around since ancient times and is now recognized as a common mental disorder. It is defined as a psychiatric disorder that affects individuals in many different psychological areas such as depressed mood, anhedonia, feeling worthless, and physiological areas such as loss of appetite, sleep problems, fatigue, and lack of concentration.[1]

According to Freud, one of the important antecedents of depression is the experience of loss in the early stages of life. This experience of loss can mean the death of an important person or the deprivation of needs during the developmental stages.[2]

Object Relations Theory within the psychodynamic school is a theory developed by other psychoanalysts to transfer new models of the self-due to the inadequacy of the psychoanalysis developed by Sigmund Freud to explain his personality theory. The theorists focus on the relationship of individuals with their environment and people. The theorists argue that impulses are not the main motive; the main motive is that individuals are in search of relationships and that the individual's personality and attitudes are shaped as a result of interactions with the outside world.[3]

Human beings grow and develop in relationships from the moment they are born. The relationship with the mother, which is the first relationship established, is expressed as the determinant of the relationships to be formed in the following years. Object relations theorists examine this relationship between mother and baby and the characteristics of this relationship.[4] According to the Object Relations Theory, the individual is in search of relationships and the main human motivation is

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explained as the need to be in contact with the object.4 While the need for a caregiver is greater in the early stages of life, the need for individualization increases in later periods.[5] As a result of the failure to complete the healthy separation and individuation process in the child, the intense anxiety and clinging that occurs when the child separates and moves away from the parent causes separation anxiety.[6] Separation anxiety is defined as feeling excessive anxiety in case of separation from parents or caregivers in a period that is not appropriate for the developmental period.[7] Separation anxiety may have been defined in childhood and continued into adulthood or may have first started in adulthood.8 Problems in the individuation process are explained in relation to recurrent self-harming behaviors and depressive disorders.[9,10]

Relationship addiction was first used to describe the spouses of individuals with alcohol addiction.[11] Later, it was interpreted and expanded as a state of being overly interested in an object or person.[12] People with relationship addiction have a focus on others by ignoring their own needs due to excessive fear of abandonment and feelings of guilt directed towards themselves in negative situations.[13] From a phenomenological point of view, relationship addiction is analogous to mood disorders. While attachment to the object of love manifests itself in euphoria such as hypomania, the individual who is separated from the object may experience a depressive state.[14] Object Relations theory is a theory that explains the understanding of the relationships that individuals establish with other people throughout their lives.[15] According to Object Relations, children internalize the individuals and relationships they relate to and create images. These internalized images shape their relationships in adulthood.[16] In this context, it is thought that it is important to examine the emergence of relationship addiction within the framework of object relations theory.

This study mainly aims to examine the relationship between object relations, relationship dependence and separation anxiety in adults with depressive tendencies. Object Relations Theory deals with the relationship with the primary caregiver. The relationship with the caregiver is important for the quality of the relationship with other people in the future. The goal of this study is to draw attention to object relations in people with depressive tendencies and to raise awareness about relationship addiction and separation anxiety, which are important problems today. In line with these goals, it is thought that there is a significant relationship between object relations, relationship dependence and separation anxiety with the study conducted on depressive prone people and it is planned to examine these relationships.

One of the hypotheses of the study is that there is a statistically significant relationship between object relations and relationship addiction. The other hypothesis of the study is that there is a statistically significant relationship between object relations and separation anxiety.

Although the variables of object relations, relationship dependency and separation anxiety have been the subject of different studies, the sample did not consist of depressive individuals and these three variables were not found together. There are many studies showings that the relationship with the caregiver is important and determinant for the relationships with other people in the future, but there are not many studies that examine separation anxiety and relationship dependency from the object relations theory framework. Since the sample of the study includes individuals with depressive tendencies and there is no other study that addresses the three variables together, it is thought to be important and will contribute to the literature.

METHODS

Permission was received for this study from İstanbul Nişantaşı University Ethics Committee (Date: 04.05.2023, Decision No: 2023/18). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The research is a descriptive study in the relational screening model, which aims to examine the relationship between object relations, relationship dependence and separation anxiety of male and female participants with depressive tendencies in the 18-60 age group on a voluntary basis. Convenience sampling is a non-probability sampling technique widely used in social science research, particularly in situations where the researcher seeks to obtain quick and easy access to participants. This method involves selecting individuals who are readily available and accessible to the researcher, often based on their proximity or willingness to participate.[17]

The sample consists of adults between the ages of 18 and 60 with depressive tendencies using the Beck Depression Inventory (BDI). The sample number is 404 people. It consists of 287 women, 116 men and 2 other participants. As exclusion criteria, those under the age of 18, over the age of 60, and those with a diagnosis of psychiatric disorders other than depression were excluded from the study. The study included 113 individuals with moderate and severe depressive tendencies with the Beck Depression Inventory. The Beck Depression Inventory (BDI) is a widely used 21-item self-report questionnaire for assessing the severity of depression in both normal and psychiatric populations.[18] It has been validated and found to be a sound tool for detecting depression in

patients with medical conditions.[19] The BDI has been used in various clinical settings, including in psychiatric outpatients with various psychiatric disorders[20] and in the assessment of depressive symptoms in clinically depressed patients.[21] Additionally, it has been used in the assessment and treatment of depressed older adults in primary care.[22] BDI can be used for the measurement of depressive tendencies. Depressive tendencies encompass a range of cognitive, emotional, and behavioral patterns that are indicative of a predisposition towards experiencing depressive symptoms. These tendencies may manifest as cognitive responses to success and failure, cognitive reactivity, and rumination, which are linked to depression.[23,24]

The Demographic Information Form was used to determine the sociodemographic characteristics of the participants, the Beck Depression Inventory was used to recruit depressive adults, the Bell Object Relations and Reality Testing Inventory was used to assess object relations, the Spann-Fischer Relationship Dependency Scale was used to assess relationship dependency, and the Adult Separation Anxiety Scale was used to assess separation anxiety.

Socio-Demographic Information Form

In the study, a Socio-Demographic Information Form consisting of 11 items prepared by the researcher and the thesis advisor, including demographic characteristics of the participants, was used. The form included questions about gender, age, marital status, education level, ongoing romantic relationship or marriage, relationship with mother, relationship with father, caregiver during childhood, whether they were separated from their parents for a long time during childhood, diagnosed psychiatric disorder and whether they had lifelong self-harming behavior.

Beck Depression Inventory (BDI)

Beck Depression Inventory is a 21-item scale developed by Beck and colleagues in 1961 to measure the severity of depression in individuals. It is a 4-point Likert-type scale. The highest score that can be obtained from the scale is 63, with each option receiving a score between 0 and 3. In a study conducted by Nesrin Hisli on the validity and reliability of the Beck Depression Inventory for university students, the correlation coefficient was found to be 0.74.[25]

Bell Object Relations and Reality Testing Inventory (BORRTI)

Bell Object Relations and Reality Testing Inventory (BORRTI) Developed by Morris D. Bell in 1995, the reliability and validity study of the scale in Turkiye was conducted by Sait Uluç, Zeynep Tüzün, Manolya Haselden, and Serap Piri Erbaş in 2015. The scale consists of 90 items and is answered as true-false. It consists of two sub-dimensions: Object Relations and Reality Appraisal. The current ego functions of individuals and the fact that the characteristics of their established relationships are shaped according to early object relations are evaluated in the scale.[26] The internal consistency Cronbach's alpha value of the object relations sub-dimension is between .70 and .80. The internal consistency Cronbach's alpha value of the Reality Assessment sub-dimension is between .54 and .77.[27]

Spann-Fischer Relationship Dependence Scale (SFRDS)

Spann-Fischer Relationship Dependency Scale (SFRDS) developed by Fischer, Spann and Crawford.[28] In Turkiye was conducted by Fuat Tanhan and Gamze Mukba in 2014. It is a 6-point Likert-type scale consisting of 16 questions.[29] The maximum score is 96 and the minimum score is 16. In the study, the reliability value was calculated by calculating the Cronbach's Alpha coefficient and the internal consistency coefficient was determined as 0.65 and a reliable result was obtained.[30]

Adult Separation Anxiety Scale

Adult Separation Anxiety Scale was developed by Manicavasagar, Silove, Wagner, and Drobny[31] and the validity and reliability study in Turkiye was conducted by Meliha Diriöz. It is a 4-point Likert-type scale consisting of 27 items.[32] Each item is answered as "never", "rarely", "often" and "very often". An increase in the scores obtained from the scale means an increase in the level of adult separation anxiety. The reliability and validity of the test were determined as a result of internal consistency, item analysis and test-retest consistency. In the study, the reliability value was calculated by calculating the Cronbach Alpha coefficient and a reliable result was obtained by determining the internal consistency coefficient as 0.93.[32]

Statistical Analysis

SPSS 26.0 software program was used to analyze the data. The severity of depressive symptoms was determined by the Beck Depression Inventory Pearson correlation was applied to examine the relationship between adult separation anxiety, relationship attachment, object relations and reality testing levels in individuals with moderate and severe depression. The significance level was taken at 0.05 and 0.001 levels.

RESULTS

Preliminary analyses regarding the participants' sociodemographic variables and measurement tools are given below (**Table 1**).

Table 1. Descriptive statistics of sociodemographic variables

Sociodemographic Variables Groups	F	%
Gender		
Male	28	24.8
Female	85	75.2
Age		
18-28	55	48.7
29-39	19	16.8
40-50	18	15.9
51-60	21	18.6
Marital status		
Single	44	38.9
Married	48	42.5
In a relationship	21	18.6
Education level		
Primary school-secondary school	5	4.4
High school	14	12.4
Undergraduate	59	52.2
Master's degree	35	31.0
Romantic relationship		
Yes	72	63.7
No	41	36.3
Relationship with mother		
Very good	42	37.2
Good	46	40.7
Poor	2	1.8
Fair	23	20.4
Relationship with the father		
Very good	29	25.7
Good.	40	35.4
Poor	10	8.8
Fair	34	30.1
Who provided care during childhood		
My mom	28	24.8
Mom and dad	74	65.5
My father	2	1.8
Other	9	8.0
Childhood separation from parents		
Yes	29	25.7
No	84	74.3
Diagnosed psychiatric disorder		
Yes	7	6.2
No	106	93.8
Self-harming behavior		
Yes	16	14.2
No	97	85.8
Depression Level (n=404)		
Minimal	218	53.9
Mild	73	18.0
Moderate	89	22.0
Severe	24	5.9
Total	113	100.0

When the frequency distributions of sociodemographic variables are analyzed in Table 1, 24.8% of the 113 participants with moderate-to-severe depressive symptoms were male and 75.2% were female. When the age groups are analyzed, 18-28 years old 48.7%, 29-39 years old 16.8%, 40-50 years old 15.9% and 51-60 years old 18.6%. According to marital status, 38.9% were single, 42.5% were married and 18.6% were in a relationship. The rate of those with primary education was 4.4%, high school was 12.4%, associate or bachelor's degree was 52.2% and master's degree was 31.0%. The rate of participants who are currently in a romantic relationship is 63.7%. Those who had a very good relationship with their mother were 37.2%, 40.7% had a good relationship, 20.4% had a moderate relationship and 1.8% had a poor relationship. Those whose relationship with the father was very good were 25.7%, good was 35.4%, fair was 30.1% and poor was 8.8%. Those who were cared for by their mother during childhood were 24.8%, those who were cared for by their parents were 65.5%, those who were cared for by their father were 1.8% and those who answered "other" were 8.0%. The rate of participants who were separated from their parents for a long period of time (not less than 1 month) during childhood was 25.7%. The rate of participants who stated that they had a diagnosed psychiatric disorder was 6.2%. The proportion of participants who had self-harm behavior throughout their lives was 14.2%. When the depression levels of all participants were analyzed, minimal depression was 59.3%, mild depression was 18.0%, moderate depression was 22.0% and severe depression was 5.9%.

Descriptive statistics for the mean values of the measurement tools used in the study were given above. For the normality test, skewness and kurtosis values of the mean values were examined (Table 2). In line with the values found to be within the range of ±1.5 recommended by Tabachnick and Fidell, parametric tests were found appropriate for this study.[33] However, the kurtosis values of the BORRTI distortion of reality and BORRTI delusional hallucination sub-dimensions were found to be 3.24 and 2.73. As stated by Kline, kurtosis values up to 10 were considered acceptable.[34]

As seen in Table 3, according to the findings of Pearson correlation to examine the relationship between adult separation anxiety, relationship dependency and object relations scores of depressive-prone individuals, adult separation anxiety and BORRTI alienation (r=.27; p<0.01), BORRTI insecure attachment (r=.28; p<0.01), BORRTI egocentrism (r=.25; p<0.01), BORRTI distortion of reality (r=.30; p<0.01), BORRTI uncertainty of perceptions (r=.28; p<0.01), BORRTI delusion hallucination (r=.29; p<0.01) and BORRTI object relations (r=.28; p<0.01) dimensions (Table 3).

A positive and significant relationship was found between SFBIQ scores and BORRTI insecure attachment (r=.29; p<0.01), BORRTI egocentrism (r=.21; p<0.01), BORRTI social inadequacy (r=.20; p<0.01) and BORRTI object relations (r=.25; p<0.01) (Table 3).

Table 2. Descriptive statistics for the mean values of the measurement tools used in the study

Variables	N	XX	Ss	Skewness	Kurtosis
BDI total	113	23.78	6.626	1.266	1.019
ASAS	113	38.52	17.036	.226	-.514
SFRDS	113	34.41	16.454	-.415	-.398
BORRTI reality testing	113	11.27	6.948	1.387	1.997
BORRTI alienation	113	8.74	3.807	.160	-.931
BORRTI insecure attachment	113	7.10	2.906	.275	-.445
BORRTI egocentrism	113	5.11	2.674	.461	-.371
BORRTI social incompetence	113	2.16	1.771	.589	-.840
BORRTI distortion of reality	113	5.57	4.476	1.604	3.241
BORRTI the ambiguity of perceptions	113	6.44	2.525	.213	-.368
BORRTI delusion hallucination	113	2.79	3.231	1.764	2.738
BORRTI object relations	113	15.38	6.070	.305	-.395

ASAS=Adult Separation Anxiety Scale, SFRDS=Spann-Fischer Relationship Dependency Scale, BORRTI=Bell Object Relationships and Reality Testing Inventory

DISCUSSION

As explained in the introduction, the aim of this study is to answer the question "Is there a relationship between object relations, relationship dependency and separation anxiety in depressive prone adults?". In line with the purpose of the study, the findings obtained in this section are interpreted and discussed within the framework of the literature.

As a result of Pearson correlation findings in depressive-prone individuals, a positive and significant relationship was found between adult separation anxiety and BORRTI object relations and BORTTI alienation, insecure attachment, egocentrism, distortion of reality, uncertainty of perceptions, and delusion hallucination sub-dimensions.

In study, the positive and significant relationship between Adult Separation Anxiety and the alienation sub-dimension of BORTTI object relationships is consistent with the literature. When the sub-dimensions of object relations are examined, it is stated that people with high

Table 3. Examining the relationship between participants' adult separation anxiety, relationship dependence and object relations scores

		1	2	3	4	5	6	7	8	9	10
ASAS	r	1									
	p										
	N	113									
SFRDS	r	-.155	1								
	p	.100									
	N	113	113								
BORRTI alienation	r	.267**	.159	1							
	p	.004	.092								
	N	113	113	113							
BORRTI insecure attachment	r	.278**	.297**	.731**	1						
	p	.003	.001	.000							
	N	113	113	113	113						
BORRTI egocentrism	r	.246**	.214*	.636**	.643**	1					
	p	.009	.023	.000	.000						
	N	113	113	113	113	113					
BORRTI social incompetence	r	.101	.204*	.811**	.571**	.404**	1				
	p	.285	.030	.000	.000	.000					
	N	113	113	113	113	113	113				
BORRTI distortion of reality	r	.304**	.016	.550**	.573**	.631**	.414**	1			
	p	.001	.862	.000	.000	.000	.000				
	N	113	113	113	113	113	113	113			
BORRTI The ambiguity of perceptions	r	.289**	.118	.519**	.559**	.515**	.388**	.625**	1		
	p	.002	.214	.000	.000	.000	.000	.000			
	N	113	113	113	113	113	113	113	113		
BORRTI delusion	r	.289**	-.114	.417**	.443**	.485**	.315**	.903**	.606**	1	
	p	.002	.228	.000	.000	.000	.001	.000	.000		
	N	113	113	113	113	113	113	113	113	113	
BORRTI object relations	r	.284**	.251**	.901**	.878**	.823**	.729**	.666**	.598**	.524**	1
	p	.002	.007	.000	.000	.000	.000	.000	.000	.000	
	N	113	113	113	113	113	113	113	113	113	113

ASAS=Adult Separation Anxiety Scale, SFRDS=Spann-Fischer Relationship Dependency Scale, BORRTI=Bell Object Relationships and Reality Testing Inventory **p<0.01*p<0.05, Pearson Correlation Analysis

scores in the alienation sub-dimension have a lack of trust in their relationships with others. It is stated that there may be difficulties in realizing emotional intimacy, establishing lasting relationships and the satisfaction of these relationships. It is said that there may be situations such as artificiality in social relationships and inability to feel belonging.[26] In this context, it is thought that these characteristics seen in individuals affect the separation anxiety they experience in their relationships. As the alienation sub-dimension scores increase, an increase in adult separation anxiety is observed. As a result of the study conducted by Durmaz,[35] object relations and the level of adult separation anxiety were found to be directly proportional. As a result of the examination of the relationship between object relations and separation anxiety by Günhan,[36] a significant and positive relationship was found in three sub-dimensions (alienation, egocentrism, insecure attachment) except for the "social incompetence" sub-dimension.

In study, the positive and significant relationship between Adult Separation Anxiety and insecure attachment sub-dimension of BORTTI Object Relationships is consistent with the literature. Individuals with high insecure attachment subscale scores emphasize the need for closeness and sensitivity to rejection in their relationships. The fact that these individuals desperately long for the feeling of closeness in their relationships with significant others indicates how important relationships are for them. Their tolerance for separation, loss and loneliness is very low and they are constantly on guard against the possibility that others will abandon them. Feelings such as constant anxiety, intense worry, guilt and jealousy may accompany relationships.[26] The diagnostic criteria for adult separation anxiety (DSM-5) such as intense anxiety about losing the people they are attached to, being alone, being reluctant to be alone and having severe fear, having nightmares about separation overlap with the characteristics of individuals with high scores in the insecure attachment sub-dimension. If the parent is insecure and rejecting, the child feels unlovable and worthless. Accordingly, the child acts dependent on the parent and seeks to make sure that the parent is present. As a result of this search, separation anxiety occurs. As the level of insecure attachment increases, anxiety disorder develops.[37] When the level of secure attachment decreases, the level of separation anxiety increases.[38] It is stated that the loss of the mother, who is the first protector, and the safe space and feelings of attachment she has created are effective in the formation of anxiety and its continuation in adulthood.[39]

In study, as the scores obtained from the BORRTI sub-dimensions of uncertainty of perceptions, delusion hallucination and distortion of reality increase, adult separation anxiety scores increase. It is stated that people with high scores in the distortion of reality sub-dimension may have paranoid beliefs such as being punished and being conspired against. High scores from the uncertainty of perceptions sub-dimension indicate that they may have doubts in interpreting events as a result of the mistakes they have made in their relationships with people.[26] Looking at the literature, it is stated that adult separation anxiety causes loss of functionality and increases disability when combined with additional diagnoses.[40,41] As a result of an epidemiologic study, it is stated that 28% of people with adult separation anxiety have significant impairment in personal relationships and 31.5% in social relationships.[40]

In the study, a positive and significant relationship was found between Spann-Fischer Relationship Dependence Scale scores and BORRTI insecure attachment, social inadequacy, egocentrism sub-dimensions and BORRTI object relations dimension.

The finding of a positive and significant relationship between the insecure attachment sub-dimension and relationship dependence scores in the study is consistent with the literature. As a result of the study conducted by Cengiz[42] with university students, it was found that the relationship addiction scores of participants with insecure attachment were higher than those of participants with secure attachment.As a result of the study conducted by Havaçeliği,[43] relationship addiction scores of participants with secure attachment style among relationship addiction and parental attachment styles were found to be lower. The fact that people with secure attachment style have positive perceptions of themselves and others, think that they are worthy of being loved, and that the people they relate to are reliable, supportive and well-intentioned; it shows that they can both establish relationships and manage to be independent.[44] Harrison and Grey[45] state that the biggest fear of individuals with relationship addiction is the fear of the end of their relationships.

A significant positive relationship was found between social inadequacy and relationship addiction. It is stated that individuals with high scores on the social inadequacy sub-dimension are timid and tense in their relationships and have difficulty in interacting with the opposite sex and forming friendships. These individuals tend to perceive themselves as socially inadequate and their relationships with other people as overwhelming and unpredictable.[26] In individuals with relationship addiction, characteristics such as being too responsible or irresponsible, fearing loss of control, not accepting compliments, not being able to say no, having feelings of worthlessness, making efforts to please others, and anger are expressed.[46] In this context, it is thought that people

with social inadequacy and relationship addiction have difficulty in establishing healthy relationships, that they are related to lack of self-confidence, and that a cycle may occur in which both affect each other.

When the literature is examined, Gürek[47] found a significant positive relationship between object relations and relationship addiction. There are also studies in the literature examining the relationship between different variables and relationship addiction. Mukba,[48] examined relationship addiction in terms of some variables and found a significant difference between relationship addiction and intra-family relationships. As a result of the findings, it was concluded that there was an increase in the levels of relationship dependency in line with the perception of family relationships from "very good" to "bad". Berlin and Dodge[49] stated that people who were emotionally neglected and abused as children may perceive emotional threat, trauma and relationship addiction in their later relationships. They stated that children who did not receive enough love from their parents and whose needs were not met may have "excessive caregiving" and "controlling tendency" attitudes observed in relationship addiction when they reach adulthood.

As a result of the findings, the effect of object relations on adult separation anxiety and relationship addiction was supported by our study, and the results were found to be compatible with the literature. It is thought that it will contribute to the literature since the studies on relationship addiction and separation anxiety are limited.

Limitations

This research is limited to people with depressive tendencies between the ages of 18-60 in Istanbul. The relevant data in the study are limited to the values measured by the scales. It is thought that collecting data from a larger sample will yield more reliable results. For this reason, more participants can be reached in new studies. The study covers the age range of 18-60 years. In future studies, groups under the age of 18 can also be included in the study.

CONCLUSION

This study was conducted to examine whether there is a relationship between object relations, relationship dependence and separation anxiety in depressive individuals between the ages of 18-60. According to the results of this study, a positive and significant relationship was found between object relations sub-dimensions and adult separation anxiety in adults with depressive tendencies. A positive and significant relationship was found between object relations and relationship dependence in depressive adults. The results of the study are consistent with literature, and it is thought that the findings obtained will contribute to similar studies to be conducted in the future and to the literature.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of İstanbul Nişantaşı University Ethics Committee (Date: 04.05.2023, Decision No: 2023/18).

Informed Consent

All participants signed free of charge and informed consent form.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluation of occupational radiation dose due to ^{99m}Tc and ^{131}I based examinations

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ABSTRACT

Aims: This study investigates the individual organ doses and the impact on effective dose of radiation emitted from radioactive sources.

Methods: In the conducted research, the standing ICRP adult male phantom defined as the phantom material in the Visual Monte Carlo dose calculation program (VMC) was used. Subsequently, doses incurred were calculated by defining different doses, distances, and durations for ^{99m}Tc and ^{131}I radioactive sources.

Results: Simulation durations (exposure durations) were set at 1 minute and 5 minutes for comparison. The results indicated that both in 1-minute and 5-minute exposures, the doses remained below the ICRP's recommended annual dose limit of 50 mSv/year for occupational exposure.

Conclusion: It was observed that the organ dose and effective dose vary with the source strength and exposure duration. Regardless of how low the doses may be, individuals working in radiation fields must make greater efforts to reduce radiation doses by adhering to the ALARA principles.

Keywords: Effective dose, Monte Carlo, VMC program, ICRP Phantom

INTRODUCTION

Nuclear medicine emerges as a crucial medical discipline where radioactive substances are utilized in diagnostic and therapeutic processes.[1] However, professionals working in this field, known as radiation workers, face the risk of radiation exposure. The radiation doses of personnel working in nuclear medicine units are of critical importance both for the health of healthcare workers and the quality of patient care. Determining the radiation doses to which personnel in nuclear medicine departments are exposed is essential for radiation safety, including understanding the effects of these doses and implementing necessary precautions. Healthcare professionals working in this field are at risk of exposure to radiation due to their occupational activities. Radioactive substances such as Technetium-^{99m} (^{99m}Tc) and iodine-131 (^{131}I) are frequently used for diagnostic and therapeutic purposes in the field of nuclear medicine. During the administration of ^{99m}Tc and ^{131}I, the radiation doses incurred by personnel during patient imaging or admission to the treatment room must be meticulously monitored.[2] Compounds labeled with ^{99m}Tc are commonly employed to diagnose various diseases, including cardiac, endocrine, orthopedic, inflammatory, urological, and other pathologies.[3-5] The use of radioactive iodine is the most frequently employed method in the treatment of thyroid diseases. Following the administration of ^{131}I, it is absorbed in thyroid tissues, and clearance mainly occurs through the intestines, resulting in a whole-body dose in addition to uptake in the glandular tissues of the thyroid.[6] In patients with thyroid cancer treated with ^{131}I, the clearance of the administered activity is faster due to the surgical removal of thyroid tissues, allowing for the administration of higher doses of ^{131}I (1850-7400 MBq) in treatments.[7] With the increasing use of radiation for diagnostic and therapeutic purposes in medicine, the doses incurred by radiation workers in this field have gained significance. Personal radiation doses resulting from low-level exposure may lead to potential somatic health effects such as heart disease and cataracts.[8] Chodick et al.[9], conducted a prospective study on American technicians, reporting an increased risk of cataracts with cumulative doses of 10 mGy. Therefore, it is crucial to maintain radiation doses at acceptable levels to prevent the likelihood of stochastic effects, thus enhancing local practices and improving the implementation of radiation protection principles.[10-14]

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Diagnostic and therapeutic procedures in nuclear medicine are rapidly expanding, and an increase in the use of new imaging agents is anticipated. Additionally, the nuclear medicine department holds particular significance as it represents one of the most crucial radiobiological models where response probability is associated with radiation dose.[14] The aim of this study is to investigate the individual organ doses and the impact on effective dose of radiation emitted from ^{99m}Tc and ^{131}I radioactive sources at different doses (10 mCi, 24 mCi, 100 mCi), durations (1 and 5 minute), and distances (50 cm, 100 cm, and 200 cm) using.

Visual Monte Carlo (VMC) dose program. Furthermore, this study aims to contribute to the establishment of safe working environments in nuclear medicine units. Thus, the dose values obtained as a result of these exposures can serve as a guide for implementing safety measures.

METHODS

Since the conducted research is not related to either human or animal use, there is no need for an ethics committee approval.

VMC Dose Calculation Program

The VMC dose calculation program is designed for the calculation of tissue and effective doses. In this simulation program, ICRP voxel phantoms of male and female human bodies are provided. Results obtained from comparisons and validations indicate that doses calculated with VMC can be accepted within the range of ±5% of actual doses. The size and shape differences between the exposed real person and mathematical phantoms constitute the main source of uncertainty.[15] In this study, the standing ICRP adult male phantom was used as the phantom material within the VMC dose calculation program.

Some Radiopharmaceuticals used in the Study

^{99m}Tc is used in various nuclear medicine imaging procedures, including myocardial perfusion scintigraphy using MIBI (methoxyisobutyl isonitrile), bone scans using MDP (methylene diphosphonate), dynamic kidney scintigraphy using DTPA (diethylene triamine penta-acetate), and many other nuclear medicine imaging procedures. In nuclear medicine imaging procedures (thyroid, kidney, bone, and heart scintigraphy), ^{99m}Tc-labeled radiopharmaceuticals are utilized, and doses vary between 1 and 40 mCi depending on the patient's age and weight. Patients may spend between 20 minutes to 4 hours in the nuclear medicine department based on the imaging procedure. In the simulation program, a 10 mCi source (representing the average imaging procedure dose) and a 24 mCi source (representing the average stress protocol for myocardial perfusion scintigraphy) of ^{99m}Tc were defined at distances of 50 cm, 100 cm, and 200 cm from the ICRP adult male phantom. Thus, the doses incurred under different doses and different radioactive sources were examined. ^{131}I ablation is a common procedure used in patients with differentiated thyroid cancer to eliminate functional residual tissues following the initial thyroid surgery.[16] Before administering ^{131}I therapy, patients are informed about all the necessary steps and requirements. Nuclear medicine departments are equipped with specially designed isolation rooms and communication systems that reduce close contact between patients and staff. Since each patient may not have the same characteristics, radioactive iodine treatment is administered in different doses. Some patients may require 20 mCi, some 50 mCi, or others 100 mCi. In this study, a simulation program defined a 100 mCi ^{131}I radioactive source from the ICRP adult male phantom at distances of 50 cm, 100 cm, and 200 cm. Table 1 shows the input parameters used in the simulation.

Table 1. Input parameters

Radionuclide	**^{99m}Tc**	**^{131}I**
Parameter	Value chosen	Value chosen
Source	External Source	External Source
Radionuclide activity	10 mCi and 24 mCi	100 mCi
Phantom	ICRP adult male	ICRP adult male
Geometry	Point source	Point source
Exposure time	1 and 5 minute	1 and 5 minute
History	10×10^6	10×10^6

RESULTS

Figure 1 displays the simulation image created for different radioactive sources and distances. At a distance of 50 cm, the radiation dose received at different doses and durations is shown in Figure 2. Tables 2, 3, and 4 present the estimated radiation dose values received by organs and effective doses at different sources, distances, and durations.

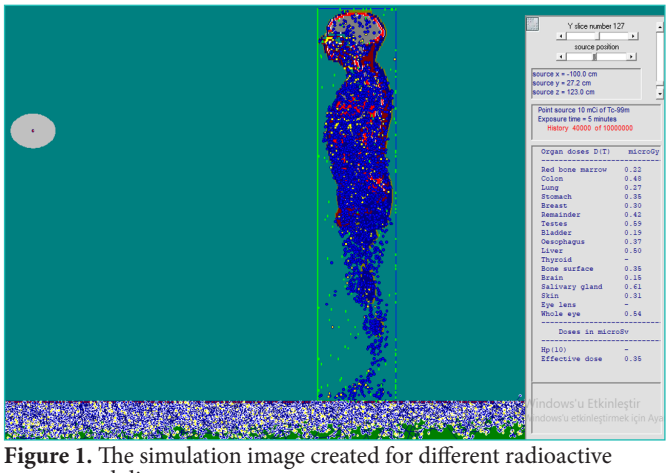

Figure 1. The simulation image created for different radioactive sources and distances.

Table 2. Radiation dose exposure at different durations and distances for ^{99m}Tc- 10 mCi

	5-minute µGy			1-minute µGy		
Organ doses D(T)	50 cm	100 cm	200 cm	50 cm	100 cm	200 cm
Red bone marrow	0.81	0.30	0.10	0.16	0.06	0.02
Colon	1.29	0.44	0.14	0.26	0.09	0.03
Lung	1.09	0.38	0.12	0.22	0.08	0.02
Stomach	1.43	0.48	0.15	0.30	0.10	0.03
Breast	1.99	0.68	0.16	0.40	0.14	0.04
Remainder	1.01	0.36	0.12	0.20	0.07	0.02
Testes	1.03	0.45	0.16	0.21	0.09	0.03
Bladder	1.18	0.46	0.15	0.24	0.09	0.03
Oesophagus	0.97	0.38	0.11	0.19	0.08	0.02
Liver	1.21	0.39	0.12	0.24	0.08	0.02
Thyroid	1.50	0.61	0.20	0.29	0.12	0.04
Bone surface	0.74	0.34	0.12	0.15	0.07	0.02
Brain	0.43	0.20	0.07	0.09	0.04	0.01
Salivary gland	1.02	0.37	0.12	0.20	0.07	0.02
Skin	0.82	0.34	0.12	0.16	0.07	0.02
Adrenals	0.54	0.22	0.06	0.11	0.04	0.01
Extrathor airways	1.04	0.40	0.13	0.21	0.08	0.03
Gall bladder	1.20	0.36	0.17	0.24	0.07	0.02
Heart	1.28	0.43	0.14	0.26	0.09	0.03
Kidneys	0.65	0.23	0.07	0.13	0.05	0.02
Lymphatic nodes	1.17	0.45	0.13	0.23	0.09	0.03
Muscle	0.75	0.32	0.11	0.15	0.06	0.02
Oral mucosa	1.05	0.37	0.11	0.21	0.07	0.02
Pancreas	1.25	0.40	0.14	0.25	0.08	0.03
Prostate	0.76	0.32	0.11	0.15	0.06	0.02
Small intestine	1.32	0.46	0.15	0.26	0.09	0.03
Spleen	0.66	0.23	0.08	0.13	0.05	0.02
Thymus	1.43	0.50	0.16	0.29	0.10	0.03
Eye lens	0.68	0.40	0.15	0.14	0.15	0.03
Whole eye	1.27	0.52	0.14	0.25	0.10	0.03
Hp(10) (µSv)	2.39	0.73	0.36	0.28	0.14	0.03
Effective dose (µSv)	1.23	0.44	0.14	0.25	0.08	0.02

In Table 2, when a 10 mCi radioactive source of ^{99m}Tc was used at 50 cm, the highest doses within a 5-minute duration were received by the breast (1.99 µGy), thyroid (1.50 µGy), and stomach (1.43 µGy), respectively. When this duration was reduced to 1 minute at the same distance, the highest received doses decreased to 0.40 µGy, 0.29 µGy, and 0.30 µGy for the breast, thyroid, and stomach, respectively. In Table 2, when a 10 mCi radioactive source of ^{99m}Tc was used at a distance of 100 cm, the highest doses within a 5-minute duration were received by the breast (0.68 µGy), thyroid (0.61 µGy), and stomach (0.48 µGy). When this duration was reduced to 1 minute at the same distance, the highest received doses decreased to 0.14 µGy, 0.12 µGy, and 0.10 µGy for the breast, thyroid, and stomach, respectively. In Table 2, when a 10 mCi radioactive source of ^{99m}Tc was used at a distance of 200 cm, the highest doses within a 5-minute duration were received by the thyroid (0.20 µGy), breast (0.16 µGy), and gall bladder (0.17 µGy), respectively. When this time was reduced to 1 minute at the same distance, the highest dose received was 0.04 µGy for breast, thyroid, while the doses received by other organs ranged between 0.03 µGy and 0.02 µGy. When a 10 mCi radioactive source of ^{99m}Tc was used, the effective doses obtained within a 5-minute duration at distances of 50 cm, 100 cm, and 200 cm were found to be 1.23 µSv, 0.44 µSv, and 0.14 µSv, respectively. When the duration was reduced to 1 minute at the same activity and distances, the effective doses were found to be 0.25 µSv, 0.08 µSv, and 0.02 µSv, respectively. As seen from Table 2, the radiation dose is inversely proportional to the distance. The intensity of radiation decreases as the square of the distance, i.e., radiation intensity diminishes proportionally with the square of the distance from the radiation source.[17] As the distance increases, the effect of the radiation dose decreases. Therefore, personnel working in the radiation field should operate as far away as possible from the radiation source.

In Table 3, when a 24 mCi radioactive source of ^{99m}Tc was used at a distance of 50 cm, the highest doses within a 5-minute duration were received by the breast (4.78 µGy), stomach (3.59 µGy), and thyroid (3.42 µGy), respectively. When this duration was reduced to 1 minute at the same distance, the highest received doses decreased to 0.96 µGy, 0.69 µGy, and 0.72 µGy for the breast, thyroid, and stomach, respectively. In Table 3, when a 24 mCi radioactive source of ^{99m}Tc was used at a distance of 100 cm, the highest doses within a 5-minute duration were received by the breast (1.58 µGy), thyroid (1.19 µGy), and stomach (1.13 µGy), respectively. When this duration was reduced to 1 minute at the same distance, the highest received doses decreased to 0.33 µGy, 0.29 µGy, and 0.23 µGy for the breast, thyroid, and stomach, respectively. In Table 3, when a 24 mCi radioactive source of ^{99m}Tc was used at a distance of 200 cm, the highest doses within a 5-minute duration were received by the thyroid (0.46 µGy), breast (0.42 µGy), and Thymus (0.40 µGy), respectively. When this time was reduced to 1 minute at the same distance, the highest dose received was 0.09 µGy and 0.08 µGy for thyroid and breast, respectively, while the doses received by other organs ranged between 0.04 µGy and 0.08 µGy. For a 24 mCi radioactive source of ^{99m}Tc, the effective doses obtained within a 5-minute duration at distances of 50 cm, 100 cm, and 200 cm were found to be 2.94 µSv, 1.03 µSv, and 0.32 µSv, respectively. When the duration was reduced to 1 minute at the same activity and distances, the effective doses were found to be 0.59 µSv, 0.21 µSv, and 0.06 µSv, respectively. In nuclear medicine departments, myocardial perfusion scintigraphy rest and stress imaging procedures are often performed on the same day (same-day protocol), but they can also be done on different days.[18,19] In stress protocols, patients are typically administered doses in the range of 555 MBq–1.11 GBq. Standard exercise protocols involve injecting the radiopharmaceutical at the peak of exercise.[20] During the injection of the radiopharmaceutical, there are doctors, technicians, and nurses in the room. Radiation workers in the room are exposed to radiation

during the injection of the radiopharmaceutical. In the study, the ^{99m}Tc radiation source was additionally selected at 24 mCi to estimate the doses incurred by radiation workers. As the radiation dose increases, exposure and the effects of radiation on the body also increase. However, the farther the radiation source is from the body, the lower the dose that will be incurred. As shown in Table 3, there is a significant difference in dose between the results obtained at distances of 50 cm and 200 cm.

