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EDITORIAL

Our Dear Colleagues,

As you know, Journal of Health Sciences and Medicine (JHSM) is publish 6 times per a year. We are proud to publish this first issue of JHSM in 2023, in its 7th year. With think that great works and important achievements are only possible with teamwork, we strive to act in full teamwork with senior academicians, in addition to the typesetting, layout and design team, in order to bring the journal to better positions. The quality of the articles is increasing day by day in our journal. We want to contribute to the international literature at an increasing level and increase the success of our journal by entering valuable international indexes such as SCI-Expanded, Scopus, ESCI, Pubmed. We would like to thank all editors and authors.

We would also like to thank everyone who contributed to the journal at every stage.

Sincerely,

Prof. Aydın ÇİFCİ
Editor in Chief

CONTENTS

Original Articles

CONTENTS

Original Articles

Reviews

Case Report

Original Article

HEALTH SCIENCES
MEDICINE

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Evaluation of the knowledge, attitudes, and behaviors of school-age children on oral and dental health

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ABSTRACT

Aims: It is essential to teach children behaviors about oral and dental health in the early period. This study aimed to evaluate school-age children's knowledge, attitudes, and behaviors about oral and dental health.

Methods: The population of this research consists of 782 school-age literate individuals who applied to the Fırat University Faculty of Dentistry Department of Pedodontics. A questionnaire consisting of 20 questions was administered to the participants.

Results: 45.5% of the participants were boys, and 54.5% were girls. The mean age of the participants was 10.53±1.95. While 42.7% of the participants answered in the morning and before going to bed, 24.7% stated that they did not brush regularly. The rate of those who indicated that they touched only in the morning was 11.6%, and those who said that they brushed only in the evening was 21.6%. Also, "When do you brush your teeth?" there is a significant difference in the answers given among the participants based on their gender ($p<0.05$). The female participants answered that they brush their teeth in the morning and before bed at a higher rate than the male participants. In addition, 77.9% of the female and 70.5% of the male participants think that routine dental check-ups should be done before our teeth hurt.

Conclusion: As a result of this study, it was seen that the individual's knowledge, attitudes, and behaviors on oral and dental health were similar to other studies in the literature. We think the behaviors taught to school-age children about oral and dental health can be effective throughout an individual's life.

Keywords: Oral and dental health, school-age child, knowledge and behavior

INTRODUCTION

While oral and dental health constitutes an important part of general health, it also plays an important role in maintaining general health. Problems related to oral and dental health are among the common public health problems globally. These problems can affect individuals not only physically but also spiritually and socially, due to pain and school absences due to pain, learning difficulties, discomfort and negative effects on aesthetic appearance. A healthy mouth enables the individual to speak, eat and socialize without experiencing illness, discomfort or embarrassment. In addition, poor oral and dental health affects the growth and development of the child. For this reason, problems related to oral and dental health negatively affect the quality of life of individuals.[1-5]

In developing countries, including ours, problems related to oral and dental health bring economic and social issues.[6] Considering the effects on public health, studies on oral and dental health should be intensified from early life. It is seen that the school-age period constitutes an essential part of the studies in this field due to the population it covers and the broad age range. Many studies have been conducted on oral and dental health in school-age children in our country and the world. The nature of these studies conducted in our country is similar to those undertaken in other countries. The results of the studies on the subject will guide health professionals in terms of target setting and planning preventive interventions in the field of oral and dental health within school health services.[7,8]

In recent years, there has been a decrease in the severity and prevalence of oral diseases in developed countries. Dental care is organized systematically to improve dental health attitudes among children and young people.[9-11]

As a result of these developments, it has been observed that there is an improvement in children's dental health and the forms of dental caries that affect the teeth.[12]

In addition, gaining these habits will help the individual to continue his dental health in adulthood.[13]

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This study aims to evaluate the knowledge, attitudes, and behaviors of school-aged individuals who apply to Fırat University, Faculty of Dentistry, Department of Pedodontics, and to raise awareness of oral and dental health in children indirectly.

METHODS

The study was carried out with the permission of the Fırat University Non-invasive Research Ethics Committee (Date: 25.05.2023, Decision No: 2023/ 07-06). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In our study, a questionnaire consisting of 20 questions was applied to evaluate the knowledge, attitudes, and behaviors of 782 school-age literate individuals who used the Pedodontics Department of the Faculty of Dentistry of Fırat University. The participants, Al-Omiri et al.[14] were asked to answer all the questionnaire questions created using his work.

Analysis of Data

The data collected from 782 participants were evaluated within the scope of the research. Statistical analyzes were performed with SPSS 26.0 (SPPS Inc, Chicago, IL, USA) statistical program. Basic descriptive statistics are presented as frequency percentages. The chi-square test was used to compare the participants' knowledge, attitudes, and oral and dental health behaviors according to their gender. The statistical significance level in the study was determined as $p<0.05$.

According to the results of the power analysis performed through the G*Power (G * Power 3.1 software; Heinrich Heine University, Düsseldorf, Germany) program, In the study where x^2: goodness of fit test, contingency tables analysis will be performed, it was decided that a total of 847 samples were suitable with α (error margin) = 0.05, 0.15 effect (w) 0.95 power (1-β).

RESULTS

45.5% of the participants are boys, and 54.5% are girls. The mean age of the participants was 10.53±1.95 (Table 1).

No statistically significant difference was found in terms of the average age of the participants according to their gender ($p>0.05$). The mean age of male participants was 10.56±2.02, and that of female participants was 10.50±1.89 (Table 2).

Which oral hygiene method do you use? It was stated that they could give multiple answers to the question. While the most frequent response was a toothbrush and paste provided by 95.4% of the participants, 5.9% used mouthwash, 4.9% used toothpicks, and 2% floss. There was no significant difference in the answers among the participants based on gender ($p>0.05$). The answer given by female and male participants with the highest rate is toothbrush and paste.

Table 1. Demographic characteristics of the participants

	F	%
Gender		
Male	356	45.5
Female	426	54.5
Age		
6	2	0.3
7	23	2.9
8	68	8.7
9	174	22.3
10	196	25.1
11	80	10.2
12	97	12.4
13	67	8.6
14	50	6.4
15	25	3.2

Table 2. Age distribution of the participants by gender

	N	Ort.	S.S.	P
Age				0.686
Male	356	10.56	2.02	
Female	426	10.50	1.89	
General (Total)	782	10.53	1.95	

When do you brush your teeth? Multiple answers can be given to the question. While 42.7% of the participants answered in the morning and before going to bed, 24.7% stated that they did not brush regularly. The rate of those who indicated that they touched only in the morning was 11.6%, and those who said that they brushed only in the evening was 21.6%.

What does bleeding gums mean? It was stated that the participants could give multiple answers to the question. The participants' most common response was gum disease with 42.3%, while 40.7% indicated that they did not know. There is a significant difference in the answers given among the participants based on gender ($p<0.05$). Female participants gave a higher rate of gum disease response than male participants.

Which one protects our gum health? Participants were able to give multiple answers to the question. The most frequent response by the participants was brushing, with 62% participation; 10.2% gave soft foods, 14.5% gave vitamin C responses, and 18.3% stated that they did not know. (Table 3)

Table 3. Knowledge, attitudes, and behaviors on oral and dental health - multiple answer questions

	Answers		Percentage of people
	N	%	
Which oral hygiene method do you use?			
Toothbrush and paste	746	88.2%	95.4%
Floss	16	1.9%	2.0%
Toothpick	38	4.5%	4.9%
Mouthwashes	46	5.4%	5.9%
Total	846	100.0%	108.2%
When do you brush your teeth?			
Morning	91	11.6%	11.6%
Before bed	169	21.5%	21.6%
In the morning and before bed	334	42.4%	42.7%
I do not brush regularly	193	24.5%	24.7%
Total	787	100.0%	100.6%
What does bleeding gums mean?			
Gum disease	331	42.1%	42.3%
Healthy gums	29	3.7%	3.7%
Receding gums	109	13.9%	13.9%
I don't know	318	40.4%	40.7%
Total	787	100.0%	100.6%
Which one protects our gum health?			
Brushing	484	59.0%	62.0%
Soft foods	80	9.8%	10.2%
Vitamin C	113	13.8%	14.5%
I don't know	143	17.4%	18.3%
Total	820	100.0%	105.0%

The participants were asked how many primary teeth there are in the mouth; 14.3% answered 20, while 85.7% answered I don't know. When asked how many permanent teeth there are in the mouth, 42.5% answered 32, while 57.5% answered I don't know. While 12.3% of the participants defined dental plaque as a soft attachment on the teeth, 18.5% as a rigid attachment on the teeth, 4.2% as the coloration of the teeth, and 64.9% answered I do not know. While 66.6% of the participants stated that caries would affect the aesthetics of the teeth, 9.8% indicated that it would not, and 23.6% said that they did not know. Does frequent sugar and junk food intake affect dental health? 94% of the participants answered yes, 4% no, 2% did not know. Do carbonated drinks affect dental health? To the question, 79.2% of the participants answered yes, 11.6% answered no, 9.2% did not know. Does our oral health affect our body health? 58.1% of the participants answered yes, 21.4% no, and 20.5% did not know. Is the treatment of toothache as crucial as our other organs? To the question, 66% of the participants answered yes, 11.6% no, 22.4% did not know. Should we go to the dentist for routine check-ups before our teeth hurt? To the question, 74.6% of the participants answered yes, 12.4% no, and 13% did not know. While 95.1% of the participants replied to the suggestion that the dentist examines their patients and takes care of them, 4.5% answered no, and 0.4% did not know. Does brushing teeth prevent tooth decay? 93% of the participants answered yes to the question, and 7% answered no. Is it necessary to go to the dentist regularly? 87% of the participants answered yes to the question, and 13% answered no. Are you afraid of going to the dentist? 30.7% of the participants answered yes, and 69.3% answered no. We were wondering if you have rot in your mouth. 70.3% of the participants answered yes, and 29.7% answered no. (Table 4)

Table 4. Knowledge, attitudes, and behaviors on oral and dental health

	F	%
How many primary teeth do we have in our mouth?		
20	112	14.3
I don't know	670	85.7
How many permanent teeth do we have in our mouth?		
32	332	42.5
I don't know	450	57.5
What does dental plaque mean?		
Soft attachment on teeth	96	12.3
Hard attachment on teeth	145	18.5
Teeth discoloration	33	4.2
I don't know	508	64.9
Do caries affect our dental aesthetics?		
Yes	521	66.6
No	77	9.8
I don't know	184	23.6
Does frequent sugar and junk food intake affect our dental health?		
Yes	735	94.0
No	31	4.0
I don't know	16	2.0
Do carbonated drinks affect our dental health?		
Yes	619	79.2
No	91	11.6
I don't know	72	9.2
Does our oral health affect our body health?		
Yes	454	58.1
No	167	21.4
I don't know	161	20.5
Is the treatment of the dental network as important as our other organs?		
Yes	516	66.0
No	91	11.6
I don't know	175	22.4
Should we visit the dentist for routine check-ups before our teeth hurt?		
Yes	583	74.6
No	97	12.4
I don't know	102	13.0
Does brushing prevent tooth decay?		
Yes	727	93.0
No	55	7.0
Is it necessary to go to the dentist regularly?		
Yes	680	87.0
No	102	13.0
Are you afraid of going to the dentist?		
Yes	240	30.7
No	542	69.3
Do you wonder if he has rot in his mouth?		
Yes	550	70.3
No	232	29.7

Among the participants, "Does caries affect our dental aesthetics?" In terms of the answers given to the question, there is a significant difference based on their gender (p<0.05). 70% of the female and 62.6% of the male participants answered yes.

Among the participants, "Is the treatment of toothache as important as our other organs?" In terms of the answers given to the question, there is a significant difference based on their gender (p<0.05). 70.2% of the female and 61% of the male participants answered yes.

Among the participants, "Should we go to the dentist for routine check-ups before our teeth hurt?" Regarding the answers to the question, there was no significant difference based on their gender (p>0.05). However, 77.9% of the female and 70.5% of the male participants answered yes. The rate of responding yes to female participants is higher than that of males.

"When do you brush your teeth?" Multiple answers can be given to the question, and there is a significant difference in the answers given among the participants based on their gender (p<0.05). Female participants answered at a higher rate in the morning and at bedtime than male participants. (Table 5)

Table 5. When do you brush your teeth by gender? Comparison of the evaluation of the question

		Gender		P
		Male	Female	
When do you brush your teeth?				0,016
Morning	n %	33 9.3%	58 13.6%	
Before bed	n %	82 23.0%	87 20.4%	
In the morning and before bed	n %	136 38.2%	198 46.5%	
I do not brush regularly.	n %	105 29.5%	88 20.7%	

Among the participants, "Are you afraid of going to the dentist?" In terms of the answers given to the question, there is a significant difference based on their gender (p<0.05). 34.5% of the female and 26.1% of the male participants answered yes. The rate of those who stated they feared female participants was higher.

Among the participants, "Do you wonder if you have a bruise in your mouth?" Regarding the answers to the question, there was no significant difference based on their gender (p>0.05). However, 72.5% of the female and 67.7% of the male participants answered yes. The rate of responding yes to female participants is higher than that of males.

"Which oral hygiene method do you use?" Multiple answers can be given to the question, and there is no significant difference in the answers given among the participants based on their gender (p>0.05). The answer given by female and male participants at the highest rate is toothbrush and paste.

Among the participants, "Should we go to the dentist for routine check-ups before our teeth hurt?" Regarding the answers to the question, there was no significant difference based on their gender (p>0.05). However, 77.9% of the female and 70.5% of the male participants answered yes. The rate of responding yes to female participants is higher than that of males. (Table 6)

Table 6. According to gender, should we go to the dentist for routine check-ups before our teeth hurt? Comparison of the evaluation of the question

		Gender		Total	P
		Male	Female		
Should we visit the dentist for routine check-ups before our teeth hurt?					0.064
Yes	n %	251 70.5%	332 77.9%	583 74.6%	
No	n %	49 13.8%	48 11.3%	97 12.4%	
I don't know	n %	56 15.7%	45 10.6%	102 13.0%	
Total	n %	356 100.0%	426 100.0%	782 100.0%	

"What does bleeding gums mean?" Multiple answers can be given to the question, and there is a significant difference in the answers given among the participants based on their gender (p<0.05). Female participants gave a higher rate of gum disease response than male participants.

DISCUSSION

This study provides a comprehensive perspective on literate school-age children's oral and dental health behaviors, knowledge, and attitudes. The results of this study allow us to make comparisons with the studies carried out.[14-17]

Health and education institutions should work together to improve society's oral and dental health. Oral and dental health institutions should become more functional in providing only clinical and therapeutic services, as well as the implementation of preventive dentistry. In the 1978 Alma-Ata Declaration (Basic Health Services Declaration), which was approved by the member states of WHO, the duties of all world societies in the protection and improvement of human health and the units responsible for health and development were specified. The priority given to safety in the declaration was considered one of the most critical approaches. The ayes have it.[18] The services provided and the measures taken to protect and develop oral and dental health in our country are not at the desired level. In a study conducted on children in the 6-8 age group, it was found that body mass index decreased as the frequency of tooth decay increased in children with oral

and dental health problems.[4] This study emphasizes the importance of the subject for individuals who continue to grow and develop at school age.

Since the patients do not go to the dentist before their dental complaints begin, dentists cannot reach the group when they deem it necessary to perform preventive applications.[19] In this study, whether we should go to the dentist for routine check-ups before our teeth hurt was at least more promising, with 74.6% of the participants saying yes, 12.4% no, and 13% I don't know.

In a study conducted by the Health Project Coordinator of the Ministry of Health in 1992, it was seen that the frequency of going to the dentist in developed countries was five times a year, while it was 0.7 in Turkiye.[20]

According to the data on the official website of the Turkish Dental Association, it has been determined that 47.11% of the population in our country has never been to a dentist in the last year, and 12.5% of them have never been to a dentist in their lifetime.[21] Likewise, in the research conducted by the Turkish Dental Association, the frequency of going to the dentist in developed countries is two or more per year, while this rate is approximately once every two years in our country. In addition, although the number of toothbrushes per capita has increased in recent years, annual toothbrush consumption is still below one.[22] According to studies in Turkiye, it has been reported that most parents take their children away when they have toothache complaints.[23,24]

However, in this study, 87% of the participants answered yes, and 13% said no to whether it is necessary to visit the dentist regularly. Thus, it was seen that he was aware of this information.

It has been observed that the prevalence of caries is lower in Western European countries than in Baltic and Eastern European countries because they attach more importance to oral health and state policies. Comprehensive oral health education programs aimed at professionals and the public were directed to raise this awareness in adults and youth. In addition, the government financially supported these programs by making necessary reforms.[15,25]

Karabekiroglu et al.[26] in their study, no relationship was found between gender at the dentist visit. However, it has been observed that girls visit the dentist by percentage compared to boys. In this study, 77.9% of the female participants and 70.5% of the males answered yes that a routine dental visit is required before our teeth hurt.

This study is similar to previous studies in that individuals do not have sufficient knowledge and awareness of periodontal problems.[14,27-28] In addition to brushing, 5.9% of the participants used mouthwash, 4.9% used toothpicks, and 2% used dental floss. This low rate may be due to insufficient information or the expense of these products. While 42.3% stated that they were aware of gingival bleeding as an indicator of periodontal disease, 40.7% of the participants indicated that they did not know. The fact that this rate is lower compared to previous studies is attributed to the small average age of our participants.[14,27]

"Most participants stated they were unafraid of going to the dentist. Compared to other studies, we think that the high rate in this study may be because we applied the treatment to a population that accepted or was convinced.[14,27]

In a study conducted with primary school students in Nevşehir city center, Yaramış et al.[29] found that 30.6% brushed their teeth 1-2 times daily. In another study, after oral hygiene education was given, 36% of the students brushed their teeth twice daily and 32% once daily. He was seen brushing once.[30] In addition, in the study conducted by Doğan et al.[5] on school-age children, it was seen that socio-demographic characteristics affected both eating habits and tooth brushing habits.

In this study, 42.7% of the participants answered in the morning and before bed, while 24.7% stated that they did not brush regularly. The rate of those who said they brushed only in the morning is 11.6%, and those who said that they brushed only in the evening is 21.6%.

It was observed that the participants' perspectives on dentistry and oral health were generally positive. They also stated that oral and dental health is as important as the rest of the body and affects aesthetics. This shows that positive attitudes can help support school-age children's oral health care and oral care practices.

CONCLUSION

We think oral and dental health training to improve oral health practices in society is very important. We also suggest that this study can be applied to a larger population. In recent years, applications in oral and dental health and preventive dentistry have become widespread in our country. In parallel, we think that there is a need for comprehensive education programs to be implemented in society and that it can help evaluate the effectiveness of future public education programs.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Fırat University Non-invasive Researches Ethics Committee (Date: 25.05.2023, Decision No: 2023/ 07- 06).

Informed Consent

All patients signed the 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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Is fixation with a U-shaped staple necessary in anterior cruciate ligament reconstruction?

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ABSTRACT

Aims: This study aimed to compare the clinical and functional outcomes of patients who underwent anterior cruciate ligament reconstruction using a quadruple hamstring autograft with and without U-shaped staple fixation and tibial tunnel BioScrew fixation.

Methods: Patients who underwent arthroscopic anterior cruciate ligament (ACL) reconstruction by a single surgeon between August 2020 and June 2022 were retrospectively analyzed. The time to return to sports after surgery and the evaluation of preoperative and postoperative Lysholm Knee scores, International Knee Documentation Committee (IKDC) scores, VAS scores, and thigh diameters, were conducted. Statistical analysis of the study data was performed using SPSS 29.0 (IBM InCorp, USA).