Table 3. Radiation dose exposure at different durations and distances for ^{99m}Tc- 24 mCi

	5-minute µGy			1-minute µGy		
Organ doses D(T)	50 cm	100 cm	200 cm	50 cm	100 cm	200 cm
Red bone marrow	1.94	0.73	0.24	0.39	0.15	0.05
Colon	3.11	1.02	0.32	0.62	0.21	0.06
Lung	2.62	0.93	0.30	0.52	0.18	0.06
Stomach	3.59	1.13	0.34	0.72	0.23	0.07
Breast	4.78	1.58	0.42	0.96	0.33	0.08
Remainder	2.42	0.87	0.28	0.48	0.17	0.06
Testes	2.47	1.10	0.37	0.49	0.21	0.07
Bladder	2.82	1.13	0.37	0.56	0.22	0.07
Oesophagus	2.33	0.80	0.29	0.47	0.18	0.06
Liver	2.90	0.96	0.29	0.58	0.19	0.06
Thyroid	3.42	1.19	0.46	0.69	0.29	0.09
Bone surface	1.78	0.81	0.30	0.36	0.16	0.06
Brain	1.03	0.49	0.18	0.21	0.09	0.04
Salivary gland	2.44	0.86	0.28	0.49	0.18	0.06
Skin	1.96	0.82	0.29	0.39	0.17	0.06
Adrenals	1.31	0.46	0.17	0.26	0.10	0.03
Extrathor airways	2.50	1.08	0.37	0.50	0.19	0.07
Gall bladder	2.88	0.84	0.23	0.58	0.17	0.05
Heart	3.08	1.06	0.31	0.62	0.21	0.06
Kidneys	1.55	0.56	0.19	0.31	0.11	0.04
Lymphatic nodes	2.82	0.95	0.31	0.56	0.22	0.06
Muscle	1.79	0.77	0.27	0.36	0.15	0.05
Oral mucosa	2.53	0.99	0.29	0.51	0.18	0.06
Pancreas	3.01	0.98	0.32	0.60	0.19	0.06
Prostate	1.83	0.67	0.28	0.37	0.15	0.06
Small intestine	3.16	1.07	0.33	0.63	0.22	0.07
Spleen	1.58	0.56	0.20	0.32	0.11	0.04
Thymus	3.43	1.29	0.40	0.68	0.24	0.08
Eye lens	1.63	2.29	0.33	0.33	0.37	0.07
Whole eye	3.04	1.20	0.34	0.61	0.25	0.07
Hp(10) (µSv)	5.74	1.22	0.38	1.15	0.35	0.07
Effective dose (µSv)	2.94	1.03	0.32	0.59	0.21	0.06

In Table 4, when a 100 mCi radioactive source of ^{131}I was used at a distance of 50 cm, the highest doses within a 5-minute duration were received by the breast (55.66 µGy), stomach (45.65 µGy), and thyroid (42.93 µGy), respectively. When this duration was reduced to 1 minute at the same distance, the highest received doses decreased to 11.13 µGy, 9.13 µGy, and 8.59 µGy for the breast, stomach, and thyroid, respectively. In Table 4, when a 100 mCi radioactive source of ^{131}I was used at a distance of 100 cm, the highest doses within a 1-minute duration were received by the breast (18.44 µGy), stomach (15.21 µGy), and thyroid (12.90 µGy), respectively. When this duration was reduced to 1 minute at the same distance, the highest received doses decreased to 3.69 µGy, 3.04 µGy, and 2.58 µGy for the breast, stomach, and thyroid, respectively. In Table 4, when a 100 mCi radioactive source of ^{131}I was used at a distance of 200 cm, the highest doses within a 5-minute duration were received by the testes (5.67 µGy), breast (4.74 µGy), and Thymus (4.03 µGy), respectively. When this duration was reduced to 1 minute at the same distance, the highest received doses decreased to 1.13 µGy, 1.01 µGy, and 0.95 µGy for the testes, prostate, and breast, respectively. When a 100 mCi radioactive source of ^{131}I was used, the effective doses obtained within a 5-minute duration at distances of 50 cm, 100 cm, and 200 cm were found to be 36.58 µSv, 12.53 µSv, and 3.83 µSv, respectively. When the duration was reduced to 1 minute at the same activity and distances, the effective doses were found to be 7.32 µSv, 2.51 µSv, and 0.77 µSv, respectively.

Table 4. ^{131}I – Radiation dose exposure at different durations and distances for ^{131}I - 100 mCi

	5-minute µGy			1 minute µGy		
Organ doses D(T)in	50 cm	100 cm	200 cm	50 cm	100 cm	200 cm
Red bone marrow	26.11	9.64	3.00	5.22	1.93	0.60
Colon	37.58	12.55	4.00	7.52	2.51	0.80
Lung	33.81	11.77	3.64	6.76	2.35	0.73
Stomach	45.65	15.21	3.38	9.13	3.04	0.68
Breast	55.66	18.44	4.74	11.13	3.69	0.95
Remainder	31.20	11.02	3.50	6.24	2.20	0.70
Testes	27.52	11.58	5.67	5.50	2.32	1.13
Bladder	35.08	12.93	3.78	7.02	2.59	0.76
Oesophagus	31.91	11.71	3.37	6.38	2.34	0.67
Liver	36.26	11.66	3.41	7.25	2.33	0.68
Thyroid	42.93	12.90	3.97	8.59	2.58	0.79
Bone surface	20.75	9.13	3.26	4.15	1.83	0.65
Brain	16.04	7.55	2.47	3.21	1.51	0.49
Salivary gland	31.56	12.03	3.59	6.31	2.41	0.72
Skin	24.26	10.22	3.46	4.85	2.04	0.69
Adrenals	21.64	4.55	2.27	4.33	0.91	0.45
Extrathor airways	31.65	13.42	3.20	6.33	2.68	0.64
Gall bladder	39.66	12.78	3.65	7.93	2.56	0.73
Heart	38.06	12.99	3.91	7.61	2.60	0.78
Kidneys	20.67	6.95	2.35	4.13	1.39	0.47
Lymphatic nodes	34.12	11.91	3.88	6.82	2.38	0.78
Muscle	23.66	9.78	3.33	4.73	1.96	0.67
Oral mucosa	31.37	12.27	3.52	6.27	2.45	0.70
Pancreas	38.45	11.67	3.76	7.69	2.33	0.75
Prostate	24.07	12.07	5.03	4.81	2.41	1.01
Small intestine	38.22	12.89	3.87	7.64	2.58	0.77
Spleen	21.12	6.78	2.68	4.22	1.36	0.54
Thymus	42.90	15.21	4.03	8.58	3.04	0.81
Eye lens	33.38	10.66	0.51	6.68	2.13	0.10
Whole eye	39.48	13.43	3.75	7.90	2.69	0.75
Hp(10) (µSv)	62.22	16.92	5.34	12.44	3.38	1.07
Effective dose (µSv)	36.58	12.53	3.83	7.32	2.51	0.77

DISCUSSION

Radioactive pharmaceuticals such as ^{99m}Tc and ^{131}I are utilized for both diagnostic and therapeutic purposes in nuclear medicine departments. During the administration and imaging procedures of these radiopharmaceuticals, radiation workers are constantly exposed to radiation doses. Monitoring of these doses is performed through dosimeters. The International Atomic Energy Agency (IAEA) recommends an average annual dose of <5 mSv for radiation workers employed in nuclear medicine departments.[21,22] The amount of radiation dose is directly proportional to the time of exposure. The less time one is exposed to radiation, the lower the dose received. Radiation workers should plan their tasks in advance and avoid unnecessary time spent in the radiation field. In the study conducted by Kara,[17] indicating a linear dependence of effective dose on the exposure time of the human body. Similar results were obtained in this study as well, indicating that as the exposure duration increases, the radiation dose also increases accordingly. In their study, Işıkçı et al.,[22] determined the radiation dose contribution of radiopharmaceuticals labeled with ^{99m}Tc, 18F, and 68Ga to technologists' annual occupational doses over a period of 6 years. The dose contribution of positron emission tomography/computed tomography was found to be the highest among diagnostic nuclear procedures. Albersberg et al.,[23] aimed to predict and subsequently measure the occupational radiation exposure of all personnel using [^{99m}Tc]Tc-PSMA-I&S, which has begun to be used for the identification of tumor-positive lymph nodes during salvage prostate cancer surgery. In the study, the effective dose for personnel working with [^{99m}Tc]Tc-PSMA-I&S was found to be comparable to other ^{99m}Tc radiopharmaceuticals, hence it was concluded to be safe for imaging and radioguided surgery. In our study, it was observed that the radiation dose exposure remained within the specified limit values and did not exceed the annual dose limit. In their study, Elshami et al.[24] investigated the temporal trends and variations in occupational radiation doses among nuclear medicine workers. The study emphasized the possibility of implementing measures to reduce occupational radiation exposure for nuclear medicine technologists, who constitute the most exposed group in the study. In their study, Fathy et al.[3] determined the radiation doses incurred by radiation workers in the nuclear medicine department. According to this study, the average doses per patient for cardiac stress and rest were found to be 20.4±5.0 and 16.0±3.8 μSv, respectively. For bone scans, 67Ga, 18F, and ^{131}I therapy, the average doses per patient were found to be 6.1±2.5, 6.0±1.4, 11.1±2.2, and 4.1±2.6 μSv, respectively. Analysis of data and measurements related to occupational radiation risks can lead to the development of different ways to reduce radiation exposures.[25] Evaluation and improvement of radiation protection measures are important. Similarly, assessing radiation protection practices can assist in developing measures against radiation exposure.[26]

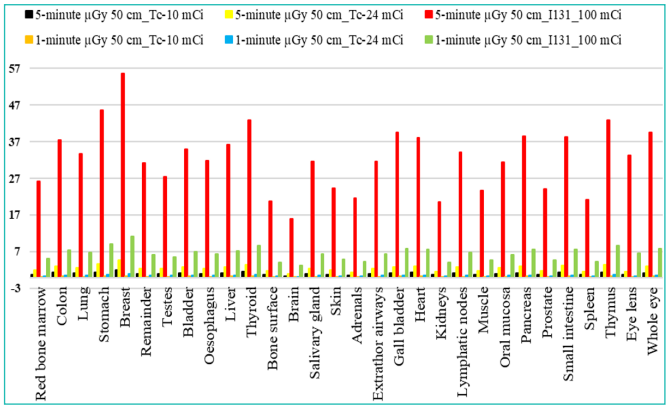

Figure 2. Radiation dose received at 50 cm distance at different doses and durations

CONCLUSION

In this study, individual organ doses and effective dose results at different distances and durations were obtained using Monte Carlo simulation for ^{99m}Tc and ^{131}I radioactive sources. It was observed that the organ dose and effective dose vary with the source strength and exposure duration. According to the ICRP 103 report, the annual effective dose for radiation workers should not exceed an average of 20 mSv over any consecutive five-year period and 50 mSv in any single year. The study demonstrates that as the distance from the radiation source increases, the recommended annual dose limit is not exceeded. Regardless of how low the doses may be, individuals working in radiation fields must make greater efforts to reduce radiation doses using the ALARA principles.

ETHICAL DECLARATIONS

Ethics Committee Approval

Since the conducted research is not related to either human or animal use, there is no need for an ethics committee approval.

Informed Consent

Informed consent is not required due to the design of the study.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Comparison of TAPP and TEP in laparoscopic inguinal hernia repair

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ABSTRACT

Aims: The aim of this study was to compare TAPP and TEP techniques, which are laparoscopic inguinal hernia repair techniques. In this study, it was tried to determine whether one technique has an advantage over the other in terms of surgery, hospitalization and recovery times, recurrence, postoperative bleeding and testicular edema.

Methods: Totally 62 patients who underwent laparoscopic inguinal hernia repair between January 2015 and January 2020 were included in this retrospective study.

Results: Among the patients who underwent TAPP and TEP operations; it was determined that there was no significant difference in terms of operation time, recovery time, hospital stay, recurrence and complications ($p>0.05$).

Conclusion: Based on the results of this study, TEP and TAPP are equally effective and safe as laparoscopic hernia repair surgery. The choice of which approach to perform can be made according to the skill and preference of the surgeon.

Keywords: Inguinal hernia, laparoscopy, TAPP, TEP

INTRODUCTION

One of the frequently performed surgeries is the repair of inguinal hernia for adults throughout the world.[1] Inguinal hernia makes up 75% of all abdominal wall hernias, %97 of which are inguinal and 3% are femoral hernia. 90.2% of inguinal hernias are present amongst men, and 70.2% of femoral hernias occur in women.[2] As an inguinal hernia can easily be detected as a palpable mass on the inguinal region, it is often diagnosed on time. It is generally treated successfully via surgery and doesn't threaten life. Emergency surgery might be required in cases of strangulation due to possible complications like bowel necrosis, diffuse peritonitis, and septic shock.[3] One of the primary concerns associated with inguinal hernia repair is the possibility of relapse, but it has been diminished by the adoption of a uniform surgical technique and the production of artificial mesh.[4] When it comes to laparoscopic inguinal hernia repair, transabdominal preperitoneal (TAPP) repair and totally extraperitoneal (TEP) repair are the two most commonly utilized techniques. In TAPP management, the peritoneal cavity must be penetrated in order to insert the mesh via the incision in the peritoneum. Synthetic mesh is placed in the peritoneal cavity to cover all of the possible hernia spots in the inguinal region. Later, a peritoneal mesh is covered so that the mesh is inserted between the abdominal wall and preperitoneal tissue, where it will fuse with fibrous tissue. In TEP management, contrary to TAPP, the peritoneal cavity is not penetrated, and mesh is utilized outside the periton to provide coverage for the hernia. Despite being considered a more difficult method than TAPP, the chances of complications such as adhesion formation and internal organ damage leading to intestinal obstruction are less likely with this approach.[5]

This study aims to conduct a comparison of the surgery time, postoperative bleeding ratio, time of hospital stay, time of recovery, relapse ratio, and testicular edema related to TEP and TAPP inguinal hernia repair.

METHODS

Between January 2015 and January 2020, a retrospective analysis of laparoscopic inguinal hernia repair was conducted on a total of 62 patients. Using the cohort method, the patients who received TAPP and TEP treatments were categorized into two groups and compared. Surgery time, testicular edema, postoperative

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bleeding, time of hospital stay, time for recovery, and relapse ratios of the two groups were evaluated. Ethics committee approval was provided by the faculty before starting the study Dicle University Medical Faculty Non-interventional Studies Ethics Committee (Date: 26.11.2020, Decision No:11). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All of the patients were informed about the study, and written consent was provided, declaring they agreed to be a part of the study. Written consent of parents was taken for the patients who were not of full age legally. Individuals aged 15 years or above, both male and female, who were diagnosed with inguinal hernia and agreed to surgery were enrolled in the study. Patients with cases of contradiction for laparoscopic inguinal hernia repair (high-risk for general anesthesia, intraabdominal extensive ascites, intraabdominal active infection, story of open pelvic surgery, large scrotal hernia, coagulation disorder that is refractory to treatment, strangulation), patients who were on immunosuppressive agents for any indication, patients with acute or chronic infections were not included in the study.

Statistical Analysis

Statistical analysis was performed by the SPSS program. As the data distribution was not homogenous, analysis was performed using non-parametrical tests. The Mann-Whitney U test or the Kruskal-Wallis H test was utilized to compare continuous parameters. Categorical parameters were evaluated by the Chi-square test. P values that were lower than 0.05 were regarded as indicative of statistical significance.

RESULTS

27(43.5%) of 62 patients operated for inguinal hernia were operated using the TEP method, and 35 (56.5%) were operated by the TAPP approach. Of the patients with TEP operation, 24(38.7%) had unilateral, 3(4.8%) had bilateral inguinal hernia; 28(45.2%) of TAPP patients had unilateral, and 7(11.3%) had a bilateral inguinal hernia operation. 2(3.2%) of the patients were female and 60(96.8%) were male. Patients who were operated with TEP were all male, while 2(5.7%) of the patients in the TAPP group were women, and 33(94.3%) of them were men (Table 1).

The mean age of the patients was 41±14.29 (17-66) years old in the TEP group, and 43.06±15.97 (19-78) years old in the TAPP group; The mean age did not differ significantly between the two groups (Table 1) (p=0.601).

It was found that 7 (11.3%) of the patients had a relapse, and 55 (88.7%) didn't. While relapse occurred in 2 (7.4%) of the TEP groups patients and 5(14.3%) of those in the TAPP group, the comparison between the groups did not reveal any significant difference concerning the rate of relapse (p=0.396). Evaluating the two groups as subgroups of each; 1 (4.2%) of the patients with unilateral TEP, 1 (33.3%) of those with bilateral TEP, 3 (10.7%) of patients with unilateral TAPP, and 2 (%28.6) of those with bilateral TAPP had a relapse (Table 2).

Postoperative bleeding was observed in 3 of the patients (4.8%), while 59 (95.2%) of them did not have such a condition. 1 (3.7%) of the patients in the TEP group and 2 (5.7%) of those in the TAPP group had bleeding in the postoperative period; The comparison between the two groups did not show any significant difference concerning postoperative bleeding (p=0.715). 1 (4.2%) of the patients with unilateral TEP had postoperative bleeding while 2 (7.1%) of those with unilateral TAPP did. None of the patients going through bilateral inguinal hernia repair surgery had bleeding in the postoperative period (Table 2).

Testicular edema was observed in 3(4.8%) of the patients and not in 59 (95.2%) of them. In the TEP group, 2 (74%) of the patients had testicle edema while 1 (2.9%) of those in the TAPP group did; therefore, the comparison between the two groups did not show any significant difference concerning testicular edema incidence (p=0.480).

Table 1. Distribution of patients in terms of operation method and age

	TEP		TAPP	
	Unilateral TEP (n, %)	Bilateral TEP (n, %)	Unilateral TAPP (n, %)	Bilateral TAPP (n, %)
Surgery type	24 (38.7%)	3 (4.8%)	28 (45.2%)	7 (11.3%)
Age (mean.±sd)	41±14.29 (min: 17-max: 66)		43.06±15.97 (min: 19-max: 78)	

TAPP: transabdominal preperitoneal repair, TEP: totally extraperitoneal repair, SD: standard deviation

Table 2. Rate of incidence of relapse, postoperative bleeding, and testicular edema in patients considering surgery type

	Unilateral TEP (n, %)	Bilateral TEP (n, %)	Unilateral TAPP (n, %)	Bilateral TAPP (n, %)	Total (n, %)
Relapse	1 (4.2%)	1 (33.3%)	3 (10.7%)	2 (28.6%)	7 (11.3%)
Postoperative bleeding	1 (4.2%)	0	2 (7.1%)	0	3 (4.8%)
Testical edema	1 (4.2%)	1 (33.3%)	0	1 (14.3%)	3 (4.8%)
Total (n, %)	24 (100%)	3 (100%)	28 (100%)	7 (100%)	62 (100%)

TAPP: transabdominal preperitoneal repair, TEP: totally extraperitoneal repair

1 (4.2%) of the patients operated with unilateral TEP presented with testicular edema, 1 (33.3%) of those who had bilateral TEP, and 1 (14.3%) with bilateral TAPP had testicular edema. None of the patients operated unilaterally with TAPP had testicular edema (Table 2).

Time from the first skin incision until the last suture was recorded during the surgeries. It was found that the TEP groups average surgery time was 138.15±49.22 minutes, and the surgery time ranged from 60 minutes, which was the shortest, to 238 minutes, which was the longest. In the TAPP group, the mean surgery time was 136.49±49.24 minutes, the range of surgery time was from 28 minutes, which was the quickest, to 295 minutes, which was the longest. Comparing the TEP and TAPP groups, no statistically significant variation was observed concerning surgery time (p=0.896) (Table 3).

Evaluating the groups regarding time for the hospital stay, it was found that the mean time for hospital stay in the TEP group was 1.85±1.03 days. The hospitalization period varied between one day, which was the least, and five days, which was the most. In the TAPP group, the mean time for hospital stay was 2.03±1.38 days, the shortest time was one day, and the longest stay lasted for eight days. Between TEP and TAPP groups, no statistically significant disparity was detected concerning the length of hospitalization (p=0.580) (Table 3).

Analyzing two groups in terms of recovery parameters, it was seen that the mean time for recovery in the TEP group was 7.67±3.78 days, while the shortest time was 3 days and the longest was 21 days. In the TAPP group, the mean time for recovery was 9.51±4.98 days, the range of time for recovery was from 5 days, which was the quickest, to 18 days, which was the slowest. Between TEP and TAPP groups, no statistically significant disparity was detected concerning the time taken for recovery (p=0.114) (Table 3).

Evaluating 62 patients operated on for inguinal hernia regarding subgroups of the operation type they had, it was found that the mean surgery time in the unilateral TEP group was 135.62±48.46 minutes, the range of surgery time was between 60 minutes, which was the least, and 238 minutes, which was the most. In the bilateral TEP group, the mean surgery time was 158.33±61.71 minutes, and the range of surgery time was between 60 minutes, which was the least, and 238 minutes, which was the most. In the unilateral TAPP group, the mean time of surgery was 129.36±43.90 minutes, the shortest surgery time was 28 minutes, and the longest one was 225 minutes. In the bilateral TAPP group, the mean surgery time was 165±62.28 minutes, while the shortest surgery duration was 112 minutes, and the longest one was 295 minutes. The comparison of surgery time between the groups undergoing unilateral TEP, bilateral TEP, unilateral TAPP, and bilateral TAPP did not reveal any statistically significant difference (p>0.05) (Table 4).

The average time of hospital stay for these four groups was found to be 1.92±1.06 days upon comparison, and varied between 1 day, which was the least, and 5 days, which was the most in the unilateral TEP group. On the other hand, in the bilateral TEP group, the mean time of hospital stay was 1.33±0.58 days, the shortest stay was for 1 day, and the longest one lasted for 2 days. In the unilateral TAPP group, the mean time of hospital stay was 1.85±1.03 days, and the shortest time of stay was 1 day, while the longest stay lasted for 8 days. In the bilateral TAPP group, the mean time of stay was 2±1 days, the shortest one was for 1 day, and the longest stay lasted for 3 days. The comparison between the groups undergoing unilateral and bilateral TEP, and unilateral and bilateral TAPP, did not reveal any statistically significant difference in hospital stay duration (p>0.05) (Table 4).

These four groups were further analyzed for recovery parameters in the hospital. In the unilateral TEP group, the mean time of recovery was 6.92±2.72 days, The shortest recorded recovery time was 1 day, while the longest was 15 days. In the bilateral TEP group, the mean time of recovery was 13.67±6.35 days. The range of time for recovery was from 10 days, which was the quickest, to 21 days, which was the slowest. In the unilateral TAPP group, the mean time of recovery was 9.57±3.10 days, the shortest time was 3 days and the longest one took 30 days. In the bilateral TAPP group, the mean time of recovery was 8.71±4.56 days. The duration of recovery varied between 5 days, which was the quickest, and 15 days, which was the slowest. The comparison between the groups undergoing unilateral and bilateral TEP, and unilateral and bilateral TAPP, did not reveal any statistically significant difference in recovery time (p=0.012) (Table 4).

Table 3. Comparison of TEP and TAPP groups in terms of surgery time, hospital stay period, and recovery time

	Operation type	Number (n)	Mean.±SD	Min-max	P
Surgery Time	TEP	27	138.15±49.22min	60-238 min	p>0.05
	TAPP	35	136.49±49.24 min	28-295 min	
Time of hospital stay	TEP	27	1.85±1.03 days	1-5 days	p>0.05
	TAPP	35	2.03±1.38 days	1-8 days	
Time of recovery	TEP	27	7.67±3.78 days	3-21 days	p>0.05
	TAPP	35	9.51±4.98 days	5-18 days	

TAPP: transabdominal preperitoneal repair,TEP:totally extraperitoneal repair,SD:standard deviation

Table 4. Comparison of surgery time, time of hospital stay, and time of recovery between unilateral and bilateral TEP and TAPP groups

Operation type	Number (n)	Mean.±SD	Min-max	P
Surgery time				0.529
Unilateral TEP	24	135.62±48.46 min	60-238 min	
Bilateral TEP	3	158.33±61.71 min	90-210 min	
Unilateral TAPP	28	129.36±43.90 min	28-225 min	
Bilateral TAPP	7	165.0±62.28 min	112-295 min	
Time of hospital stay				0.746
Unilateral TEP	24	1.92±1.06 days	1-5 days	
Bilateral TEP	3	1.33±0.58 days	1-2 days	
Unilateral TAPP	28	2.04±1.48 days	1-8 days	
Bilateral TAPP	7	2±1 days	1-3 days	
Time of recovery				0.012
Unilateral TEP	24	6.92±2.72 days	3-15 days	
Bilateral TEP	3	13.67±6.35 days	10-21 days	
Unilateral TAPP	28	9.57±3.10 days	3-30 days	
Bilateral TAPP	7	8.71±4.56 days	days	

TAPP: Transabdominal preperitoneal repair, TEP: Totally extraperitoneal repair, SD: Standard deviation

DISCUSSION

Inguinal hernia is a common problem, significantly lowering the quality of life. Inguinal herniorrhaphy is one of the most prevalent general surgical operations worldwide and emergency surgery might be required in cases of obstructed or strangulated inguinal hernia.[5] Although inguinal hernia is encountered commonly and in spite of several studies comparing different techniques of repair, a consensus is not reached on the ideal repairing technique yet. Currently, research has been continuing on the topic, and the opinion that the ideal surgical technique is the one personalized for the patient by the operating surgeon is dominant.[6] In inguinal hernia repair, a technique that is easy and simple to perform, that requires minimal incision and dissection, providing enough vision and minimizing relapse must be preferred. Deciding on the method, patients comfort, cost of the surgery, duration of hospitalization, and time taken to resume work must be considered as well.[5,7] Inguinal hernia can be repaired by laparoscopic and open techniques.[5]

Comparing TAPP and TEP, two laparoscopic techniques used to repair inguinal hernias, is the aim of this study. It was also studied whether one of the techniques is advantageous over the other regarding surgery time, time of hospital stay and recovery, relapse, postoperative bleeding, and testicular edema incidence.

In the world population, approximately 90% of all inguinal hernias are present in males, while around 10% are seen in females.[8] In this study, it was found that 96.8% of patients operated for inguinal hernia are male, which was consistent with the existing literature. Similar to our study, Çelik and Erbil[9] and Köckerling et al.[10] have compared patients operated with TEP and TAPP procedures, and the comparison between the groups did not reveal any statistically significant disparity in age and gender.

Although it was found in this study that TEP surgery takes a longer time, the comparison between the two procedures did not reveal any significant difference in terms of the time of surgery. Supporting our study, Çelik and Erbil[9] found that although operation time was slightly longer for patients operated with the TEP procedure, the difference was not significant. The possible justification for the fact that the TEP procedure takes a long time for surgery is that because there is a limited area for work, so possible anatomical landmarks might be misinterpreted, and wider dissection gets to be performed. On the other hand, In the randomized controlled study carried out by Krishna et al.,[11] they detected that TAPP results in a longer mean time of surgery than TEP. According to those who carried out the study, the reason TAPP application takes longer surgery time is suggested to be due to the time required for suturing the periton that covers the mesh.

Even though the difference is not found significant in this study, the average time of surgery was longer for bilateral TAPP (165±62.28 min) than bilateral TEP (158.33±61.71 min). The possible explanation for this might be that in bilateral hernia repair, dissection is performed as a shift from one side to the other on the plane of work during TEP. At the same time, a separate flap formation might be needed for each side in TAPP. Actually, a new flap formation on the second site of operation during TAPP doesn't take a long time because the medial (retro-pubic) dissection is already completed on the first site of operation.[12] This fact could be the explanation for not detecting a significant difference between bilateral inguinal hernia operation times of TEP and TAPP in this study. On the other hand, the shortest time of recovery after laparoscopic hernia repair was seen with unilateral TEP, while the longest time was seen with bilateral TEP. Another explanation of this result could be variations in the epidemiological profile of the patients.

In the meta-analysis by Wei et al.[13] experienced surgeons (ones who had performed TEP in approximately 30-100 cases) it was discovered that there was no statistically significant difference between TAPP and TEP in relation to clinical outcomes. Additionally, when subgroup analysis is applied, it was revealed that the surgeons' level of experience does not affect the incidence of complication but only affects the operation time. This finding was clarified in their meta-analysis, suggesting that although surgeons of different levels of experience had done the evaluation, all of them were experienced enough to perform the surgery securely.[13] Similarly,

the fact that rates of complication in both TEP and TAPP groups were low in this study could be because the operating surgeons were experienced and qualified enough.

In the study carried out by Vãrcuæ et al.[14] they detected the time of hospital stay for patients operated using both TEP and TAPP techniques as approximately 2 days, supporting our article. The results of this study indicated that there was no difference between the TAPP and TEP groups in terms of hospital stay duration. Similarly, in a study by Rao et al.[15] TAPP and TEP groups were not found to be significantly different regarding hospital stay and return to daily activities.

In laparoscopic inguinal hernia repair, prominent outcome parameters are complications related with surgical wound and incidence of relapse.[12] In this study, consistent with Özkaya's research, no significant difference was detected between TAPP and TEP groups in terms of relapse.[16] In favor of our article, existing meta-analysis do not show difference between TAPP and TEP in terms of relapse, either. For any hernia surgery, recurrence is accepted as the most critical endpoint.[5,17,18] For a long time, the absence of a recurrence was the only factor considered to evaluate the success of hernia repair. Compared with unilateral hernia repair, bilateral repair surgeries were found to have a higher recurrence rate in this study. The incidence of two or more recurrences after inguinal hernia repair was significantly higher in those with a defect size larger than 3cm.19 Similarly, the larger defect in bilateral inguinal hernia repair may have increased the recurrence rate.

After the laparoscopic repair of an inguinal hernia, scrotal edema or hematoma are common complications.[5,17] In this study, the rate of incidence of edema was 7.4% inTEP group and 2.9% in the TAPP group. Similarly, in the study carried out by Jaiswal and his friends, it was reported that scrotal edema was encountered by 8.9% of patients in the TAPP group and 8.9% of those in TEP group.[17] On the other hand, the incidence of scrotal edema in TAPP group was reported to be 34% and in TEP group as 9.4%.[11] However, scrotal edema incidence was found to be as high as 33.3% amongst patients treated with bilateral TEP. In the study by Hidalgo et al.[20] advanced age, large hernia defect, complete inguinal hernia and presence of dital indirect sac are reported as risk factors related with scrotal edema formation. The justification for difference of the results of the mentioned studies in terms of rates of scrotal edema formation could be because the epidemiological and clinical factors are not similar.

In this study, it has been found that there is no significant difference between TEP and TAPP surgical procedures, considering postoperative bleeding. Consistent with our study, Jaiswal et al.[17] have reported that although postoperative hematoma incidence is higher in TAPP group than that in TEP group, this difference is not statistically significant.

Similar to this study, Wei et al.[13] have confirmed through the meta-analysis comparing TAPP and TEP for laparoscopic hernia repair, that no significant disparity was detected between TAPP and TEPP in terms of recovery time, operation time, hospital stay duration, and total complications.

CONCLUSION

This studys results indicate that TEP and TAPP are equally efficient and safe as laparoscopic hernia surgery procedures. It was anticipated that because it is not required during TEP to penetrate into the abdominal cavity, it would lower incidence of complications and enhance the clinical results, but the results did not support this idea.

During inguinal herniorapphy, both laparoscopic techniques can be favored. The decision of which approach to use, is dependent on the surgeon and their surgical skills.

Further comprehensive studies can be planned, including the surgeons learning curves and levels of experience, evaluating the postoperative pain scores and cost-efficiency analysis of TEP and TAPP surgical procedures.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Dicle University Medical Faculty Non-interventional Studies Ethics Committee (Date: 26.11.2020, Decision No:11).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Evaluation of styloid process elongation: a cross-sectional study

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ABSTRACT

Aims: The styloid process is a cylindrical projection of the temporal bone. When the length of the styloid process exceeds 30 mm, it is considered elongated. The aim of this study was to investigate the length, thickness and morphological pattern of the styloid process of patients using panoramic radiography and to evaluate the relationship with age and gender.

Methods: The study is designed retrospective cross-sectional and consisted of 3012 panoramic radiographs from patients routinely referred within the last six months, in which the borders of the styloid process region were clearly visualised on radiographs. Age and gender data of the patients were recorded. Styloid process was evaluated in terms of length, thickness and Langeais classification.

Results: The mean age of the patients was 38.32 years. 34.4% (n:1156) were male and 61.6% (n:1856) were female. The mean styloid process length was >30 for 32% of the patients. The thickness of the right styloid process was 2.73±1.56 and that of the left styloid process was 2.69±1.5. The length of the styloid process ranged between 0-79.98 mm with a mean of 26.12±10.78 mm. Most of the patients were in Langeais Type 1 class in right and left styloid process. There was a statistically significant correlation between the length, thickness and Langeais classification of right and left styloid process ($p<0.01$). There was a statistically significant correlation between age and styloid process length and thickness. Also, styloid process length and thickness were statistically greater in males than females ($p<0.01$).

Conclusion: In this study, the highest prevalence of elongation styloid process in Türkiye evaluated with panoramic radiographs is reported. Although styloid process elongation is mostly asymptomatic, more multicenter studies are needed to investigate the reasons for such a high prevalence.

Keywords: Styloid process, styloid process elongation, prevalence

INTRODUCTION

Styloid process (SP) is a cylindrical projection of the temporal bone located anterior to the stylo-mastoid foramen.[1] SP apex lies between the internal and external carotid arteries anteriorly and inferiorly. This anatomical location is a clinically important formation because it extends lateral to the pharyngeal wall and tonsillar fossa, and the ligaments to which it originates have functions such as mastication and swallowing.[2-5] The length of the SP is between 20-30 mm in the general population, and the SP is considered elongated when it is longer than 30 mm.[1] Panoramic radiographs can be used to determine SP elongation and elongated SP can be classified according to its morphology.[6,7] Langlais et al.[7] classified elongated SP as Type I (Elongated), Type II (Pseudo-articular), Type III (Segmented) based on its morphology.

Typically, an elongated SP is not associated with any pathology. However, in some cases, an elongated SP may be linked to various symptoms such as a sensation of a foreign body in the throat, pain when moving the head, dizziness, dysphagia, facial pain, tinnitus, and trismus. This cluster of symptoms associated with an elongated SP is referred to as Eagle syndrome.[8]

The aim of this study is to investigate the length, thickness, and morphological pattern of the SP using panoramic radiography in patients residing in the Kütahya province of Türkiye, and to evaluate its association with age and gender.

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METHODS

Ethics

The study, structured as a retrospective cross-sectional analysis, involved patients who regularly sought treatment at the Kütahya Health Sciences University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery. Obtaining approval from the Kütahya Health Sciences University Non-interventional Clinical Researches Ethics Committee, permission with decision number 2022/06-22 was secured. Conducted in accordance with the guidelines outlined in the Helsinki Declaration of Human Rights, the study obtained written consent from each patient.

Sample Size

While power analysis was performed; Since it was a prevalence (%, prevalence) study, the incidence of any event (prevalence of SP prolongation for this study) was taken into account. Referencing the study by Şişman et al.[20] a tolerance level (acceptable error) of d=0.05, and a significance level of 0.05 (95% confidence level) were employed for a prevalence of 15. The sample size was calculated with a power value of 80% and the study was planned to include at least 265 patients.

Study Design

During the last six months (January 2022 to July 2022), the study involved the examination of 4151 panoramic radiographs from patients who regularly visited the Kütahya Health Sciences University Faculty of Dentistry, specifically the Department of Oral and Maxillofacial Surgery. 1139 patients in whom the borders of the SP region could not be seen on panoramic radiograph were excluded from the present study. The study was conducted with 3012 panoramic radiographs. Age and gender data of the patients were recorded. SP were evaluated in terms of length, thickness and Langeais classification. The length of the SP was assessed from the tympanic cavity to its endpoint, categorizing those surpassing 30 mm as elongated. The thickness of the SP was measured at its thickest part. The classification of the SP morphology into Type 1, Type 2, and Type 3 was based on the Langeais criteria (Figure 1).

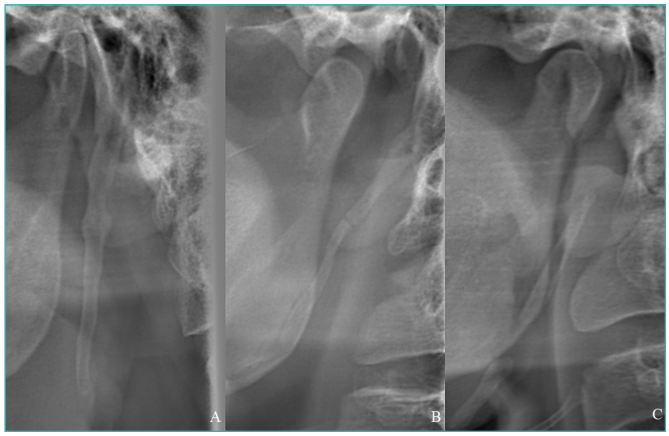

Figure 1. A. Type I SP, B. Type II SP, C. Type III SP

Statistical Analysis

Statistical analyses were performed using IBM SPSS 20 program. Descriptive statistics including minimum, maximum, mean and standard deviation values were used for numerical data. Frequency tables including frequency and percentage values were used for categorical data. Kolmogorov-Smirnov and Shapiro-Wilk tests were evaluated for the suitability of the distribution of the data of numerical variables to normal distribution. Kolmogorov-Smirnov and Shapiro-Wilk tests were evaluated for the suitability of the distribution of the data of numerical variables to normal distribution. Since the sample size was large enough, parametric hypothesis tests were used on data suitable for normal distribution. Independent-Samples T Test was used to show the difference between the two groups in terms of the mean of the numerical variable analyzed. Chi-square test was used to examine the variation and differences between two categorical variables. Pearson Correlation coefficient was calculated to express the direction and level of the relationship between two numerical variables. Phi and Cramer V correlation coefficients were used to calculate the degree and direction of the relationship between two categorical variables. In addition, histograms and bar graphs were used to obtain information about the distribution of the data of the variables. $p<0.05$ and $p<0.01$ were considered statistically significant.