Results: A total of 100 patients (77% male) who underwent arthroscopic ACL reconstruction were included in the analysis. There was no significant difference in Lysholm knee scores and IKDC scores between patients undergoing fixation with or without staples. However, VAS scores were significantly lower in the non staple group.

Conclusion: The present study found that fixation with a staple in addition to tibial BioScrew fixation of the autograft in the tibial tunnel resulted in more pain in the patients, and there was no significant difference in clinical and functional outcomes between the staple and non staple groups.

Keywords: Anterior cruciate ligament, U-shaped staple, pain, VAS

INTRODUCTION

The anterior cruciate ligament (ACL) is one of the crucial ligaments stabilizing the knee joint. The ACL, which is an extra-synovial ligament, is located in the intercondylar space, alongside the intra-synovial posterior cruciate ligament. ACL injury is the most common ligament injury in the knee, with a prevalence of approximately 1 in 3,000 in the general population. Approximately 70% of ACL injuries occur during sports activities. While conservative therapies and surgery have a place in treating ACL rupture, surgery is the primary option for young patients with a complete ACL tear who actively participate in sports activities. Aligning with the advancements in technology, arthroscopic surgery has become the most commonly preferred method for ACL reconstructions.[1-3]

Graft selection and graft fixation methods are among the most important factors affecting clinical and functional outcomes in anterior cruciate ligament reconstruction. When a complete soft tissue autograft such as a hamstring tendon is used, the graft fixation method gains great importance in ACL surgeries.[4,5]

In addition, the length and position of the femoral and tibial tunnels also play a crucial role in determining the outcomes of ACL reconstruction.[6] It has been demonstrated that achieving an anatomically appropriate positioning of the graft and the femoral and tibial tunnels is crucial for successful ACL reconstruction.[7] However, various modern fixation systems necessitate a minimum tunnel length to anchor the graft and facilitate its successful integration securely.[8] Studies have demonstrated that tibial fixation in ACL reconstruction involves the utilization of various methods, such as interference screws, BioScrews, fixation with washers and screws, staples, endobuttons with suspension systems, or a combination of these techniques.[9]

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This study aimed to compare the clinical and functional outcomes of patients who underwent arthroscopic single-strand anterior cruciate ligament reconstruction using a quadruple hamstring autograft and the Ziploop method with Doratek lifting system, with and without staple fixation, in addition to tibial tunnel BioScrew fixation.

METHODS

This study was approved by the Necmettin Erbakan University Non-drug and Non-medical Device Researches Ethics Committee (Date: 07.07.2023, Decision No: 2023/4427). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We conducted a retrospective review of the medical charts of patients who underwent arthroscopic ACL reconstruction using a double-layer (four-strand) hamstring tendon graft for ACL rupture between August 2020 and June 2022.

Out of 102 patients with adequate follow-up time and complete medical records, the study included 100 patients. The study excluded patients with previous ACL surgery, a history of infection in the knee joint, inflammatory or rheumatologic conditions, severe degenerative osteoarthritis, multiple ligament injuries and graft without hamstring tendons. Two patients were excluded from the study: one with concurrent posterior cruciate ligament (PCL) rupture and another with a history of peroneus longus tendon autograft harvesting.

Surgical Technique and Patient Classification

Semitendinosus and gracilis tendon autografts were harvested for ACL reconstruction, and both ends were sutured using reinforced sutures. The autografts were held taut in traction with the help of the tensioners in the system, ensuring appropriate tension. The femoral tunnels were placed in an anatomically appropriate location, while the tibial tunnels were opened using a 55-degree angled tibial guide. A 25-mm long femoral tunnel of standard size was created using femoral reamers matching the diameter of the tendon graft. All patients underwent the same method for graft fixation in the femoral tunnel, using the Ziploop technique with a lifting system. However, patients were divided into two groups based on the differences in the fixation method used in the tibial tunnel. Fifty patients who underwent fixation with a staple in addition to BioScrew fixation in the tibial tunnel were classified as Group 1, while another 50 patients who underwent only BioScrew fixation in the tibial tunnel without using staple were classified as Group 2. Any additional pathologies seen in the knee joint during knee arthroscopy were noted. Postoperative complications (wound infection, hematoma, re-rupture, etc.) were recorded.

Clinical and Radiological Evaluation

Demographic characteristics (age, gender, follow-up time, laterality and comorbidity), trauma etiology, operatione time, and length of hospital stay were evaluated. Postoperative time to return to sports, daily sports activity, and change in thigh circumference were recorded. The visual analog scale (VAS), International Knee Documentation Committee (IKDC) knee evaluation score and Lysholm scores were used to evaluate the clinical and functional outcomes of the patients preoperatively and during the last follow-up visit. Patients with persistent postoperative knee joint complaints and those with a history of trauma underwent follow-up magnetic resonance imaging (MRI) to assess ACL re-rupture, graft loosening, or other potential reasons, and the results were recorded.

Statistical Analysis

Descriptive statistics are presented as frequency (percentage) and mean ± SD or median with minimum and maximum values. The normality of numerical variables was analyzed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Nonparametric tests were employed for comparisons since it was observed that the scores did not adhere to a normal distribution. The Mann-Whitney U test was used to analyze two independent groups, and the Wilcoxon signed-rank test was used for paired comparisons. The relationships between categorical variables were analyzed using the Chi-square test with Monte Carlo correction. A p-value of less than 0.05 was considered statistically significant in the analyses.

RESULTS

This study was completed by retrospectively investigating 100 out of 102 patients who underwent ACL surgery. Of the study patients, 77% were male, and the mean age was 32.05±9.48 (median 33; 16-51) years. The primary surgical procedure was performed by a single surgeon, and all patients underwent arthroscopic ACL reconstruction using hamstring autografts. Sixty percent of the surgeries were performed on the right knee, with meniscal repair in 18 patients (18%) and microfracture in 9 patients (9%). The most common cause of trauma was football (51%), followed by falls (42%), basketball (accounting for 5%), and kickboxing and wrestling (accounting for one patient). Of the patients, 41% had an ASA score of 1 (normal), and 53% had an ASA score of 2 (mild systemic disorder). Most patients (76%) were hospitalized for three days postoperatively. As additional procedures, two patients in Group 1 underwent closed mobilization, and one patient underwent arthroscopic graft exchange, while only two patients in Group 2 underwent superficial wound debridement procedures (**Table 1**).

Table 1. Demographic and clinical characteristics of patients according to Staple Fixation

	Group 1 n (%)	Group 2 n (%)	Total n (%)	p
Sex				
Male	38 (76)	39 (78)	77	0.813
Female	12 (24)	11 (22)	23	
Trauma				
Fall	17 (34)	25 (50)	42	0.119
Basketball	2 (4)	3 (6)	5	
Football	30 (60)	21 (42)	51	
Kick-Box	1 (2)	0 (0)	1	
Wrestling	0 (0)	1 (2)	1	
ASA				
1	27 (54)	14 (28)	41	0.028*
2	20 (40)	33 (66)	53	
3	3 (6)	3 (6)	6	
Hospitalization				
2	11 (22)	9 (18)	20	0.667
3	37 (74)	39 (78)	76	
4	2 (4)	2 (4)	4	
Surgery				
Primary	49 (98)	50 (100)	99	0.317
Revision	1 (2)	0 (0)	1	
Side				
Right	31 (62)	29 (58)	60	0.685
Left	19 (38)	21 (42)	40	
Additional processing				
No	40 (80)	32 (64)	72	0.113
Meniscal Repair	7 (14)	11 (22)	18	
Microfracture	2 (4)	7 (14)	9	
Additional Surgery				
No	45 (90)	48 (96)	93	0.668
Mobilisation	2 (4)	0 (0)	2	
Arthroscopy-intact	2 (4)	0 (0)	2	
Tunnel Grafting	1 (2)	0 (0)	1	
Debridement	0 (0)	2 (4)	2	

*: Significant at 0.05 level according to Monte Carlo Exact Chi-Square test

Gender (p=0.813), type of trauma (p=0.119), length of hospital stay (p = 0.667), and distribution of comorbidities (p=0.369) were not significantly different between the groups. In contrast, the rate of patients with an ASA score of 1 (normal) was higher, and the rate of those with an ASA score of 2 (mild systemic disorder) was lower in Group 1 (p=0.028). Other clinical features did not differ significantly between the groups. The ages of the patients did not differ significantly between the groups (p=0.959). Preoperative sports duration was not different between the groups, but postoperative sports duration was significantly shorter in Group 1 (p=0.011). Both preoperative and postoperative VAS scores significantly differed between the groups (p=0.001). Both scores were higher in the staple group. The median preoperative VAS score was 8 (range 5-9) in the staple group and 7 (range 5-9) in the non staple group. The median postoperative VAS score was 3 (range 1-5) in the staple group and 2 (range 1-4) in the non staple group. The Lysholm and IKDC scores did not differ significantly between the groups. Preoperative and postoperative thigh circumference values were not significantly different between the two groups (p >0.05) (Table 2).

When the preoperative and postoperative values were compared between the groups, the duration of sports after surgery showed a significant decrease in Group 1 compared to postoperative values (p= 0.001). In Group 2, the duration of sports decreased to a lesser extent after surgery (p=0.046). Preoperative and postoperative VAS scores differed significantly (p <0.001). Postoperative thigh circumference values slightly compared to preoperative values in both groups (p <0.001) (Table 3).