RESULTS

The ages of the patients ranged between 12-90 years with a mean age of 38.32 years. 34.4% (n:1156) of the patients were male and 61.6% (n:1856) were female. The length of the SP ranged between 0-79.98 mm with a mean length of 26.12±10.78 mm. For the mean SP length, the proportion of >30 was calculated as 32%. There were 192 (6.4%) patients with 0 for the length of the right SP and 0 for the thickness of the right SP. There were 203 (6.7%) patients with 0 length of the left SP and 0 thickness of the left SP.

The mean length of the right SP was 26.04±11.46; the mean length of the left SP was 26.20±11.42. There was a positive correlation between the length of the right SP and the length of the left SP at the 77.6% level and it was statistically significant ($p<0.01$; Table 1). The proportion of patients with right SP length >30 was 32.8% and the proportion of patients with left SP length >30 was 33.5%. When the length of the right SP and the length of the left SP were divided into two categories as ≤30 and >30 and the relationship between them was examined; there was a relationship of 64.9% and it was statistically significant ($p<0.01$; Table 2). The rate of those with both right and left >30 is 77.4%. The rate of those with ≤30 in both right and left is 87.9%.

Table 1. Relationships between Length and Thickness of SP According to Regions

	Length of the right SP and length of the left SP	Thickness of the right SP and thickness of the left SP	Length of the right SP and thickness of the right SP	Length of the left SP and thickness of the left SP
Pearson correlation	0.776**	0.478**	0.335**	0.349**
p-value	0.000	0.000	0.000	0.000

**: Correlation is significant at the 0.01 level

Table 2. Relationships between length and classification types of SP

	Length of the right SP and the length of the left SP		Right and left SP classification type
Phi	0.649**	Cramer`s V	0.537**
χ^2	1270.432	χ^2	1588.546
p-value	0.000	p-value	0.000

**: Correlation is significant at the 0.01 level

The thickness of the right SP was 2.73±1.56; the thickness of the left SP was 2.69±1.50. There is a positive correlation between the thickness of the right SP and the thickness of the left SP at 47.8% level and it is statistically significant (p<0.01; Table 1). There is a positive correlation between the length of the right SP and the thickness of the right SP at the level of 33.5%; there is a positive correlation between the length of the left SP and the thickness of the left SP at the level of 34.9% and it is statistically significant (p<0.01; Table 1).

There is a low positive correlation between age and the length of the right SP, the length of the left SP, and the average SP length at approximately 9%, 11%, and 10%, respectively (p<0.01; Table 3). There is a low positive correlation between age and thickness of the right SP, thickness of the left SP, thickness of the average SP with approximately 13%, 14%, 16%, respectively (p<0.01; Table 3).

Table 3. Relationships between age and, length and thickness of SP

	Age	
	Pearson Correlation	p-value
Length of the Right SP	0.087**	0.000
Length of the Left SP	0.107**	0.000
Length of the Left SP	0.103**	0.000
Thickness of the Right SP	0.132**	0.000
Thickness of the Left SP	0.144**	0.000
Thickness of the Average SP	0.161**	0.000

**: Correlation is significant at the 0.01 level

There is a statistically significant difference in the mean age value between individuals with a length of the right SP of 30 and individuals with a length of >30 (p<0.01). The mean age of individuals with a right SP length of >30 was calculated as 39.93±15.30, which was older than the mean age of individuals with a right SP length of ≤30. There was a statistically significant difference in mean age between individuals with a left SP length ≤30 and individuals with a left SP length >30 (p<0.01). The mean age of individuals with a left SP length of >30 was calculated as 39.62±15.49, which was greater than the mean age of individuals with a left SP length of ≤30.

There is a statistically significant difference between female and male individuals in terms of mean right SP length, left SP length and mean SP length values (p<0.01). SP length values were found to be larger in males. There is a statistically significant difference between female and male individuals in terms of mean right SP thickness, left SP thickness and mean SP thickness values (p<0.01). SP thickness values were found to be larger in males.

When the Langeais classification of the right SP was analyzed; the majority of the patients were in Type 1 class. This was followed by Type 3 and Type 2 (Figure 2). 78.4% of the right SP were longer than 30 mm and were Type 1. 8.2% of the right SP were Type 2 and 13.4% were Type 3. When the Langeais classification of the left SP was analyzed; the majority of the patients were in Type 1 class. This was followed by Type 3 and Type 2 (Figure 3). 70.6% of left SP longer than 30 mm were Type 1, 8% were Type 2, and 12.4% were Type 3. There was a 53.7% correlation between right and left SP classification type and it was statistically significant (p<0.01; Table 2). In both right and left SP classification types, 91.9% had type 1, 51.1% had type 2, and 61.3% had type 3.

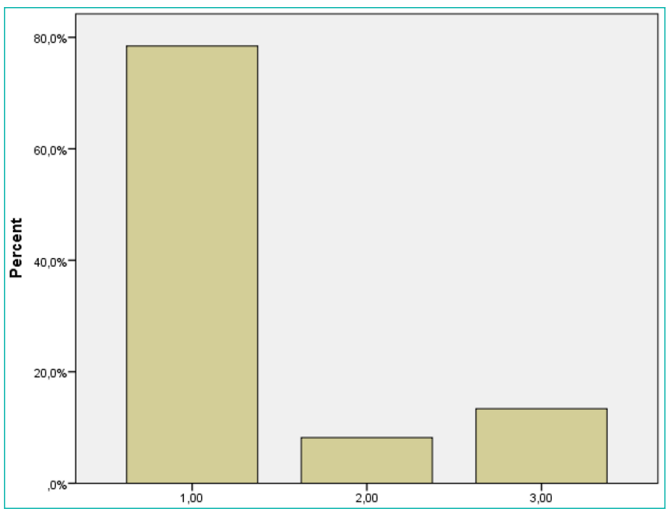

Figure 2. Rates of right SP langeais classification

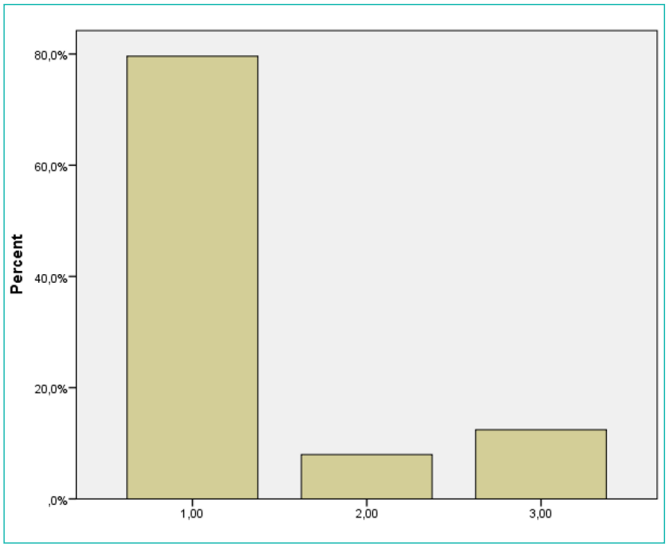

Figure 3. Rates of left SP langeais classification

DISCUSSION

According to the results obtained from present study, the prevalence of SP elongation was found to be 32% in Kütahya, Türkiye. It was observed that the length and thickness of SP increased with increasing age, and this increase was higher in males. Right and left SP length, thickness, and calcification type were related to each other. This study is one of the rare studies evaluating the SP thickness and the relationship between right and left SP length, thickness and morphological type. To the best of our knowledge, it is also the first study that evaluated the highest number of patients, reported the highest prevalence rate, and evaluated SP thickness using panoramic radiographs in Türkiye.

Although elongated SP is usually asymptomatic, various theories have been proposed to clarify its cause.[4,9-11] Studies have reported that congenital etiology is the cause of elongated SP. However, traumatic events in the pharyngeal region such as tonsillectomy, ageing, endocrine disorders, local chronic irritation or inflammation have also been reported to be factors.[4,9,10] Systemic factors related to abnormal serum calcium and phosphorus levels may also be associated with elongated SP.[11] A recent study reported that intraoral inflammatory events associated with root canal treatments may have an association with SP.[12] Renal transplant patients on haemodialysis may also experience elongated SP due to changes in phosphate balance and PTH levels.[13] The exact aetiology of elongated SP is unknown, although many hypotheses have been proposed.[14] According to some authors, SP elongation may be considered a physiological phenomenon.[15] The finding that the right and left SP were morphologically related in this study may indicate that the elongation of SP is influenced by systemic conditions.

The incidence of elongated SP varies in the population.[16] Studies have shown that the incidence of SP formation on panoramic radiographs varies between 4% and 33.4% and the majority of these patients are asymptomatic.[1,8,17,18] In studies conducted in Türkiye, the prevalence of elongated SP ranges from 3.7 % to 7.7%.[19,20] The present study presents a higher prevalence rate compared to studies evaluating patients in Türkiye using panoramic radiographs. In a study evaluating patients in Türkiye with cone beam computed tomography (CBCT), 13% had left, 8% right and 33% bilateral SP elongation.[21]

In a study of patients with temporomandibular disorder (TMD), it was reported that 76% of patients showed elongation of at least one of the SP and the incidence of SP may be high in patients with this disorder.[22] In a study of patients with torus palatinus, it was reported that 15% had SP on at least one side.[23] SP elongation may also be higher in edentulous patients.[24]

The relationship between SP elongation and age is controversial in the literature. SP elongation has been reported to be associated with age and to increase with age in studies.[6,20] However, there are also studies that suggest that it is not associated with age.[25,26] A recent systematic review reported that the length of the SP was longer in females than in males.[27] However, there are also studies that reported that it was similar between genders[3,20,21] or that it was greater in males.[6,28] In the present study, SP length was associated with age and male gender, similar to the results of Shaik MA et al.[6]

Limitations

The limitations of this study include the inability to clinically evaluate patients and the failure to exclude individuals with diseases that may affect bone metabolism. This circumstance prevented direct observation or assessment of clinical symptoms or signs of SP elongation. Moreover, the inclusion of individuals with conditions potentially influencing bone metabolism could impact the generalizability of the results. Hence, future study designs may focus on better controlling factors that may influence bone metabolism.

CONCLUSION

To the best of the authors' knowledge, this study reports the highest prevalence of elongated SP in Türkiye, with a rate of 32%. While elongated SP is predominantly asymptomatic, further research is warranted to explore the underlying reasons for this elevated prevalence. Given the potential involvement of various systemic and environmental factors in SP elongation, multicenter studies involving a larger patient cohort and comprehensive clinical evaluations, particularly focusing on maxillofacial symptoms, are warranted.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Kütahya Health Sciences University Non-invasive Clinical Researches Ethics Committee (Date: 25.05.2022, Decision No: 2022/06-22).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Association between pancreatic lipase levels and coronavirus disease-2019

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ABSTRACT

Aims: Elevated pancreatic enzyme can be observed in the course of coronavirus disease-2019 (COVID-19) due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Here, we aimed to determine the frequency of lipase elevation in the course of COVID-19 and examine its effect on disease outcomes.

Methods: Of 42742 patients with the positivity of SARS-CoV-2 reverse transcriptase-polymerase chain reaction test (RT-PCR), 3167 undergoing lipase tests were included. The relationship between patients' clinical features, development of acute pancreatitis (AP), and mortality rates was investigated.

Results: Higher lipase levels than normal limits were found in 399 (12.6%) patients. Lipase levels were three times higher than the normal limit in 119 (3.8%) patients; compared to the rest of the patients, patients' age (62.8±17.9 vs 52.1±17.9 years, p<0.001), and rates of male patients (58% vs 45%, p=0.006) and mortality (17.6% vs 8%, p=0.001 respectively) were higher. Thirty-two (1.01%) patients were diagnosed with acute pancreatitis (AP). As lipase levels elevated, hospitalization (p<0.001) and requirement for intensive care unit (p=0.002) also increased. A total of 264 (8.3%) patients died, and mortality rates were higher in males (11% vs 6%, p<0.001). The dead were older (72.0±12.3 years vs 50.7±17.4 years, p<0.001). There was a linear positive correlation between patients' age (p<0.001), lipase levels (p<0.001), and mortality. Mortality was increased among men and in the presence of AP (p<0.001).

Conclusion: Pancreatic enzyme levels should be measured in the course of COVID-19 due to increased mortality in patients of advanced age, males with AP, and high lipase levels.

Keywords: Coronavirus disease 2019, COVID-19, lipase, pancreas, pancreatitis, SARS-CoV-2

INTRODUCTION

Although coronavirus disease 2019 (COVID-19) due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) primarily affects the respiratory and coagulation systems, the involvement of the gastrointestinal system at various levels, including endocrine and exocrine pancreas, can be observed in the course of the disease.[1-3] The elevation of pancreatic enzymes and the development of acute pancreatitis (AP) have been reported in the course of COVID-19 disease in both adults and children.[4-16] Although the exact mechanism is unknown, the SARS-CoV-2 virus enters cells via angiotensin-converting enzyme-2 (ACE-2) and transmembrane protease serine-2 (TMPRSS-2).[1] Compared to pancreatic islet cells, exocrine pancreatic ducts have been shown to express more ACE-2 and TMPRSS-2.[1] Acute pancreatitis is a serious clinical condition, and gallstones, alcohol, and such viruses as coxsackie B and mumps have often been blamed for its etiology.[17-19] Although SARS-CoV-2 is considered responsible for the etiology in a very small portion of the patients followed up due to the diagnosis of AP[20] it has been reported that the elevations of lipase and amylase are present at different levels in 1-2% of moderate and 17% of severe COVID-19 cases.[11-15] It has also been emphasized that the accompanying elevation of lipase in the course of COVID-19 is an indicator of the disease severity; those with hyperlipasemia are three times more likely to have severe COVID-19,[12] and those with high lipase levels have a higher risk of hospitalization.[10] On the other hand, the SARS-CoV-2 virus has been blamed in only one (2.3%) of 43 cases with AP.[20]

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In the present study, we aimed to determine the frequency of pancreatic enzyme elevation developing during the COVID-19 pandemic and to investigate its effect on the disease outcome.

METHODS

During the recent pandemic, our hospital served as a tertiary health facility where COVID-19 patients were diagnosed and treated. The cases where serum lipase levels were examined with positive SARS-CoV-2 reverse transcriptase-polymerase chain reaction (RT-PCR) test and followed-up due to COVID-19 between 31st March 2020, and 9th July 2021, were included in the analysis. The present study was retrospectively designed as a cohort study. The study was carried out with the permission of the Usak University School of Medicine Scientific Researches Evaluation and Ethics Committee (Date: 25.05.2022, Decision No: 89-89-12). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In our hospital, a total of 42742 patients were diagnosed with the positivity of SARS-CoV-2 through the RT-PCR test during the study period. A total of 100453 lipase tests were performed in the study period. During the study period, lipase levels were measured by the spectrophotometric method using quinone staining with the Architect c 8000 model device (Abbott Diagnostic, Lake Forest, IL, USA). The reference limits for lipase levels were 13-60 U/L. The SARS-CoV-2 RT-PCR test results of 3167 cases were found to be positive, and after performing the measurements during the COVID-19 pandemic, 399 were determined to have higher lipase levels above the laboratory limit (>60 U/L). Therefore, the patients' group with "high lipase level" was created from these cases; 119 cases (3.8%, the rate among PCR positive patients was 0.27%) had higher lipase levels three times higher than the laboratory limit (≥180 U/L), and so the group with "three times higher lipase level" was created from these cases. The "control-1" group was constituted of the rest 2768 patients with normal lipase levels, and, the "control-2" group was composed of the rest 3048 patients with normal lipase levels and lipase levels three times lower than the upper laboratory limit. The cohort diagram of the study population is shown in Figure. The study was registered by the ClinicalTrials.gov Protocol and Results System (ClinicalTrials.gov identifier: NCT05601258).

Demographic features of the patients, such as age, gender, symptoms, and lipase levels on admission, and imaging methods were scanned from the hospital's automation system and recorded on the patient's charts. During the study period, the cases with AP were diagnosed through the device of computerized tomography (CT) (TOSHIBA Aquilion 16 CT Scanner in Kyoto, Japan) and the 1.5 T magnetic resonance imaging (MRI) scanner (Siemens Magnetom Aera, Erlangen, Germany) in our hospital. If the patients had at least two of the three features of the condition under the Atlanta Criteria, they were diagnosed with AP.[21] The "control 3" group was created from the 3135 patients whose lipase levels were monitored but didn't develop AP.

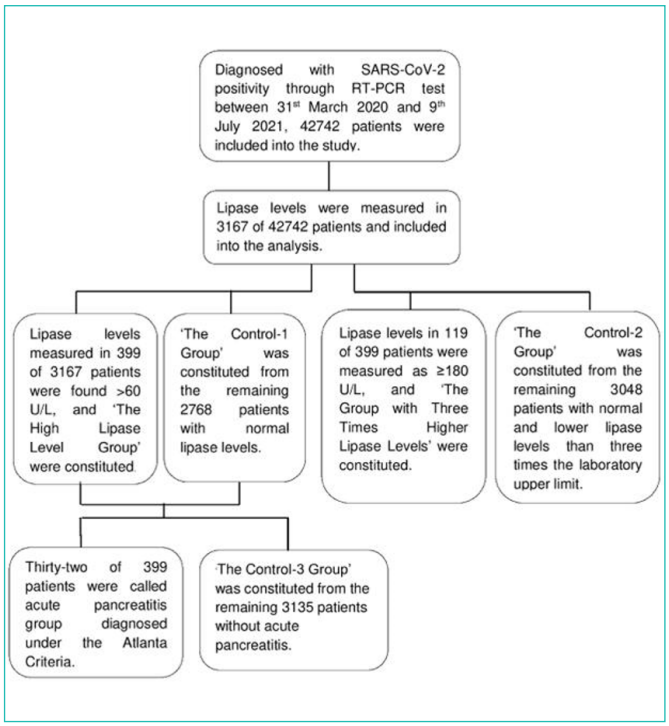

Figure. COHORT diagram of the study population

SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2, RT-PCR: Reverse transcriptase-polymerase chain reaction.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 25.0 (SPSS, IBM Corp., Armonk, NY, U.S., 2017). While the continuous data were expressed as mean±standard deviation (SD), the categorical data were referred to as percentages. In the comparison of the differences in terms of gender, mortality, and age levels in the groups with normal and high lipase levels, the Pearson chi-square test was used. However, in comparing the variables such as the time elapsed after the PCR test and age, the Mann-Whitney U test was used, considering the results of the Shapiro–Wilk normality test. A logistic regression model was created, in which lipase levels were considered normal (≤60) and high (>60) binary dependent variables, age, gender, and time elapsed after PCR test as independent variables. In addition to giving raw p values in Tables, the minimum statistical significance level was determined as 0.05.

RESULTS

Of 3167 patients included in the study, 399 (12.6%) (the rate was 0.9% among all SARS-CoV-2 PCR positive patients) had lipase levels above the laboratory upper limits; the group with "high lipase level" was constituted from these patients and compared with the "control-1 group" with lipase levels within normal limits. Both the descriptive statistics of the data and the relationships between lipase levels and other variables are given In Table 1. The age level of those in the group with "high lipase level" was higher than that of the controls (60.6±16.8 years vs 51.3±17.9, p<0.001, respectively). Considering the age variable categorically, a significant linear-by-linear association was found between the increasing age and lipase levels (p<0.001). The highest lipase levels were detected at 41.0±68.4 days after the positivity of SARS-CoV-2 PCR. The rate of male patients in the group with high lipase levels was higher than that in the control-1 (54.6% vs 44.6%, p=0.009). During the follow-up period, 263 patients (8.3%) died, and the mortality rate was higher in the group with high lipase levels [55 (13.8%) vs 208 (7.5%, respectively), p<0.001]. Given the findings of multivariate logistic regression analysis, the male gender was found to increase the risk 1.288 times more for the detection of high lipase, compared to female patients (p=0.046). In addition, considering the 18-24 age segment as the reference range, it was determined that the risk of encountering an increase in lipase levels increased in the 25-44 age, 45-59 age, 60-74 age, 75-90 age and >90 age categories (p=0.048, 0.007, <0.001, <0.001, and 0.007, respectively). (Table 2)

Table 1. Characteristics of patients with normal and high lipase levels

Variables	Normal lipase level Group/n (%) (%) 2768 (87.4)	High lipase level Group/n (%) (%) 399 (12.6)	p value
Sex			0.009[3]
Female	1533 (55.4%) (88.8%)	193 (48.4%) (11.2%)	
Male	1235 (44.6%) (85.7%)	206 (51.6%) (14.3%)	
Mortality			<0.001[3]
Survivors	2560 (92.5%) (88.2%)	343 (86.0%) (11.8%)	
Exitus	208 (7.5%) (78.8%)	56 (14.0%) (21.2%)	
Age (years)			<0.001[3]
18-24	189 (6.8%) (96.4%)	7 (1.8%) (3.6%)	
25-44	880 (92.8%) (89.3%)	68 (7.2%) (10.7%)	
45-59	766 (27.7%) (81.3%)	92 (23.1%) (18.7%)	
60-74	595 (21.5%) (77.6%)	137 (22.4%) (22.4%)	
75-89	316 (11.4%) (84.6%)	91 (22.8%) (15.4%)	
90+	22 (0.8%) (87.4%)	4 (1.0%) (12.6%)	
	Median (Min-Max)	**Median (Min-Max)**	
Time after PCR (days)	9 (0-382)	5 (2-103)	<0.001[4]
Age (years)	63 (18-94)	50 (18-99)	<0.001[4]

[1]In-group comparisons, [2]Intergroup comparisons, [3]Pearson chi-square test, [4]Mann Whitney U test, PCR: Polymerase chain reaction

Table 2. Characteristics of threefold high lipase levels of the study population

	Lipase Levels		
Variables	**Lipase Normal+High n (%)1 (%)2 3048 (96.2)**	**Lipase Threefold High n (%)1 (%)2 119 (3.8)**	**p value**
Sex			0.006[3]
Female	1676 (55.0) (97.1)	50 (42.0) (2.9)	
Male	1372 (45.0) (95.2)	69 (58.0) (4.8)	
Mortality			0.001[3]
Survivor	2805 (92.0) (96.6)	98 (82.4) (3.4)	
Exitus	243 (8.0) (92)	21 (17.6) (8)	
Age (years)			<0.001[3]
18-24	194 (6.4) (99)	2 (1.7) (1)	
25-44	927 (30.4) (97.8)	21 (17.6) (2.2)	
45-59	840 (27.6) (97.9)	18 (15.1) (2.1)	
60-74	691 (22.7) (94.4)	41 (34.5) (5.6)	
75-89	372 (12.2) (92.3)	35 (29.4) (7.7)	
90 +	24 (0.8) (96.2)	2 (1.7) (3.8)	
Clinical Spectrum			
No	-	86 (72.3)	
Abdominal pain	-	33 (27.7)	
Stones			
No	-	101 (84.9)	
Yes	-	18 (15.1)	
Imaging			
Negative	-	32 (26.9)	
Positive/ compatible with pancreatitis	-	13 (10.9)	
No imaging	-	74 (62.2)	
	Median (Min-Max)	**Median (Min-Max)**	
Time after PCR (days)	12 (0-382)	8 (0-244)	<0.0014
Age (years)	66 (18-94)	51 (18-99)	<0.0014

[1]In-group comparisons, [2]Intergroup comparisons, [3]Pearson chi-square test, [4]Mann Whitney U test, PCR: Polymerase chain reaction

In the group with lipase ≥3x, the age rate was higher in the patients' group than that in the controls (62.8±17.9 years vs 52.1±17.9 years, p<0.001), as well as including more male patients (58% vs 45%, p=0.006) (Table 2). While the mortality rates were 17.6% in the group with lipase ≥3x, the rates were found as 8% in the controls (p=0.001). As the age rate increased, lipase levels were detected to increase three times in more cases (p<0.001). Clinical findings, presence of gallstones, and imaging results only belonging to the group with three times higher lipase levels are given in Table 2. The time elapsed after PCR was longer in the group with lipase ≥3x (p<0.001). Given the findings of multivariate logistic regression analysis, it was observed that males were 1.641 times more likely than females to have lipase levels ≥3x (p=0.013). In addition, when the 18-

24 age group was taken as the reference, the probability of lipase levels ≥3x was found to increase 6,745 times between 60-74 years of age and 9,912 times between 75-90 years; however, lipase level was likely to be 9,833 times higher in the 90 and over age group (p=0.011, 0.002 and 0.030, respectively).

Thirty-two patients (in 1.01% of the cases where lipase levels were measured, and 0.07% of those with the positivity of PCR) were diagnosed with AP (Table 3). There was no difference in patients developing AP in terms of gender and mortality; however, it was found that AP patients were older, and the time elapsed after PCR was longer (p<0.001) (Table 3). When the multivariate logistic regression was performed, it was detected that the age rate and gender difference had no role in the development of pancreatitis, but only the increase in lipase levels was a risk factor for the development of pancreatitis [Odds ratio (OR) 1.005, 95% Confidence interval (CI) for OR lower-upper=1.003-1.007, p<0.001]. Hospitalization was evaluated only in those with high lipase levels. While the rate of hospitalization was found as 52.5% in the patients' group with lipase levels one to three times higher, the rate was 76.5% in those with three times higher lipase levels (p<0.001). Similarly, the requirement for the intensive care unit (ICU) was 12.1% in those with lipase levels one to three times higher while the rate was observed as 25.2% in the group with three times higher lipase levels (p=0.002).

Table 3. Clinical characteristics of patients with and without acute pancreatitis

Variables	Patients with AP 32 (1%) n (%)1 (%)2	Patients without AP (Control 3 Group) 3135 (99%) n (%)1 (%)2	p value
Sex			0.476[3]
Female	15 (46.9%) (0.9%)	193 (48.4%) (99.1%)	
Male	17 (53.1%) (1.2%)	206 (51.6%) (98.8%)	
Mortality			0.668[3]
Survivor	30 (93.7%) (1%)	2873 (91.6%) (99%)	
Exitus	2 (6.3%) (0.8%)	262 (8.4%) (99.2%)	
Age (years)			<0.001[3]
18-24	1 (3.1%) (0.5%)	195 (6.2%) (99.5%)	
25-44	6 (18.8%) (0.6%)	942 (30%) (99.4%)	
45-59	1 (3.1%) (0.1%)	857 (27.3%) (99.9%)	
60-74	10 (31.3%) (1.4%)	722 (23%) (98.6%)	
75-89	13 (40.6%) (3.2%)	394 (12.6%) (96.8%)	
90 +	1 (3.1%) (3.8%)	25 (0.8%) (96.2%)	
	Median (Min-Max)	**Median (Min-Max)**	
Time after PCR (days)	74.5 (0-382)	8 (0-256)	<0.001[4]
Age (years)	69.5 (22-94)	52 (18-99)	<0.001[4]

AP: Acute pancreatitis, [1]In-group comparisons, [2]Intergroup comparisons, [3]Pearson chi-square test, [4]Mann Whitney U test, PCR: Polymerase chain reaction

During the study period, 264 (8.3%) cases died, and the cases dying were seen to be older (72.0±12.3 years vs. 50.7±17.4 years, p<0.001), and the mortality rates were found higher in males (11% and 6% in males and females, p<0.001) (Table 4). Descriptive statistics of mortality are demonstrated in Table 4. Given the findings of multivariate logistic regression analysis, it was found that a one-year increase in age led mortality to increase by 8.5% (p<0.001), and male gender caused mortality to increase by 1.610 times (61%) (p=0.001). Considering normal lipase levels as the reference category, although high lipase levels increased the mortality rate 1.514 (51.4%) times compared to the normal limit, the increase did not reach a statistically significant level. On the other hand, while three times higher lipase levels increased the mortality rate 1.924 (92.4%) times compared to the normal limit (p=0.031), the presence of AP was detected to increase the mortality rate 2.136 times (p=0.033).

Table 4. Descriptive statistics of mortality accepted as a dependent variable

Variables	Exitus n (%)1 (%)2 264 (8.3)	Survivor n (%)1 (%)2 2903 (91.7)	p value
Sex			<0.001[3]
Female	105 (39.8) (6.1)	1622 (55.8) (93.9)	
Male	159 (60.2) (11)	1282 (44.2) (89)	
Age (yrs)			<0.001[3]
18-24	3 (1.1) (1.5)	193 (6.6)(98.5)	
25-44	5 (1.9) (0.5)	943 (32.5) (99.5)	
45-59	24 (9.1) (2.8)	834 (28.7) (97.2)	
60-74	105 (39.8) (14.3)	627 (21.6) (85.7)	
75-89	119 (45.1) (29.2)	288 (9.9) (70.8)	
90 +	8 (3) (30.8)	18 (0.6) (69.2)	
Lipase Levels			<0.001[3]
Normal	208 (78.8) (7.5)	2560 (88.2) (92.5)	
High	35 (13.3) (12.5)	245 (8.4) (87.5)	
Threefold High	21 (8.0) (17.6)	98 (3.4) (82.4)	
Clinical Spectrum			0.058[4]
No	19 (90.5) (22.1)	67 (68.4) (77.9)	
Abdominal Pain	2 (9.5) (6.1)	31 (31.6) (93.9)	
Stone			0.737[4]
No	19 (90.5) (18.8)	82 (83.7) (81.2)	
Yes	2 (9.5) (11.1)	16 (16.3) (88.9)	
Imaging			0.321[4]
Negative	4 (19) (12.5)	28 (28.6) (87.5)	
Positive/compatible with pancreatitis	1 (4.8) (7.7)	12 (12.2) (92.3)	
No imaging	16 (76.2) (21.6)	58 (59.2) (78.4)	
	Median (Min-Max)	**Median (Min-Max)**	
Time after PCR	9.5 (0-106)	9 (0-382)	0.177[5]
Age (years)	74 (18-94)	50 (18-99)	<0.001[5]

[1]Ingroup comparisons, [2]Intergroup comparisons, [3]Pearson chi square test, [4]Fisher exact test, [5]Mann Whitney U test

DISCUSSION

In the present study, it was detected that 12.6% of the patients whose lipase levels were measured during the COVID-19 pandemic had higher lipase levels than the upper limit of the normal; however, 3.8% had lipase levels three times higher than the upper limit of the normal. In addition, it was also found that there was a greater increase in lipase and mortality levels in males and elderly patients, and the presence of concomitant AP caused mortality to increase even further.

Although alcohol consumption and gallstones often come to the fore in the etiology of AP, many viruses may contribute to the development of AP through unknown mechanisms.[17-19] It has been reported that the invasion of pancreatic islet cells by viruses and the proliferation of the virus there may contribute to the development of diabetes mellitus (DM) and AP by triggering autoimmune events;[22] the SARS-CoV-2 virus has been blamed in only one (2.3%) of 43 cases diagnosed with AP.[20] Increased lipase levels developing in the course of SARS-CoV-2 infection due to AP cause lipotoxicity by leading to the breakdown of triacylglycerol from adipose tissues and increasing the release of unsaturated fatty acids, and have a toxic effect on mitochondria, which in turn causes the development of cytokine storm by increasing cytokine release, can be contributors.[23]

There are many studies evaluating lipase levels during the course of COVID-19. In the study where 756 patients with COVID-19 were evaluated by Hemant Goyal et al.[12] it was stated that the frequency of concomitant elevation in lipase levels was 11.7%; the cases with hyperlipasemia were 3.143 times more likely to have severe COVID-19, and therefore, high lipase levels are a determining criterion for the severity of COVID-19 along with the presence of AP. In another study involving 52 patients with COVID-19 pneumonia, Wang et al.[8] reported that although amylase or lipase levels were high in nine (17%) cases and the lipase levels were not three times higher than the upper laboratory limit. In another study by Barlass et al.[10] where 1003 patients with COVID-19 were included, and the lipase levels were measured in only 83 cases, the lipase levels were reported to be three times higher than the normal limit in 14 (16.8%) of the cases. In the present study, the lipase level was measured in only 7.4% of the patients undergoing PCR for SARS-CoV-2 within the relevant period. While the lipase level was higher in 0.9% and higher ≥3x in 0.27% of all PCR-positive patients than the normal limit, the lipase level was found to be higher in 12.6% and higher ≥3x than the normal limit in 3.8% of the cases where the lipase level was measured.

In the present study, 32 (1.01%) of 3167 patients evaluated through the measurements of the lipase levels were detected to have AP under the Atlanta Criteria. In a prospective study involving 316 patients with COVID-19 pneumonia, Akarsu et al.[13] stated that approximately 12.6% of the patients had AP; while no AP was witnessed in mild cases, 7.6% of severe cases and 32.5% of critical cases were found to have AP, and the rates of hospitalization and mortality were higher among those with AP.[13] In another study, however, Bulthuis et al.[4] reported that five (1.2%) of 433 patients with COVID-19 developed pancreatitis; all of the cases developing pancreatitis had also had severe COVID-19 disease, and three of the patients died. In the same study, the researchers suggested that in the development of AP, pancreatitis might be due to hypoperfusion rather than the direct effect of the virus. In the study where the patients with three times higher lipase levels were compared with those without by Barlass et al.[10] it was emphasized that intubation (78.6 and 23.5%, respectively) and requirement for ICU (92.9 and 32.8%, respectively) were higher among those with three times higher lipase levels.[10] Similarly, Akarsu et al.[13] determined that hospitalization and mortality rates were high in patients with COVID-19 developing AP. On the other hand, it was reported that the lipase levels were three times higher than the normal levels in 12 (31.6%) of 38 patients developing acute respiratory distress syndrome due to COVID-19; however, none of the patients displayed pancreatic pathology in imaging and were not diagnosed with AP, and the elevation of lipase may have also been related to the deterioration in microcirculation due to severe disease[15]. In the present study, it was found that increased lipase levels led to an increase in hospitalization and requirement for ICU; even three-quarters of the patients with three times higher enzyme levels required hospitalization; mortality rates were higher among male patients and the elderly, and mortality rose linearly with an increase in lipase levels. (**Tables 5** and **6**). In the present study, when the patients were categorized concerning their age levels, and each group was compared with the reference age segment of 18-24 years, it was found that the risk of encountering high lipase levels increased in each age group; the risk of having three times higher lipase levels than the normal was even higher, especially in the patients aged 60 years and above, and even the risk increased up to ten times in those at the age of 75 years and over. However, although lipase levels increased with age, and lipase levels were higher in males, we detected no effects of age and gender on the development of AP. On the other hand, the presence of AP was seen to cause a 2.1-fold increase in mortality.

Table 5. Clinical characteristics of patients with and without acute pancreatitis

Variables	Patients with AP 32 (1%) n (%)1 (%)2	Patients without AP (Control 3 Group) 3135 (99%) n (%)1 (%)2	p-value
Sex			0.476[3]
Female	15 (46.9%) (0.9%)	193 (48.4%) (99.1%)	
Male	17 (53.1%) (1.2%)	206 (51.6%) (98.8%)	
Mortality			0.668[3]
Survivor	30 (93.7%) (1%)	2873 (91.6%) (99%)	
Exitus	2 (6.3%) (0.8%)	262 (8.4%) (99.2%)	
Age (years)			<0.001[3]
18-24	1 (3.1%) (0.5%)	195 (6.2%) (99.5%)	
25-44	6 (18.8%) (0.6%)	942 (30%) (99.4%)	
45-59	1 (3.1%) (0.1%)	857 (27.3%) (99.9%)	
60-74	10 (31.3%) (1.4%)	722 (23%) (98.6%)	
75-89	13 (40.6%) (3.2%)	394 (12.6%) (96.8%)	
90 +	1 (3.1%) (3.8%)	25 (0.8%) (96.2%)	
	Median (Min-Max)	**Median (Min-Max)**	
Time after PCR (days)	74.5 (0-382)	8 (0-256)	<0.001[4]
Age (years)	69.5 (22-94)	52 (18-99)	<0.001[4]

AP: Acute pancreatitis, [1]In-group comparisons, [2]Intergroup comparisons, [3]Pearson chi-square test, [4]Mann Whitney U test, PCR: Polymerase chain reaction

Table 6. Rates of patients with high and ≥3X levels of lipase in need of hospitalization and admission to the intensive care unit

		Lipase Levels One-threefold High	Lipase Levels ≥3X High	p value
Hospitalization n (%)1 (%)2	Yes	133 (47.5) (82.6)	28 (23.5) (17.4)	<0.001
	No	147 (52.5) (61.8)	91 (76.5) (31.2)	
Admission to ICU n (%)1 (%)2	Yes	246 (87.9) (73.4)	89 (74.8) (26.6)	0.002
	No	34 (12.1) (53.1)	30 (25.2) (46.9)	

ICU: Intensive care unit

In the present study, we demonstrated that 8.3% of the patients with high lipase levels died; the mortality rate was higher in the males and the elderly, and the mortality increased linearly with increasing age. It is known that COVID-19 becomes more symptomatic with advancing age and is more fatal in the elderly.[11,13,24] Akarsu et al.[13] reported that while the female gender was dominant in the cases with mild COVID-19 pneumonia, the male gender was dominant in severe cases; AP was seen higher in those with high involvement in thorax CT; O_2 saturation was also lower in the cases with AP than the controls, and while the support of O_2, need for mechanical ventilation and requirement for ICU were higher, the length of hospital stay was longer, and the mortality was higher (32.5% vs 7.9%, respectively); however, it was reported that there was no effect of gender differences on the development of AP and mortality. On the other hand, in the study by Barlas et al.[10] the patient group with three times higher lipase levels was reported to include more male patients (78.6% vs 38.3%) than those with normal and lower lipase levels. In a retrospective cohort study conducted in our clinic, which is one of the first studies on the subject, we determined that 15.7% of COVID-19 patients whose lipase levels were measured had higher lipase levels than the normal limit; AP developed in only two patients, and the presence of the history of DM increased the risk of encountering hyperlipasemia 4.63 times.[6] In addition, we also determined in the same study that the patients with hyperlipasemia had lower oxygen saturation, higher C-reactive protein (CRP) and D-dimer levels, higher length of hospital stay, and higher requirement for ICU on admission.[6]

Limitations

SARS-CoV-2 is a novel virus not well-known by physicians. Therefore, although some physicians treating and following up COVID-19 patients in our hospital ordered pancreatic enzymes to be measured whether or not the patients had the symptoms suggestive of pancreatic pathology, such factors as the fact that other physicians did not order these tests, periodic monitoring of enzyme levels was not performed in some cases with elevated enzyme levels, or pancreatic imaging has yet to be performed are the limitations of the study. As another limitation, although it is known that many drugs, such as steroids and antibiotics, may contribute to the development of AP,[25] we included none of these drugs in the present analysis. The possibility that the physicians were unwilling to stay face-to-face with the patients for a long time due to the concerns about virus contamination during the SARS-CoV-2 pandemic, and the disease symptoms and other symptoms such as abdominal pain and nausea were ignored to be questioned in detail can also be regarded as another limitation of the study.