Table 3. Pre-op and post-op comparisons of clinical measurements

	Pre-op Median±SS (Median; Min-Max)	Post-op Median±SS (Median; Min-Max)	p
Group 1			
Spor	0.87±0.89 (1; 0-4)	0.64±0.71(0.75; 0-3)	0.001*
VAS	7.48±0.91 (8; 5-9)	3.34±0.8 (3; 1-5)	<0.001*
Thigh	51.68±4.3 (52; 38-65)	50.6±4.4 (50.5; 36-64)	<0.001*
Group 2			
Spor	1.14±0.9 (1; 0-4)	1.06±0.89 (1; 0-4)	0.046*
VAS	7.18±1.11 (7; 5-9)	1.76±0.85 (2; 1-4)	<0.001*
Thigh	51.98±4.48 (52; 35-61)	50.86±4.65 (51; 34-60)	<0.001*

*: significant at 0.05 level according to Wilcoxon Signed Rank test

Table 2. Clinical measurements of patients according to Staple fixation

	Group 1 Median±SS (Median; Min-Max)	Group 2 Median ±SS (Median; Min-Max)	Total Median ±SS (Median; Min-Max)	p
Spor preop	0.87±0.89 (1; 0-4)	1.14±0.9 (1; 0-4)	1.01±0.9 (1; 0-4)	0.111
Spor postop	0.64±0.71(0.75; 0-3)	1.06±0.89 (1; 0-4)	0.85±0.83 (1; 0-4)	0.011*
Return to spor	4.42±4.31 (6; 0-12)	5.14±3.43 (6; 0-10)	4.78±3.89 (6; 0-12)	0.713
VAS preop	7.48±0.91 (8; 5-9)	7.18±1.11 (7; 5-9)	7.33±1.1 (8; 5-9)	0.140
VAS postop	3.34±0.8 (3; 1-5)	1.76±0.85 (2; 1-4)	2.55±1.14 (3; 1-5)	<0.001*
Lysholm	81.84±10.3 (84; 35-100)	84.94±8.25 (86; 65-100)	83.39±9.42 (85; 35-100)	0.194
IKDC	75.5±9.45 (77.5; 33-90)	78.68±7.68 (80; 60-90)	77.09±8.71 (79; 33-90)	0.054
Thigh preop	51.68±4.3 (52; 38-65)	51.98±4.48 (52; 35-61)	51.83±4.37 (52; 35-65)	0.588
Thigh postop	50.6±4.4 (50.5; 36-64)	50.86±4.65 (51; 34-60)	50.73±4.51(51; 34-64)	0.679
Age	32.02±9.53 (32; 16-51)	32.08±9.53(33; 17-51)	32.05±9.48 (33; 16-51)	0.959

*: significant at 0.05 level according to Mann-Whitney U test

DISCUSSION

The goals of ACL reconstruction are to prevent osteoarthritis and restore knee kinematics. The favorable result of ACL reconstruction is significantly impacted by two critical factors: rigid and stable graft fixation and anatomic positioned tunnels.[10,11]

Recently, significant progress has been made in ACL surgery, thanks to advancements in arthroscopic techniques and instruments. Although the studies in the literature have provided better insights into the biology, biomechanics, and pathophysiology of ACL tears, there still needs to be a standard consensus on its treatment.[10,11] The present study revealed that in arthroscopic ACL reconstruction using the Ziploop method with a lifting system, there were no significant differences in clinical and functional outcomes between patients undergoing fixation with a staple and those undergoing fixation without a staple, in addition to tibial BioScrew fixation of the autograft in the tibial tunnel However, the use of staple fixation resulted in more pain for the patients.

Rigid and stable graft fixation and anatomically precise placement of the femoral and tibial tunnels are among the most critical factors influencing the successful outcome of ACL reconstruction.[1,2] With the increasing use of soft tissue grafts in ACL reconstruction, such as hamstring autografts, cortical suspension devices have gained popularity as a means of fixation for the femoral and tibial tunnels. Furthermore, biomechanical studies have shown that cortical suspension devices exhibit superior tensile strength and less elongation during cyclic loading when compared to interference and transfixation devices.[12-14]

In femoral fixation systems with suspensions, the femoral tunnel is drilled more than 6-10 mm, depending on the characteristics of the system and the surgeon's experience, to enable the implant's placement in the femoral cortex using a "flip" movement. It has been frequently reported that femoral tunnel enlargement may occur as the graft moves within the femoral tunnel. Recent femoral fixation implant designs are aimed at minimizing movement over the femoral cortical surface. In addition, improved loop materials aim to reduce the micro-movement of the graft within the tunnel. The Toggle Loc with Zip Loop (TL-ZL) system (Biomet, Warsaw, IN) is similar to the standard application of the Endobutton CL (E-CL) (Smith & Nephew, Andover, MA), while the TL-ZL is attached to the ceiling of the femoral tunnel with a mechanism similar to a graft suspensor due to improved loop formation. Thus, the objective is to fill the hole underneath the femoral tunnel ceiling.[15] For this reason, the Zip Loop system was used for femoral tunnel fixation as the standard of care in all patients in the present study.

Although there is currently no consensus on the appropriate length of the femoral tunnel, several studies have suggested minimum lengths ranging from 15 mm to 30 mm.[16,17] Recently, adjustable-loop cortical suspension implants have been increasingly used to extend the graft length within the tunnel. These implants have effectively filled the tunnel, making the procedure technically easier to perform and eliminating the need for tedious calculations during the surgery. They also increased the graft-bone interface, providing a larger surface area for graft integration and healing. Although adjustable ring devices have theoretical advantages in promoting graft healing, recent studies have raised concerns about poor mechanical properties and the potential for elongation of adjustable rings under cyclic loading.[18,19] In this study, standard 25 mm long femoral tunnels were created in anatomically appropriate locations.

Stable graft fixation is required during biological union to prevent graft elongation and failure.[20] This is particularly required for early range of motion, weight-bearing, and the likelihood of returning to sports after ACL reconstruction.[21] Although studies have demonstrated higher ultimate failure loads for hamstring graft compared to bone-patellar tendon-bone (BPTB) graft, the fixation of hamstring graft to the tibial bone has often been identified as a potential cause of failure due to the weaker tibial metaphyseal bone compared to the femur.[22,23]

Due to the absence of gold standard material and technique for tibial fixation in ACL reconstruction using hamstring graft and the ongoing uncertainty regarding this matter, a variety of devices (such as interference screws, BioScrews, washer and screw fixation, staples, endobuttons with suspension systems, or a combination of these) are widely employed for this procedure. Consequently, this particular matter continues to be the focus of further research.[24] In their study, Wang et al.[25] investigated the safety of utilizing the Rigidfix cross-pin system through various tibial tunnels for tibial fixation in ACL reconstruction. When employing the Rigidfix cross-pin system for ACL reconstruction at the tibial fixation site, the study concluded that it is crucial to avoid placing the external opening of the tibial tunnel in the extreme posterosuperior region to prevent injuries to the medial collateral ligament (MCL) and tibial plateau cartilage (TPC).

In another study, tibial fixation with an interference screw demonstrated superior biomechanical properties in cyclic loading tests compared to the suspension button and tape locking screw. The ultimate failure loads did not differ between the groups, and no significant difference was found in biomechanics between the suspension button and tape-locking locking screw fixation devices.[26] In addition to these factors, determining the preferred tibial fixation structure remains challenging due to limitations in clinical trials, such as variability in the reporting of outcomes and the use of various surgical

techniques. Clinical studies comparing various tibial fixation methods have shown no difference in clinical outcomes.[27]

In a study by Chadwick et al.[28] the Lysholm score was reported to be 94.5 after ACL reconstruction using a hamstring tendon graft and the Endobutton-CL technique. In a study conducted by Cansever et al.[29] the mean Lysholm score was 92.5 in the Endobutton-CL group and 94 in the Ziploop with Elevator System group. In a study conducted by Peter et al.[30] using the Endobutton-CL, it was reported that, in the first postoperative year, 6 out of 46 patients received A scores, 30 received B scores, and 9 received C scores in the IKDC evaluation. The study results demonstrated that the outcomes were favorable compared to the preoperative clinical status. Gobbi[31] utilized IKDC scoring in his study and reported that he found A-B scores in 72 patients, C scores in 7 patients, and D scores in 1 out of 80 patients after 36 months. In the study conducted by Cansever et al.[29] it was observed that a total of 24 (96%) patients scored A or B in both the Endobutton-CL group and the Ziploop with Lifting System group, and no patients scored D. The results were comparable in both groups. Furthermore, in the study conducted by Çınar et al.[32] no significant difference was found between the RigidFix and Endobutton groups postoperatively. Similarly, in the study by Mayr et al.[33] comparing BioScrew and suspension implants with tibia and femoral tunnel fixation in ACL reconstruction, no significant difference was detected in terms of knee function outcomes (Lysholm and IKDC scores).[33] In our study, the Lysholm and IKDC scores were not significantly different between the groups, which are consistent with the findings in the literature.

In the clinical study by Lai et al.[34] 4 out of 34 patients in the adjustable suspensory devices and interference screw (ASIS) group experienced anterior knee pain, while 11 of 32 patients in the cortical screw post along with the interference screw (CSIS) group experienced anterior knee pain at least one year after the operation. Although the risk of experiencing anterior knee pain (VAS=3) was lower in the adjustable suspensory devices and interference screw (ASIS) group, no significant difference was observed between the groups. In addition, other studies have also reported anterior knee pain due to the use of cortical screw posts in hybrid tibial fixation.[35,36] In the present study, the VAS score was lower in Group 1 than in Group 2, and there was a significant difference in the postoperative VAS scores of patients in Group 1 compared to preoperative values.

As limitations of this study, more meaningful results could have been obtained by including 3D computed tomography and magnetic resonance imaging as radiological evaluations to assess tibial tunnel widening in addition to the clinical and functional outcomes.

CONCLUSION

In line with the existing literature, the findings of this study indicate that ACL reconstruction utilizing hamstring tendon autograft and the Ziploop technique with a lifting system is an effective and reliable method, offering the advantage of avoiding the complexity of tunnel size calculation and yielding favorable functional outcomes. In addition, autograft fixation in the tibial tunnel using a staple, in addition to tibial BioScrew fixation, caused more pain in patients, and there was no significant difference in clinical and functional outcomes between the groups undergoing reconstruction with and without staple fixation.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by the Necmettin Erbakan University Non-drug and Non-medical Device Researches Ethics Committee (Date: 07.07.2023, Decision No: 2023/4427).

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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Incidence of acute endophthalmitis after intravitreal bevacizumab injection in a tertiary hospital

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ABSTRACT

Aims: To investigate the incidence of acute endophthalmitis after intravitreal bevacizumab injections.

Methods: Patients who received treatment with intravitreal bevacizumab (1.25 mg/0.05 ml) injections for various indications between November 2020 and March 2022 were included in this retrospective study. The patients were followed up for 4 weeks after the injection in terms of acute endophthalmitis symptoms and signs.

Results: Acute endophthalmitis developed in 1 patient after 4593 intravitreal bevacizumab injections were administered to 1427 eyes of 1026 patients, and the incidence was found to be 0.0217%. The patient who developed acute endophthalmitis underwent pars plana vitrectomy and after 3 months, a significant improvement in visual acuity was observed.

Conclusion: Development of endophthalmitis postoperatively was found to be moderately low after intravitreal bevacizumab injection. It was concluded that following asepsis rules and optimal bevacizumab preparation conditions could further reduce this.

Keywords: Bevacizumab, intravitreal injections, endophthalmitis, complications

INTRODUCTION

Bevacizumab (Avastin, Genetech inc., San Francisco, California, USA) is a recombinant human monoclonal antibody that can bind to all forms of vascular endothelial growth factor (VEGF).[1] It is an approved drug for the treatment of colorectal cancers.[2] However, despite being off-label it is also widely used intravitreally in the treatment of ocular diseases such as macular edema due to diabetic retinopathy (DRP), choroidal neovascularization (CNV), retinal vein occlusion (RVO), retinopathy of prematurity (ROP), age-related macular degeneration (AMD) and degenerative myopia.[3-7] Intravitreal bevacizumab (IVB) administration has ocular side effects such as subconjunctival hemorrhage, transient intraocular pressure (IOP), vitreous hemorrhage, and retinal detachment, as well as serious side effects that cause permanent vision loss such as acute endophthalmitis.[8,9]

With the worldwide increase in retinal diseases that cause vision loss, off-label use of intravitreal bevacizumab (IVB) is increasing, due to it being more cost-effective.[10] Risk factors for the development of post-injection acute endophthalmitis include immune system diseases, failure of physicians and auxiliary personnel to comply or pay attention to asepsis techniques, withdrawal of more than one dose of medication from a single vial, presence of chronic ocular infection in patients, and patients' failure to pay attention to post-procedural hygiene.[9] Asepsis training of physicians and auxiliary personnel and explaining hygiene rules to patients can reduce the risk of acute endophthalmitis that may occur after injection.

The aim of our study was to evaluate the incidence, management, and visual results of acute endophthalmitis after IVB injections in our ophthalmology clinic.

METHODS

The study was carried out with the permission of Gaziantep Islamic Science and Technology University Ethics Committee (Date: 07.06.2022, Decision No: 129.17.18). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this retrospective study, the records of 1026 patients who presented to our ophthalmology clinic with the complaint of low vision, who were treated with IVB (1.25 mg/0.05 ml) injection for various indications between November 2020 and March 2022, were retrospectively reviewed. Only patients who received IVB were included in the study.

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The best corrected and uncorrected visual acuities of all patients were measured with the Snellen chart, intraocular pressure status was measured with a Goldman applanation tonometer and anterior segment examinations were performed with slit lamp biomicroscopy. Fundus examination was performed with a 90-diopter lens. The retina and choroid were evaluated with optical coherence tomography (OCT, Optovue RTVue XR, Optovue Inc., Fremont, CA) and fundus fluorescein angiography (FFA, Topcon TRC-50DX, Topcon Corporation, Japan). Patients diagnosed with macular edema due to diabetic retinopathy, choroidal neovascularization and fluid due to AMD, and macular edema due to RVO were administered intravitreal bevacizumab injection at 1-month intervals. A separate vial is applied for each injection. All intravitreal injections were administered by 2 surgeons with 2 years of experience in this field (MB, GGS). Before the application, the surgeon and assistant nurse wore a surgical mask, cap, and surgical attire. Disposable shoe covers, caps, and sterile disposable gowns were worn by the patients. 0.5% proparacaine drops were installed, and the eye and its surroundings were cleaned with 10% povidone-iodine. The eye was then covered with a sterile disposable drape and the lids were retracted with a blepharostat device.

The diagnosis of acute endophthalmitis after intravitreal injection was defined as symptoms and clinical findings of sudden vision loss, eye pain, redness, chemosis, iritis, vitritis, and hypopyon within 4 weeks following the injection. In this period, the patients who applied with the above-mentioned symptoms and signs were accepted as acute endophthalmitis and treated accordingly.

RESULTS

The data of 1026 patients, treated with intravitreal bevacizumab for various indications, were retrospectively analyzed. The mean age of patients was 63.5±10 years. Of the patients, 477 (46%) were female and 549 (54%) were male. The total number of injections administered was 4593, of which 3062 were repeat injections. These 4593 injections were administered to 1427 eyes. The distribution of patients according to the indications for intravitreal bevacizumab is shown in Table 1. Among the indications for intravitreal bevacizumab, DRP was the most common (66.7%), followed by AMD (24.3%). The rate of cases with RVO was 9%.

Table 1. Distribution of patients by intravitreal bevacizumab indication

Disease	Number of patients	(%)
Diabetic retinopathy	684	66.7
Age related macular degeneration	249	24.3
Retinal vein occlusion	93	9
Total	1026	100

Of the 4593 intravitreal injections of bevacizumab, only 1 patient developed acute endophthalmitis. Thus, the incidence of acute endophthalmitis after intravitreal injection of bevacizumab was 0.0217% (at 18 months) and 0.014% per year. The infection rate for each eye was 0.07%. The patient who developed acute endophthalmitis after IVB injection, was a 62-year-old male, with a diabetes diagnosis of 12 years duration, who presented with complaints of pain, redness, watering, and sudden vision loss in his left eye one day after the injection. Visual acuity in the affected eye was hand motion. On examination, there was intense conjunctival hyperemia and chemosis, a fibrin reaction in the anterior chamber, and intense condensation in the vitreous of the left eye. B-scan ultrasonography revealed vitreous condensation and diffuse choroidal inflammation findings. The patient had no history of previous eye surgery. The patient was diagnosed with acute endophthalmitis and an emergency pars plana vitrectomy was carried out on the same day. His visual acuity increased to 6/60 at 3 months postoperatively.

DISCUSSION

Bevacizumab inhibits angiogenesis with its anti-VEGF effect in retinal vascular diseases such as DRP, AMD, and RVO. Studies have shown that it has ocular and systemic side effects.[8] Acute endophthalmitis is the most serious of the ocular complications. Acute endophthalmitis can cause sudden vision loss and severe eye pain.[11] Several studies have been conducted in different regions regarding the incidence of acute endophthalmitis after intravitreal injection of bevacizumab.[9,12-16] Our study found the incidence of acute endophthalmitis to be 0.0217%, 0.014% per year, and 0.07% per eye, comparable to other studies.

Ahmed et al.[9] reported the incidence of acute endophthalmitis as 0.0328%, with a rate of 0.018% per year, and a rate of 0.09% per eye after 3051 IVB injections (single-use prefilled sterile syringe) were administered to 1104 eyes of 743 patients with various indications in their prospective study conducted in Lahore, Pakistan. Haider et al.[12] found the incidence of acute endophthalmitis to be 0.19% after single-use prefilled sterile syringe IVB injection in the city of Lahore, which they administered in the office environment and claimed that there was no difference in safety between the administration in the office environment and the operating room environment.

Artunay et al.[13] in their study, they grouped ten patients together for treatment to provide multiple doses from a single vial and they applied 3022 IVB injections to 1822 eyes and reported the incidence of acute endophthalmitis as 0.006%. Karimi et al.[14] reported that post-injection acute endophthalmitis developed in 9 patients in their retrospective study in which they administered

28,085 IVB injections (They applied it to each patient using a separate vial), and reported the incidence as 0.032%. Pradhan et al.[15] in their retrospective study they administered a separate vial to each patient, found the prevalence of acute endophthalmitis to be 0.048% after 4182 IVB injections. Falvarjani et al.[16] in their retrospective study in Iran, found the incidence of acute endophthalmitis to be 0.1% after 5901 IVB injections in 3975 eyes. In the treatment of acute endophthalmitis, early pars plana vitrectomy is important for prognosis.[17] One day after the injection, acute endophthalmitis developed in the left eye of 1 patient and PPV treatment was urgently administered.

In order to reduce the incidence of acute endophthalmitis after IVB injection, it is important for the team performing the application to comply with the conditions of asepsis hygiene prerequisites for patients, as well as the optimal preparation conditions of bevacizumab. In some centers, applications are made by creating multiple doses from a single vial, and there are studies showing that this causes cluster endophthalmitis.[18] Bavinger et al.[19] stated that bevacizumab filled syringes were associated with a lower risk of endophthalmitis as compared to the other multiple doses. In our study, a separate vial was opened for each IVB injection patient. Patient wearing a mask during injection does not reduce the risk of endophthalmitis.[20] In our study, masks were used in all patients who received injections. Like other eye surgeries, applying povidone-iodine to the eye and covering it with a sterile drape during intravitreal injections reduces the risk of endophthalmitis.[21] We applied povidone-iodine to all eyes and used sterile drape.

Limitations of our study were the small number of patients, the fact that the study was carried out as a single-center study, and the coverage of only one region.

CONCLUSION

We found the incidence of acute endophthalmitis after 4593 IVB injections in 1427 eyes of 1026 patients to be very low (0.0217%), which was similar to other studies. In order to reduce the incidence of acute endophthalmitis, which causes severe vision loss, it is of great importance when using IVB for intravitreal injections to prepare bevacizumab suitable for single use and to comply with the rules of asepsis.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Gaziantep Islamic Science and Technology University Ethics Committee (Date: 07.06.2022, Decision No: 129.17.18).