CONCLUSION

The SARS-CoV-2 virus can affect the pancreas, as well as many organs and tissues. Although the risk of detecting elevated lipase levels is high, especially in the elderly and male patients in the course of SARS-CoV-2 virus infection, and the relationship between age and gender, and the development of AP has yet to be demonstrated, it will be appropriate to measure the pancreatic enzymes even in the slightest suspicion in the course of COVID-19 to detect the pancreatic pathology at an earlier period in AP patients and those with high lipase levels due to the increased mortality, even if there are no typical symptoms.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Uşak University Faculty of Medicine Scientific Researches Evaluation and Ethics Committee (Date: 25.05.2022, Decision No: 89-89-12).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Radiopacity evaluations of the novel calcium-silicate and glass-ionomer-based materials

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ABSTRACT

Aims: Radiopacity is a crucial property for a liner or base material, and these materials should provide an optimal contrast for detecting secondary caries in radiographic examinations. The purpose of this study was to assess the radiopacity characteristics of four calcium-silicate-based and two glass-ionomer-based materials used as a liner or base in direct or indirect vital pulp therapy.

Methods: A total of 60 cylindrical-shaped and 1 mm thick specimens were prepared from a calcium-silicate (Biodentine, Septodont), a calcium-silicate (MTA, Angelus), a light-cured resin-modified calcium silicate (TheraCal LC, Bisco), a dual-cured resin-modified calcium silicate (TheraCal PT, Bisco), a glass hybrid glass-ionomer (Equia Forte HT, GC), and a resin-modified glass ionomer (Glass Liner, Wp Dental) material (n=10). Digital radiographic images of the specimens, a molar tooth section with 1 mm thickness, and an aluminum step wedge were obtained by a digital radiography system (Heliodent Plus, Dentsply Sirona) with 60 kV voltage, 7 mA current, and 0.25 seconds exposure time. The mean gray values (MGV) of digital images were determined using the ImageJ software program (National Institute of Health, Bethesda, MD, USA). Kruskal-Wallis and Mann-Whitney tests ($p<0.05$) were used to analyze the data.

Results: Among the tested materials, the highest radiopacity value was found in MTA, and the lowest radiopacity value was obtained in Glass Liner. The radiopacity levels of the materials studied were MTA>Biodentine>Equia Forte HT>Theracal PT>Theracal LC>Glass Liner, respectively. All the tested liner or base materials exhibited significantly greater radiopacity values when compared to those of dentin ($p<0.05$). MTA has statistically significantly higher, Biodentine, Theracal PT, Theracal LC, and Glass Liner have statistically significantly lower radiopacity values than enamel ($p<0.05$).

Conclusion: All the restorative materials tested exhibited higher radiopacity than dentin, with ThereCal LC and Glass Liner displaying lower radiopacity than enamel, ThereCal PT, Biodentine, and Equia showing equivalent radiopacity to enamel, and MTA demonstrating higher radiopacity than enamel.

Keywords: Radiopacity, dental materials, biocompatible materials, calcium silicate cement, dental radiography

INTRODUCTION

In operative dentistry, the main goal with deep caries teeth is to preserve the pulp vitality. Pulp-capping treatments involve the covering of the pulp with a biocompatible material after the removal of carious tissue, aiming to preserve the pulp vitality in order to prevent bacterial leakage and promote dentin bridge formation.[1,2] Liners and bases have been employed as one of the approaches for pulp capping.

Currently, a diverse selection of pulp capping agents possessing distinct characteristics, benefits, and limitations are accessible in the field of dentistry.[3] Recently, novel biomaterials known as calcium silicate-based materials have been introduced, serving both as sealers and cements. Bioceramics, commonly known as calcium silicate-based cements, have emerged as a substitute for the traditional application of calcium hydroxide.[4,5] In modern dentistry, there is a broad range of calcium silicate-based cements available, which are used for a variety of indications, including direct and indirect pulp capping, regenerative endodontic treatments. These cements are favored for their notable biocompatibility, bioactivity, and biomineralization properties.[6]

Mineral trioxide aggregate (MTA), primarily consisting of calcium oxide, bismuth oxide, and silica, is a hydrophilic cement that offers numerous advantages,

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including biocompatibility, low solubility, prevention of bacterial leakage, and the capacity to release calcium hydroxide. Nevertheless, there are some disadvantages such as difficulty in handling and extended setting time, requiring layering with other restorative materials before the final restoration is completed, which may cause marginal loss of adaptation and leakage.[2,7]

As an alternative to MTA, Biodentine (Septodont, Saint-Maur-des-Fossés, France), a tri-calcium silicate material consisting of zirconium oxide, tricalcium silicate, calcium chloride, calcium carbonate and water, has been introduced to the market. Biodentine is highly recommended as a dentin substitute due to its favorable characteristics, including excellent sealing properties, high compressive strength, a rapid hardening rate (typically within 9-12 minutes), as well as its biocompatibility, bioactivity, and remineralization properties.[8]

TheraCal LC is a novel calcium silicate cement which serves as a liner and base beneath composite restorations. It is light-cured and MTA filled. Its composition consists of around 45% Portland cement, around 10% radiopaque components like bismuth oxide, about 5% hydrophilic thickening agent (fumed silica), and nearly 40% resin content.[8] TheraCal LC, in comparison to MTA, demonstrates a quicker setting time, reduced solubility, and enhanced flowability.[9]

The new resin-based chemical formulation, TheraCal PT (Bisco Inc., Schaumburg, IL, USA), releases calcium because of hydrophilic structure of the matrix. The manufacturer recommends its use as a liner and for indirect/direct pulp capping purposes.[10] It offers advantages such as dual-cure polymerization, with a maximum setting time of 5 minutes. This enables the application of a permanent restorative material in a single session. Additionally, TheraCal PT exhibits properties similar to Angelus MTA and Biodentine on human dental pulp stem cells, setting it apart from TheraCal LC.[11]

Glass ionomers and resin-modified glass ionomers (RMGIs) represents examples of lining materials that can release fluoride and form an ionic bond with the tooth structure.[1] Nowadays, resin-modified glass ionomers are commonly preferred due to their lower solubility, fluoride-releasing capability, excellent bonding properties, and higher resistance compared to traditional glass ionomers.[7]

Radiopacity property is accepted as a crucial characteristic for restorative materials. It refers to the relative resistance of a material to the passage of electromagnetic radiation, such as radio waves and X-ray photons, resulting in a white appearance on a radiograph. This feature enables a clear contrast between the surrounding structures and the restorative material, ensuring proper visualization on X-ray images. Thanks to radiopacity, secondary caries beneath restorations, overhangs in proximal restorations, and open margins, as well as gaps within the restoration, which are some of the primary causes of restoration failure, can be assessed. Furthermore, radiopacity also enables the assessment of restoration integrity at recall appointments, proximity to the pulp chamber, and proximal contacts. There are several factors that influence the radiopacity of a material, including its thickness, composition, type of filler (fillers with lower atomic numbers are more radiolucent compared to those with higher atomic numbers), and weight percentage. Additionally, the presence of opacifying agents such as barium, zirconium, lanthanum, strontium, bismuth oxide, carbonates, and sulfates, also contributes to the radiopacity of the material.[12]

To the best of our knowledge, there is no published radiopacity study comparing Therecal PT to other liners. The purpose of this study was to compare the radiopacity of six different liner materials (four calcium silicate-based cements and two glass ionomer materials). The objective of this research was to evaluate the radiopacity of MTA, Biodentine, ThereCal LC, ThereCal PT, Equia Forte HT and Glass Liner using digital radiography. The null hypothesis under examination was that there would be no statistically significant differences among the radiopacity values of the six tested liner materials.

METHODS

Preperation of Study Samples

The protocol for the study was approved by Bahçeşehir University Clinical Researches Ethics Committee (Decision No: 2023-16/01). All procedures were carried out in accordance with the ethical rules and the principles. The study tested a range of materials, including calcium-silicate (Biodentine, Septodont, Saint-Maur-des-Fossés, France), a calcium-silicate (MTA, Angelus), light-cured resin-modified calcium silicate (TheraCal LC, Bisco), dual-cured resin-modified calcium silicate (TheraCal PT, Bisco), glass hybrid glass-ionomer (Equia Forte HT, GC), and resin-modified glass ionomer (Glass Liner, Wp Dental). **Table 1** provides information on the materials examined in the study, their corresponding study codes, and their characteristics. The sample size of the study was determined using G*Power Ver. 3.1 software (Franz Faul, Universität Kiel, Kiel, Germany). The calculations were conducted using an error probability (alpha) of 0.05, an effect size of 0.55, and a power level of 95%.

Table 1. Types, manufacturers, composition and application procedures of the materials used in the study

Material	Manufacturer	Type	Composition	Application step
Angelus MTA	Angelus, Londrina, Brazil	CSC (Calcium silicate cement)	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, silicon oxide, potassium oxide, aluminum oxide, sodium oxide, iron oxide, calcium oxide, bismuth oxide, magnesium oxide, insoluble residues of crystalline silica Liquid: water	Powder + liquid (mixed manually)
Biodentine	Septodont. France	CSC	Powder: tricalcium silicate, dicalcium silicate, calcium oxide, calcium carbonate, zirconium oxide, iron oxide Liquid: calcium chloride, water-soluble polymer, water	0.7 g capsule of powder + 5 drops of liquid (30 s; 4000–4200 rpm) Pour five drops liquid from into the capsule. Place the capsule on a mixing device and mix 30 s.
TheraCal LC	Bisco, Schaumburg, IL, USA	CSC	Light-curing single paste: resin bis-phenyl glycidyl methacrylate (BisGMA) & polyethylene glycol dimethacrylate (PEGD) modified calcium silicate filled with CaO, calcium silicate particles (type III Portland cement), Sr glass, fumed silica, barium sulphate, barium zirconate	Dispensed directly from a flowable syringe (no mixing) Inject the material into the mold in 1 mm increments Light cure each increment for 20 s.
TheraCal PT	Bisco, Schaumburg, IL, USA	CSC	Silicate glass-mix cement (50–75 wt%, Polyethylene Glycol Dimethacrylate (10–30 wt%) Bis-GMA (5–10 wt%), Barium zirconate (1–5 wt%), Ytterbium fluoride (CAS no. 13760-80-0), Initiator.	Dispensed directly from a flowable syringe (mixing) Inject the material into the mold in 1 mm increments Light cure each increment for 20 s.
Glass Liner	WP Dental		Qualitative composition: Glass ceramic, glass ionomer powder, silica, camphorquinone, hexanediol dimethacrylate, Bis-GMA, BHT, DMTBA Quantitative composition: Fillers 65%; activators, accelerators and stabilisers 1%, dimethacrylates 34%	The material, at a thickness of 1 mm, was polymerized using an LED light curing device.
Equia HT	GC Tokyo Japan	Glass Hybrid	Surface-treated FAS glass, highly 1901091 reactive surface-treated fine FAS glass, high-molecular-weight polyacrylic acid, polyacrylic acid	The capsule material was mixed in a capsule mixer for 10 s. Then capsule was placed into the mold with applier as 1mm.

Ten disk-shaped samples (total of 60 samples) were prepared using a circular metal mold with a 10 mm diameter and 1 mm thickness, ensuring a precise thickness of 1±0.1 mm for each sample. A freshly extracted third molar tooth intended for orthodontic use, was used to acquire samples of enamel and dentin. The extracted tooth was thoroughly cleansed, and the roots were excised below the cementoenamel junction. Subsequently, the crown of the tooth was carefully sectioned longitudinally using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluf, IL, USA) with water cooling, resulting in enamel and dentin samples with 1 mm thickness. These enamel and dentin samples were then placed in light-proof containers filled with distilled water and maintained at a temperature of 37°C for a period of 24 hours before the radiographic examination.

Digital Radiography and Radiopacity Calculation

A 12-step aluminum (Al) step wedge, with increments of 0.5-mm thickness, was crafted from a high-purity Al alloy (1050, 99.5% purity) for the purpose of standardizing and calibrating the radiographic images. This step wedge also served to gauge the radiographic density of the samples in terms of millimeters equivalent of aluminum (mm Al).

To conduct the radiographic assessments, the Al step wedge, enamel and dentin samples, and one sample of every restorative material were positioned onto a phosphor plate sized 2+(PSP VistaScan® Sytem, Dürr Dental, Bietigheim-Bissingen, Germany). The plate was then exposed to X-rays using a wall-mounted X-ray device with 2.5 mm aluminum equivalent filtration (Heliodent Plus, Dentsply Sirona, Bensheim, Germany) at a source-to-object distance of 30 cm. The exposure parameters were set at 60 kVp, 7 mA, and an exposure time of 0.25-second in accordance with the manufacturer's instructions. This procedure was repeated twice for every sample within all the groups. Subsequently, the phosphor plates (PSPs) were promptly scanned using a theoretical spatial resolution of 25 line pairs per millimeter (lp/mm) with the VistaScan Mini View system (Dürr Dental, Bietigheim-Bissingen, Germany). All the resulting images were converted in 8-bit TIFF format using the DBSWIN 5.2.0 software (Dürr Dental, Germany).

We employed ImageJ v1.5e, a freely available image analysis software (National Institute of Health, Bethesda, MD, USA) to determine the mean gray values (MGVs) of both the specimens and the stepwedge. ImageJ is a recognized and open-access tool that has been commonly chosen in previous studies to evaluate the radiopacity levels of restorative materials. To perform the measurements, a 10×10-pixel region of interest (ROI) was randomly chosen in five distinct areas on each specimen and on each aluminum step. MGV values were recorded for each ROI, and the average of these values from the five sections was calculated for every specimen in every radiograph (**Figure 1**).

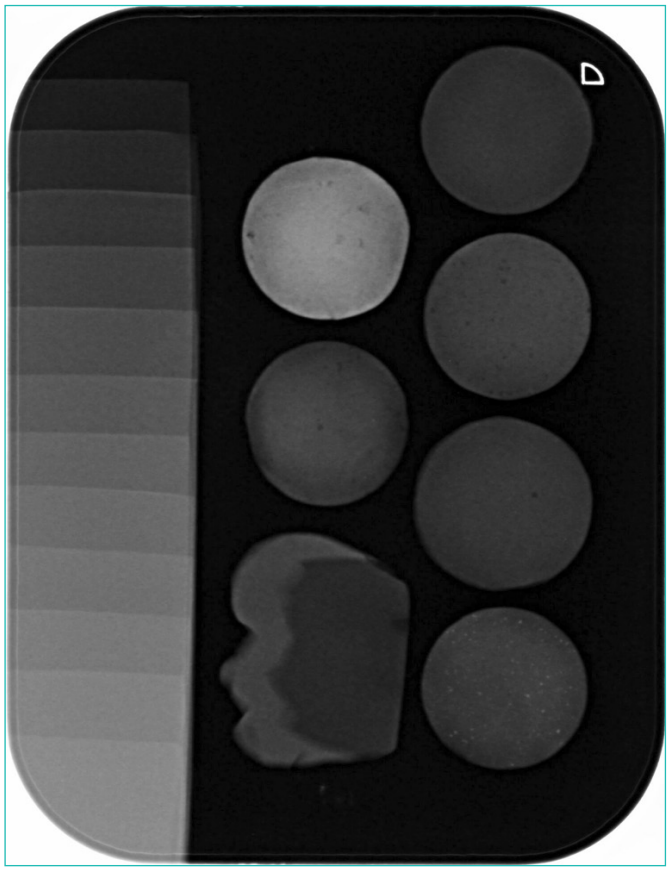

Figure 1. Digital radiographic image of using calcium silicate cement samples, glass ionomer cements, enamel, dentin and aluminum step-wedge

A calibration protocol was established for each radiographic image using the mean gray values (MGVs) derived from the aluminum (Al) stepwedge present in each image. This involved performing linear interpolation on the MGV values associated with aluminum and the corresponding stepwedge thickness. Subsequently, the resultant interpolation function was employed to compute the radiopacity of individual specimens, quantified in millimeters equivalent of aluminum (mm Al).

Statistical analysis

Statistical analyses were performed using SPSS version 25.0 (IBM SPSS Statistics, Chicago, IL, USA). The Shapiro-Wilk test was used to assess the normality of data included in the study. Comparison tests were performed at a significance level (p) of 0.05. Given that the variables did not exhibit normal distribution (p>0.05), the analytical approach proceeded with non-parametric test methodologies. Group comparisons were conducted employing the Kruskal-Wallis test. The assessments of intra-group disparities were carried out utilizing the Mann-Whitney test and decisions regarding the outcomes were determined in relation to the Bonferroni-corrected p-value.

RESULTS

The Shapiro-Wilks test showed that radiopacity (in mm Al) values did not follow a normal distribution (p<0.05). According to the Kruskal-Wallis test, there were statistically significant differences among the tested materials (p<0.05). The means and standard deviations for the mean MGV and radiopacity values of the studied materials, enamel, and dentin are shown in Figure 2 and Table 2. The enamel and dentin radiopacity values were 2.13±0.07 and 1.13±0.06 mm Al, respectively. The radiopacity values of all materials tested were higher than the radiopacity of dentin. Among the tested materials, glass liner had the lowest radiopacity value (1.49±0.23). MTA (5.15±0.69) was significantly more radiopaque than all other calcium silicate-based or glass ionomer materials, as well as enamel (P<0.05). Biodentine, Equia Forte HT, and TheraCal PT exhibited radiopacity values similar to that of enamel (P>0.05). Biodentine, Theracal PT, and ThereCal LC have a statistically higher radiopacity than Glass Liner (P<0.05), but similar to Equia Forte HT (P>0.05). Radiopacity of calcium silicate materials ranged from 1.66 to 5.15 mm Al, while glass ionomer materials ranged from 1.49 to 2.28.

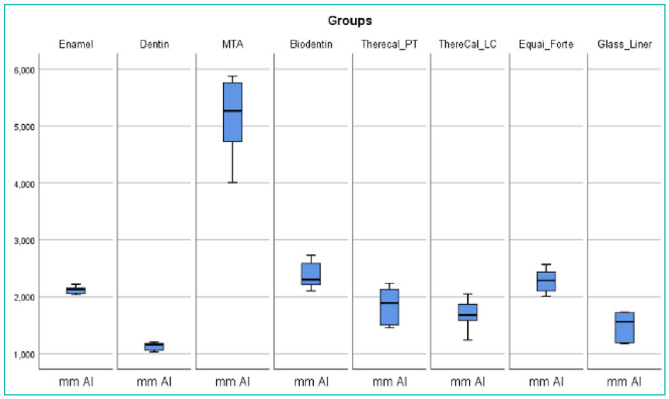

Figure 2. Graphical representation of mmAl radiopacity values of the experimental groups

Table 2. Radiopacity and mean gray value (MGV) of the studied materials, enamel, and dentin (mean±SD)

Material	Mean Radiopacity Value (mm Al)	Mean MGV
MTA	5.15±0.69[a]	124.84±13.42
Biodentin	2.38±0.24[bx]	69.99±4.73
Equia_Forte	2.28±0.2 [bx]	67.84±2.99
Enamel	2.13±0.07 [bcx]	63.87±1.48
Therecal_PT	1.83±0.33[cd]	56.09±7.92
ThereCal_LC	1.66±0.26 [d]	51.58±5.81
Glass liner	1.49±0.23 [e]	47.04±4.44
Dentin	1.13±0.06 [f]	37.35±1.55

* Means in a column followed by the same letter are not significantly different by the Kruskal-Wallis test at α=0.05.

DISCUSSION

In this study, the radiopacity properties of MTA, Biodentine, TheraCal LC, TheraCal PT, Glass Liner, and Equia HT were compared. The null hypothesis stating that there would be no statistically significant difference in radiopacity value among the six tested materials was evaluated and ultimately rejected.

Despite recent advancements in radiological diagnostic tools and the increasing integration of Cone Beam Computed Tomography (CBCT) into clinical practice, two-dimensional periapical radiographs continue to be the standard method for assessing the condition of teeth and the quality of applied restorations.[13] Suitable radiopacity is crucial for the detectability of restorative materials on a radiograph, allowing them to be readily differentiated from the adjacent anatomical structures.[14] Poorterman et al.[15] emphasized the importance of radiographic examination by noting that less than 15% of insufficient restorations can only be detected through clinical examination.

The presence of secondary caries serves as a primary reason for the replacement of restorations. It is imperative for base and liner materials to possess optimal radiopacity to create contrast with recurring caries, thus facilitating accurate diagnosis. Furthermore, it is expected that the interface between the tooth and the restoration should be radiopaque enough to be distinguished from the tooth structure, enhancing the clarity of diagnostic imaging. It has been reported that materials with moderate radiopacity are more suitable in this regard, and that the optimal radiopacity should be slightly higher than that of enamel.[16,17] In the present study, Biodentine and Equia Forte HT fitted this description. Additionally, MTA showed greater radiopacity compared to enamel, however, there might be another concern with high radiopacity levels. In radiographic evaluations, a high contrast between radiopaque region adjacent to a less radiopaque area may lead an visual illusion called the Mach Band phenomenon and may obstruct the detection of caries on surfaces neighboring the restoration.[16]

In the current study, only TheraCal LC and Glass Liner exhibited radiopacity, which was significantly lower than that of enamel. Having lower radiopacity than enamel may lead to misdiagnosis by clinicians, resulting in the mistaken identification of these materials as dentin, pulp, caries, or gaps.[17] The radiopacity value of TheraCal LC, as found in the current study, was 1.66±0.26, which is lower compared to the radiopacity value reported by Corral et al.[18] (2018) (2.17±0.17 mm Al) and higher compared to those reported by Gandolfi et al.[9] (2012) (1.07±0.17 mm Al). These discrepancies among studies highlight the need for further investigation. In addition, there is a lack of available literature findings on the radiopacity of TheraCal PT. Although these materials were not compared with others in various studies, the fact that they are more radiopaque than dentin but less so than enamel in our study suggests the need for further investigation. This also indicates that radiographic evaluations should be conducted with caution.

In their study, Yaylacı et al.[19] compared the radiopacity of 17 different restorative materials at thicknesses of 1mm, 2 mm, and 4 mm and identified the radiopacity of 1mm Equia Forte HT as 2.10±0.16, which was statistically similar to that of enamel. In accordance with this study, the present study found the radiopacity of the Equia Forte HT group to be 2.28±0.2, which was statistically akin to that of enamel. Furthermore, the presence of fluoroaluminosilicate glass and iron oxide in Equia Forte HT's composition may have contributed to this result.

ISO 4049 establishes pure aluminum as the benchmark for assessing the radiopacity of dental restorative materials, with enamel and dentin recommended as secondary standards.[20] This study, aligning with several in vitro research on the radiopacity of restorative materials, utilized both a pure aluminum step wedge as well as the hard tissues of human teeth as standards for comparison. Williams and Billington have reported that the radiopacity of enamel at 1 mm thickness matches that of aluminum at 2.1 mm thickness, and that dentin has the same radiopacity as aluminum of equivalent thickness.[21] The results of the current research also indicate that the radiopacity of dentin (1.13±0.06) is similar to that of aluminum of the same thickness. In contrast the radiopacity of enamel (2.13±0.07) is approximately twice that of aluminum of the same thickness.

It has been revealed that the minimum radiopacity value recommended for the identification and distinguishing of endodontic sealing materials is equal to 3 mm of aluminum, as required by the ISO 6876/2001 specifications. Additionally, it has been reported that materials with a radiopacity value lower than 3 mm of aluminum will be difficult to distinguish from dentin.[22] In the comparison of calcium silicate cements used in this study, the MTA group, with a radiopacity value of 5.15±0.69, was the only one found to meet the required minimum radiopacity threshold. Furthermore, in alignment with previous studies, the MTA group has demonstrated the highest radiopacity values.[23,24]

The radiopacity of a material is directly linked to the atomic numbers of the elements that constitute it; this represents the quantity of protons in the nucleus of the composing atoms, which defines the electrical charge and thus the force that attaches an electron to its orbit.[13] Given that bismuth has an atomic number of 83, it

can be considered more radiopaque in comparison to zirconium and tantalum, with atomic numbers of 40 and 73, respectively.[25] Thus, Angelus MTA, which includes bismuth oxide as a radiopacifying agent, demonstrates enhanced radiopacity due to the high atomic number of bismuth.

In the current study, the radiopacity value of Biodentine was determined to be 2.38±0.24. While Angelus MTA incorporates bismuth as its radiopacifying agent,[24] Biodentine utilizes zirconium oxide, as per the information provided by its manufacturer.[23] This variance in radiopacity between Biodentine and ProRoot MTA can be attributed to their use of distinct radiopacifiers. Kaup et al.[23] reported the radiopacity value of Biodentine as equivalent to 1.5 mm of aluminum, Tanalp et al.[26] found it to be 2.8±0.48 mm of aluminum, and Grech et al.[27] measured Biodentine's radiopacity at 1 and 28 days post-preparation, finding values of 3.3 and 4.1 mm of aluminum, respectively. These findings from literature demonstrate variations from the manufacturer's claim that Biodentine possesses a radiopacity equal to 3.5 mm of aluminum.

The wide range of radiopacity values observed for Biodentine, from 1.5 to 4.1 mm aluminium, may be due to the lack of standardization in the production of the material as previously reported and methodological differences in other studies, such as the film-focusing distance.[23] The storage conditions of the samples may also have played a role.[18] Additionally, the variance could be attributed to the quantity of zirconium oxide in Biodentine, which is reported to be superior in biocompatibility compared to bismuth oxide.[28] Further research is needed to determine the reasons for these discrepancies in radiopacity measurements.

Limitations

One significant limitation of this study is the inability to fully replicate the conditions of the oral environment. The radiopacity of restorative materials may be influenced by various factors present in the oral environment, including oral fluids, soft tissues, and adjacent dental structures. Furthermore, the leaching of ions from radiopacifiers within the material into an aqueous environment could diminish its radiopacity. Further research is needed to evaluate restorative materials under conditions that more closely resemble the oral environment, including studies on the effects of aging.

CONCLUSION

All the restorative materials tested exhibited higher radiopacity than dentin, with ThereCal LC and Glass Liner displaying lower radiopacity than enamel, ThereCal PT, Biodentine, and Equia Forte HT showing equivalent radiopacity to enamel, and MTA demonstrating higher radiopacity than enamel. The radiopacity of materials can be influenced by their structural characteristics as well as their types.

Currently, there are also commercial materials used as lining for restorations that exhibit insufficient radiopacity. As manufacturers continuously reformulate their products to enhance characteristics and reduce costs, there is a growing need for studies to assess the radiopacity of these lining materials.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Bahçeşehir University Clinical Researches Ethics Committee (Date: 20.09.2023, Decision No: 2023-16/01).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The relationship between homocysteine and no-reflow phenomenon in patients undergoing primary percutaneous coronary intervention

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ABSTRACT

Aims: The current study aimed to investigate the relationship between homocysteine and no-reflow phenomenon in patients undergoing primary percutaneous coronary intervention (pPCI).

Methods: Patients with ST-elevation myocardial infarctions (STEMI) who underwent pPCI in our center between May 01, 2022, and 20 August 2023 were included in this cross-sectional observational study. Patients were classified into two groups according to the occurrence of no-reflow during pPCI. Findings were compared between the two groups.

Results: A total of 332 patients [male, 75 (%82.8)] with STEMI undergoing pPCI, were included. Among them, 35 (10.5%) patients developed no-reflow. Homocysteine level was significantly higher in the no-reflow(+) group than the no-reflow(-) group [median (IQR), 19.02 (16.11-22.23 vs. 12.45 (10.99-14.93), p=0.019]. According to the multivariate analysis, homocysteine level, TIMI risk score, and postdilatation were independent predictors of no-reflow occurrence [Odds Ratio (95% CI), 1.127 (1.042-1.218), p=0.003, 1.385 (1.157-1.659), p<0.001, and 2.396 (1.092-5.257), p=0.029, respectively]. Considering the ROC curve analysis for homocysteine predicting no-reflow, the area under the curve (AUC) was 0.714 with an optimal cut-off value of 14.1 (sensitivity of 71%, specificity 62%).

Conclusion: Higher admission homocysteine levels were associated with no-reflow development in STEMI patients during pPCI. Higher levels of homocysteine may identify a subset of patients at a higher risk of no-reflow development during pPCI.

Keywords: No-reflow, homocysteine, STEMI, PCI

INTRODUCTION

Primary percutaneous coronary intervention (pPCI) is the most effective and gold-standard treatment for ST-elevation myocardial infarction (STEMI).[1] In addition to ensuring quick antegrade blood flow, it also lowers myocardial necrosis and raises survival rates.[2] However, this advantageous effect may remain incapable when a patient develops a no-reflow phenomenon.[3] No-reflow phenomenon, shortly no-reflow, is a post-PCI complication characterized by insufficient myocardial perfusion in the coronary arteries without any angiographic indications of dissection, obstruction, or spasm in the epicardial vessels.[4] This phenomenon is related to the functional and structural change of the coronary microcirculation and, as a result, cardiovascular mortality.[5] The pathophysiological etiology is thought to be associated with distal atherothrombotic embolization, ischemic damage, reperfusion injury, microcirculation abnormality, inflammatory response, individual susceptibility, and endothelial dysfunction.[5,6]

Homocysteine, a sulfur-containing amino acid, is produced as a result of the catabolism of methionine.[7] This molecule can cause endothelial dysfunction and oxidative stress by producing free radicals.[7,8] Both oxidative stress and endothelial dysfunction increase cardiovascular risk.[8,9] A vast number of studies investigated the relationship between homocysteine and cardiovascular diseases.[6,8-11] Higher homocysteine levels were associated with poor cardiovascular outcomes, in these researches. However, the impact of homocysteine on no-reflow has not been examined well. The relationship between homocysteine and the no-reflow phenomenon after pPCI

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has remained unclear. The current study aimed to address this gap, examining the relationship between homocysteine and no-reflow phenomenon in STEMI patients during pPCI.

METHODS

Patients

Patients with STEMI who underwent pPCI in our center between May 01, 2022, and 20 August 2023 were included in this cross-sectional observational study. Diagnosis and treatment of STEMI were based on guideline recommendations.[12] The research excluded individuals with acute infections, malignancies, coagulopathy, and patients receiving anticoagulant therapy. Our endpoint was the development of no-reflow during pPCI. Patients were classified into two groups according to the occurrence of no-reflow during pPCI. Findings were compared between the two groups.

Ethics

The protocol of the study was approved by the ethics committee of Kafkas University (Date: 27.04.2022, Decision No: 80576354-050-99/128) according to the Declaration of Helsinki and Good Clinical Practice guidelines.

Blood Sample

Routine complete blood cell count and blood biochemical measurements were performed on a blood sample obtained on admission. Plasma total homocysteine at admission was measured using a colorimetric assay test kit from Elabscience Biotechnology, Wuhan, China. The measurement protocol was obtained from Elabscience and the results were determined using a spectrophotometer (Epoch, Biotech, USA).

Coronary Angiography

The percutaneous trans-femoral (Judkins) approach was followed in the performance of both coronary angiography and pPCI. All patients received anticoagulation therapy with unfractionated heparin of 70–100 units/kg (maximal dose 10.000 units) and antiplatelet therapy with acetylsalicylic acid (300 mg). Also, a loading dose of clopidogrel (300–600 mg) or (ticagrelor 180 mg) was given before the pPCI. Intravenous nitroglycerine and Glycoprotein IIb/IIIa inhibitors were introduced if necessary. Coronary blood flow was defined per TIMI flow classification which classifies coronary flow as follows: TIMI grade 0, no perfusion (no antegrade flow beyond the point of occlusion); TIMI grade 1, penetration without perfusion (the contrast material passes beyond the area of obstruction, but fails to opacify the entire coronary bed distal to the obstruction for the duration of the cine run); TIMI grade 2, perfusion of the entire infarct vessel into the distal bed but with delayed flow compared with a normal artery; TIMI grade 3, full perfusion of the infarct vessel with normal flow.[13,14] On this base, patients with TIMI flow below grade 3 was defined as no-reflow in the current study. Thrombus burden was assessed according to the TIMI thrombus grading scale that ranged from grade 0 (no thrombus) to grade 5 (very large thrombus causing vessel occlusion).[14]

Syntax II score was calculated using six clinical variables including age, gender, left ventricular ejection fraction (LVEF), chronic obstructive pulmonary disease, peripheral arterial disease, and creatinine clearance, as well as two anatomical variables including Syntax score and the existence of left main coronary artery disease.[15] The Thrombolysis in Myocardial Infarction (TIMI) risk score was calculated in conformity with Marrow et al.[16] Cockroft–Gault formula was performed for the calculation of glomerular filtration rate (eGFR).

Statistical Analysis

IBM SPSS Statistics for Windows, Version 20.0 was used for the statistical analyses. Continuous variables are expressed as mean ± standard deviation (SD), minimum and maximum values. Variables that did not show normal distribution were presented as median [interquartile range (IQR)], mean, minimum, and maximum values. Frequencies and percentages were used to represent the categorical variables. The Kolmogorov-Smirnov test was used to test the normality distribution of continuous variables. While the chi-square or Fisher exact test was utilized for the comparison of the categorical data, the Student t-test or Mann–Whitney U test was used to compare continuous variables between the two groups. The Pearson correlation test was employed to assess the association between age and homocysteine levels. A univariate regression analysis was performed including factors potentially related to no-reflow as shown in **Table 1**. Also, a multivariate logistic regression analysis (forward likelihood ratio method) with a significance level of 0.05 was used to determine the independent determinants of no-reflow. Using a receiver operating characteristic (ROC) curve analysis, an optimal homocysteine value for predicting no-reflow was found. For statistical significance, a two-sided p-value of less than 0.05 was established as the cut-off point.