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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Correlation between antibody levels and long-term symptoms in survivors of COVID-19: health outcomes and societal implications

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ABSTRACT

Aims: It was aimed to evaluate the relationship between antibody levels, demographic characteristics, and ongoing symptoms of people who have positive COVID-19 real-time PCR (RT-PCR) tests and donated plasma after the disease.

Methods: Patients who voluntarily donated plasma were called by phone, and 105 patients who wanted to participate in the survey were included in the study. Ongoing symptoms, antibody test results, demographic characteristics, and other data of the participants were recorded.

Results: IgM was high in patients whose first complaint was fever and muscle pain at the onset of the disease and who used favipiravir for treatment (p=0.030, p=0.035, p=0.007). In those who survived the disease, it was determined that the IgM level decreased after the peak in the first month and the IgG level in the third month; the results were statistically significant. The IgG level decreased with the elapsed time and smoking, and the IgG level was found to be high in those who used favipiravir, hydroxychloroquine, or both during the disease and those in the AB blood type.

Conclusion: Some symptoms may persist even after the COVID-19 infection has been overcome. This study will contribute to a better understanding of this disease and the process after it.

Keywords: COVID-19, IgM and IgG antibodies, post-COVID symptoms, cigarette, blood type

INTRODUCTION

COVID-19 disease caused by the SARS-CoV-2 virus, which affected the whole world in late December 2019, was first identified in Wuhan, Hubei Province, China.[1] The disease can be recovered from with no or mild symptoms, or it can result in severe illness and death. Common symptoms are cough, fever, shortness of breath, weakness, fatigue, muscle pain, loss of taste and/or smell.[2-6]

The disease is diagnosed with clinical symptoms, serological tests, and lung imaging.[7,8] The sensitivity of ELISA-based IgM and IgG-detecting antibody tests used in the diagnosis of COVID-19 is 77-83%, and the specificity is >95%. When a serum sample taken two weeks after the first positive RT-PCR test is studied, the accuracy of serological tests increases even further.[9]

COVID-19 antibody level is used epidemiologically to determine those who have recovered from the disease. As a result of the follow-ups, it has been shown that some complaints persist even months after the illness.[10,11]

This study aimed to evaluate the correlation between antibody levels, demographic characteristics, and ongoing symptoms in healthy volunteers who tested positive for an RT-PCR test diagnosed with COVID-19. Then, the PCR test turned negative, and they wanted to donate Convalescent plasma (CP). Patients who volunteered to donate CP were contacted by phone, and those who agreed to participate in the survey were included in the study.

METHODS

The study was carried out with the permission of Adıyaman University Non-interventional Clinical Researches Ethics Committee (Date: 19.01.2021, Decision No: 2021/01-4). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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In this retrospective descriptive type study, those who applied to the Adıyaman University Training and Research Hospital blood center, who had a positive COVID-19 RT-PCR test before, whose PCR test was negative in their follow-up, who voluntarily donated CP, and whose IgG antibodies turned positive, were called by phone. Those who accepted to participate in the survey were included in the study.

The study participants' symptoms, antibody test results, epidemiological characteristics, and other data were registered between the time the first PCR test was positive and the time they donated. In the antibody study, 5 cc blood taken from volunteer CP donors into a biochemistry tube was centrifuged and quantitatively studied using the micro-ELISA method with the GRIFOLS brand TIRITURUS model device.

In the survey, 105 people participated. The participants were asked about their blood group, the date on which they were PCR positive, IgM and IgG levels, time of illness, first seen complaints, hospitalization or outpatient treatment, how many days they received treatment, drugs used in the treatment of COVID-19 disease, presence of ongoing complaints, if complaints not continued, what and when the last complaint was, smoking, whether there is any other person (spouse, children, mother, father, siblings) who had COVID-19 disease in the family, whether there is a person (spouse, children, mother, father, siblings) who died due to COVID-19 disease in the family.

Statistical Analysis

Predictive Analytics Software (PASW) 18 (2009) program was used for statistical analysis. The cases where the Type-1 error level is below 5% were interpreted as statistically significant. The conformity of the variables to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov / Shapiro-Wilkt tests).

Descriptive statistics were presented as numbers and percentages for categorical variables, median, percentile 25, and percentile 75 for numerical variables. The Kruskal Wallis test was used as the normal distribution condition was not met in the multi-group comparison analysis in numerical variables, and the Mann Whitney U test was used as the normal distribution condition was not met in the paired group comparison analysis.

Mann-Whitney U test with Bonferroni correction was used for post hoc analysis. Spearman's rho test was used to examine the correlation between numerical variables. Patient characteristics are summarized in Table 1.

Table 1. Demographic characteristics

Age, mean±SD median (Q1-Q3)	105	38.08±10.56 37 (29-47)
IgM level, mean±SD median (Q1-Q3)	105	8.57±6.69 6 (4-11.2)
IgG level, mean±SD median (Q1-Q3)	105	39.41±25.02 41.2 (21-48)
Blood group, n (%)	105	
A		44 (41.9)
B		24 (22.9)
AB		7 (6.7)
0		30 (28.6)
Time of illness, n (%)	105	
1 month ago		19 (18.1)
2 months ago		32 (30.5)
3 months ago		31 (29.5)
4 months ago		5 (4.8)
5 months ago		12 (11.4)
6 months ago		6 (5.7)
First complaint, n (%)	105	
Fever		8 (7.6)
Fever+other complaints		2 (1.9)
Fever+weakness		12 (11.4)
Fever+muscle pain		2 (1.9)
Fever+muscle pain+weakness		1 (1)
Fever + cough		1 (1)
Fever+cough+weakness		2 (1.9)
Other complaints		17 (16.2)
Weakness		28 (26.7)
Weakness+other complaints		2 (1.9)
Muscle pain		14 (13.3)
Muscle pain+other complaints		1 (1)
Muscle pain+weakness		8 (7.6)
Shortness of breath		1 (1)
Cough		4 (3.8)
Cough+weakness		2 (1.9)
First complaint, n (%)		
Fever	105	28 (26.7)
Cough	105	9 (8.6)
Shortness of breath	105	1 (1)
Muscle pain	105	26 (24.8)
Weakness	105	55 (52.4)
Other complaints	105	22 (21)
Hospitalization status, n (%)	105	7 (6.7)
Day of treatment received, n (%)	105	
5 days		80 (76.2)
7 days		1 (1)
10 days		11 (10.5)
I did not receive any treatment		13 (12.4)
Treatment used, n (%)	105	
Favipiravir		35 (33.3)
Favipiravir+Plaquenil		56 (53.3)
Plaquenil		1 (1)
I did not use any drugs		13 (12.4)
Treatment used, n (%)		
Favipiravir	105	91 (86.7)
Plaquenil	105	57 (54.3)

Table 1. Demographic characteristics (continued)		
Complaint continuation status, n (%)	105	15 (14.3)
Time of the last complaint, n (%)	105	
10 Days ago		5 (4.8)
20 Days ago		11 (10.5)
1 Month ago		5 (4.8)
Over 1 month		69 (65.7)
My complaint continues		15 (14.3)
Latest resolved complaint, n (%)		
Weakness		14 (13.3)
Muscle pain		7 (6.7)
Muscle pain+other complaints		1 (1)
Muscle pain+weakness		1 (1)
Muscle pain+fatigue		1 (1)
Shortness of breath		4 (3.8)
Fatigue		14 (13.3)
Fatigue+other complaints		2 (1.9)
Fatigue+shortness of breath		1 (1)
Other complaints	105	60 (57.1)
Latest resolved complaint, n (%)		
Muscle pain	105	10 (9.5)
Weakness	105	15 (14.3)
Fatigue	105	18 (17.1)
Shortness of breath	105	5 (4.8)
Other complaints	105	63 (60)
Smoking status, n (%)	105	14 (13.3)
Presence of another person who had COVID-19 disease in the family, n (%)	105	41 (39)
Presence of a person who died due to COVID-19 disease in the family, n (%)	105	5 (4.8)

RESULTS

A total of 105 people participated in our study. We found that females were 16 (15.2%), males were 89 (84.8%), and the mean age was 37 (29-47). It was determined that 44 (41.9%) people had A blood group, 30 (28.6%) people had O blood group, 24 (22.9%) people had B blood group, and 7 (6.7%) people had AB blood group. It was observed that 32 (30.5%) people had illness two months ago, 31 (29.5%) people three months ago, 19 (18.1%) people one month ago, 12 (11.4%) people five months ago, 6 (5.7%) people six months ago and 5 (4.8%) people four months ago. We found 28 (26.7%) people whose first complaint was weakness and fatigue, 17 (16.2%) with other complaints (other than weakness, fatigue, muscle pain, fever, cough, and shortness of breath), 14 (13%) people with muscle pain, 14 (13.3%) people with fever and fatigue complaints, 8 (7.6%) people with complaints of weakness and muscle pain, 8 (7.6%) people with only fever. It was found that 98 (93.3%) people survived the disease at home, and 7 (6.7%) people were hospitalized. During the illness, 80 (76.2%) people received treatment for five days, 11 (10.5%) people for ten days, 1 (1%) person for one day, and 13 (12.4%) people recovered without treatment. While 56 people (53.3%) were found to use favipiravir and hydroxychloroquine together in the treatment of the disease, 35 people (33.3%) were found to use favipiravir, only one person (1%) was found to use hydroxychloroquine,13 (12.4%) were found not to receive any medical treatment.

It was observed that the complaints of the disease continued in 15 (14.3%) people after surviving the disease. For those who did not have a complaint, the most recent complaints were seen more than a month ago in 69 (65.7%) people, 20 days ago in 11 (10.5%) people, one month ago in 5 (4.8%) people, and ten days ago in 5 (4.8%) people. The most recently recovered complaints were found to include fatigue in 18 people (17.1%), weakness in 15 people (14.3%), muscle pain in 10 people (9.5%), shortness of breath in 5 people (4.8%), other complaints (other than fatigue, weakness, muscle pain, and shortness of breath) in 63 people (60%).