RESULTS

A total of 332 patients [male, 75 (%82.8)] with STEMI undergoing pPCI, were included. Among them, 35 (10.5%) patients developed no-reflow. **Table 2** shows the comparison of demographics, lesion features, and procedural aspects, based on the presence of no-reflow. The no-reflow (+) group was older (mean+SD, 61.7±12.4 vs. 55.9±10.7, p=0.003) and had significantly higher

Table 1. Univariate and multivariate regression analysis for predicting no-reflow

	Univariate		Multivariate	
	OR (95% CI)	**p value**	**OR (95% CI)**	**p value**
Homocysteine	1.151 (1.069-1.239)	<0.001	1.127 (1.042-1.218)	0.003
Pain-to-ballon time	1.004 (1.002-1.007)	0.001	-	-
TIMI risk score	1.361 (1.150-1.611)	<0.001	1.385 (1.157-1.659)	<0.001
Postdilatation	2.280 (1.123-4.629)	0.023	2.396 (1.092-5.257)	0.029
Age	1.048 (1.016-1.082)	0.004	-	-
Syntax II score	1.155 (0.987-1.351)	0.072	-	-

CI, confidence interval; OR, odds ratio; TIMI, thrombolysis in myocardial infarction

Table 2. Comparison of demographics, lesion features, and procedural aspects, based on the presence of no-reflow

	Total (n=332)	**No-reflow (+) (n=35)**	**No-reflow (-) (n=297)**	**P-value**
Male, n(%)	275 (82.8)	25 (71.4)	250 (84.2)	0.059
Age (years), mean±SD (min-max)	56.5±11 (27-90)	61.7±12.4(41-90)	55.9±10.7(27-86)	0.003
SBP (mmHg), median (IQR), mean (min-max)	135 (120-147) 136 (66-245)	140 (128-177) 146 (69-240)	133 (120-143) 135 (66-245)	0.027
DBP (mmHg), mean±SD (min-max)	80±18 (32-145)	85±23 (32-130)	80±18 (35-145)	0.120
Heart rate (beat/minute), mean±SD (min-max)	78±16 (27-132)	81±18 (37-114)	78±16 (27-132)	0.255
Syntax II score, median (IQR), mean (min-max)	31.3 (25.4-39.0) 33.4(15.5-73.3)	43.5 (31.5-52.8) 42.9 (26.1-71)	30.4 (24.7-37.6) 32.3 (15.5-73.3)	0.036
TIMI risk score, median (IQR), mean (min-max)	2 (1-4) 2.57 (0-10)	4 (2-5) 3.66 (1-7)	2 (1-3) 2.44(0-10)	<0.001
TIMI thrombus grade, mean±SD (min-max)	4.90±0.44 (2-5)	4.97±0.16 (4-5)	4.89±0.47 (2-5)	0.304
Killip class, mean±SD (min-max)	1.25±0.55 (1-4)	1.57±0.74 (1-3)	1.21±0.52 (1-4)	<0.001
Procedural features				
Predilatation, n(%)	303 (91)	34 (97)	269 (90)	0.193
Stent length (mm), median (IQR), mean (min-max)	20 (16-28) 23 (10-95)	25 (19-35.5) 28 (10-95)	20 (16-28) 23 (10-78)	0.037
Stent diameter (mm), median (IQR), mean (min-max)	3 (3-3.25) 3.08 (2.5-4)	3 (3-3.5) 3.16 (2.5-4)	3 (3-3.25) 3.07 (2.5-4)	0.185
Postdilataion, n (%)	104 (31,3)	17 (48,6)	87 (29.3)	0.020
Door-to-balloon time (minute), median (IQR), mean (min-max)	30 (25-35) 31 (18-213)	32 (28-35) 32 (20-48)	30 (25-35) 31 (18-213)	0.207
Pain-to-balloon time (minute), median (IQR), mean (min-max)	150 (80-240) 176 (25-590)	230 (155-297) 241 (50-590)	150 (80-230) 168 (25-590)	<0.001
Maximum balloon inflation pressure (atmosphere), median (IQR), mean (min-max)	14 (14-16) 14.8 (10-22)	15 (14-16) 15.4 (10-22)	14 (14-16) 14.7 (10-22)	0.115
Visible distal embolization, n(%)	14 (4.2)	7 (20)	7 (2.4)	<0.001
lesion location (proximal), n(%)	236 (71.1)	31 (88.6)	205 (69)	0.016
Infarc related artery ectasia, n(%)	14 (4.2)	1 (2.9)	13 (4.4)	0.672
Multivessel disease, n(%)	226 (68)	21 (60)	205 (69)	0.279
Comorbities				
Hypertension, n (%)	151 (45.5)	22 (62.9)	129 (43.4)	0.029
Diabetes, n (%)	77 (23.2)	12 (34.3)	65 (21.9)	0.100
Smoking, n (%)	188 (56.6)	15 (42.9)	173 (58.2)	0.082
COPD, n (%)	19 (5.7)	1 (2.6)	18 (6.1)	0.440
Dyslipidemia, n (%)	138 (41.6)	10 (28.6)	128 (43.1)	0.099
Coronary artery disease (n)	3	0	3	
Family history, n(%)	66 (19.9)	7 (20)	59 (19.9)	0.985
Chronic renal disease, n(%)	9 (2.7)	3 (8.6)	6 (2)	0.024

COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; IQR, interquartile range; SBP, systolic blood pressure; SD, standard deviation; TIMI, Thrombolysis in Myocardial Infarction; TIMI, thrombolysis in myocardial infarction

systolic blood pressure, Syntax II risk score, and TIMI risk score compared to the no-reflow (-) group [median (IQR), 140 (128-177) vs. 133 (120-143), p=0.027, 43.5 (31.5-52.8) vs. 30.4 (24.7-37.6), p=0.036 and 4 (2-5) vs. 2 (1-3), p<0.001, respectively]. killips class was also significantly higher in the no-reflow (+) group (mean±SD, 1.57±0.74 vs. 1.21±0.52, p<0.001). For comorbidities, hypertension and chronic renal disease were more frequent in patients with no-reflow [n (%), 22 (62.9) vs. 129 (43.4), p=0.029 and 3 (8.6) vs. 6(2), p=0.024, respectively]. Regarding lesion and procedural features, in the no-reflow(+) group stent length and pain-to-balloon time were significantly longer than in the no-reflow(-) group [median (IQR), 25 mm (19-35.5 mm) vs 20 mm (16-28 mm), p=0.037 and 230 minutes (155- 297) vs. 150 minutes (80-230), p<0.001, respectively]. Also, proportions of postdilatation, distal embolization, and stent implantation to the proximal of the artery rather than mid or distal were significantly higher in the no-reflow (+) group [n (%), 17 (48.6) vs. 87 (29.3), p=0.020, 7 (20) vs. 7 (2.4), p<0.001 and 31 (88.6) vs. 205 (69), p=0.016, respectively].

A comparison of laboratory findings is presented in **Table 3**. Homocysteine level was significantly higher in the no-reflow (+) group than the no-reflow (-) group [median (IQR), 19.02 (16.11-22.23) vs. 12.45 (10.99-14.93), p=0.019]. Levels of Troponin, CK-MD, CK, MPV, and glucose were also significantly higher in the no-reflow (+) group. [median (IQR), 9.8 (3.6-13.8) vs. 2.3 (0.8-5.3), p<0.001, 54 (38-62) vs. 34 (24-45), p<0.001, 564 (292-854) vs. 332 (195-568), p=0.005, 9.4 (8.75-10.55) vs. 9 (8.2-9.8), p=0.034 151 and (121-207) vs. 128 (108-167), p=0.009, respectively]. As well as, neutrophil and uric acid levels showed significantly higher means in the same group (mean+SD, 11.75±3.85 vs. 10.26±2.87, p=0.006 and 5.75±1.91 vs. 5.12±1.42, p=0.023, respectively).

Table 3. Comparison of laboratory findings based on the presence of no-reflow

	Total (n=332)	No-reflow (+) (n=35)	No-reflow (-) (n=297)	P-value
Laboratory				
Homocysteine (µmol/L), median (IQR), mean (min-max)	12.56 (11.04-16.30) 13.72 (1.92-42.8)	19.02 (16.11-22.23) 16.84 (8.69-42.8)	12.45 (10.99-14.93) 13.35 (1.92-34.69)	0.019
Hemoglobin (g/dl),mean±SD (min-max)	13.92±1.67 (8.5-19)	13.49±1.60 (9.6-17)	13.9±1.67 (8.5-19)	0.104
WBC (×10³/µL), median (IQR), mean (min-max)	12.83 (11.14-14.59) 13.2 (6.2-25)	14 (11.45-17.45) 14.4 (8.2-25)	12.7 (11.1-14.4) 13.0 (6.2-25)	0.056
Neutrophil (×10³/µL), mean±SD (min-max)	10.42±3.01 (4-21.9)	11.75±3.85 (5.2-21.9)	10.26±2.87 (4-21.3)	0.006
Lymphocyte(×10³/µL), median (IQR), mean (min-max)	1.8 (1.3-2.5), 1.95 (0.4-6.11)	1.6 (1.2-2.2), 1.86 (0.5-4.7)	1.8 (1.3-2.5), 1.96 (0.4-6.11)	0.381
Platelet (×10³/µL), mean±SD (min-max)	262±63 (105-494)	257±82 (105-465)	262±60 (119-494)	0.645
PDW (fL), mean±SD (min-max)	16.07±1.32 (10-18.1)	16.08±1.32 (12.8-18)	16.06±1.32 (10-18.1)	0.953
MPV (fL), median (IQR), mean (min-max)	9 (8.2-9.8), 9.1(6.5-14.3)	9.4 (8.75-10.55), 9 (7.1-13)	9 (8.2-9.8), 9(6.5-14.3)	0.034
Troponin I (ng/ml), median (IQR), mean (min-max)	2.67 (0.81-5.82), 5.48 (0.001-64)	9.8 (3.6-13.8), 10.82(0.5-57)	2.3 (0.8-5.3), 4.84 (0.001-64)	<0.001
CK-MB (ng/ml), median (IQR)], mean (min-max)	35.5 (25-47), 40.7 (7-259)	54 (38-62), 57 (10-211)	34 (24-45), 38.8 (7-259)	<0.001
CK (ng/ml), median (IQR), mean (min-max)	349 (206-643), 448 (26-2876)	564 (292-854), 653 (39-2876)	332 (195-568), 423 (26-1675)	0.005
Creatinine (mg/dl), median (IQR), mean (min-max)	0.90 (0.79-1.03), 0.93 (0.48-2.3)	0.85 (0.80-1.10), 0.96 (0.58-1.9)	0.90 (0.79-1.03), 0.93 (0.48-2.3)	0.955
eGFR, median (IQR), mean (min-max)	85 (70-100), 85 (25-160)	75 (67-99), 79 (34-135)	86 (71-100), 86 (25-160)	0.099
Glucose (mg/dl), median (IQR), mean (min-max)	130 (109-172), 151 (44-493)	151 (121-207), 149 (67-335)	128 (108-167), 149 (44-493)	0.009
Total protein (g/dl), mean±SD (min-max)	6.63±0.67 (4.7-9.3)	6.63±0.65 (5.2-7.7)	6.63±0.67 (4.7-9.3)	0.984
Albumin (g/dl), median (IQR), mean (min-max)	3.8 (3.5-4.08), 3.81 (2.4-6.2)	3.7 (3.3-4.04), 3.71 (2.5-5.17)	3.8 (3.5-4.08), 3.83 (2.4-6.2)	0.289
Uric acid (mg/dl), mean±SD (min-max)	5.19±1.50 (1.7-11.9)	5.75±1.91 (3-11.7)	5.12±1.42 (1.7-11.9)	0.023
Total cholesterol (mg/dl), median (IQR), mean (min-max)	183 (156-210), 186 (87-386)	176 (143-191), 176 (94-321)	183 (159-211), 187 (87-386)	0.126
LDL (mg/dl), mean±SD (min-max)	119±38 (34-246)	112±41 (45-207)	120±37 (34-246)	0.296
HDL (mg/dl), mean±SD (min-max)	39.8±12.0 (18-83)	41.7±12.9 (20-73)	39.5±11.9 (18-83)	0.348
Triglycerides (mg/dl), median (IQR), mean (min-max)	121 (86-166), 136 (27-936)	112 (80-147), 112 (39-213)	124 (87-167), 139 (27-936)	0.123

CK, creatine kinase; CK-MB, creatine kinase MB; eGFR, estimated glomerular filtration rate; fL, femtolitre; IQR, interquartile range; LDL, low-density lipoprotein; HDL, high density lipoprotein; MPV, mean platelet volume; SD, standard deviation; PDW, platelet distribution width; WBC, white blood count;

According to the multivariate analysis, homocysteine, TIMI risk score, and postdilatation were independent predictors of no-reflow occurrence [Odds Ratio (95% CI), 1.127 (1.042-1.218), p=0.003, 1.385 (1.157-1.659), p<0.001, and 2.396 (1.092-5.257), p=0.029, respectively] (**Table 1**). Considering the ROC curve analysis for homocysteine predicting no-reflow, the area under the curve (AUC) was 0.714 with an optimal cut-off value of 14.1 (sensitivity of 71%, specificity 62%) (**Figure**). Last but not least, homocysteine level was significantly correlated with age (R2=0.001, p=0.038).

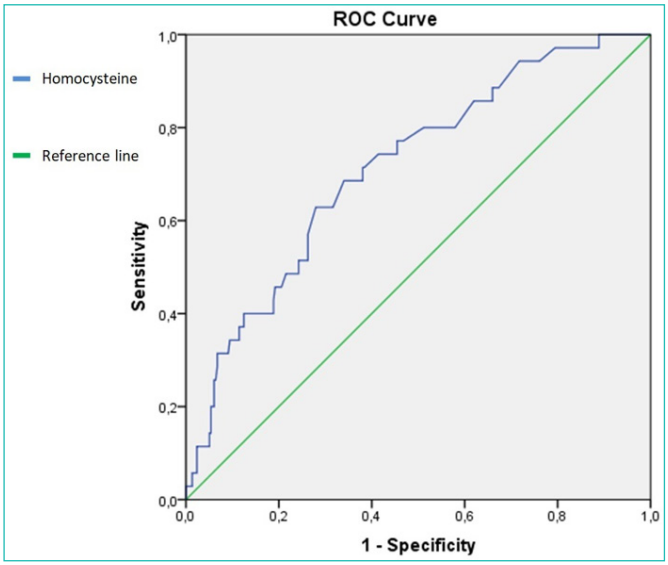

Figure. Diagnostic accuracy of homocysteine value on no-reflow development during pPCI by ROC curve.

pPCI, primary percutaneous intervention; ROC, receiver operating characteristic curve

DISCUSSION

In this study, we focused on the potential relationship between homocysteine and no-reflow development in patients undergoing pPCI. Our results demonstrated a significant relation between serum homocysteine level and the occurrence of no-reflow in this population. Patients with higher levels of homocysteine developed more frequent no-reflow. Furthermore, homocysteine along with the TIMI risk score and postdilatation was an independent predictor of no-reflow in the current work. Besides, no-reflow was significantly associated with age, higher values of systolic blood pressure, Syntax II risk score, TIMI risk score, Killips class, neutrophil, MPV, troponin I, CK-MD, CK, glucose, uric acid, longer stent length and pain-to-balloon time.

No-reflow is linked to a higher risk of rehospitalization, negative ventricular remodeling, malignant arrhythmias, heart failure, and mortality.[17] Endothelial cell dysfunction and microvascular damage are well-established mechanisms of no-reflow.[18,19]

In the meantime, the relationship of homocysteine with endothelial cell dysfunction and microvascular damage has been demonstrated in many studies.[20,21] Homocysteine may trigger microcirculation dysfunction and endothelial cell injury through several mechanisms. Elevated homocysteine can contribute to significant oxidative stress and the production of oxygen radicals.[22] By inhibiting nitric oxide synthase, oxygen free radicals can decrease the synthesis of nitric oxide (NO), which can seriously harm vascular endothelial cells.[23] Higher homocysteine values might also cause thrombin regulatory protein activity by increasing low-density lipoprotein's natural oxidation, further damaging endothelial cells.[24] Moreover, elevated levels of homocysteine have the potential to cause the production of cyclins D and A, hence inducing vascular smooth muscle proliferation and enhancing vascular resistance.[25] The expression of thrombomodulin, von Willebrand factor, and cell adhesion molecules may also be promoted by high homocysteine values. Consequently, they may raise vascular resistance by damaging vascular endothelial cells while stimulating the proliferation of smooth muscle cells.[23] Our findings support the role of homocysteine in the pathogenesis of no-reflow in patients with coronary STEMI undergoing pPCI. Li et al.[26] also showed a close relationship between elevated Hcy levels and no-reflow. They included 54 patients with no-reflow who underwent non-emergency coronary angiography. The patients were compared with 101 control group with normal coronary angiography. However, our study population included patients with STEMI who underwent pPCI. In other words, the comparison was made based on no-reflow development in a homogeneous population in our study. Moreover, we found homocysteine as an independent predictor of no-reflow occurrence.

In a similar line to a past study, our findings demonstrated a significant relationship between TIMI risk score and no-reflow.[27] Of note, we found the TIMI risk score as an independent predictor of no-reflow. TIMI risk score includes clinical variables like age, diabetes mellitus/hypertension/angina, blood pressure, heart rate, Killip class, weight, anterior ST-elevation or left bundle branch block, and time to treatment.[28] Among these, age and hypertension were also associated with no-reflow in the present study. Along the lines of previous reports, distal embolization,[29] ischemia time,[30] stent length,[31] and postdilatation[31] were also associated with no-reflow in the current work.

Early estimation of no-reflow risk factors may have an impact on the prevention of this phenomenon. According to the present findings, higher levels of homocysteine may identify a subset of patients at a higher risk of no-reflow development during pPCI. Therefore, It should be kept in

mind that STEMI patients with higher homocysteine levels may be more susceptible to no-reflow during pPCI. Further studies are necessary to confirm the present findings and elucidate the mechanisms behind these findings.

Limitations

There are some important limitations in the current study. This is an observational single-center study. Therefore, it is not appropriate to apply these results to other populations. The sample size included in the study is quite small.

CONCLUSION

Higher homocysteine levels, higher TIMI risk scores, and postdilatation were associated with no-reflow development during pPCI. Higher levels of homocysteine may identify a subset of patients at a higher risk of no-reflow development during pPCI. More prospective multicenter studies involving a wider population are required to review the significance of the current findings.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Ethics Committe of Kafkas University (Date: 27.04.2022, Decision No: 80576354-050-99/128).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Exercise benefits and barriers perceived by hemodialysis patients: relationship with fatigue and physical activity level

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ABSTRACT

Aims: The aim of our study was to determine the attitudes of hemodialysis (HD) patients towards exercise and to determine the relationship between physical activity level (PAL) and fatigue.

Methods: This cross-sectional study was conducted with 113 HD patients (57.8±5.96) aged 18-65 years. Personal information form, fatigue impact scale (FIS), international physical activity questionnaire short form (IPAQ-SF), and dialysis patient-perceived exercise benefits and barriers scale (DPEBBS) were used as data collection tools.

Results: The mean DPEBBS total score was 64.35±6.15 and PAL was low. The most commonly perceived exercise benefits were preventing muscle atrophy and maintaining a stable body weight, while the most common exercise barriers (EB) were not understanding how to exercise and fatigue. There was a weak negative correlation between the PAL of the patients with the Perceived Exercise Barriers subscale (r=-0.275, p=0.003) and a weak positive correlation between the total scale score (r=0.318, p=0.001). There was a weakly significant positive correlation between the Cognitive, Physical, Psychosocial sub-dimension of Fatigue and Total FIS with the Perceived Exercise Barriers sub-dimension (r=0.337, p=0.000; r=0.358, p=0.000; r=0.334, p=0.000; r=0.387, p=0.000). A very weakly significant negative correlation was found between the Fatigue Cognitive, Physical, Psychosocial sub-dimension and Fatigue Total Impact Dimension with the total scale score (r=-0.247, p=0.008; r=-0.234, p=0.013), r=-0.222, p=0.018, r=-0.243, p=0.003) .

Conclusion: HD patients had higher perceptions of the benefits of exercise. It was concluded that the perception of EB decreased as PAL increased and the perception of EB increased as fatigue levels increased. It is recommended that specialized physiotherapists evaluate HD groups at risk in detail and create individual interventions that support HD patients' compliance with exercise.

Keywords: Dialysis, exercise, sedantary behavior, fatigue

INTRODUCTION

Chronic kidney disease (CKD) is a progressive public health problem affecting more than 10% of the general population and more than 800 million people worldwide.[1] Most patients with CKD eventually progress to end-stage renal disease (ESRD) and require renal replacement therapy (RRT) such as peritoneal dialysis (PD), haemodialysis (HD) or kidney transplantation to survive.[2]

The most commonly used RRT method in the treatment of ESRD is HD.[3] Although the rapid development of HD technology can lead to a significant increase in the life expectancy of patients with ESRD and alleviate the uremic symptoms of CKD, it does not change the underlying disease process.[4]

Fatigue is a common symptom in HD patients and its prevalence ranges from 60-97%. The reduced level of physical activity, low functional capacity and general muscle weakness in these patients lead to a general feeling of fatigue.[5] Fatigue reduces self-care activities, disrupts family and social roles, reduces the ability to perform routine activities, and can lead to unemployment and increased dependence on healthcare. This situation negatively affects the patient's quality of life and self-confidence.[6]

Exercise is one of the most effective strategies used to control or eliminate dialysis symptoms and complications experienced by patients. It is highly beneficial for the physical health of dialysis patients. It improves cardiovascular function, blood pressure, muscle strength, nutritional status, dialysis quality, reduces negative emotions such as

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anxiety and depression, makes them feel good, improves social interaction of patients and their families.[7] Safe aerobic and resistance exercise programs that can be applied intradialytically and interdialytically have been developed to prevent lack of physical function in the HD population.[8] Meta-analyses have reported that aerobic exercise during HD reduces fatigue, improves resting blood pressure, urea clearance, muscle cramps and dialysis-related symptoms such as fatigue[7,9], and resistance exercise increases muscle strength, power and muscle endurance.[8]

Even though the benefits of exercise are clearly documented in the literature, most patients with ESRD have sedentary lifestyles and only 6% of HD patients are shown to have physical activity 4 to 5 days a week.[10] With the progression of kidney disease, the level of physical activity continuously decreases and reaches its lowest level in hemodialysis patients. This lowest level is particularly pronounced in elderly patients.[11] In addition, physical inactivity of HD patients leads to muscle atrophy, decreased muscle strength, and dysfunction.[11]

Although many benefits of exercise for dialysis patients are known, it should be investigated why most dialysis patients have insufficient physical activity and do not exercise. The findings of the study will provide data for the holistic evaluation of patients and the planning to be made to improve exercise behaviors. The aim of our study was to determine the effect of fatigue and physical activity level on exercise perception of hemodialysis patients and to determine the attitude of dialysis patients towards exercise.

METHODS

Ethics

The study was carried out with the permission of the Sakarya University of Applied Sciences Ethics Committee (Date: 07.07.2023, Decision No: 33/4). We obtained an informed consent form from all patients for procedure. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

This cross-sectional study was carried out on HD patients treated at Dialysis Center in Sakarya province between September and October 2023. Participants between the ages of 18-65 years, who had the cognitive ability to understand the tests and assessments, could communicate verbally, and had been on HD treatment for at least 3 months were included in the study. As exclusion criteria: It was stated that lower extremity functions were affected due to any orthopedic problem or neurological disease (as it would affect the physical activity level), incomplete or invalid filling of the scale/questionnaire. Information was given before the study and signed informed consent was obtained from the participant who agreed to participate in the study.

Data Collection

"Personal Information Form, Fatigue Impact Scale, International Physical Activity Questionnaire Short Form and Exercise Benefits/Barriers Scale" were used as data collection tools. Data were collected in approximately 30 minutes by face-to-face questionnaire method. The Personal Information Form consisted of questions determining the participants' characteristics such as age, gender, body mass index, disease history, job and marital status.

Fatigue Impact Scale was used to define how fatigue creates problems in daily life in HD patients.[12] It is a scale consisting of a total of 40 questions, including 20 questions on psychosocial effects, 10 questions on social effects and 10 questions on cognitive effects. Each question is scored between 0-4 and the maximum score is 160. High scores indicate functional limitations due to fatigue.[12]

The International Physical Activity Questionnaire Short Form (IPAQ Short Form), which has Turkish validity and reliability, was used to determine the physical activity level of HD patients.[13] In the IPAQ, the criterion was that physical activities should be performed for at least 10 minutes (min) at a time. The questionnaire asked the duration of sitting, walking, moderate and vigorous physical activity in minutes in the last 7 days. These durations were converted to metabolic equivalents (MET). According to the total physical activity score, the physical activity levels of the individuals were categorized as "low, moderate and high". According to IPAQ, above 3000 MET-min/week(wk) is classified as high level, 600-3000 MET-min/wk as moderate level and below 600 MET-min/wk as low level physical activity.[13]

Dialysis Patient-perceived Exercise Benefits and Barriers Scale (DPEBBS) was used. Turkish validity and reliability were performed by Taş and Akyol.[14] The scale consists of 24 items, 2 open-ended questions and six sub-dimensions. Twelve of the 24 items (1, 2, 3, 4, 6, 7, 10, 13, 16, 20, 22 and 23) consisted of statements about the benefits of exercise, while the other 12 (5, 8, 9, 11, 12, 14, 15, 17-19, 21 and 24) consisted of statements that prevent exercise. Negative items were reversed and coded. The scale is a 4-point Likert scale (1=Strongly disagree, 2=Disagree, 3=Agree, and 4=Strongly agree) with a minimum score of 24 and a maximum score of 96. Higher scores indicate more perceived exercise benefits and fewer exercise barriers.[14]

Statistical Analysis

The effect size obtained in the reference study was found to be at a medium level (r=0.548).15 Considering that a lower effect size could be obtained (r=0.3), as a result of the power analysis; It was calculated that 90%

power could be achieved at a 95% confidence level if at least 109 people were included in the study. SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)) package program was used for data analysis. Continuous variables were expressed as mean±standard deviation and categorical variables as number and percentage. Correlation analyses were used to examine the relationships between numerical variables. In all examinations, $p<0.05$ was considered statistically significant.15

RESULTS

Sociodemographic characteristics of the HD patients who participated in the study are shown in Table 1. The mean age of the participants was 57.8±5.96 years and 53.1% were male. It was determined that 72.6% of the participants were married, 49.6% were primary school graduates, 42.5% were retired and 37.2% were not working.

Table 1. Sociodemographic characteristics

	Mean±S.D	Med (IQR)	Min-Max	
Age, year	57.8±5.96	60 (55-61.5)	38-70	
			n	**%**
Gender				
Male			60	53.1
Woman			53	46.9
Marital Status				
Married			82	72.6
Single			11	9.7
Widow			20	17.7
Education Status				
Literate only			10	8.8
Primary school graduate			56	49.6
Secondary school graduate			27	23.9
High school graduate			16	14.2
Higher education graduate			2	1.8
University graduate			2	1.8
Job Status				
Works full time			4	3.5
Works part-time			1	.9
Not working-unemployed			42	37.2
Retired			48	42.5
Disability pensioner			18	15.9

HD: Hemodialysis, SD: Standard deviation, Med: Median, IQR: Interquartile range, min-max: minimum-maximum

The clinical characteristics of HD patients are shown in Table 2. The etiology of CKD was hypertension in 46.9% and diabetes mellitus in 25.7%, 90.3% had an additional chronic disease, 32.7% had hypertension as a comorbidity, and 85% had a dominant right extremity. The mean duration of CKD was 7.75±6.09 years, the mean duration of HD was 7.17±5.92 years, the mean BMI was 25.25±3.82 kg/m2, 43.4% were overweight and It was found that 48.7% had a low level of physical activity.

Table 2. Clinical features of the HD patients

	Mean±SD	Med (IQR)	Min-Max	
BMI, kg/m^2	25.25±3.82	25.39 (22.89-27.51)	16.85-39.19	
Duration of CKD, years	7.75±6.09	6 (3-10)	1-32	
HD duration, years	7.17±5.92	6 (3-9.5)	1-30	
			n	**%**
BMI classification				
<18.5 kg/m^2 Weak			6	5.3
18.5-24.9 kg/m^2 Normal			48	42.5
25.0-29.9 kg/m^2 Overweight			49	43.4
30.0-34.9 kg/m^2 Grade I obesity			9	8.0
35.0-39.9 kg/m^2 Grade II obesity			1	.9
Dominant hand				
Right			96	85.0
Left			17	15.0
Etiology of CKD				
Hypertension			53	46.9
Glomerulonephritis			7	6.2
Diabetes			29	25.7
Urinary tract infections			5	4.4
Urinary stones			2	1.8
Polycystic kidney disease			8	7.1
Idiopathic			6	5.3
Urethral stricture			1	.9
Vesicoureteral reflux			1	.9
Long-term medication use			1	.9
Comorbidete status				
Diabetes			7	6.2
HT			37	32.7
Peripheral vascular disease			4	3.5
Chronic heart disease			6	5.3
Chronic lung disease			4	3.5
Cancer			3	2.7
Diabetes+HT			22	19.5
HT+chronic Lung Disease			2	1.8
Atherosclerosis			3	2.7
HT+atherosclerosis			2	1.8
HT+chronic heart disease			8	7.1
DM+ atherosclerosis			2	1.8
HT+COPD			2	1.8
No			11	9.7
Physical activity level				
Low level activity			55	48.7
Moderate activity			53	46.9
High level of activity			5	4.4

HD: Hemodialysis, BMI: Body Mass Index, CKD: Chronic kidney disease, HD: Hemodialisis, HT: Hypertension, COPD: Chronic Obstructive Pulmonary Disease, kg: kilogram, m: metre, SD: Standard deviation, Med: Median, IQR: Interquartile range, min -max: minimum-maximum

The mean scores of the scales used in the study are shown in Table 3. The mean IPAQ score was 925.54±921.93 and the mean total score of the exercise benefits/barriers scale was 64.35±6.15. While the mean of fatigue total effect dimension was 5±3.91, the mean of fatigue cognitive

sub-dimension (12.9±13.71) was higher than the mean of physical (3.04±2.19) and psychosocial (6.54±8.7) fatigue sub-dimensions.

Table 3. Average scores of the scales (n=113)

	Mean±S.D	Med (IQR)	min-max
IPAQ value	925.54±921.93	610 (274.5-1386)	66-4751
Exercise benefits/ barriers scale total score	64.35±6.15	65 (60-69)	45-81
Exercise benefits	27.58±4.25	28 (25-30)	15-40
Exercise barriers	36.77±5.5	37 (33-40)	20-51
Fatigue total impact dimension score	5±3.91	3.8 (2,56-6.15)	1.63-22.85
Fatigue cognitive	12.9±13.71	5.71 (3.33-20)	1.73-40
Fatigue physical	3.04±2.19	2.5 (1.9-3.63)	1.02-20
Fatigue psychosocial	6.54±8.7	4.7 (2.71-6.66)	1.7-80

IPAQ: International Physical Activity Questionnaire, SD: Standard deviation, Med: Median, IQR: Interquartile range, min -max: minimum-maximum

Table 4 shows the distributions of the item means of the DPEBBS scale. Among the twelve statements regarding the benefits of exercise, "exercise prevents muscle atrophy" received the highest score (2.96±0.78), followed by "exercise can keep my body weight at a constant level" (2.94±0.7). The statements "Exercise will help reduce my total health expenditures" and "Exercise will protect me from developing other diseases" received the lowest scores (2.23±0.81; 2.36±0.89, respectively). Among the twelve statements that prevent exercise, "I lack understanding of how exercise is done" (3.28±0.85) received the highest score, followed by "frequent fatigue prevents me from participating in exercise" (3.08±0.92). The statements "I need my family to be with me when I am outside, so exercising outdoors puts a burden on my family" and "exercise negatively affects the health of dialysis patients" received the lowest exercise barriers score (1.96±0.83; 2.19±0.77, respectively).

The relationship between patients' DPEBBS and IPAQ and FIS is shown in Table 5. There was a weak negative correlation between the Physical Activity Level of the patients with the Perceived Exercise Barriers subscale (r=-0.275, p=0.003) and a weak positive correlation between the total scale score (r=0.318, p=0.001), (p<0.05). There was a weakly significant positive correlation between the Cognitive, Physical, Psychosocial sub-dimension of Fatigue and Total Impact of Fatigue with the Perceived Exercise Barriers sub-dimension (r=0.337, p=0.000; r=0.358, p=0.000; r=0.334, p=0.000; r=0.387, p=0.000) (p<0.05). A very weakly significant negative correlation was found between the Fatigue Cognitive, Physical, Psychosocial sub-dimension and Fatigue Total Impact Dimension with the total scale score (r=-0.247, p=0.008; r=-0.234, p=0.013), r=-0.222, p=0.018, r=-0.243, p=0.003) (p<0.05).

Table 4. Distribution of DPEBBS item averages

	Mean±S.D	Med (IQR)	min-max
Benefits			
Q1. Exercise helps reduce my total medical costs.	2.23±0.81	2 (2-3)	1-4
Q2. Exercise helps reduce my body pain.	2.68±0.84	3 (2-3)	1-4
Q3. Exercise can postpone a decline in body function.	2.64±0.79	3 (2-3)	1-4
Q4. Exercise prevents muscular wasting.	2.96±0.78	3 (3-3)	1-4
Q6. Exercise improves my mood.	2.71±0.72	3 (2-3)	1-4
Q7. Exercise improves bone disease.	2.78±0.73	3 (2-3)	1-4
Q10. Exercise improves my appetite.	2.77±0.87	3 (2-3)	1-4
Q13. Exercise helps me to lead an optimistic and active life.	2.79±0.56	3 (2,5-3)	1-4
Q16. Exercise improves my quality of life.	2.82±0.49	3 (3-3)	1-4
Q20. Exercise can keep my body weight at a steady level.	2.94±0.7	3 (3-3)	1-4
Q22. Exercise helps enhance my self-care abilities.	2.65±0.56	3 (2-3)	1-4
Q23. Exercise will keep me free from having other diseases (e.g., cold).	2.36±0.89	2 (2-3)	1-4
Barriers			
Q5. Frequent tiredness impedes my exercise participation.	3.08±0.92	3 (3-4)	1-4
Q8. Exercise is adverse to health of dialysis patients.	2.19±0.77	2 (2-3)	1-4
Q9. I worry about a fall during exercise.	2.58±1.09	3 (2-3)	1-4
Q11. Frequent lower-extremity muscle fatigue impedes my exercise	2.96±0.89	3 (2-4)	1-4
Q12. I lack an understanding of the benefits of exercise.	2.98±0.93	3 (2-4)	1-4
Q14. Exercise is not suitable for me since I have other medical conditions.	2.45±0.81	2 (2-3)	1-4
Q15. Body pain impedes my exercise participation.	2.85±0.84	3 (2-3)	1-4
Q17. I lack an understanding of the knowledge on how to carry out exercise.	3.28±0.85	3 (3-4)	1-4
Q18. I worry that exercise may make me feel thirsty.	2.86±1.07	3 (2-4)	1-4
Q19. Exercise is not suitable for me since I have kidney disease	2.41±0.82	2 (2-3)	1-4
Q21. I worry that exercise may affect my arteriovenous fistula.	2.98±1.03	3 (2-4)	1-4
Q24. Outdoor exercise adds burden to my family (since I need their company while I am out).	1.96±0.83	2 (1-2)	1-4

DPEBBS: Dialysis patient-perceived exercise benefits and barriers scale

Table 5. Relationship between patients' DPEBBS and IPAQ and FIS

		Exercise benefits	Exercise barriers	Total scale score
Physical activity level	r	-0.010	-0.275*	0.318*
	p	0.916	0.003	0.001
Fatigue cognitive	r	0.135	0.337*	-0.247*
	p	0.155	0.000	0.008
Fatigue physical	r	0.192	0.358*	-0.234*
	p	0.052	0.000	0.013
Fatigue psychosocial	r	0.163	0.334*	-0.222*
	p	0.085	0.000	0.018
Fatigue total impact Dimension	r	0.177	0.387*	-0.243*
	p	0.060	0.000	0.003

Fatigue Impact Scale (FIS), International Physical Activity Questionnaire Short Form (IPAQ-SF), Exercise Benefits/Barriers Scale ((DPEBBS), p<0,05

DISCUSSION

In this study, which was conducted to determine the attitudes of hemodialysis patients towards exercise and to determine the relationship between physical activity level and fatigue, it was found that the patients' perceptions of the benefits of exercise were at a moderate level according to the DPEBBS value and the patients were stated to have low levels of physical activity. A significant relationship was found between the total scale score of benefits and barriers of exercise and the dimensions of physical activity and fatigue.

Considering that the mean total score of the Exercise Benefits and Barriers Scale in this study shows that higher scores indicate more perception of exercise benefits and less perception of exercise barriers (the lowest score that can be obtained from DPEBBS is 24 and the highest score is 96), it can be said that their perception of the benefits of exercise is at a moderate level. When the relevant studies conducted in hemodialysis patients in the literature were examined, it was shown that the mean DPEBBS score was 61.76±13.97 in the study of Kılıc and Uzdil[15], while the mean DPEBBS score was 62.47±10.60 in the study of Dogru and Kasal[10] and these scores were similar to the results of our study. The results of these studies revealed that most of the patients receiving HD treatment had a positive perception of the benefits of exercise and stated that exercise could be beneficial for their health. However, in our study, the most frequently perceived exercise benefits were preventing muscle atrophy and keeping body weight at a constant level, while the most common exercise barriers were lack of understanding of how to do exercise and fatigue prevents me from participating in exercise. In Darawad and Khalil's[16] study, the most frequently perceived benefits of exercise in hemodialysis patients were prevention of muscle atrophy and improvement of mood, while fatigue and lower extremity fatigue were the most common barriers to exercise. Jayaseelan et al.[17] reported that most patients reported that exercise was positive in terms of preventing bone disease, maintaining body weight at a stable level, improving mood, quality of life and barriers to exercise such as fear of falling, family burden, vascular access. Lightfoot et al.[18] 53-76% of patients reported "lack of knowledge about how to exercise in dialysis patients" and "lack of understanding of the benefits of exercise". Although patients' knowledge of the benefits of exercise is high, their knowledge of exercise in HD is relatively poor and the results of this study provide evidence for this view. When examining HD patients' perceptions of exercise, the dialysis team should focus on the direct benefits and barriers of exercise, identify exercises appropriate for the individual, and encourage patients to exercise regularly.

Sarcopenia, muscle wasting, nutritional deficiencies, decreased exercise tolerance, significant arterial hypotension during HD sessions and fatigue after HD sessions cause individuals with ESRD to be sedentary.[19,20] In this study, 48.7% of HD patients had low physical activity levels. Musolino et al.[21] found that ESRD patients were severely sedentary and METs of these patients was 590. Observational and epidemiologic studies have reported that 45% of patients with ESRD do not exercise at all and individuals with CKD participate in physical activity 9 days per month.[22] In addition, physical inactivity has been reported as a strong predictor of mortality in hemodialysis patients.[23] A cohort study in Taiwan found that patients with CKD who switched from a highly active to a less active state were at risk for all-cause mortality, ESRD and unintentional cardiovascular events.[24] In a study by Matsuzawa et al.[23] in which physical activity was objectively assessed, it was found that every 10 minutes of daily increase in physical activity decreased the risk of death by 22% in hemodialysis patients. However, in our study, it was found that as the physical activity level of the patients increased, the perceived Exercise Barriers subscale score decreased and the total scale score increased. In another study conducted in the literature to compare the perception of exercise in HD patients with different physical activity levels, fatigue, muscle fatigue and lack of exercise knowledge were found to be common barriers and these barriers were shown to have a significant effect on the physical activity level of the patient.[25] Regolisti et al.[26] problems such as experiencing fatigue and pain on HD days, lack of motivation, and feeling helpless are reported as the main barriers perceived by patients in physical activity. In addition to lack of confidence due to lack of guidelines, the dialysis medical team does not encourage HD patients to exercise due to lack of time.[26]

Currently, the concept of physical literacy in CKD, which includes confidence, ability and motivation to engage in physical activity, has entered the literature.[27] Physical literacy involves learning in the psychomotor, cognitive and emotional domains; therefore, interventions should be supported by effective cognitive strategies to enable inactive middle-aged and older adults with CKD to learn the value of physical activity. From this perspective, patients' physical activity status should be assessed and managed as part of routine renal care.[25]

In our study, it was found that as the fatigue sub-dimensions and total scores of the patients increased, the perceived Barriers to Exercise score increased and the total scale score of Exercise Benefits/Barriers decreased. In previous studies, symptom-related fatigue and hemodialysis were found to be the most common barriers to exercise. In a literature review by Hannan

and Bronas[28], fatigue and low energy levels were the most commonly reported barriers to regular exercise in patients with CKD or receiving dialysis treatment. Kılıc and Uzdil15 reported that the majority of participants were unable to exercise due to fatigue, and other studies in the literature emphasize that fatigue is the biggest barrier to exercise.[29,30] Considering this information, if fatigue is managed effectively in HD patients, the fatigue factor that prevents exercise can be eliminated and the opportunity to exercise can be created for them.

In this study, the most frequently perceived exercise benefits were preventing muscle atrophy and maintaining body weight at a constant level, whereas the most common exercise barriers were lack of understanding of how exercise is performed and fatigue prevents me from participating in exercise. Over time, aerobic capacity and muscle strength decline in CKD. Meta-analyses have reported that aerobic exercise during HD reduces fatigue, improves resting blood pressure, urea clearance, muscle cramps and dialysis-related symptoms, and resistance exercise increases muscle strength and muscular endurance.[7,9] However, in our study, as the physical activity level of the patients increased, the perceived Exercise Barriers subscale score decreased and the total scale score increased. In addition, it was found that as the fatigue sub-dimensions and total scores of the patients increased, the perceived Exercise Barriers score increased and the Exercise Benefits/Barriers total scale score decreased. According to the findings of the study, physical activity and fatigue levels of HD patients are associated with exercise perception. For this reason, physical activity and fatigue levels of HD patients should be evaluated and individualized treatment interventions should be developed.

Finally, in this study, patients' perceived exercise benefits were found to be higher than exercise barriers. In another study, exercise was perceived positively for HD recipients in the majority of participants.[8] Although this finding is considered to be positive, it is thought that additional research is needed to determine the barriers that patients perceive in exercise and that interventions specific to each patient should be developed to overcome these barriers.

Limitations

The limitations of the study are that the study was conducted in a single center and self-report scales were used.

CONCLUSION

Our study found that HD patients had higher perceptions of the benefits of exercise and low physical activity levels. As physical activity levels increased, the perception of exercise barriers decreased and concluded that the perception of exercise barriers increased as fatigue levels increased. It is recommended that specialized physiotherapists evaluate HD groups at risk in detail and create individual interventions that support HD patients' compliance with exercise.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Sakarya University of Applied Sciences Ethics Committee (Date: 07.07.2023, Decision No: 33/4).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Effect of the COVID-19 pandemic on rotavirus infection frequency in children

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ABSTRACT

Aims: During the COVID-19 pandemic, measures such as the wearing of masks, social distancing, enhanced hygiene practices, closures of workplaces and schools, and lockdowns influenced the spread of various infectious diseases. This study aimed to compare the frequency of rotavirus infections during the pandemic to that of the pre-pandemic period.

Methods: This retrospective study included 2912 patients diagnosed with acute gastroenteritis who were admitted to the Pediatric Health and Diseases Department of Hisar Intercontinental Hospital between January 2018 and August 2022. For the diagnosis of rotavirus infection, the Rota-Adeno Ag Rapid Test-Cassette was applied to stool samples as an immunochromatographic method. Patients were divided into two groups based on their hospital admission dates: before the COVID-19 pandemic (1 January 2018 to 10 March 2020) and during the COVID-19 pandemic (11 March 2020 to 30 August 2022).

Results: The prevalence of rotavirus infection in the entire population was 9.5% (n=277). The rate of cases of rotavirus infection was higher among patients during the COVID-19 pandemic compared to the group of patients before the COVID-19 pandemic (10.9% vs. 8.7%, p=0.050). A sharp decline in the frequency of rotavirus infection was observed at the beginning of the COVID-19 pandemic compared to the pre-COVID-19 pandemic period, followed by a sharp increase. In 2022, the frequency of rotavirus infections exceeded the pre-COVID-19 pandemic levels.

Conclusion: The provision of the rotavirus vaccine for free by health authorities, especially for at-risk infants, together with adherence to hand washing, hygiene, and sanitation rules can significantly reduce the frequency of rotavirus infections during both pandemic and non-pandemic periods.

Keywords: COVID-19, pandemic, rotavirus, viral gastroenteritis

INTRODUCTION

Acute gastroenteritis, characterized by the sudden onset of diarrhea and vomiting, remains a significant public health challenge globally, contributing to substantial morbidity and mortality, especially among children under 5 years of age.[1] Among various pathogens, the genus Rotavirus emerges as the primary cause of serious diarrhea in young children on a global scale, leading to significant morbidity and mortality. Vaccines for rotavirus have been available worldwide for more than 10 years, but these infections continue to cause over 200,000 deaths each year, predominantly in countries of lower economic status.[2]

Rotavirus transmission primarily occurs via the fecal-oral route, facilitated by the virus's high infectivity and environmental stability. The persistence of rotavirus in environments frequented by children amplifies its transmission potential, making it a formidable challenge in public health.[3] The onset of the COVID-19 pandemic brought about widespread implementation of non-pharmaceutical interventions such as social distancing, hand hygiene, and the use of masks. These measures, while primarily aimed at curbing the spread of SARS-CoV-2, also affected the transmission dynamics of other infectious agents, including rotavirus.[4-7] Limited studies have shown that the measures taken to control COVID-19 led to significant reductions in the incidence of rotavirus and other enteric viral infections[8-15], highlighting the collateral benefits of such public health interventions. However, comprehensive data on changes in the frequency of rotavirus infections before and during the COVID-19 pandemic are still lacking.

We hypothesized that the public health interventions implemented in response to the COVID-19 pandemic,

aimed at curtailing the spread of the virus, influenced the transmission dynamics of rotavirus, thereby altering its incidence rates. This study aimed to compare the frequency of rotavirus infections during the pandemic to the pre-pandemic period.

METHODS

This retrospective study was conducted with patients who were admitted to the Department of Pediatric Health and Diseases of Hisar Intercontinental Hospital between June 2018 and June 2023. The study was approved by the Hisar Intercontinental Hospital Ethics Committee (Date: 12.01.2024, Decision No: 24-01) and was carried out in accordance with the relevant ethical guidelines and the Declaration of Helsinki (2013 Brazil revision).

A total of 2912 pediatric patients who were admitted to the hospital with a diagnosis of acute gastroenteritis were included in the study. The inclusion criteria were patients being under 18 years of age and diagnosed with acute gastroenteritis. Patients over the age of 18, those with a history of rotavirus, those who had received the rotavirus vaccine, and those diagnosed with co-infections of other enteric pathogens were excluded from the study.

Stool samples were collected from patients for rotavirus infection diagnosis and sent to the hospital's microbiology laboratory. All samples were packaged and stored at -80 °C, and then thawed for processing. Stool specimens were studied using an immunochromatographic method for the presence of rotavirus (Rota-Adeno Ag Rapid Test-Cassette, CTK Biotech, USA) in line with the recommendations of the device's manufacturer. The sensitivity and specificity of this test were reported by the manufacturer as 99.9% and >97.8% for rotavirus. This immunochromatographic assay method relies on identifying rotavirus and adenovirus antigens in feces using an anti-rotavirus antibody (R test line) on the assay membrane. The antigen testing involved blending fecal samples with an extraction buffer and applying three drops (approximately 100 µL) into the kit's sample section. Following 5-15 minutes of incubation at ambient temperature, the appearance of colored lines in the R (rotavirus) and control (C) areas indicated a positive result, while the absence of a colored line in the R area was interpreted as negative.

The demographic and clinical data of the patients were collected from patient files or the hospital's electronic information system. In Turkiye, the first COVID-19 case was detected on 11 March 2020. Subsequently, strict policy measures were introduced to combat the outbreak, including compulsory wearing of masks, social isolation, suspension of air travel, curfews, a transition to online learning, the temporary shutdown of cafes and restaurants, and the cancellation of public events.[16,17] Given the assumption that these measures could influence the transmission pattern of rotavirus and lead to variations in its prevalence, patients were divided into two groups based on their hospital admission dates: before the COVID-19 pandemic (1 January 2018 to 10 March 2020) and during the COVID-19 pandemic (11 March 2020 to 30 August 2022).

Statistical Analysis

All data were analyzed with IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Numerical data determined to be normally distributed based on the results of Kolmogorov-Smirnov tests are given as mean ± standard deviation while non-normally distributed variables are given as median (minimum–maximum). For comparisons between groups, the Student t-test or Mann-Whitney U test were used in line with the normality of the considered distribution. Categorical variables are given as numbers and percentages, and inter-group comparisons were conducted with chi-square and Fisher exact tests. Significance was accepted at $p<0.05$ (*) for all statistical analyses.

RESULTS

The study population consisted of a total of 2912 pediatric patients, including 1271 girls (43.6%) and 1641 boys (56.4%). The median age of the patients was 3 years (range: 1 month to 18 years), with the majority being between the ages of 1 and 4 years (44.9%). It was determined that the majority of patients (30.6%) presented to the hospital during the spring season. The prevalence of rotavirus infection in the entire population was 9.5% (n=277) (Table 1).

The distributions of sex and age between the groups were similar. In spring, the rates of hospital admissions were higher during the COVID-19 pandemic compared to the group of patients before the COVID-19 pandemic (37.1% vs. 26.7%, $p<0.001$), while admission rates were lower in winter (14.7% vs. 31.4%, $p<0.001$). In summer, the rates of hospital admissions were found to be higher during the COVID-19 pandemic compared to patients before COVID-19 (31.8% vs. 19.5%, $p<0.001$). The rate of cases of rotavirus infection was higher in the group of patients during the COVID-19 pandemic compared to patients before COVID-19 (10.9% vs. 8.7%, $p=0.050$) (Table 1).

The fluctuations in the frequency of rotavirus infection across the years are depicted in Figure. A sharp decline in frequency was observed at the beginning of the COVID-19 pandemic compared to the pre-COVID-19 period, followed by a sharp increase. In 2022, the frequency of rotavirus infections exceeded the pre-COVID-19 pandemic levels (Figure). Prior to the COVID-19

Table 1. Demographic and clinical characteristics of patients				
Variables	**All population**	**COVID-19 pandemic**		**p**
Sex, n (%)	n=2912	Before n=1827	During n=1085	
Girl	1271 (43.6)	790 (43.2)	481 (44.3)	0.566
Boy	1641 (56.4)	1037 (56.8)	604 (55.7)	
Age, years	3 (1-7)	3 (1-7)	3 (1-6)	0.671
<1 years, n (%)	463 (15.9)	290 (15.9)	173 (15.9)	0.072
1-4 years, n (%)	1307 (44.9)	816 (44.7)	491 (45.3)	
5-9 years, n (%)	812 (27.9)	495 (27.1)	317 (29.2)	
10-14 years, n (%)	260 (8.9)	184 (10.1)	76 (7.0)	
15-18 years, n (%)	70 (2.4)	42 (2.3)	28 (2.6)	
Season of presentation, n (%)				
Summer	702 (24.1)	357 (19.5)	345 (31.8)	<0.001*
Autumn	587 (20.2)	409 (22.4)	178 (16.4)	
Winter	733 (25.2)	573 (31.4)	160 (14.7)	
Spring	890 (30.6)	488 (26.7)	402 (37.1)	
Rotavirus infection, n (%)				
No	2635 (90.5)	1668 (91.3)	967 (89.1)	0.049*
Yes	277 (9.5)	159 (8.7)	118 (10.9)	

Numerical variables were shown as mean ± standard deviation or median (IQR). Categorical variables were shown as numbers (%). * P<0.05 shows statistical significance

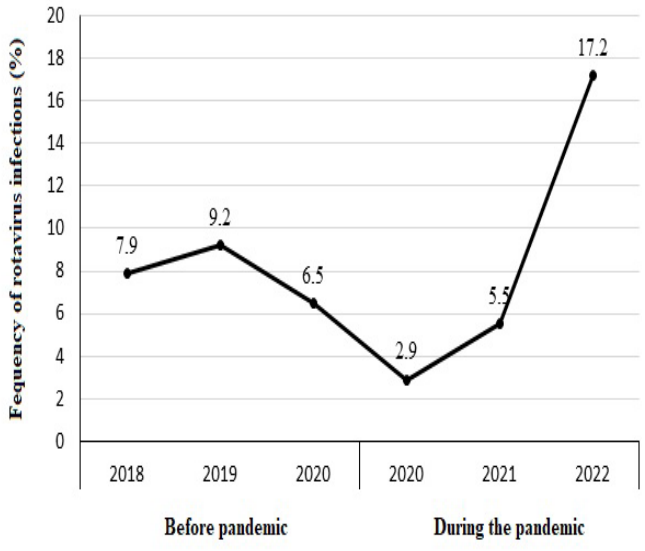

Figure. Yearly distribution of rotavirus infections in children before and during the COVID-19 pandemic

pandemic, peaks in rotavirus infection frequency were noted at the end of winter and during spring, while during the early COVID-19 pandemic period, infection rates peaked at the beginnings of summer and autumn. During the late phase of the COVID-19 pandemic, alongside pronounced surges in winter and spring, there were also notable increases at the end of summer (Table 2).

The frequency of rotavirus infections in girls did not show a significant difference before and after the beginning of the COVID-19 pandemic (9.9% vs. 10.6%, p=0.676). However, a significant increase in the frequency of rotavirus infections was detected in boys after the COVID-19 pandemic began (7.8% vs. 11.1%, p=0.025). Although an increasing trend in the frequency of rotavirus infections was observed in children over the age of 1 year after the beginning of the COVID-19 pandemic, statistical significance was only detected for children aged 1 to 4 years (Table 3).

DISCUSSION

Rotavirus is the leading cause of viral gastroenteritis among newborns and children under the age of 5, representing a major cause of mortality and morbidity within this age group.[18,19] The rates of hospitalization associated with rotavirus range from 7% to 81%, with the average rate of admission being approximately 40%.20,21 On the other hand, the hospitalization rate for diarrhea cases caused by rotavirus varies between 30% and 50%, and it is reported that mortality occurs in 90% of cases in low-income countries.[2] The primary transmission route of rotavirus infection is the fecal-oral route, but it can also spread through contact with contaminated surfaces.[22-24] Therefore, in the prevention of rotavirus, sanitation, hand hygiene, and immunization play significant roles.[25]

In our study, the median age of the patients was 3 years, with the majority of cases being seen among patients aged 1-4 years. The overall prevalence of rotavirus infection

Table 2. Monthly trends of rotavirus infections among children

Months	2018		2019		2020		2021		2022	
	Rotavirus (-)/(+), n	%	Rotavirus (-)/(+), n	%	Rotavirus (-)/(+), n	%	Rotavirus (-)/(+), n	%	Rotavirus (-)/(+), n	%
January	58/2	3.3	58/6	9.4	89/5	5.3	12/0	0	39/4	9.3
February	38/8	17.4	49/21	30.0	57/10	14.9	7/0	0	21/11	34.4
March	47/6	11.3	74/17	18.7	51/6	10.5	15/2	11.8	43/25	36.8
April	42/8	16.0	72/10	12.2	9/2	22.2	12/0	0	97/24	19.8
May	73/12	14.1	64/4	5.9	25/0	0	21/0	0	106/23	17.8
June	52/1	1.9	50/4	7.4	30/0	0	22/3	12.0	75/3	3.8
July	54/8	12.9	55/3	5.2	32/2	5.9	26/2	7.1	64/2	3.0
August	65/4	5.8	57/4	6.6	40/0	0	32/1	3.0	9/2	18.2
September	76/5	6.2	75/0	0	31/1	3.1	39/5	11.4	-	-
October	51/0	0	75/2	2.6	18/1	5.3	43/2	4.4	-	-
November	71/1	1.4	50/3	5.7	11/0	0	26/1	3.7	-	-
December	80/6	7.0	83/3	3.5	12/0	0	52/2	3.7	-	-

among these groups of patients presenting to our hospital was determined to be 9.5% (277/2912). This is similar to the rates of 8-10% reported in the literature.[2,21] Rotavirus can cause nosocomial gastroenteritis in the pediatric wards of hospitals.[26] The prevalence of acute gastroenteritis varies between 25% and 40%, often peaking during winter months.[20] Although a decrease in rotavirus infection frequency towards the summer months is reported in developed countries, seasonal trends may not be observed in developing countries.[27,28]

Rotavirus infections are frequently reported during the winter months, but in our study they were found to peak towards the end of winter. From a seasonal perspective, the rates were significantly higher in the spring months. Moreover, in our study, the rate of rotavirus infection, which was significantly elevated during the COVID-19 pandemic compared to the pre-pandemic period, also exhibited variations in spring. There are limited studies on this topic. In a study conducted in Israel, it was reported that there was an off-season increase in rotavirus cases during the second year of the pandemic.[29] A study in China documented a marked reduction in the incidence of rotavirus infections at the onset of the pandemic, with the greatest disparities noted during

Table 3. Distribution of patient with rotavirus infection by gender, age, and season

Variables	Before pandemic		During the pandemic		p
	Rotavirus (-)/(+)	%	Rotavirus (-)/(+)	%	
Sex, n (%)					
Girl	712/78	9.9	430/51	10.6	0.676
Boy	956/81	7.8	537/67	11.1	0.025*
Age, n (%)					
<1 years	257/33	11.4	162/11	6.4	0.075
1-4 years	745/71	8.7	429/62	12.6	0.023*
5-9 years	457/38	7.7	281/36	11.4	0.076
10-14 years	168/16	8.7	68/8	10.5	0.643
15-18 years	41/1	2.4	27/1	3.6	0.058
Season of infection, n (%)					
Summer	333/24	6.7	330/15	4.3	0.170
Autumn	398/11	2.7	168/10	5.6	0.079
Winter	512/61	10.6	143/17	10.6	0.994
Spring	425/63	12.9	326/73	18.9	0.014*

Numerical variables were shown as mean ± standard deviation or median (IQR). Categorical variables were shown as numbers (%). * P<0.05 shows statistical significance

February, March, and April.[11] Similar results have been observed in another study carried out in China.[30] In a study conducted in Brazil, it was reported that there was a significant increase in the frequency of rotavirus infections during the later stages of the pandemic, and it was shown to peak during the summer months.[7] In a study conducted in Turkiye, it was demonstrated that the frequency of rotavirus infections during the later stages of the pandemic was similar to the rates observed before the pandemic.[12] The possible reasons for these findings may include reduced visits to health facilities for newborn and childhood vaccinations due to the anxiety created by the pandemic in the community, inadequate follow-up of infants and mothers, and the relaxation of measures towards the end of the pandemic. It has been reported that the COVID-19 pandemic caused serious problems in accessing essential health services, such as vaccinations, due to the inadequacies of health systems in responding to the outbreak.[31] Additionally, it has been reported that the reduced circulation of microbial agents during the COVID-19 pandemic, along with disruptions in vaccine supply and administration, may have led to decreased stimulation of the immune system, resulting in adverse outcomes after the pandemic and potentially leading to future outbreaks. It has been emphasized that the implementation of an effective vaccination program is necessary to prevent the resurgence of vaccine-preventable diseases.[32]

The rotavirus vaccine has been shown to significantly reduce hospitalization and mortality rates associated with the infection and indirectly stimulate herd immunity. Therefore, it is recommended to include the rotavirus vaccine in childhood immunization programs, especially in countries with a high prevalence of infection.[24] The rotavirus vaccine is a live oral vaccine and there are two types available: one is the monovalent human rotavirus vaccine, and the other is the pentavalent human-bovine reassortant rotavirus vaccine. The most effective method for preventing highly contagious rotavirus diarrhea is rotavirus vaccination. However, these vaccines are not yet routinely administered in all countries. Since the introduction of rotavirus vaccines in 2006, there has been a significant reduction in disease burden and hospitalization rates.[33] Additionally, although hygiene practices have led to a decrease in rotavirus infection rates, it has been noted that infections can be effectively controlled with the universal rotavirus vaccine.[23] On the other hand, secretory IgA found in breast milk also plays a significant role in the immune response against rotavirus.[34]

Limitations

There are notable limitations to this study. Due to its nature as a retrospective observational study, there was no opportunity to access the long-term follow-up outcomes of the patients. Therefore, clinical data related to patients' long-term clinical courses, rates of recurrent infections, and vaccination statuses could not be obtained. Despite this, our study contributes to the literature by including a large number of patients and determining the prevalence and seasonal occurrence of rotavirus before and after the pandemic.

CONCLUSION

The provision of the rotavirus vaccine for free by health authorities, especially for at-risk infants, together with adherence to hand washing, hygiene, and sanitation rules can significantly reduce the frequency of rotavirus infections during both pandemic and non-pandemic periods. Additionally, it is necessary for doctors to advise mothers with newborn babies to breastfeed their children until at least the age of 2 and to ensure that their vaccinations against rotavirus are up to date to protect against rotavirus gastroenteritis.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Hisar Intercontinental Hospital Ethics Committee (Date: 12.01.2024, Decision No: 24-01).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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Original Article

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A comprehensive look at inflammation in RLS: assessing NLR, MLR, PLR, SII, SIRI, and microR

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ABSTRACT

Aims: Restless legs syndrome (RLS) has been linked to systemic inflammation. The number of studies investigating inflammation in RLS patients is extremely limited. The purpose of this study is to examine the possible role of proinflammatory parameters in RLS, specifically neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), systemic inflammatory response index (SIRI), and microR.

Methods: The study included 100 patients admitted to the neurology outpatient clinic diagnosed with RLS using the International Restless Legs Syndrome Study Group ((IRLSSG) scale and 100 healthy controls. Hemogram results were obtained from both RLS patients and healthy controls, while ferritin, folate, vitamin D and B12, and C-reactive protein (CRP) levels were obtained only from RLS patients.

Results: The median age of the patient group was 52.50 (43-60.75), while the median age of the healthy group was 51.00 (50-53). The patient group is 37% male, while the healthy group is 34% male. It doesn't vary by age or gender (p=0.658). The two groups showed significant differences in PLR (<0.001), MLR (0.035), microR (p=0.023), and SIRI (p=0.022). There was no statistically significant difference in NLR, SII, and macroR levels between the two groups.

Conclusion: In the current study, the inflammatory variables PLR, MLR, and microR were significantly lower, and SIRI was significantly higher from healthy control groups.

Keywords: Restless legs syndrome, NLR, PLR, MLR, SII, SIRI, microR

INTRODUCTION

Restless legs syndrome (RLS), also known as Willis-Ekbom illness, was first identified in 1945 by Dr Karl Ekbom. RLS is a sensory-motor neurological condition characterized by an impulse to move the legs, aberrant feelings in the legs, and dysaesthesia while at rest.[1] RLS affects 3-10% of the population. The etiology of RLS remained unknown until recently. Dopaminergic dysfunction is the most commonly accepted explanation for the etiology of RLS. RLS can be classified as idiopathic or secondary. Secondary RLS can have multiple reasons. The primary causes include iron deficiency, terminal renal failure, Parkinson's disease, polyneuropathy, pregnancy, and medications. Certain medications, including antiemetics, antipsychotics, antihistamines, antiepileptics, and antidepressants, can induce or aggravate RLS.[2]

Although the pathomechanism is clearly unknown, dopaminergic dysfunction, brain iron deficit, and inflammation are likely to be key contributions to the pathophysiology of idiopathic RLS. Neuroinflammation and oxidative stress have been linked to the development and progression of chronic neurodegenerative diseases.[2] RLS is related to systemic inflammation.[3] The number of studies examining inflammation and oxidative stress in RLS patients is extremely low. A study demonstrated high C-reactive protein (CRP) levels and enhanced inflammation in patients with RLS.[4] A recent study discovered a high neutrophil/lymphocyte ratio (NLR) in RLS patients compared to controls, highlighting the role of inflammation in illness pathogenesis.[5] However, to our knowledge, no study has been conducted in the literature to investigate the relationship between RLS and inflammatory parameters monocyte lymphocyte ratio (MLR), platelet lymphocyte ratio (PLR), systemic immune-inflammation index (SII), system inflammation response index (SIRI), and microR.

In this study, we investigated the potential role of inflammatory parameters NLR, MLR, PLR, SII, SIRI, and microR in RLS.

METHODS

The study was carried out with the permission of the Kastamonu University Clinical Researches Ethics Committee (Date:12.06.2023, Decision No:2023-KAEK-156). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All study participants provided informed consent forms. This study was conducted in Kastamonu Training and Research Hospital between January 2024 and February 2024. RLS was diagnosed using the International Restless Legs Syndrome Study Group (IRLSSG) questionnaire, which includes four questions: (a) Do you have an urge to move your legs accompanied by uncomfortable or disagreeable feelings? (b) Do the uncomfortable or disagreeable feelings begin or worsen during inactive periods? (c) Do the uncomfortable or disagreeable feelings decrease with activity? (d) Are the uncomfortable or disagreeable feelings more prominent in the evening or at bedtime? Patients who replied 'yes' to every question were diagnosed with RLS. Exclusion criteria included polyneuropathy, lumbosacral radiculopathy, malignancy, acute infection, severe liver or renal failure, or being younger than 18 and older than 65.

Hemogram values, ferritin, folate, vitamin D and B12, and CRP levels were measured. According to IRLSSG the severity scale consisted of ten items, each graded from 0 to 4, for a total score of 0 to 40. An IRLS score of 1 to 10 correlates to mild RLS, 11 to 20 moderate, 21 to 30 severe, and 31 to 40 very severe RLS. Patients were divided into two groups based on their scores: mild-moderate-severe disease (0-30 points) and very severe disease (31-40 points). To strengthen the statistical power of the study, we defined two categories in the IRLSSG score subgroup (mild-moderate-severe as group 1 and very severe as group 2). The NLR, MLR, PLR, SII, and SIRI were computed as follows:

NLR=Neutrophil count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

MLR=Monocyte count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

PLR=Platelet count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

SII=Platelet count ($x10^9$/L) x NLR

SIRI=Neutrophil count ($x10^9$/L) x MLR.

Statistical Analysis

Data were analyzed with IBM SPSS V23. Compliance with normal distribution was examined using Shapiro-Wilk and Kolmogorov-Smirnov tests. The chi-square test was used to compare categorical variables according to groups. Independent two-sample t-test was used to compare normally distributed data according to binary groups, and Mann-Whitney U test was used to compare non-normally distributed data. The Pearson correlation coefficient was used to analyze relationships between normally distributed data, whereas Spearman's rho correlation coefficient was used to examine relationships between non-normally distributed data. The significance level was taken as $p<0.050$.

RESULTS

One hundred patients and 100 healthy controls were included in our study. Median age (p=0.133) and gender (p=0.658) do not differ according to the groups. The average time to onset of disease symptoms in the patient group was 6.79 years. 86 (86%) of the patient group is married (Table 1).

A comparison of hemogram parameters and immune response-related markers (NLR, MLR, LMR, PLR, SII,

Table 1. Demographic characteristics of each group

	Patients	Healthy Controls	Total	Test Statistic	p
Age	51.31±11.05	50.72±0.45	51.02±7.80	U=4402.000	0.133
	52.50 (21.00-73.00)	51.00 (50.00-51.00)	51.00 (21.00-73.00)		
Gender					
Male	37 (37)	34 (34)	71 (35.5)	x^2=0.197	0.658
Female	63 (63)	66 (66)	129 (64.5)		
Year	6.79±6.11	----	6.79±6.11	----	---
	5.00 (0.00-30.00)	----	5.00 (0.00-30.00)		
Marital Status					
Married	86 (86)	----	86 (86)	----	---
Single	14 (14)	----	14 (14)		

U: Mann-Whitney U test statistic, x^2: Chi-square test statistic, frequency (percentage), mean±s. deviation, median (minimum-maximum)

and SIRI) in the patient and control groups is given in Table 2. A statistically significant difference was found between the two groups in WBC (10^3/uL) (p=0), PLT (10^3/uL) (p=0.029), PCT(%) (p=0.038), NEUT (10^3/uL) (p=0), LYMPH (10^3/uL) (p=0), MONO (10^3/uL) (p=0.001), EO (10^3/uL) (p=0.007) , BASO (10^3/uL) (p=0.007), IG (10^3/uL) distributions (p=0.038), microR (%) (p=0.023), SIRI (p=0.022) No statistically significant difference was detected in the other data stated in the table. In Table 3, patients are divided into two groups according to the UHBSSG score: mild-moderate disease (0-30 points) and severe disease (31-40 points) and compared. In addition to the data given in Table 2, serum ferritin, folate, vitamin B12, vitamin D, albumin, and CRP levels were also examined in both groups. A statistically significant difference was found between the creatine medians according to the UHBSSG score groups in both groups (p=0.029). A statistically significant difference was found between HGB (g/dL) and HCT (%) averages according to UHBSSG score groups (p=0.01, p=0.023).

DISCUSSION

In this study, PLR, MLR, and microR were significantly lower, and SIRI was significantly higher from healthy control groups. We observed a statistically significant relationship in HGB and HCT between the groupings mild-moderate-severe and very severe according to the IRLSSG rating scale. We also found a significant association between CRP and the total score of the IRLSSG rating scale.

The most common explanations of RLS etiology are dopamine dysregulation and iron deficiency.[6] Although there is various research on the subject, the association between systemic inflammation and RLS has just recently been investigated. Many inflammatory and autoimmune diseases, such as chronic liver disease, Sjogren's syndrome, rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus, inflammatory bowel disease, and multiple sclerosis, have been linked to an increased risk of developing RLS.[7,8] Additionally, patients with recurrent and severe RLS have consistently been observed to have these infectious-inflammatory conditions.[7,8] These findings improve the possibility that inflammatory factors play a role in the etiopathogenesis of RLS.

The association between NLR and several neurological disorders has been established in the literature. NLR has been demonstrated to be effective in predicting neurological disorders as well as prognosis and death following critical neurological diseases.[9,10] Two cross-sectional investigations found increased NLR in RLS than in controls.[4,11] However, Dowsett et al.[12] observed no connection between RLS and NLR in Danish blood donors after controlling for sex, age, alcohol use, smoking status, and BMI. Furthermore, Tak et al.[13] found no statistically significant link between NLR and RLS in their research of

Table 2. Comparison of parameters according to groups

	Patients		Healthy Controls			
	Means±S. Deviation	Median (min.-max.)	Mean±S. Deviation	Median (min.-max.)	Test Statistic	p
WBC(10^3/uL)	7.64±2.50	7.20 (4.67-24.91)	6.29±3.32	4.63 (2.78-12.13)	U=2419	<0.001
RBC(10^6/uL)	4.93±0.44	4.88 (4.05-6.64)	4.87±0.53	4.89 (2.99-6.42)	t=0.842	0.401
HGB(g/dL)	14.05±1.38	14.00 (9.60-17.40)	13.64±1.59	13.75 (9.10-18.60)	t=1.912	0.057
HCT(%)	42.67±3.72	42.80 (32.20-52.80)	41.65±4.29	41.85 (26.50-54.50)	t=1.727	0.086
MCV(fL)	86.62±4.33	87.00 (70.80-94.40)	85.68±4.90	85.45 (68.60-99.00)	U=3781.5	0.075
PLT(10^3/uL)	268.12±82.04	256.00 (116.00-748.00)	248.20±77.37	230.00 (37.00-499.00)	U=3632	0.029
NEUT#(10^3/uL)	4.362±1.934	3.920 (1.570-17.810)	3.579±2.209	2.520 (1.180-9.910)	U=2631	<0.001
LYMPH#(10^3/uL)	2.430±0.673	2.360 (1.200-4.950)	1.995±1.105	1.575 (0.690-6.990)	U=2486.5	<0.001
MONO#(10^3/uL)	0.592±0.233	0.550 (0.320-1.980)	0.518±0.232	0.430 (0.240-1.280)	U=3187	0.001
EO#(10^3/uL)	0.212±0.207	0.150 (0.030-1.370)	0.158±0.176	0.100 (0.000-1.240)	U=3431	0.007
BASO#(10^3/uL)	0.048±0.026	0.040 (0.010-0.140)	0.040±0.027	0.030 (0.000-0.150)	U=3447.5	0.007
MicroR(%)	2.902±3.697	1.900 (0.400-27.400)	3.502±4.194	2.500 (0.300-29.400)	U=3600	0.023
MacroR(%)	3.961±0.428	3.900 (2.900-5.600)	3.918±0.580	3.850 (3.000-7.800)	U=3975.5	0.205
NLR	1.889±0.751	1.750 (0.610-4.320)	1.901±1.055	1.680 (0.570-8.930)	U=4160	0.440
PLR	117.35±46.15	107.18 (55.14-387.56)	143.70±55.38	138.76 (30.76-305.41)	U=3035.5	<0.001
MLR	0.255±0.097	0.240 (0.120-0.600)	0.292±0.127	0.270 (0.080-0.890)	U=3658.5	0.035
SII	520.50±325.72	451.68 (97.66-2464.91)	473.15±306.87	413.63 (57.81-2446.25)	U=3891	0.136
SIRI	1.190±0.934	0.940 (0.270-7.120)	1.044±0.862	0.730 (0.260-4.600)	U=3588	0.022

t: Independent two sample t test statistic, U: Mann-Whitney U test statistic

Table 3. Comparison results according to UHBSSG score groups in the patient group

	Mild-Moderate-Severe		Very Severe			
	Mean±s. deviation	Median (min.-max.)	Mean±s. deviation	Median (min.-max.)	Test statistic	p
FERRITIN (ng/mL)	45.92±43.90	27.15 (3.70-157.40)	35.66±34.52	18.00 (2.00-109.00)	U=727	0.211
FOLATE (ng/mL)	9.364±4.529	8.000 (4.500-23.500)	9.646±4.345	9.000 (3.200-23.500)	U=801	0.553
VITAMIN B12 (pg/mL)	307.06±218.74	262.50 (0.51-1500.00)	246.32±120.46	235.00 (102.00-656.00)	U=687	0.109
VİTAMIN D (ng/dL)	20.06±17.36	15.00 (3.00-116.00)	17.63±9.47	15.00 (9.00-52.00)	U=705.5	0.813
CRP (mg/L)	5.287±8.582	2.325 (0.150-53.000)	5.799±5.710	3.230 (0.430-22.000)	U=611.5	0.160
WBC(10^3/uL)	7.649±2.835	7.080 (4.670-24.910)	7.632±1.756	7.680 (4.780-12.380)	U=814	0.464
RBC(10^6/uL)	4.986±0.451	4.915 (4.180-6.640)	4.831±0.402	4.730 (4.050-5.480)	t=1.599	0.113
HGB(g/dL)	14.33±1.34	14.40 (10.70-17.40)	13.54±1.32	13.50 (9.60-15.60)	t=2.641	0.010
HCT(%)	43.32±3.63	43.35 (35.60-52.80)	41.45±3.62	41.10 (32.20-47.70)	t=2.321	0.023
MCV(fL)	87.00±3.67	87.25 (75.70-94.40)	85.92±5.36	86.60 (70.80-94.20)	U=788.5	0.341
PLT(10^3/uL)	266.36±93.57	248.00 (116.00-748.00)	271.42±55.59	260.00 (173.00-406.00)	U=744	0.182
NEUT#(10^3/uL)	4.362±2.208	3.830 (1.570-17.810)	4.363±1.308	4.080 (2.620-8.090)	U=822	0.507
LYMPH#(10^3/uL)	2.443±0.684	2.405 (1.280-4.950)	2.405±0.663	2.350 (1.200-3.900)	t=0.25	0.803
MONO#(10^3/uL)	0.604±0.261	0.550 (0.320-1.980)	0.568±0.170	0.550 (0.340-0.980)	U=853.5	0.695
EO#(10^3/uL)	0.191±0.133	0.140 (0.040-0.630)	0.251±0.299	0.160 (0.030-1.370)	U=889	0.931
BASO#(10^3/uL)	0.049±0.027	0.040 (0.010-0.140)	0.045±0.024	0.040 (0.010-0.120)	U=791	0.346
MicroR(%)	2.393±2.272	1.800 (0.400-13.200)	3.855±5.370	1.900 (0.500-27.400)	U=768	0.259
MacroR(%)	3.964±0.414	3.900 (3.100-5.600)	3.955±0.460	3.900 (2.900-5.100)	U=881.5	0.880
NLR	1.853±0.715	1.740 (0.610-3.600)	1.956±0.822	1.750 (0.870-4.320)	U=866.5	0.780
PLR	116.77±52.87	103.47 (55.14-387.56)	118.45±30.58	109.50 (67.26-210.32)	U=763	0.242
MLR	0.256±0.096	0.245 (0.130-0.600)	0.253±0.101	0.230 (0.120-0.530)	U=869	0.796
SII	521.41±373.68	445.04 (97.66-2464.91)	518.79±214.21	465.24 (188.33-1144.13)	U=787.5	0.337
SIRI	1.202±1.029	0.920 (0.270-7.120)	1.166±0.738	0.950 (0.360-3.230)	U=886	0.911

t: Independent two sample t test statistic, U: Mann-Whitney U test statistic

RLS patients and healthy controls. Our investigation did not reveal a significant link between NLR and RLS. We attributed this to the fact that, as the neutrophil count rises in RLS patients, the lymphocyte count also rises.

Several studies indicated a link between neurological diseases and PLR. PLR was exhibited to be useful in predicting the prognosis and mortality of neurological illnesses.[14] Furthermore, in individuals with epilepsy, PLR level has been found to be connected to seizures.[15] Ozdemir et al.[16] observed that patients with Guillain Barre syndrome (GBS) had a high PLT level, which is associated with inflammation. Consistent with the literature, we observed that PLR levels were lower in RLS patients than in healthy controls. This was explained by the patient population's notably elevated lymphocyte count.

SII has been demonstrated to be helpful in predicting prognosis and mortality in a variety of neurological illnesses. High SII was found to be substantially related to poor outcomes in stroke patients.[17] Furthermore, Liu et al.[18] found that high SII levels were related to a poor prognosis in GBS patients. Additionally, it was determined that increased SII was an independent predictor of stenosis severity in carotid artery stenosis.[19] To our knowledge, no studies have been performed within the literature to investigate the link between RLS and SII. However, our investigation showed no significant difference in SII levels between RLS patients and the healthy control group.