There were 14 smokers (13.3%) and 91 non-smokers (86.7%). It was found that 41 (39%) people had a person in their family (spouse, children, mother, father, siblings) other than themselves with COVID-19 disease. It was observed that 5 (4.8%) people died in the family due to COVID-19 disease (Table 1).

In our survey study, 105 healthy volunteers who previously had a positive COVID-19 PCR test and had a negative COVID-19 PCR test in their follow-up and who donated CP participated. The difference between the antibody levels between the time of illness and the time of the last complaint was statistically significant.

IgM levels were higher in those who had the disease one month ago, and IgG levels were higher in those who had the disease three months ago ($p=0.040$, $p<0.001$, respectively). IgM and IgG levels were higher in those whose last complaint was ten days ago ($p=0.030$, $p<0.001$, respectively). The difference between the treatment received groups and antibody level was found to be statistically significant. Higher IgM level was found to be statistically significant in patients using favipiravir or favipiravir and Plaquenil compared to those not receiving treatment ($p=0.011$, $p=0.007$). The IgM level was higher in patients whose first complaint was fever and muscle pain and who used favipiravir for treatment ($p=0.030$, $p=0.035$, respectively). The IgM level was found to be low in patients whose last recovered complaints were not weakness, fatigue, or shortness of breath ($p=0.011$), and the results were found to be statistically significant.

The risk of SARS-CoV-2 infection was the highest in people with A blood group (44 people 41.9%), and the risk of SARS-CoV-2 infection was the lowest in people with AB blood group (7 people 6.7%). Although the antibody level was high in the AB blood group, it was observed that there was no statistically significant difference between the other groups.

Antibody levels were found to be higher in hospitalized patients. There was no statistically significant difference between those who were not hospitalized. The IgM level was high in those treated for ten days during the disease, and the IgG level was high in those treated for five days. The results were not found to be statistically significant between the treatment periods.

Patients whose complaints did not continue after recovering from the disease were found to have high IgG levels; there was no statistically significant difference in antibody levels between those who continued their complaints after recovering from the disease and those who did not. IgG levels were found to be high in non-smokers. No statistically significant difference was found between smoker and non-smoker antibody levels.

There was no statistically significant difference in antibody levels between those with family members other than themselves (spouse, children, mother, father, siblings) with COVID-19 disease and those who do not. IgM was higher in those with family members (spouse, children, mother, father, siblings) who died due to COVID-19 disease; no statistically significant difference was found between the results (**Table 2**).

Table 2. Comparison of various findings with antibody level

	IgM Level				IgG Level			
	N	**Mean±SD**	**Median (Q1-Q3)**	**p**	**N**	**Mean±SD**	**Median (Q1-Q3)**	**p**
Blood group				0.640*				0.080*
A	44	7.57±4.91	5.5 (4.05-11)		44	33.43±15.12	34.6 (21.35-41.75)	
B	24	10.74±9.35	7.15 (4.9-13.85)		24	42.3±19	41.9 (34.5-45.2)	
AB	7	7.01±2.38	7.2 (5-9)		7	52.31±31.16	59 (17-63.6)	
0	30	8.67±6.94	6.45 (3.4-12)		30	42.87±36.12	45.65 (17.6-48.3)	
Time of illness				0.004*				<0.001*
1 month ago	19	11.62±9.36	11 (4.3-17)		19	39.34±33.05	37.7 (23.7-42)	
2 months ago	32	7.11±4.81	5 (3.8-9.55)		32	36.45±25.7	40.5 (18.85-44.45)	
3 months ago	31	10.75±7.01	9 (6-12.7)		31	53.95±17.75	53 (42-62)	
4 months ago	5	4.94±3.05	4.2 (2.5-6)		5	26.94±10.08	23 (19.9-32.2)	
5 months ago	12	5.03±1.39	5.1 (3.65-6.15)		12	24.7±12.68	22 (15-35.6)	
6 months ago	6	5.55±5.63	3.24 (2.9-4.1)		6	20.17±13.48	13.5 (11.5-32)	
First complaint								
Fever				0.030**				0.879**
No	77	7.87±6.16	5.6 (3.6-10.4)		77	39.03±24.53	41.2 (20.7-48)	
Yes	28	10.5±7.75	9.1 (5.35-13.35)		28	40.45±26.76	40.8 (26.85-45.65)	
Cough				0.643**				0.152**
No	96	8.73±6.84	6.1 (4.05-11.65)		96	40.48±25.51	41.45 (23.5-48)	
Yes	9	6.87±4.65	6 (3.7-6.6)		9	27.96±15.86	18 (17-42)	
Shortness of breath				-				-
No	104	8.61±6.71	6.1 (4-11.4)		104	39.43±25.14	41.2 (20.9-48)	
Yes	1	4.9±0	4.9 (4.9-4.9)		1	37.7±0	37.7 (37.7-37.7)	
Muscle pain				0.035**				0.062**
No	79	7.88±6.08	6 (3.5-11)		79	36.3±21.93	37.7 (17.6-48)	
Yes	26	10.67±8.05	9.05 (4.9-13.4)		26	48.87±31.31	41.8 (32.4-48)	
Weakness				0.447**				0.649**
No	50	9.19±7.5	6.3 (4.2-11.6)		50	39.24±27.04	37.85 (19.9-48)	
Yes	55	8.01±5.87	6 (3.6-11.2)		55	39.57±23.29	41.7 (26-48)	
Other complaints				0.651**				0.386**
No	83	8.3±6.51	6 (4-11.2)		83	40.94±26.81	41.7 (23-48)	
Yes	22	9.58±7.38	7 (3.5-13.5)		22	33.65±15.82	32.1 (19.7-46)	
Hospitalization status				0.576**				0.714**
Yes	7	11.46±13.27	6.3 (4.9-11)		7	36.11±17.74	41.2 (14.2-46)	
No	98	8.37±6.03	6 (4-11.6)		98	39.65±25.51	41.1 (21-48)	
Day of treatment received				0.646*				0.379*
5 Days	80	9.31±7.24	6.4 (4.15-12.35)		80	41.87±26.77	41.7 (23.85-48.15)	
7 Days	1	4.2±0	4.2 (4.2-4.2)		1	32.2±0	32.2 (32.2-32.2)	
10 Days	11	8.51±3.91	7.2 (6.3-11)		11	31.31±19.23	26 (14.2-46)	
Over 10 days	0	0±0	0 (0-0)		0	0±0	0 (0-0)	
I did not receive any treatment	13	4.45±2.37	3.6 (2.9-5.2)		13	31.72±15.18	24.2 (20.7-42)	

Table 2. Comparison of various findings with antibody level (continued)

	IgM Level				IgG Level			
	N	Mean±SD	Median (Q1-Q3)	p	N	Mean±SD	Median (Q1-Q3)	p
Treatment used								
Favipiravir				0.007**				0.089**
No	14	4.94±3.23	3.85 (2.9-5.6)		14	29.25±16.28	23.95 (19.7-42)	
Yes	91	9.13±6.91	6.6 (4.2-12)		91	40.97±25.82	41.7 (23.3-48)	
Plaquenil				0.082**				0.108**
No	48	7.96±7.48	5.45 (3.5-10)		48	33.51±17.72	31.35 (18.35-47.5)	
Yes	57	9.09±5.96	8 (4.6-12)		57	44.38±29.05	41.7 (28-48)	
Treatment used				0.011*				0.146*
Favipiravir	35	9.22±8.25	6.3 (3.7-11.7)		35	35.31±17.95	40.4 (17-48.3)	
Favipiravir+plaquenil	56	9.07±6.01	7.6 (4.6-12.35)		56	44.52±29.3	41.7 (27.1-48)	
I did not use any drugs	13	4.55±3	3.5 (2.9-5,2)		13	28.68±16.8	23.7 (19.7-42)	
Plaquenil	1	10			1	36.6		
Complaint continuation status				0.314**				0.492**
Yes	15	8.67±10.09	5 (3.5-11)		15	33.82±16.43	37 (20.7-45.3)	
No	90	8.56±6.02	6.3 (4.1-11.6)		90	40.34±26.13	41.2 (21-48)	
Time of last complaint				0.030*				<0.001*
10 days ago	5	13.74±6.04	13 (10.4-17)		5	108.2±60.46	107 (105-159)	
20 days ago	11	8.25±3.26	8 (6-9)		11	57.04±15.7	62 (61-62)	
1 Month ago	5	6.82±3.67	5 (4.9-8)		5	55.34±2.74	55 (53-58)	
Over 1 month	69	8.7±6.42	6 (4-11.6)		69	33.63±12.9	38 (21-42)	
My complaint continues	15	7.07±9.85	4.2 (2.9-5.4)		15	24.83±14.22	23.7 (10.2-41.7)	
Latest resolved complaint								
Muscle pain				0.290**				0.172**
No	95	8.42±6.75	6 (3.7-11.2)		95	38.58±24.88	40.4 (20.7-48)	
Yes	10	10.02±6.23	8.5 (5-17)		10	47.26±26.3	43.9 (26-62)	
Weakness				0.425**				0.826**
No	90	8.5±6.93	6 (4-11.2)		90	38.9±22.98	41.2 (23-48)	
Yes	15	8.99±5.17	8 (4.1-13)		15	42.46±35.78	37.7 (17-48)	
Fatigue				0.109**				0.936**
No	87	7.79±5.35	6 (3.6-11)		87	39.94±26.96	41.2 (19.7-48.3)	
Yes	18	12.34±10.51	9 (4.3-17)		18	36.86±12.03	37.5 (28-45.1)	
Shortness of breath				0.077**				0.320**
No	100	8.4±6.73	6 (3.85-11.1)		100	39.22±25.46	40.7 (20.9-47.5)	
Yes	5	12.04±5	11.6 (9-17)		5	43.28±14.58	48 (41.7-48)	
Other complaints				0.011**				0.351**
No	42	10.08±6.35	9 (4.9-15)		42	41.9±25.46	41.7 (26.2-48)	
Yes	63	7.57±6.77	5.9 (3.5-9.7)		63	37.75±24.78	41 (17.6-48)	
Smoking status				0.720**				0.685**
Yes	14	8.4±4.99	7.5 (4.2-12.7)		14	44.67±37.48	36.4 (23-58.3)	
No	91	8.6±6.93	6 (4-11.2)		91	38.6±22.7	41.2 (20.8-47)	
Presence of another person who had COVID-19 disease in the family				0.380**				0.430**
Yes	41	7.78±5.82	5.6 (4-11)		41	36.44±19.55	40.4 (20.7-45.3)	
No	64	9.08±7.18	6.45 (3.95-11.8)		64	41.31±27.95	41.45 (22-48)	
Presence of a person who died due to COVID-19 disease in the family				0.059**				0.910**
Yes	5	13.26±5.34	14.8 (13.4-17)		5	36.82±7.02	41.7 (32-41.8)	
No	100	8.34±6.68	6 (3.85-11)		100	39.54±25.6	41.1 (20.75-48)	