The association between SIRI, an inflammatory parameter, and some neurological disorders has been studied. Han et al.[20] observed that SIRI predicted worse functional outcomes in ischemic stroke patients. Furthermore, a study showed that SIRI was linked to respiratory failure in GBS patients.[21] In our study, SIRI levels were significantly higher in RLS patients than in the healthy control group.

The microR and macroR parameters are indices of red blood cells that allow for a thorough morphological evaluation of erythrocytes. MicroR represents the percentage of microcytic RBC with a volume less than 60 fL, while macroR represents the percentage of macrocytic RBC with a volume greater

than 120 fL.[22] MicroR and macroR levels have not received significant consideration in studies. To our knowledge, there are very few investigations on this topic in the literature. Çığrı et al.[23] observed microR to be linked with newborn sepsis. In the present research, the microR value was significantly lower than the healthy control groups, but there was no significant difference in the macroR value.

Limitations

However, some limitations regarding our study should be mentioned. Several variables can influence the hemogram parameters. As a result, a thorough analysis is required. Second, the sample size was relatively small. More multi-center research with more participants are needed on this topic in the future.

CONCLUSION

In the present investigation, the inflammatory variables PLR, MLR, SIRI, and microR distinguished significantly from healthy control groups, suggesting that inflammation plays a key role in RLS.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Kastamonu University Clinical Researches Ethics Committee (Date: 12.06.2023, Decision No:2023-KAEK-156).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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ChatGPT in dentomaxillofacial radiology education

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ABSTRACT

Aims: Artificial intelligence refers to the ability of computer systems or machines to perform cognitive functions and tasks that are similar to humans'. The aim of this study is to assess the knowledge and interpretative abilities of ChatGPT-versions by administering a dentomaxillofacial-radiology exam, comparing its performance with that of dentistry-students in Türkiye, and questioning the effectiveness of different languages.

Methods: It is a descriptive research comparing the data of ChatGPT versions 3.5 and 4 in both Turkish and English.

Results: Firstly 20 test-questions were evaluated. There is a significant difference($p<0.05$) between the ChatGPT answer-sheets. ChatGPT-4 in English demonstrated the highest performance. Answer-sheets of chatGPT-4 in Turkish and English demonstrated the best performance with 5 correct answers in open-ended-questions. Based on the answers of 89 students to 20 test-questions, a class-profile was created. ChatGPT answer-sheets and class-profile were analyzed($p<0.05$). Class-profile ranked first as ChatGPT-4 in English. A significant difference was found between the answer-sheets of ChatGPT and the class-profile for open-ended-questions($p<0.10$). The most successful results were obtained from ChatGPT-4 in Turkish and English, as well as the class-profile.

Conclusion: It is important to mention that ChatGPT 3.5's knowledge and perception in the field of dentomaxillofacial radiology are not sufficient, particularly for use in examinations.

Keywords: Artificial intelligence, dentistry, education, oral radiology

INTRODUCTION

Artificial intelligence (AI) refers to the ability of computer systems or machines to perform cognitive functions and tasks that are similar to those of humans. These functions include image or language recognition, learning, decision-making, problem-solving, and other processes performed by humans. AI systems utilize algorithms to execute tasks that were previously exclusive to human intelligence. As AI technology continues to evolve, its applications are becoming increasingly prevalent across various fields.[1-4]

A Large Language Model (LLM) is a type of machine learning model that employs deep-learning algorithms. They are also referred to as auto-regressive language models, and ChatGPT (Chat Generative Pre-trained Transformer) is the latest iteration within this category. Developed by OpenAI in San Francisco, California, ChatGPT utilizes the GPT architecture. It is engineered to generate responses resembling those of humans when prompted with questions or prompts, employing sophisticated algorithms to comprehend and process natural language. The conversational abilities of ChatGPT stem directly from its AI capabilities, positioning it as a chatbot facilitating advanced and natural interactions between humans and machines. ChatGPT finds application in various domains, including customer service, chatbots, text-based dialogue systems, automatic text completion tools, among others.[5-8]

Shortly after the announcement of the first version of the model, GPT-1 in 2018, GPT-2 was introduced in 2019 with a larger dataset and increased analytical power. With the release of GPT-3 in 2020, a series of models capable of understanding and generating natural language was established, making it the largest language model to date. The current default version, GPT-3.5, has incorporated

code understanding and generation capabilities in addition to its superior language comprehension abilities. As of March 14, 2023, the limited beta version of GPT-4 has been released. Currently, GPT-3.5 represents an advanced version capable of handling both text and code, with potential future enhancements in image analysis capabilities.[6,8]

Over the past decade, the clinical application of AI programs in the medical profession, including dentistry, has gained significant traction. AI finds various applications in dentistry, not only in clinical practice but also in dental education and patient management. Dental education is evolving beyond traditional clinical simulator studies to include opportunities for students to reinforce or challenge theoretical knowledge. ChatGPT, as an AI tool, offers numerous benefits for dental students in their education and learning processes. Demonstrating ChatGPT's capabilities can provide crucial information for dental students regarding their education and establish the reliability of ChatGPT for learning purposes. It is essential for dental students to be able to evaluate the accuracy of medical and dental information generated by AI and to generate reliable, verified information for patients. Therefore, it is imperative to evaluate ChatGPT's ability to provide accurate answers to dental examination questions.[1,9-12]

The objective of this study is to evaluate the knowledge and interpretative capabilities of various versions of ChatGPT by administering a dentomaxillofacial radiology examination. This assessment will involve comparing the performance of ChatGPT with that of dentistry students in Turkiye and exploring the effectiveness of different language options.

The null hypothesis of this study posits that there will be no significant difference in the exam results between ChatGPT and students who have taken the dentomaxillofacial radiology course.

METHODS

This study is a descriptive research endeavor that involves comparing data obtained from dentomaxillofacial radiology exam questions routinely administered to 4th-year dental students with responses generated by ChatGPT versions 3.5 and 4, in both Turkish and English languages. Since there were no human or animal experiments conducted, no informed consent or ethical approval was required. In April 2023, a dentomaxillofacial radiology exam was conducted for 4th-year students at the University of Health Sciences, Gülhane Faculty of Dentistry. The exam questions were presented to two distinct versions of ChatGPT, with each version supporting both Turkish and English languages. The questions were administered twice in each version and language, resulting in a total of four ChatGPT answer sheets being generated.

All exam papers were evaluated by the same assessors, Associate Professor BŞ and Assistant Professor HPÖ. Answers obtained in Turkish were manually transcribed onto copies of the official exam paper and shuffled among all exam papers to ensure objectivity in the assessment conducted by the evaluators. The Turkish and English versions of the same questions were administered twice to both ChatGPT versions 3.5 and 4, and the average of the obtained results was used for the final assessment. The study involved comparing answer sheets generated by ChatGPT with the performance of dentistry students. This comparative analysis aimed to evaluate ChatGPT's performance relative to that of dentistry students and to assess variations and consistencies within the ChatGPT-generated answers. The examination comprised 28 questions, including 20 multiple-choice questions and 8 essay and short-answer questions. The 8 essay or short-answer questions were evaluated on a scale of 5 points. According to the evaluator's assessment, a score of 2.5 or higher for each question was considered correct, while a score below 2.5 was deemed incorrect in both the evaluation of students' and ChatGPT's answer sheets.

A total of 8 answer sheets generated by ChatGPT were evaluated in terms of both multiple-choice and open-ended question types. The objective was to compare the performance success in both types of questions and examine how well ChatGPT performed in each relative to the other. The topics covered in this exam were taught to students over a period of 12 weeks, comprising 24 lecture hours between November 2022 and April 2023. The total number of participating students was 89, and there were no exclusion criteria.

The overall performance of the 89 students and ChatGPT were evaluated. All statistical analyses were conducted using IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA) and MS Excel 2007. Descriptive statistics, including the number, mean, and standard deviation, were calculated for the variables.

RESULTS

The chi-square test was used to evaluate the 20 test questions. With a p-value of 0.019 ($p<0.05$), there is a significant difference between the ChatGPT answer sheets. The exam performances were further analyzed to determine the nature of this difference (Table 1).

ChatGPT-4 in English demonstrated the highest performance by correctly answering 15 out of 20 multiple-choice questions. Similarly, the answer sheet of ChatGPT-4 in Turkish and ChatGPT-3.5 in English

Table 1. ChatGPT answer sheets' correct and incorrect answers to the 20 multiple-choice questions

Group	Incorrect answers	Correct answers	Total questions	Success ranking
ChatGPT 3.5 Turkish	10	10	20	3
ChatGPT 3.5 English	**6**	**14**	**20**	**2**
ChatGPT 4 Turkish	**6**	**14**	**20**	**2**
ChatGPT 4 English	**5**	**15**	**20**	**1**

each had 14 questions correctly answered. However, ChatGPT-3.5 in Turkish showed poorer performance, with only 10 correct answers.

There is a weak significant difference between the ChatGPT answer sheets in terms of open-ended questions, with a p-value of 0.073 (p<0.10). The answer sheets of ChatGPT-4 in both Turkish and English demonstrated the best performance with 5 correct answers each. Conversely, the poorest performance in open-ended questions was observed in ChatGPT-3.5 in Turkish, with only 2 correct answers (Table 2).

Table 2. ChatGPT answer sheets' correct and incorrect answers to the 8 open-ended questions

Group	Incorrect answers	Correct answers	Total questions	Success ranking
ChatGPT 3.5 Turkish	6	2	8	3
ChatGPT 3.5 English	4	4	8	2
ChatGPT 4 Turkish	3	5	8	1
ChatGPT 4 English	3	5	8	1

Additionally, question-based success charts for ChatGPT versions' answer sheets have been generated for both multiple-choice and open-ended questions (Figure 1 and Figure 2).

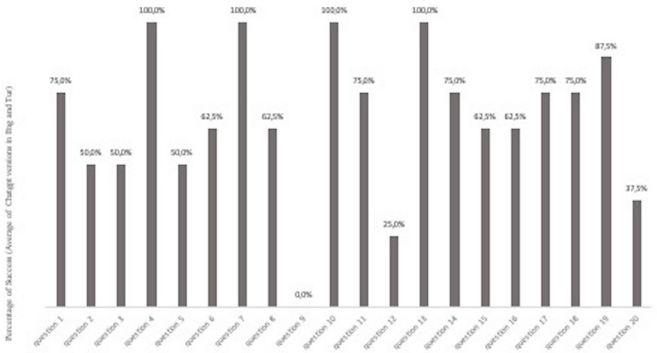

Figure 1. The correct response rates of ChatGPT versions' answer-sheets for 20 multiple-choice test questions

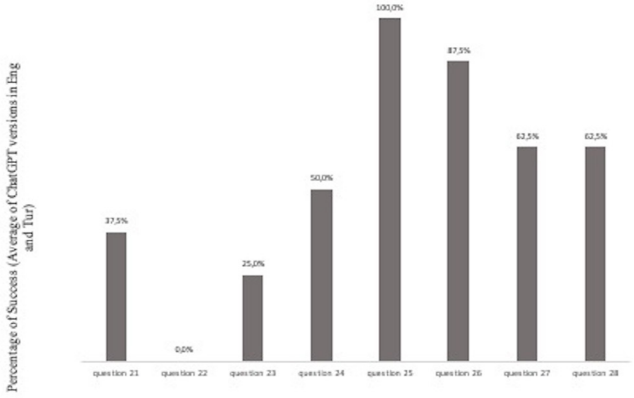

Figure 2. The correct response rates of ChatGPT versions' answer-sheets for 8 open-ended questions

Based on the answers obtained from the 89 students to the 20 test questions, a class profile was created (Table 3). The ChatGPT answer sheets and the answers of the students (class profile) for the 20 test questions were analyzed using the Kruskal-Wallis test. The p-value of 0.018 (p<0.05) indicates a significant difference. The class profile ranked first with 15 correct answers, matching the performance of ChatGPT-4 in English.

Table 3. The total scores of class-profile, ChatGPT 3.5 and 4

Answer-Sheet	Correct answers to the test-questions N/S	Correct answers to open-ended questions N/S	Totally Score S
Class-Profile(Students)	15/45	5/25	70
Chatgpt 3.5/ Turkish	10/30	2/10	40
Chatgpt 3.5/ English	14/42	4/20	62
Chatgpt 4/ Turkish	14/42	5/25	67
Chatgpt 4/ English	15/45	5/25	70

Another class profile was created for the open-ended questions of the 89 students. The class profile's question-based success chart has been developed for both multiple-choice and open-ended questions (Figure 3 and Figure 4). The Kruskal-Wallis test found a significant difference between the answer sheets of ChatGPT models and the class profile's answers for open-ended questions (p=0.076, p<0.10), indicating a weak significant difference.

Students achieved similar performance with some answer sheets of ChatGPT. The most successful results in terms of open-ended questions were obtained from ChatGPT-4 in both Turkish and English, as well as the class profile, with each correctly answering 5 out of the 8 open-ended questions.

When the answers were evaluated in total in the present study, the class profile and the English version of ChatGPT-4 achieved the highest performance, obtaining

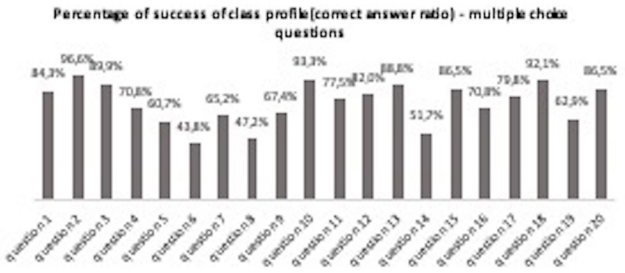

Figure 3. The correct response rates of students(class profile) for 20 multiple-choice test questions

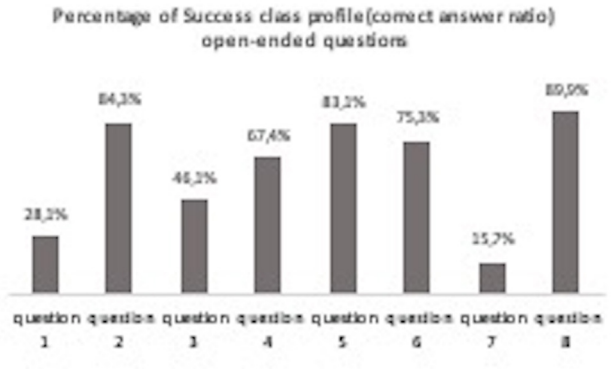

Figure 4. The correct response rates of students(class profile) for 8 open-ended questions

70 out of 100. Following closely, the Turkish version of ChatGPT-4 obtained 67 out of 100, demonstrating the second-highest performance. On the other hand, the lowest performance was observed in ChatGPT-3.5 in Turkish, obtaining 40 out of 100. The correct and incorrect answers given to both multiple-choice and open-ended questions, along with the total scores obtained, are provided in Table 3.

The performance of ChatGPT-3.5 and ChatGPT-4 in the English language remained consistent, exhibiting unchanged performance across both initial and subsequent evaluations. In open-ended questions, ChatGPT-4 demonstrated consistent results between its initial and subsequent evaluations in English, while ChatGPT-3.5 exhibited improved performance in the second evaluation compared to the first. In multiple-choice questions, the initial evaluation of ChatGPT-3.5 in Turkish displayed superior performance compared to the subsequent evaluation. However, both evaluations yielded similar results in open-ended questions. Conversely, in version-4, the second evaluation conducted in Turkish yielded improved results in both multiple-choice and open-ended questions compared to the initial evaluation. Consequently, these findings suggest that ChatGPT exhibited greater consistency in providing results in English during the Dentomaxillofacial Radiology exam.

DISCUSSION

The null hypothesis of this study, which posited that ChatGPT's exam results would outperform those of students, was partially rejected. In multiple-choice questions, the English version of ChatGPT-4 exhibited the highest performance, yet it was comparable to the performance of the student cohort. Conversely, in open-ended questions, both the English and Turkish versions of ChatGPT-4, as well as the student cohort, produced similar results.

As of January 2023, ChatGPT has amassed a user base of 100 million, solidifying its position as the AI program with the highest user ratio.[13] For researchers and practitioners to effectively utilize ChatGPT and mitigate potential issues, it is imperative to gain a comprehensive understanding of its capabilities and constraints.[7]

However, there exists a scarcity of studies that have explored ChatGPT within the realms of medical and dental fields thus far.[14]

In a similar study, medical school students' responses were compared to those generated by ChatGPT in an examination centered on medical parasitology. The performance of medical school students was found to surpass that of ChatGPT. In the current study, success was evaluated based on two question types: open-ended questions and test questions. Furthermore, two distinct versions of ChatGPT, in English and Turkish, underwent the examination twice each. Unlike Huh's study, ChatGPT-4 in English achieved the highest academic performance alongside the class-profile, both in terms of the overall exam score and the accuracy of responses to the test questions.[1]

In open-ended questions, both students and ChatGPT-4 in Turkish and English achieved comparable levels of success. However, ChatGPT-3.5 yielded varying results for Turkish and English. This discrepancy may stem from the complexity of these models, the training data utilized, and the inherent characteristics of languages. Consequently, it is believed that certain language models may generate different responses across different languages, and achieving perfect consistency in responses may not always be feasible.

The study has unveiled another noteworthy finding: the consistency between the results of the first and second evaluations of exams conducted in English by ChatGPT surpasses that of those conducted in the Turkish language. In fact, this consistency has reached 100% in version-4.

According to the evaluated versions of ChatGPT, the highest success rate in multiple-choice questions is 75%, whereas in open-ended questions, it decreases to 62.5%.

In this study, within the field of dentomaxillofacial radiology, ChatGPT has exhibited relatively superior performance in multiple-choice questions compared to open-ended questions. Nevertheless, the results from both sets of questions suggest that there are certain areas where the knowledge of ChatGPT appears to be deficient. These findings may not be universally applicable to all fields within dentistry but are specific to the domain of dentomaxillofacial radiology. However, it can be concluded that the knowledge base of ChatGPT may not be sufficiently robust for advanced levels within this particular field.

In their study, Khurana and Vaddi[15] have identified the potential applications of ChatGPT within the realm of dentomaxillofacial radiology. They highlighted several areas where ChatGPT can be effectively utilized, including; generating oral radiology reports, responding to multiple-choice questions, aiding in scientific writing, contributing to dental education by assisting in presentation creation, providing feedback on student assignments, aiding in the preparation of academic content outlines. However, they noted that ChatGPT exhibits limitations in addressing queries involving image-based questions.

The performance of ChatGPT has been assessed in a study concerning the United States Medical Licensing Examination(USMLE) Step 1, 2, and 3. The examination comprised a combination of open-ended questions with variable inputs and multiple-choice questions, specifically single-answer questions. As observed in the present study, ChatGPT exhibited its lowest performance in the Step 1 assessment, which primarily involved open-ended questions. This was followed by its performance in Step 2 and 3. Remarkably, the outcomes of the study align with those achieved by human subjects.[16] According to the results obtained from this study, it has been confirmed that ChatGPT-4 has been enhanced and improved compared to version-3.5.

In another study, Ali and his colleagues[17] utilized ChatGPT to generate clinical letters for patients. The results revealed that the correctness rates of the letters created using ChatGPT were significantly higher compared to those generated by physicians. One of the crucial aspects of studies lies in the act of writing. This is because a study can only be effectively introduced to the literature and the realm of academia through well-crafted composition. However, it is imperative to recognize that ChatGPT should not serve as the sole source. It is advisable to seek support from ChatGPT for language enhancement and rectification of certain errors.

The most significant advantage of ChatGPT can be evaluated as its ability to rapidly comprehend given information and arrive at evidence-based conclusions more swiftly than humans. Essentially, individuals can pose questions on any topic and promptly receive satisfactory answers through ChatGPT.[14,18]

CONCLUSION

Overall, in multiple-choice questions, ChatGPT-4 in English has exhibited the highest performance, mirroring that of the class profile. However, in open-ended questions, ChatGPT-4 in both English and Turkish, as well as the class profile, have demonstrated equivalent results as the highest performance. The divergent outcomes observed in ChatGPT-3.5 evaluations in Turkish and English underscore the necessity for further research into evaluation across different languages. Therefore, new studies need to be conducted in various languages and with diverse content.

As a precautionary note to dental students, it is important to mention that ChatGPT's knowledge and understanding in the field of Dentomaxillofacial Radiology are not yet sufficient, particularly for use in examinations. Nonetheless, it is rapidly evolving and improving.

ETHICAL DECLARATIONS

Ethics Committee Approval

Since there were no human or animal experiments conducted, no ethical approval required.

Informed Consent

Since there were no human or animal experiments conducted, no informed consent required.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflict of interests to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

The authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Frequency of seronegative cases in autoimmune hepatitis and their association with the systemic immune inflammation index

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ABSTRACT

Aims: It is suggested that a deficiency in B cells plays a role in pathogenesis of seronegative autoimmune hepatitis (snAIH). The lack of B cells leads to notable changes in the variety of leukocyte types within the bloodstream. This study aimed to determine the frequency of snAIH in patients with autoimmune hepatitis, as well as to explore the relationship between snAIH and leukocyte-based inflammatory indices.

Methods: In this retrospective study, 57 patients newly diagnosed with autoimmune hepatitis were included. According to clinical and pathological findings, patients were classified into seropositive autoimmune hepatitis (spAIH) and snAIH groups. The inflammation indices included the platelet to lymphocyte ratio (PLR), the neutrophil to lymphocyte ratio (NLR), and the systemic immune-inflammation index (SII).

Results: The frequency of snAIH was 26.3%. The snAIH group exhibited higher NLR (3.0 vs. 1.5, $p<0.001$) and SII (726.1 vs. 300.8, $p<0.001$) levels, along with a lower PLR level (118.2 vs. 151.1, $p=0.001$) than the spAIH group. The threshold value of SII in predicting snAIH was ≥488.4 (sensitivity=80.9%, specificity=86.7%), and it exhibited better diagnostic performance than other inflammatory indices.

Conclusion: In autoimmune hepatitis patients, snAIH exhibits by a notable prevalence and a different inflammatory landscape. For patients suspected of autoimmune hepatitis but negative for autoantibodies, the SII could serve as a straightforward, accessible, and affordable predictor prior to liver biopsy.

Keywords: Autoantibodies, autoimmune hepatitis, inflammation, liver diseases

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic condition characterized by immune-mediated destruction of hepatic cells, leading to liver inflammation and, potentially, cirrhosis and liver failure.[1] AIH is typically identified through the presence of autoantibodies and elevated immunoglobulin levels, with a definitive diagnosis confirmed through liver biopsy. However, in a subset of patients, biopsy results are positive even though serological markers are negative. This group, referred to as seronegative autoimmune hepatitis (snAIH), poses diagnostic challenges and highlights the heterogeneity of AIH.[2]

The pathogenesis of AIH involves the interaction between specific genetic characteristics and molecular mimicry in the development of the disease. This interaction includes impaired immune regulatory mechanisms played by the CD4+T cell population, Treg cells, CD8+cytotoxicity, and B cells involved in the production of autoantibodies.[3,4] This impairment of mechanisms triggers an autoimmune reaction, characterized by liver damage from interferon-γ produced by effector T cells.[5] While the precise mechanism underlying snAIH pathogenesis remains unidentified, the absence of autoantibodies could indicate a reduction or absence of B cell activity, alongside significantly preserved T cell activity.[6] Therefore, these two main lymphocyte types (B and T cells) may exhibit differences between snAIH and seropositive AIH (spAIH) groups. Additionally, platelet products induce interactions among innate or adaptive immune cells such as neutrophils, monocytes, macrophages, T cells, and B cells.[7] These findings suggest that inflammatory responses may differ between snAIH and spAIH groups.

Recent studies have emphasized the significance of extended inflammation indices, such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte

ratio (PLR), as potential markers of inflammation and prognosis in AIH.[8-11] However, we have not encountered a study evaluating the systemic immune-inflammation index (SII) in patients with AIH. Furthermore, the diagnostic performance of these inflammation indices in distinguishing between snAIH and spAIH groups has not yet been evaluated.

Given the absence and deficiency of B cells in snAIH, we hypothesized that inflammation indices might differ in snAIH compared to spAIH. This study aimed to determine the frequency of snAIH in AIH patients, as well as to explore the relationship between snAIH and inflammatory indices such as SII, NLR, and PLR.

METHODS

This retrospective study was conducted with AIH patients who admitted to the Gastroenterology Clinic of the Health Sciences University Ümraniye Training and Research Hospital between 01.01.2015 and 16.10.2023. The present study adhered to the ethical regulations and principles as stipulated in the Declaration of Helsinki. The study received approval from the Ethical Committee of Health Sciences University Ümraniye Training and Research Hospital (Date: 21.12.2023, Decision No: 565). The requirement for obtaining informed consent was exempted by the Ethics Committee, given the retrospective design of the study.

The sample size for our study was calculated based on the reported prevalence of seronegative autoimmune hepatitis (snAIH), which ranges between 7% and 36% in the literature.[6,12,13] To determine an adequate sample size for our prevalence study, we employed the following formula: $n=Z2\times P\times(1-P)/d2$. In this formula, n denotes the sample size, Z is the statistical value associated with the confidence level (Z=1.96 for a 95% confidence interval), P indicates the anticipated prevalence [(7%+36%)/2≈22%], and d reflects precision, aligning with the effect size (14%). Based on the calculation, the estimated sample size needed, with a 95% confidence level and a 14% margin of error, is approximately 34 patients.

Study Population

We retrospectively evaluated a total of 84 patients who were diagnosed with AIH for the first time. The diagnosis of AIH was based on the diagnostic criteria established by the International Autoimmune Hepatitis Group (IAIHG).[14] Patients under the age of 18, pregnant women, those with a history of AIH, those without a liver biopsy, those with any comorbid conditions such as malignancy, inflammatory diseases, heart diseases, kidney diseases or lung diseases, and those with an acute or chronic liver diseases (such as viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, and hereditary liver diseases), and those with missing clinical data were not included in the study. Subsequent to the exclusion process, 57 patients newly diagnosed with AIH were enrolled in this study.

Study Protocol

Demographic and clinical data were collected using the hospital's electronic information system and patient files. Venous blood was drawn from all patients on the same day as their liver biopsy during their initial diagnosis. According to clinical and pathological findings, patients were classified into spAIH and snAIH groups. For a spAIH diagnosis, patients were required to exhibit at least one typical non-organ-specific antibody (anti-nuclear antibody [ANA], anti-smooth muscle antibody [ASMA], and anti-liver kidney microsomal [anti-LKM] type 1 antibody), hypergammaglobulinemia, and characteristic liver histopathologic signs (interface hepatitis, predominantly lymphoplasmacytic or resetting of the liver cells), alongside the exclusion of other liver diseases. For snAIH, the diagnostic criteria include a lack of typical non-organ-specific antibody, the presence of characteristic liver histopathologic signs and successful response to immunosuppressive therapy, excluding other hepatic diseases.[2]

Blood samples were measured using Mindray MC6800 device (Mindray, Shenzhen, China) and Architect plus device (Abbot Diagnostics, Abbot Park, Illinois, USA). The electrical impedance method was employed to measure complete blood counts. The enzymatic colorimetric test was utilized to measure the concentrations of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), and albumin. Inflammation indices were calculated as follows: PLR=platelet count to lymphocyte count ratio, NLR=neutrophil count to lymphocyte count ratio, and SII=(neutrophil count×platelet count) to lymphocyte count ratio.[15]

Statistical Analysis

All data were analyzed with IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Numerical data determined to be normally distributed based on the results of Kolmogorov-Smirnov tests are given as mean±standard deviation (SD) values while non-normally distributed variables are given as median (25th-75th quartile) values. For comparisons between groups, Student T-test and Mann-Whitney U test were used in line with the normality of the considered distribution. Categorical variables are given as numbers and percentages, and inter-group comparisons were conducted with Chi-square and Fisher exact tests. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the inflammation indices to predict snAIH. Significance was accepted at $P<0.05$ (*) for all statistical analyses.

RESULTS

The mean age of 57 AIH patients included in the study was 55.4±12.5 years, the majority of them were female (71.9%). The demographic features of AIH patients were reported in Table 1. Among the patients, 56.1% tested positive for ANA, 10.5% for AMA, 28.1% for ASMA, and 5.3% for anti-LKM. Every patient's liver biopsy revealed either interface hepatitis, a mainly lymphoplasmacytic infiltrate, or rosetting of the liver cells. The frequency of snAIH was 26.3%. Demographic findings did not differ between snAIH and spAIH groups (p>0.05) (Table 1).

Table 1. The demographic features of autoimmune hepatitis patients

Variables	All population n=57	Autoimmune hepatitis		p
		Seronegative	Seropositive	
		n=15	n=42	
Age, years	55.4±12.5	53.0±9.3	53.8±12.4	0.807
Gender, n (%)				
Female	41 (71.9)	13 (86.7)	28 (66.7)	0.139
Male	16 (28.1)	2 (13.3)	14 (33.3)	
BMI, kg/m2	28.5±8.2	29.4±7.5	28.2±8.6	0.716
Smoking, n (%)	27 (47.4)	7 (46.7)	20 (47.6)	0.949
Alcohol use, n (%)	8 (14.0)	2 (13.3)	6 (14.3)	0.927
Clinical presentation, n (%)				
Jaundice	53 (93.0)	13 (86.7)	40 (95.2)	0.598
Fatigue	23 (40.4)	7 (46.7)	16 (38.1)	0.760
Pruritus	18 (31.6)	5 (33.3)	13 (30.9)	0.991
Abdominal pain	13 (22.8)	3 (20.0)	10 (28.8)	0.994

Categorical variables were shown as number percentages. Numerical variables are mean±SD or median (IQR). BMI, body mass index.

The snAIH group exhibited higher median neutrophil counts (4.6 vs. 3.0×10^3/μL, p<0.001) and median lymphocyte counts (2.3 vs. 1.8×103/μL, p=0.001) compared to the spAIH group, with no significant differences in median platelet counts (218 vs. 234×10^3/L, p=0.154) and median monocyte counts (0.6 vs. 0.5×10^3/L, p=0.350). The snAIH group exhibited higher median levels of NLR (3.0 vs. 1.5, p<0.001) and SII (726.1 vs. 300.8, p<0.001), along with a lower median PLR level (118.2 vs. 151.1, p=0.001) compared to the spAIH group (Figure 1). Additionally, the median IgG level was significantly lower in the snAIH group (1530 vs. 1890, p<0.001). Other laboratory findings did not show significant differences between the groups (Table 2).

The diagnostic performance of inflammatory indices in predicting snAIH is shown in Figure 2. The threshold

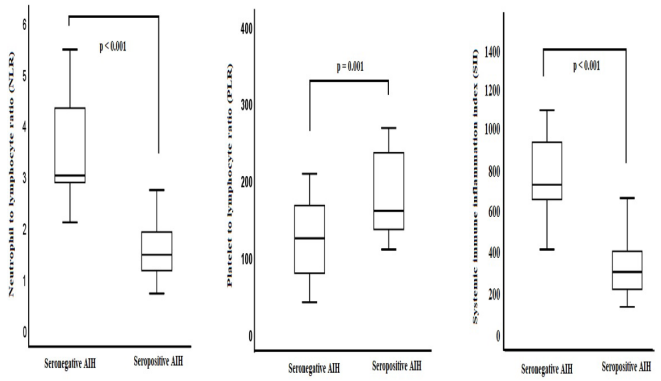

Figure 1. The distribution of immune inflammation indices among seronegative and seropositive patients with autoimmune hepatitis.

value of NLR in predicting snAIH was ≥1.9 with 71.4% sensitivity and 86.7% specificity. The threshold value of PLR in predicting snAIH was ≤100 with 73.8% sensitivity and 66.7% specificity. The threshold value of SII in predicting snAIH was ≥488.4 with 80.9% sensitivity and 86.7% specificity, and it exhibited better diagnostic performance than other inflammatory indices (Figure 2).

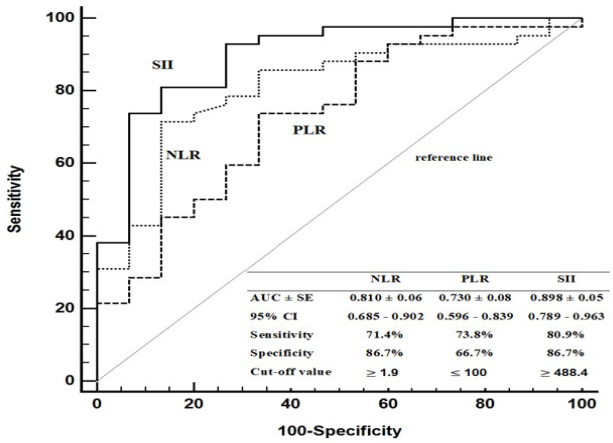

Figure 2. The diagnostic performance of immune inflammation indices in distinguishing between seronegative and seropositive autoimmune hepatitis patients

DISCUSSION

To our best knowledge, this is the first study to evaluate the diagnostic performance of the SII for distinguishing between snAIH and spAIH cases among AIH cohort. The main consequences are: 1) Higher levels of NLR and SII were observed in snAIH patients, while spAIH patients exhibited higher PLR levels. 2) In predicting snAIH patients, higher NLR levels and lower PLR levels exhibited similar sensitivity but differed in specificity. 3) SII levels demonstrated superior diagnostic performance compared to NLR and PLR in predicting snAIH patients.

Previous studies have reported that the incidence of snAIH among AIH patients varies widely, ranging from 7% to 36%.[6,12,13] In this study, the frequency of snAIH was determined to be 26.3%, consistent with the range reported in the literature. The lack of typical

Table 2. The laboratory findings of autoimmune hepatitis patients

Variables	All population n=57	Autoimmune hepatitis		p
		Seronegative	Seropositive	
		n=15	n=42	
Autoantibodies, n (%)				
ANA	32 (56.1)	-	32 (76.2)	-
AMA	6 (10.5)	-	6 (14.3)	-
ASMA	16 (28.1)	-	16 (38.1)	-
Anti-LKM	3 (5.3)	-	3 (7.1)	-
Laboratory findings				
Hemoglobin, g/dL	12.7±1.7	12.9±2.0	12.6±1.6	0.624
WBC,×103/µL	6.7±2.3	7.5±2.6	6.4±2.1	0.171
Neutrophils,×103/L	3.6 (2.7-4.6)	4.6 (3.9-5.8)	3.0 (2.6-3.8)	<0.001*
Lymphocytes,×103/µL	2.0 (1.4-2.5)	2.3 (1.1-2.0)	1.8 (1.1-2.8)	0.001*
Platelets,×103/µL	224.0 (167.5-270.4)	218.0 (145.0-276.0)	234.0 (157.0-292.0)	0.154
Monocytes,×103/µL	0.5 (0.4-0.7)	0.6 (0.4-0.8)	0.5 (0.4-0.7)	0.350
NLR	1.8 (1.2-2.7)	3.0 (2.8-4.3)	1.5 (1.2-1.9)	<0.001*
PLR	127.5 (90.3-195.4)	118.2 (86-164.3)	151.1 (130.5-233.8)	0.001*
SII	381.2 (244.1-605.3)	726.1 (620.8-949.8)	300.8 (215.8-401.4)	<0.001*
ALT, U/L	22.0 (15.0-38.0)	25.0 (17.0-38.0)	22.0 (14.2-35.5)	0.526
AST, U/L	23.0 (17.0-31.0)	23.0 (16.0-30.5)	23.5 (18.0-30.5)	0.696
ALP, U/L	85.0 (58.0-113.0)	66.0 (49.0-99.5)	85.5 (64.0-117.5)	0.205
GGT, U/L	29.0 (18.0-53.0)	31.0 (21.5-66.0)	29.0 (16.0-45.8)	0.379
IgG, mg/dL	1720 (1500-2340)	1530 (1281-2100)	1890 (1586-2615)	<0.001*
Albumin, g/dL	43.5±4.6	44.3±43.3	43.3±4.7	0.488
INR	1.1±0.2	1.0±0.1	1.1±0.2	0.339
Total bilirubin, mg/dL	0.6 (0.4-0.8)	0.4 (0.3-0.7)	0.6 (0.4-0.8)	**0.095**

Categorical variables were shown as number percentages. Numerical variables are mean±SD or median (IQR). AMA, antimitochondrial antibody; ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; anti-LKM, anti-liver kidney microsomal type 1 antibody; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; IgG, immunoglobulin G; INR, international normalized ratio; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammation index; WBC, white blood counts.

serological markers complicates the diagnosis of SnAIH, necessitating histological examination and mandating the exclusion of other liver diseases.[2] The precise mechanism underlying the pathogenesis of snAIH is still unclear. While the lack of autoantibodies constitutes the primary diagnostic distinction, this feature might not indicate a fundamental difference in pathogenesis. Instead, it could be an epiphenomenon rather than a divergence in the underlying disease mechanisms.[12] The absence of positive serology is hypothesized to be related to variations in B cell activity, as opposed to a distinct defect in generating specific autoantibodies.[6] AIH is known as a T cell-mediated disease, and these cells are associated with auto-antibody titers, immunoglobulin levels, and specific antibodies in spAIH.[16-18] It has been reported that serum IgG concentrations are significantly lower in patients with snAIH compared to those with spAIH.[19,20] This finding has also been supported in the current study. A study conducted on mice model of AIH showed that treatment with B-cell depleting antibodies (anti-CD20) reduced T-cell proliferation but did not lead to significant changes in total IgG levels or autoantibodies.[3] In AIH, B cells play a crucial role in modulating the immune response, a function they accomplish through two primary mechanisms: the secretion of cytokines and the orchestration of the recruitment of other immune cells.[21] Thus, B cells, acting as antigen-presenting cells in spAIH, might also account for the inadequate auto-antibody production in snAIH.

In a study conducted on mice with a B cell deficiency, it was reported that there was an increase in the number of neutrophils in the circulating blood.[22] Patients with snAIH exhibited higher neutrophil counts than patients with spAIH. This finding supports the suggested involvement of B cell deficiency in the pathogenic mechanism of snAIH.[6,23,24] Furthermore, an increase in lymphocyte counts was observed in snAIH patients. The decrease in peripheral neutrophil and lymphocyte counts in spAIH patients may be attributed to depletion or the migration of these cells from the bloodstream to the liver.[25] Meanwhile, patients with spAIH showed a

trend towards elevated platelet counts. Platelets, which play a significant role in the adaptive immune response, can activate peripheral blood B cells and enhance immunoglobulin production.[26] This mechanism may account for the elevated platelet and IgG levels observed in spAIH patients. On the other hand, neutrophils can influence macrophages towards an anti-inflammatory state, while platelets have the ability to modify neutrophil functions.[27] This illustrates the complex interplay within the immune system, where different cell types can significantly impact each other's roles, affecting the overall immune response and potentially the progression or resolution of inflammation.

Considering AIH is an inflammatory disease, indices derived from leukocyte subtypes might serve as better indicators for distinguishing these patients. Previous studies have reported that patients with AIH exhibit higher NLR and lower PLR levels.[8,9] However, the correlation between snAIH and these indices has not been thoroughly investigated. In this study, the observed lower NLR levels in patients with spAIH could indicate a higher migration of lymphocytes from the peripheral blood to the liver compared to neutrophils. This might also shed light on the observed higher PLR levels in these patients, in addition to the role of platelets in activating peripheral blood B cells.[26] On the other hand, the SII, which encompasses components of both NLR and PLR, could serve as a better indicator in distinguishing these patients. Patients with snAIH had higher SII levels compared to those with spAIH. Furthermore, it displayed a sensitivity of 80.9% and a specificity of 86.7% in differentiating these patient groups, outperforming both NLR and PLR in diagnostic performance. These findings are also consistent with the prognostic performance of the SII in liver diseases.[28,29] For patients suspected of AIH but negative for autoantibodies, the SII could serve as a straightforward, accessible, and affordable predictor prior to liver biopsy.

Limitations

This study had several important limitations. The main limitations were its small sample size, being conducted in a single center, and its retrospective design. Secondly, it was not possible to evaluate the extent to which inflammation indices in AIH varied in relation to healthy controls or patients with different liver conditions. This analysis might have provided further insights into the significance of these inflammation indices in AIH pathology and their efficacy in diagnosis. Finally, this study did not explore the relationship between snAIH and B cell deficiency. Hence, there is a need for more comprehensive research that includes techniques like flow cytometry to explore the impact of systemic inflammation differences in snAIH.

CONCLUSION

In patients with AIH, snAIH exhibits by a notable prevalence and a different inflammatory landscape. SnAIH exhibits different inflammatory profiles characterized by reduced PLR, as well as elevated NLR and SII. For patients suspected of AIH but negative for autoantibodies, the SII could serve as a straightforward, accessible, and affordable predictor prior to liver biopsy.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was performed in accordance with the Declaration of Helsinki, and approved by the University of Health Sciences Ümraniye Training and Research Hospital, Clinical Researches Ethics Committee (Date: 21.12.2023, Decision No: 565).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The author declared that this study has received no financial support.

Financial Disclosure

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Author Contributions

The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Review

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Meta-analytic review on the impact of factors that affect performance of malaria rapid diagnostic test in Africa

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ABSTRACT

Timely, accurate diagnosis and treatment has improved malaria case management. Malaria Rapid Diagnostic Test (mRDT) kits are largely used in malaria diagnosis. Their performance is compromised by factors related to gene deletions, parasite density, quality of the kit, poor storage conditions and end-user inefficiencies hence diagnosis gives either positive, negative, false negative (FN) or false positive (FP) which defines consequent management strategies. This review assessed reports on prevalence of the Plasmodium falciparum histidine rich protein 2/3 (Pfhrp2/3) gene deletions in malaria infected populations in Africa and the risk of mRDT failure to identify malaria positive cases. Preferred Reporting Items for Systematic Meta-Analysis (PRISMA) statement was used for data collection. Literature search was done using Google and Mendel search for data published in a malaria journal, Journal of infectious diseases, scientific reports, Annals of Ibadan postgraduate medicine, and BMC journals published between 2019 and 2023. Fifty eight reports were identified were screened and tested for eligibility. Majority of studies described the consistent use of Pfhrp2/3 mRDT for malaria diagnosis in rural health facilities in Africa and nine reports met inclusion criteria for review. Five of them certified the world health organization's sample criteria of 'more than 350 sample' to estimate the prevalence of Pfhrp2/3 gene deletions leading to declaration of false negative results of which one study posted FN outcome resulting from these deletions. Four out of nine studies did not meet this WHO criterion. This review affirmed presence of Pfhrp2/3 gene deletions challenges in Africa though other countries recorded the converse. Data was pooled using random effect models with Odds ratio and 95% confidence limit. The prevalence of the gene deletions was heterogeneous, ranging from 0% to 78.1%. The review found that an average prevalence of Pfhrp2/3 deletion as 26.2%. This was above the WHO standard recommended declaration value of 5%.; a factor that demonstrated setback to the use of mRDT in malaria endemic regions. Therefore alternative methods should be used where aspersions are cast on outcome of mRDT for it will help improve malaria treatment, tracking and management.

Keywords: Malaria diagnosis, Pfhrp2/3 gene deletions, malaria RDT

INTRODUCTION

Malaria is still a global health challenge killing thousands of people albeit governments efforts in investing heavily into its diagnosis, treatment and control.[1] This situation is worse in sub-Saharan Africa where specifically, children below five (5) years of age bear the greatest burden of morbidity and fatalities resulting from malaria infection.[2-4] To control and contain the malaria, the World Health organization has recommended the strategy of three TTTs (Testing, Treating and Tracking) of the malaria.[5-9] The TTT controls haphazard anti-malarial consumption which by extension reduces emergence of resistance to antimalarial drugs, declining malaria transmission in once considered malaria endemic areas and ultimately reduces pressure on available malaria Rapid Diagnostic Test (mRDTs) in resource constrained facilities.[10]

Initially, malaria diagnosis was done by Microscopes but lack of the required infrastructure and expertise in certain facilities has led to preferential use of malaria Mrdt.[11-13] Currently, mRDTs accounts for over 75% of malaria diagnosis in rural health facilities in Africa.[2] Facilities constrained by diagnostic infrastructure use physical examination, despite the risk of misdiagnosis, to manage malaria. This maintains a cycle of illness if not pointed out by alternative diagnosic methods and later be subjected to treatment.[1,8,10] Therefore, mRDTs offer a great potential for quick and convenient malaria diagnosis, especially in rural settings lacking alternative diagnostic methods.[7,14,15]

The mRDT detect malaria parasite antigens that are circulating in the blood stream.[10,16] The parasites' antigen

markers define the specific RDTs and hence Lactate dehydrogenase (LDH)-based and aldolase-based RDTs are said to be pan specific because they detect all human malaria species.[17] Plasmodium falciparum antigens are the most prevalent hence mRDTs specific for Pfhrp2/3 antigens are the most used in malaria diagnosis. However, the use mRDTs specific for Pfhrp2/3 antigens is sometimes challenged by occurrence of false positive or false negative results which can result to malaria misdiagnosis hence inappropriate malaria drug use or treatment failure respectively.[18]

There are many factors that influence the accurate performance of mRDT. They include Pfhrp2/3 gene deletions, mRDT sensitivity, quality of the mRDT cassettes, low parasite densities, susceptibility to the prozone effect, cross-reaction between plasmodium antigens and detection monoclonal antibodies, susceptibility to heat and humidity.[19] World health organization has called for a review and change in policy direction on malaria testing, treating and tracking in Africa.[7,8,20] Despite reports confirming that malaria transmission is declining in Africa, spatial and temporal mutability of malaria poses new challenges that impact negatively on malaria control programmes.[8,21]

Plasmodium falciparum hrp2/3 RDTs have been broadly used for diagnosis of malaria, especially in facilities lacking well established infrastructure for diagnosis. The emergene of Plasmodium falciparum mutant types has, ironically, led to mRDT negative results for malaria positive cases![4,22] This is a challenge to malaria treatment and control. The Pfhrp2/3 gene deletions protects the malaria parasite antigens causing them to evade detection by the monoclonal antibodies (Mab) of the Immunochromatographic test (ICT) resulting to false negative (FN) cases.[4,7,21,23,24] False negative patients may miss out on malaria treatment (unless confirmed otherwise). Heterogeneity in Pfhrp2/3 gene deletion prevalence could be pronounced even in different regions within the same Country. In Eritrea, North West area has a high prevalence of these deletions than the South and South west; just like the Anseba zone which also had the higher prevalence of Pfhrp2/3 deletions than the Gash Barka zones. In such like a set-up, it makes reliability of Pfhrp2/3 mRDTs not very safe.[14,25] Pfhrp2/3 antigens may also be detected in the blood circulation long after malaria parasites have been cleared off after treatment.25 The antigens will be detected by the mRDT, as if the parasites were still present. This leads to false positive (FP) malaria test interpretation and if not confirmed otherwise, may lead to consumption of anti-malarial drugs by healthy subjects.[16]

Studies have shown that, there are more Pfhrp2/3 false negative results detected at the beginning of a rainy season than during the rainy season. The above trend is reversed when the rains subside.[14,26] This is an indication that more of the expression of Pfhrp2/3 deletions are linked to low rainfall and consequently leads to low intensity of infection (Super infection).[4] Since Malaria seasonality and intensity varies widely within and between epidemiological set-ups, the choice and timing for intervention methods for malaria control should vary.[4,22] Low rainfall increases the chances of getting Pfhrp2/3 gene deleted parasites and consequently increased chance of getting false negative mRDT outcome.

The quality of the mRDT cassettes does affect their use as well since it may give inaccurate test outcome. The World Health Organisation has advised on storing RDTs kits centrally in an air-conditioned area at a temperature less than 30°C to maintain its quality, an assurance to its accuracy.[4,14] Care should also be taken to minimise the kits degradation, especially during transportation.[27] Temperatures that exceed the recommended levels are bound to compromise the quality of the test results as well.[4] The choice of RDTs should depend on the expected field conditions and end-users should be ready to control conditions in the test kit supply chain especially in the tropical and sub-tropical climates.[14,28-32] RDT Kit labels and any instructional material packed should be in tandem with the internationally accepted standard operating procedure otherwise it can compromise the mRDT accurate outcome thus slowing down malaria control in Africa.[33]

Malaria parasite transmission intensity affects the performance of mRDT. During malaria surveillance by mRDT, P. falciparum antigens are detected and isolated for malaria treatment.[34] However, there are false negative (FN) cases that are found during the use of mRDT especially when the parasites are in very low densities (<100 asexual parasites/µl or <0.002% of red blood cells infected). Some FN cases can also be detected at a very high parastaemia (hyperparastaemia) caused by the prozone effect. Hyperparastaemia is defined by the WHO as infections of >5% of red blood cells.[33] The prozone effect (sometimes referred to as high doses-hook phenomenon) is caused by a higher concentration of antigens than antibodies or the converse.[16,33,35] In this respect, high concentration of Pfhrp2/3 antigens causes exhaustion of the antibodies hence they fail to reach their expected reaction point on the mRDT consequently there will be no signal hence interpreted as a negative malaria status.[33,35-37]

The inconsistencies in mRDT outcomes caused by various factors including Pfhrp2/3 deletions, malaria parasite transmission intensity, parasite densities, host gender dynamics and seasonality have challenged malaria management strategies and consequently have

shown a knowledge gap. Review of the factors that influence performance of mRDT in Africa has not been done and hence its datais lacking. This, therefore, calls for more studies on the above factors to help realign data generated for a wider understanding and provide better malaria management strategies. This study has, therefore done a meta-analytic review of the effect of Pfhrp2/3 gene deletions to the performance of mRDT in Africa.

METHODOLOGY

Selection criteria and Search Strategy

We used Preferred Reporting Items for Systematic Meta-Analysis (PRISMA) statement to collect data from the following screened journals/sources: Malaria Journals, Parasite and Vectors, Trends in Parasitology, Malaria Microbiology and International Journals of Infectious diseases. Other journals were from Clinical Microbiology, BMC Public Health, American Journal of tropical diseases and BMJ Global Health. The five search words used for the above data extraction were Malaria RDTs, parastaemia, Pfhrp2/3 deletions, malaria seasonality and false negatives. We searched using titles, abstracts and full papers to extract the information. Only three categories of the sources fitted our prescription and they are 3 Malaria journals, a BMC public health journal and a Journal of infectious diseases. This study was approved by National Commission for Science, Technology&Innovation (NACOSTI), Kenya and the Maseno University Scientific and Ethical Review committee.

All the studies reviewed were cross-sectional surveys which aimed at assessing the prevalence of the Pfhr2/3 gene deletions and their effect to FN malaria test outcome as shown in Figure 1. These Studies were selected based on originality of the work, availability of primary data, study done in Africa and reported in 2019 or later. These studies assessed the accuracy of the mRDTs in the peripheral blood as referenced to microscopy (gold standard) and the polymerase chain reaction 'the molecular aspect' of malaria diagnosis. Participants enrolled into these studies were of all ages, infected with malaria, had no co-infections and were residents in Africa.

Extraction of data and Its Analysis

Variables under review were; authors, Country/Area/site, study design, Sample population, age, malaria diagnostic method, Phrp2/3 deletions prevalence, statistical tools used, malaria status, the type of publication, the type of journal published in and the year of publication. Malaria prevalence heterogeneity was established in all the studies under review. Chi-Square tests with 95% confidence interval and odds ratio (OD) were used to assess the risk of malaria false negative (FN) resulting from effect of Pfhrp2/3 gene deletions, malaria seasonality, status of the

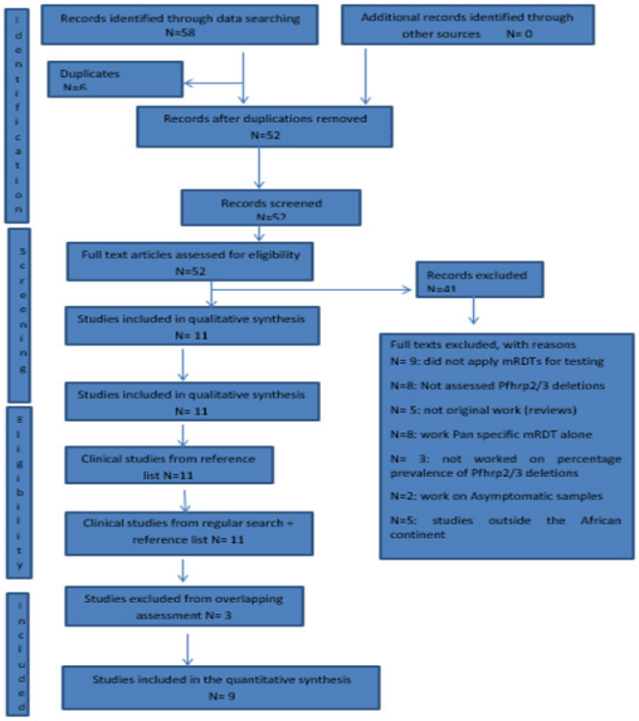

Figure 1. Analysis for inclusion of articles published for review

mRDT Kit and transmission intensity on performance of mRDT in nine (9) studies under review.

We identified Fifty eight (N=58) articles from research data with no additional article from any other source as shown in Table 1. There were six (6) duplicates from the research data which were removed from the study. Screening of the remaining fifty one (52) articles excluded forty four (41) articles from the review as they did not meet the inclusion threshold set by the team. Only eight (8) full articles were used for this review.[5,7,25,26,28,32,38,39] A total of 4482 subjects from these articles were assessed for FN status in this review study. Of the nine (9) tabulated, eight (8) studies captured the prevalence of FN cases in their study samples while one (1) article, despite assessing factors affecting mRDT performance, did not give the prevalence of FN in Nigeria.19 The Pfhrp2/3 false negative mRDT results studies in the eight countries of Africa revealed the following FN percentage prevalence: Ethiopia (78.10%), Eritria (57.90%), Tanzania (2.80%), Kenya (0.0%), Sudan (33.30%), Uganda (6.40%), DR Congo (0.27%) and Angola (0.40%) were recorded.

These studies did not lay emphasis on age as a parameter for exclusion or otherwise. Many reports on Pfhrp2/3 gene deletions in Africa were lacking and more so, those reported in the year 2019 or later were even fewer. Due to laboratory infrastructural deficiencies in malaria diagnosis (Microscopists/Microscops/PCR), the use of mRDT is so common in rural health facilities in Africa.[15,32] The thought of mRDT failure is worrying as it will wholesomely affect malaria treatment, control and

Table 1. Guiding parameters in the articles for the systemic review on impact of Pfhrp2/3 gene deletions

	Nderu et al., 2019	Michael, Olamadegun and Falade 2021	Nsobya et al., 2021	McCaffery et al., 2021	Golassa et al., 2020	Mihreteab et al. 2021	Musa et al., 2019	Thomson et al., 2019	Plucinskiet al., 2019
Design	Survey	Survey	Cross sectional survey	Cross sectional survey	Survey	Cross sectional survey	Survey	Survey	Cross sectional survey
Sample Population	400	226	1493	1109	64	715	59	176	466
Age	N/A	≤30 months	All Ages	All Ages	All Ages	All Ages	All Ages	All Ages	All Ages
Data collection site	Kenya	Nigeria	Uganda	DRC	Ethiopia	Eritrea	Sudan	Tanzania	Angola
Malaria Pfhrp2/3 confirmation method	RDT, Microscopy and PCR	RDT, Microscopy and PCR	Microscopy PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR
Statistical tools	Chi Square test %ages	SD & Geometric mean and CI	Chi Square test %ages, 95% C	Chi Square test %ages, 95% CI	Chi Square test %ages, 95% C	Chi Square test %ages, 95% CI	Chi Square test %ages, 95% C	Chi Square test %ages, 95% CI	Chi Square test %ages, 95% CI
Deletions Prevalence	1.0%	N/A	6.40%	0.27%	78.10%	57.90%	33.30%	2.80%	0.40%
Recommendations	More work on the Pfrhp2 pfhrp3 deletions	mRDT should not be used for monitoring antimalarial therapies	More work on Pfhrp2/3 gene deletions	Continuos work on pfhrp2/3 deletions encouraged	Continued surveillance in Eritrea & environs	More research on the Pfhrp2/3 deletions	More research on Pfhrp2/3 deletions	More research on Pfhrp2/3 deletions	More research on Pfhrp2/3 deletions
Publishers	Scientific Reports	Ann of Ibadan Postgraduate Medicine	Malaria Journal	Scientific Reports	Plos One	Scientific Reports	BMC Journals	Jorunal of Infectious Diseas	Jorunal of Infectious Diseas

management. Therefore, more cross-sectional surveys on impact of factors that affect malaria rapid diagnostic test performanceare is paramount to help advice governments on possibility of re-looking at the malaria testing policies and procedures of managing either malaria positive patients or negative individuals.

In Africa, the mean of the sample population from data above for Pfhrp2/3 gene deletions was 560.25 while the mean percentage prevalence Pfhrp2/2 deletion was 22.5%. as shown above in Table 2. The mean percentage prevalence is far beyond the recommended baseline of 5% (22.5%≥ 5%).33 Hence, it is advisable that for negative mRDT results, confirmatory tests should ensue to flag out FN cases. WHO recommends change in policy on Pfhrp2/3 mRDT use if deletion prevalence value is ≥5% assessed from a cross-sectional survey with a sample population of 350 or more.34 Otherwise, there should be confirmatory tests to rule out FN in Pfhrp2/3 mRDT outcome.[4,7]

The 95% confidence interval analysis and explanations: The Confidence interval (CI) refers to the range of values which we can be 95% confident includes the mean of the population from which the sample was drawn. The values were calculated at 95% and presented as shown in Table 3.

Table 2. The mean values for samples population and percentage prevalence Pfhrp2/3 gene deletions

Country	Sample population	%age Pfhrp2/3 deletions	CI
Kenya	400	1	.0037424 .0264429
Uganda	1493	6.4	.052805 .0777828
Ethiopia	64	78.1	.6601466 .8678388
Eritria	715	57.9	.5423822 .6148096
Sudan	59	33.3	.2273939 .4718864
Tanzania	176	2.8	.0117717 .0669674
DR Congo	1109	0.27	.0117717 .0669674
Angola	466	0.4	.0010682 .0170782
Mean	4482/8=560.25	180.17/8=22.5	

Analysis revealed that 1% of the population in Kenya had Pfhrp 2/3 gene deletions when tested by mRDT kit with a 95% CI (0.37-2.64), 6.4% for Uganda, 95% CI (5.28-7.78), 78.1% for Ethiopia, 95% CI (66.01-86.78), 57.9% for Eritrea, 95% CI (54.24-61.48), 33.3% for Sudan, 95%

Table 3. Confidence interval values for the prevalence of malaria across selected countries in Africa

Country	No of Cases	CI	%CI
Kenya	4	.0037424 .0264429	0.37 2.64
Uganda	96	.052805 .0777828	5.28 7.78
Ethiopia	50	.6601466 .8678388	66.01 86.78
Eritrea	414	.5423822 .6148096	54.24 61.48
Sudan	20	.2273939 .4718864	22.74 47.19
Tanzania	5	.0117717 .0669674	1.18 6.7
DR Congo	3	.0117717 .0669674	1.18 6.7
Angola	2	.0010682 .0170782	0.11 1.71

CI (22.74-47.19), 2.8% for Tanzania, 95% CI (1.18-6.7), 0.27% for DRC Congo, 95% CI (1.18-6.7) and 0.4% for Angola, 95% CI (0.11-1.71). The statistics above shows the average percentage of Pfhrp 2/3 gene deletions in Kenya sample lies between 0.37% and 2.64%. Angola had the least value of between 0.11% and 1.71 % while Ethiopia had the highest value of between 66.01% and 86.78%.

Kenya had previously reported a prevalence of 10% however in 2017, a new study which was included in this analysis (from the year 2019 and above) reported one per cent (1%) for the Pfhrp2/3 gene deletions, a concept pointed to, may be, challenges in the methodologies used for the two studies.[15,40] Uganda and Sudan reported 6.4% and 33.3% Pfhrp2/3 gene deletions respectively.[5,26] while Ethiopia and Eritrea, neighbouring countries, recorded 78.1% and 57.9% gene deletions respectively as shown in Figure 2. The two presented the highest and second highest percentage of Pfhrp2/3 gene deletions in Africa.[25,28] There are reports that the prevalence of Pfhrp2/3 deletions could even be hhigher in the North African zones.6,25 Tanzania, Angola and DR Congo had 2.8%, 0.4% and 0.27% respectively; a trend whose percentage seems to be diminishing the Pfhrp2/3 deletions Southwards of the African continent.[15,39,40]

DISCUSSION

Analysis of Demographic Health Surveys (DHS) and Malaria Indicator Surveys (MIS) has shown that the prevalence of FN-RDT is spatially heterogeneous. It occurs frequently in low malaria transmission areas and also in urban areas, areas that were initially thought to be experiencing reduced rate of malaria infection. The analysis also exposes other factors, not related directly to the parasite, which does affect mRDT performance

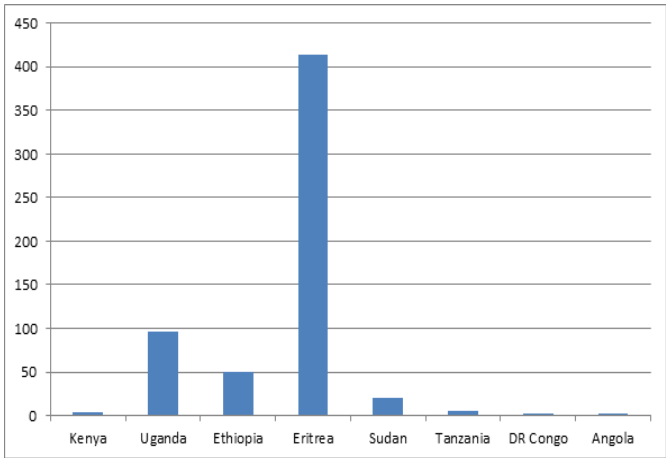

Figure 2. Prevalence of Pfhrp2/3 gene deletions

as well. False Negative-RDT results are not a preserve of mRDT product testing alone but involve other factors related to parasite density, quality of mRDT, environmental challenges up to even end-user capacity or lack of it thereof. In lieu of the above, mRDT procedure and analysis calls for accurate interpretation of the test outcome to enhance people's confidence towards their use and sustained good performance towards malaria community surveillance.

Countries that neighbour those with high percentage of Pfrp2/3 gene deletion are at risk of FN challenge-spill over. Kenya, which neighbours Ethiopia and Sudan with 78.1% and 33.3% Pfhrp2/3 gene deletions respectively may be exposed to FN challenges caused by these deletions since there is cross-border interaction's by the residents of either country.[5,28] The WHO has since then recommended routine surveillance of the Pfhrp2/3 gene deletions in such like countries to help inform national malaria diagnosis policies aimed at controlling the spread of malaria.[4,34]

In malaria endemic zones, there could be as much as FN-RDT (negative for RDT but positive for microscopy or any other molecular diagnosis) as there are FP-RDT (pfhrp2/3 antigens present well after parasite clearance after treatment) resulting from antigenaemia. The scenario above may misguide service providers to either scale down malaria treatment because of negative (FN) status or scale up treatment because of supposedly 'high malaria infections' (FP) while the converse is true. Either way, this review proposes alternative diagnostic methods to be used to confirm or disqualify results of FN or FP for proper intervention methods.[7] When analysing FN-RDT results and benchmarked with the gold standard (Microscopy) it is always assumed that the gold standard has no flaws, a concept that is challenged by presence of poorly processed blood smears, poor quality reagents, gaps in operator training and high workload for the operator to remain consistently accurate. This calls

for strict checks on standards of the whole process of microscopy to ascertain quality of test results. Molecular diagnosis (PCR) can also be used as a confirmatory test for the presence or absence of parasites.

There are three types of malaria RDT based on the antigens targeted. Both aldolase targeting and Lactate Dehydrogenase targeting mRDTs are referred to as pan specific. They are less sensitive to all malaria parasites but can be applied to diagnose all human attacking malaria parasites.[4,7,27] The Pfhrp2/3 mRDT targets the hrp2/3 antigens that are strictly generated by Plasmodium falciparum. Unlike the other two mRDT types, Pfhrp2/3 mRDTs are more sensitive, very stable and has very high specificity to Plasmodium falciparum. No wonder, Pfhrp2/3 mRDT is the method of choice in most rural endemic regions of Africa.[7] There have been recent reports that confirm that genetic variation of Plasmodium falciparum yields gene deletions in the Pfhr2/3 antigens hence failure of detection by the Pfhr2/3 based mRDT.[31] If local prevalence of Pfhrp2/3 deletions in the parasite populations is found to be 5% or more, there should be a change in policy towards mRDT use. This is recommended by WHO as it is the breakpoint at which there is high number of malaria positive cases that are missed out by mRDT jeopardising the use of mRDT in malaria endemic zones.[39]

This review found that there were Pfhrp2/3 deletions reported in most parts of the continent, especially in areas where Malaria is endemic. Eritrea is the first African country to report Pfhrp2/3 deletions and also had the highest prevalence. It had a change in policy direction by switching from the use of Phrp2/3 based RDTs to alternative pan based RDTs and microscopy as the principle mode of malaria diagnosis.[25] We isolated studies that had concentrated on malaria infected individuals who were either symptomatic or asymptomatic and tested positive for microscopy and/or PCR but testing negative for mRDT. Our findings did confirm the presence of the mRDT FN threat in Africa; with Eritria and Ethiopia having a very high prevalence of Pfrhp2/3 deletions, a trend that requires more scrutiny and characterization.[15,25] Geographically, the prevalence of these deletions tended to reduce as one moved Southwards of the above mentioned countries in Africa.[32,39,40]

From all the nine studies reviewed, there is a confirmation that the presence of Pfhrp2/3 gene deletions has been reported in a number of African Countries with a mean prevalence of 22.5%. In all the study findings, there is no proof or explanations for the genetic origin of the Pfhrp2/3 gene deletions. However, there is room for more studies and surveys to establish the probable origin and causative pressure that drives forward these deletions. Further, studies from many other countries on 'effect of these deletions' may provide an explanation on the origin, effect and mitigation steps to contain the negative effect of the deletions to malaria diagnosis and control.

There are hypotheses that have been advanced in an attempt to explain the origin and causes of these deletions. They include change in drug regiment from chloroquine and Sulfadoxine-Pyrimethamine (SP) to Artemisinin Combination Therapy, (ACT) and continuous use of Pfhrp2/3 RDT which may allow positive cases be thought to be negative (FN); which, if not flagged out by alternative diagnosis and treated, will proliferate and get spread to more populations.[22] There should be calls for more cross-sectional surveys in endemic regions where Pfhrp2/3 deletion status is unknown. There were limitations to the studies under review which included varied sampling procedures and types of surveys employed in the studies under review. This was a drawback to the conclusions anticipated that could influence tangible policy direction; however, these studies still gave cues to future studies.

This review also confirmed that there are reports on other factors that tend to compromise the accurate test results of mRDT. They were categorized as Parasite related (Parasite density and Prozone effect), Environmental and the end user challenges. Some mRDT manufacturer's may have directions that are not clear to the end user hence could fall below the expected accuracy standards. Other factors affecting mRDT performance may include poor environmental/storage conditions and end user professional capacities. Therefore, the service providers, the manufacturers, the quality assurance and standards teams should purpose to collaborate and cooperate in malaria diagnosis. Before the mRDTs are dispatched to the field, the quality assurance and standards officers should check and verify that correct instructions are included before packaging.

CONCLUSION

This review established reports on the presence of Pfhrp2/3 gene deletions in Africa though the prevalence was not homogenous. The methods used in data collection, sampling procedures, analysis and reporting were also varied which made reporting of the finding not consistent hence could not inform a change in policy direction. Very small samples used in some studies denied the studies and opportunity to give policy direction on the use of Pfhrpt2/3 mRDT. The prevalence of these gene deletions were significantly high in Ethiopia and Eritrea followed by Sudan, countries in the Northern hemisphere. Southern hemisphere countries recorded very low prevalences of these gene deletions. A Pfhrp2/3 gene deletion is not the only factor that compromises the mRDT performance. Other factors mentioned in the

studies under review were parasite density, the prozone phenomena, environmental factors and the mRDT end-user knowledge gap. All these tend to compromise the confidence and assurance in the mRDT outcome. This, therefore, calls for change of strategy in malaria diagnosis and treatment in endemic part of the continent. More funding on malaria-RDT research is paramount if the world is to eradicate malaria soon.

Recommendations

1. Microscopy testing and PCR should accompany mRDTs results reporting for proper surveillance of Pfhrp2/3 laboratory testing to ease up evolutionary pressure onto the Pfhrp2/3 gene mutations

2. Improve Monitoring, Evaluation and Reporting on the Impact of the deletions to malaria spread, surveillance and accurate geographical mapping of Pfhrp2/3 gene deleted cases in the country

3. Prevalence Pfhrp2/3 deletions of ≥5% in a particular zone should warrant change in policy direction towards the use of mRDT alone, to avoid FN missing out from on treatment.

4. There should be support in molecular research on the causes of the Pfhrp2/3 deletions and come up with mitigation measures to their influence towards mRDT FN results.

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Reviewer Evaluation Process

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The authors have no conflicts of interest to declare.

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Author Contributions

John Khamala Ongonda collected, coalesced data and drafted the manuscript under the guidance of Cyrus Ayieko from Maseno University, Kenya. Stephen Miheso (Maseno University) guided on the presentation and statistical analysis for this work.

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Letter to the Editor

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Commentary on mini dental implant insertions in children with ectodermal dysplasia

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ABSTRACT

Dental implant insertions in children raises significant concerns related to both ethical and medical aspects. On the other hand, some children suffer from several problems such as hypodontia/anodontia and malformations of teeth due to severe conditions like ectodermal dysplasia. In such cases, mini dental implants can be a promising option in enhancing the overall quality of life with positive impact on oral function. However, there are several concerns such as growth disturbances of the jaws since oral implants are considered to behave similarly to ankylosed teeth and become submerged due to the growth process associated with continued eruption of adjacent natural teeth. Currently, there is a lack of clinical research and systematic review articles in literature that evaluates and compares the outcomes, risks, and advantages of mini dental implant insertions in children with syndromes such as ectodermal dysplasia.

Keywords: Mini dental implant, ectodermal dysplasia, dental implants, growing patients

Dear Editor,

The article "Dental implants in growing patients: a systematic review" by Bohner et al.[1] presents to the readers valuable information about the protocols and clinical outcomes of dental implant placements in growing individuals. However, this report represents some limitations such as there is no evaluation on outcomes of mini dental implant (MDI) insertions in children with syndromes such as ectodermal dysplasia (ED).

The article explains the concerns about inserting implants in children and aims to search the answers for possible complications and most suitable protocols for such rehabilitations. In this review, 2133 studies were screened, and 28 studies were included. Their findings report the results of 493 dental implants placed in children between 3-18 years old with a follow up period of 1 to 20 years.[1] The most common disorders for these rehabilitations included ED and dental trauma patients. The main complications for single restorations were infra-occlusion for maxilla and rotation of the implant-supported prosthesis for mandible. The survival rate was found to be over 85%, however this finding was lower than the results reported for adult patients.[1]

Inserting implants in children raises significant concerns related to both ethical and medical aspects. Ethically, there is apprehension about the ability of children to provide informed consent, as they may lack the maturity to fully comprehend the implications of such procedures. Additionally, questions arise about the long-term impact on a child's autonomy when decisions are made for them at an early age.[2] Concerns include potential interference with normal growth and development, as well as the risk of complications that may arise as the child matures. Balancing the benefits of the implant against these potential risks is a critical aspect of ethical decision-making. Furthermore, the durability and adaptability of implants over a child's lifespan are major and most significant concerns.

On the other hand, some children suffer from several problems such as hypodontia/anodontia and malformations of teeth due to severe conditions like ED. ED is a genetic disorder affecting the development of ectodermal tissues, including teeth.[3-7] This condition often results in missing or malformed teeth, posing significant functional and aesthetic concerns.[3-5] In addition to the physical aspects, ED can have social and psychological implications.[8] Traditionally, the

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management of such children is achieved by conservative means. However, all these methods of treatment are not satisfactory and represent some concerns. MDIs play a crucial role in addressing the dental challenges faced by with ED.[3,9,10] MDIs emerge as a viable solution, offering a minimally invasive and effective means to improve oral function, facial aesthetics, and overall quality of life for these children.[3,11,12] The reduced size of MDIs minimizes the need for extensive surgical procedures, making the implantation process less invasive and more suitable for children.[11,13]

MDIs are designed to provide stability for dental prosthetics, such as dentures or crowns, offering improved masticatory function. In ED cases, where tooth development is often compromised, MDIs become essential anchors for dental restorations, facilitating proper chewing and speech development.[10,11] This not only enhances the child's nutritional intake but also contributes to their social well-being by promoting confident communication and interaction. Moreover, the placement of MDIs is generally quicker and less complex than traditional implants, making it a more manageable option for both the young patients and their caregivers. Reduced surgery time and discomfort contribute to a more positive experience for the child, fostering cooperation and compliance with necessary dental treatments.[10,11] While MDIs offer significant benefits, it is essential to consider the longevity and durability of these implants. Regular follow-up appointments with a dental professional are crucial to monitor the stability of the MDIs and address any potential complications promptly. Additionally, ongoing advancements in dental implant technology may further enhance the efficacy of MDIs, ensuring optimal outcomes for children with ED. Due to considerations for jaw development prior to termination of growth, implant placement in the anterior mandibvle at the symphyseal region should be decided with caution.

According to the results of the article by Bohner et al.[1] oral rehabilitation in children using implant-supported prosthesis is not very common all over the world, furthermore, reported follow up periods are very short to reach a conclusion. Furthermore, in this report, the results of MDI were not evaluated. There is a similar report in literature by Chrcanovic et al.[3] about dental implants in patients (including adults and growing patients) with ED that report 1472 implants (1392 conventional, 47 zygomatic, 33 MDI) with 24.6% of implants placed in children.[3] There are several concerns explained in literature such as growth disturbances of the jaws since osseointegrated implants are considered to behave similarly to ankylosed teeth and become submerged due to the growth process associated with continued eruption of adjacent natural teeth. However, some researchers report some advantages for ED patients due to physiological conservation of bone tissue, since after adolescence, severe bone resorption can be a major challenge for implant insertion in edentulous ED patient.[10] The design and type of implant system used in pediatric patients is also responsible for successful treatment outcome. Successful dental implant treatment in children can only be achieved by a multidisciplinary approach in their treatment plan.

CONCLUSION

The report by Bohner et al.[1] highlights some advantages and minor risks of dental implants in children. However, there is still a lack of clinical research and systematic review articles in literature that evaluates the outcomes of implant and MDI insertions specially in children with ED. We think that MDI is a promising option in enhancing the overall quality of life for young ED patients, due to their minimally invasive nature, improved stability, and positive impact on oral function. The status of skeletal growth, the degree of hypodontia, status of existing teeth and psychological state of each ED patient should be analyzed with caution to determine the optimal timing for implant and/or MDI insertion. Further studies should be conducted with larger sizes of samples, with longer follow up periods and with comparison of advantages, disadvantages, and complications. Currently, it is not possible to reach a recommendation on this topic since there is no clear evidence that shows MDIs can be inserted in children with ED. The objective of this work was not suggesting clinicians to insert MDIs in children with ED. Each child should be evaluated with caution as an individual case with multidisciplinary approach in their treatment plan, and possible risks and complications should be assessed with great attention. In literature more research and clinical experience are needed to reach a decision for recommendations as guidelines for insertion of MDI insertions in growing individuals with ED.

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The authors have no conflicts of interest to declare.

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Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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