* Kruskal Wallis, ** Mann-Whitney U

DISCUSSION

Research is ongoing on the COVID-19 disease that affects the world. Uncertainties in the symptoms, diagnosis, and treatment of the disease continue. In addition, it has been observed that there are ongoing symptoms even if the disease is recovered. PCR is still used in the foreground in diagnosing the disease, and antibody level is checked in epidemiological examinations. The duration and nature of the immunity that occurs in response to the infection caused by SARS-CoV-2 are unknown. The duration of immunity will determine the overall course of the pandemic and the post-pandemic dynamics. Therefore, understanding the temporal dynamics of protective immunity is critical.[11]

In the study conducted by Yang et al.[12] the data of 67 patients have been analyzed. In the first month, higher positive IgM rates have been found against SARS than IgG. The proportion of patients who performed seroconversion for IgM peaked 30 days after onset. Subsequently, it has been shown that a gradual decrease was observed in IgM levels, and IgG levels peaked in the 25th week. In our study, similar to the study of Yang et al., we found that IgM peaked in the first month in survivors, and differently, IgG peaked earlier (3rd month).

Even if the COVID-19 disease process is recovered, ongoing symptoms can be seen. In a study by Carfì et al.[13] it has been reported that patients continued Post COVID Syndrome (PCS) after an average of 60 days (72.7% of hospitalized participants had an additional disease, interstitial pneumonia), the most frequently reported symptoms were fatigue 53.1%, shortness of breath 43.4%, joint pain 27.3%, and chest pain 21.7%. On the other hand, in a study by Garrigues et al.[14] it has been reported that patients who were hospitalized due to COVID-19 had PCS for more than 100 days after discharge, the most frequently reported symptoms were fatigue 55%, dyspnea 42%, and in addition, memory loss 34%, concentration and sleep disturbances 28% and 30.8%, respectively.

In our study, PCS continued after an average of 28 days in 15 people; among those with symptoms, only one person (1%) was hospitalized, and no additional disease was found in any of the participants. The most common PCS was similar to the studies of Carfi and Garrigues; as a proportion, the symptoms were weakness and fatigue at 26.6%; differently, muscle pain was at 13.3%, fever at 7.6%, cough at 3.8%, dyspnea at 0.9%, and memory loss, concentration, and sleep disturbances were seen as other symptoms at a rate of 6.1%. Again, differently, it was found that two or more symptoms persisted at a rate of 41.7%. In addition, our study checked the IgG level according to the presence of symptoms. The IgG level was lower in those with symptoms (33.3%) than those without symptoms (40.3%). In our study, the shorter PCS incidence time compared to other studies was attributed to the fact that the participants were healthy volunteers who donated CP, did not have any additional disease, and donated in the early period.

In a study by Li et al.[15] it has been reported that people with A blood group had a significantly higher risk of SARS-CoV-2 infection, while those with O blood group had a significantly lower risk of SARS-CoV-2 infection; in another study conducted by Göker et al.16, it has been reported that while increased susceptibility to COVID-19 infection was detected in those with A blood group, O blood group may be somewhat protective. All participants in our study were followed up on an outpatient basis; similar to Li et al., while people with A blood group 41.9% had the highest risk of having COVID-19 disease, differently, we found that the risk of COVID-19 disease was the lowest in people with AB blood group, 6.7%. The lowest risk in those with the AB blood group was attributed to the lowest number of respondents with the AB blood group (6.7%).

In our study, the blood group mean antibody levels were found to be IgM 5.5 (4.05-11), IgG 34.6 (21.3-41.7) in group A, and IgM 7.2 (5-9), and IgG 59. (17-63.6) in the AB group.

It has been shown by Carlos et al.[17] that being a smoker or a former smoker is a risk factor for the worse progression of COVID-19 infection. Our study found the rate of smokers to be 13.4%. PCS continued in 4 smokers and 11 non-smokers. Among those who continued PCS, the IgG level of smokers (18.4%) was lower than that of non-smokers (40.3%).

Although many antivirals have been tried for COVID-19 disease, an effective treatment still needs to be found. In a study by Jean SS et al.[18], favipiravir, hydroxychloroquine, and azithromycin co-treatment are acceptable alternatives for treating COVID-19 patients. In the study conducted by Zarir F et al.[19] it has been determined that treatment with favipiravir may be beneficial in the clinical recovery period in patients admitted to hospital with mild (including asymptomatic) and moderate COVID-19. In another study with hydroxychloroquine, it has been mentioned that it can contribute to palliate the inflammatory response in COVID-19 patients and effectively inhibit SARS-CoV-2 infection in vitro.[20]

Participants in our study were people between the ages of 20-60 without any comorbidities. 87.62% of the participants received treatment during the illness period, and 12.4% did not. Those who received treatment were found to use favipiravir, hydroxychloroquine, or favipiravir and hydroxychloroquine together. It was observed that the level of IgG (40.3%) in those who received treatment was higher than those who did not (22.4%).

CONCLUSION

On the one hand, while the diagnosis, treatment, protection time of antibodies, and vaccination studies of COVID-19 disease continue, studies on PCS also continue in the post-disease period. Even if the disease was recovered, some symptoms continued at varying rates, and IgG levels decreased with the elapsed time and smoking. IgG levels were high in those who used favipiravir, hydroxychloroquine, or both during the treatment and those with AB blood group. Providing a framework for the possible physical symptoms of the disease after recovery in patients with COVID-19

and the results obtained when studies are conducted to determine the factors that contribute to or reduce protection will contribute to a better understanding of this disease and the subsequent process. Continuing such studies is essential to reveal the need to open a clinic for these patients in the future.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Adıyaman University Non-interventional Clinical Researches Ethics Committee (Date: 19.01.2021, Decision No: 2021/01-4).

Informed Consent

Written informed consent was obtained from all participants in this 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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Evaluation of lesions requesting biopsy according to imaging findings in breast cancer patients who have undergone breast-conserving surgery

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ABSTRACT

Aims: In patients undergoing breast-conserving surgery (BCS), the traditional follow-up imaging methods of the breast are mammography and ultrasonography. However, after BCS and radiotherapy, it becomes more difficult with imaging methods to detect the presence of recurrence or secondary focus due to the change of normal breast structure in patients. In this study, we aimed to investigate the sensitivity, specificity and malignancy prediction values of imaging methods in the follow-up of patients who underwent BCS.

Methods: 421 patients diagnosed with breast cancer who underwent BCS were retrospectively analyzed. 63 patients with histopathology results, which were categorized as BI-RADS 4 or 5 according to imaging findings in their follow-up after BCS, were included in the study. The age of diagnosis, time taken for biopsy and mammography, ultrasonography and magnetic resonance imaging findings were recorded. Patients were divided into 2 groups (benign and malignant) according to the results of biopsy. According to the pathology results, sensitivity, specificity, positive and negative predictive values and diagnostic accuracy levels of radiological imaging findings were calculated. The significance of the difference between pathology groups in terms of mean age of diagnosis and biopsy time was evaluated by Mann-Whitney U test. Categorical variables were assessed by Yates test or Fisher's exact test.

Results: Of the 63 patients, 49 (77.7%) were benign and 14 (23.3%) were malignant. There was a significant difference between the two groups in mass finding on mammography and posterior acoustic shadowing on US (p=0.011, p=0.049, respectively).

Conclusion: MRI is the most sensitive imaging method in post-BCS follow-up and mammography is the most specificity imaging method. The finding with the highest positive predictive value for malignancy detection is the presence of mass on mammography and posterior acoustic shadow on ultrasonography.

Keywords: Breast conserving surgery, biopsy, mammography, ultrasound, MRI

INTRODUCTION

Surgical treatment of breast cancer has evolved from radical mastectomy to breast conserving surgery (BCS). Currently, BCS with additional radiation therapy is the preferred treatment method for early breast cancer.[1-4]

Factors such as the fact that radical surgical treatments are not easily accepted by the patients, the good cosmetic results of BCS, advances in radiotherapy (RT) and systemic therapy, the increase in early diagnosis possibilities and the detection of breast cancer at an early stage play an important role in the widespread use of BCS.[1,5,6] While the rate of BCS application in our country was 25% before the 2000s, this rate reached 45% afterward.[7] However, with the more frequent use of BCS, multifocal and close/positive surgical margins requiring re-excision or mastectomy have become a current problem.[8]

The conventional follow-up imaging methods of the breast in patients undergoing BCS are mammography and ultrasonography.[9] However, after BCS and RT application, it becomes more difficult to detect with imaging methods the presence of recurrence or secondary focus due to the change of normal breast structure in patients.

Therefore, it is important for clinicians to determine the treatment approaches well, to know the sensitivity of imaging findings that may require re-excision after BCS and their values in predicting malignancy in order to reduce the cost and morbidity of such procedures.

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In this study, we aimed to investigate the sensitivity and malignancy prediction values of imaging methods (mammography, ultrasonography and magnetic resonance imaging) used in the follow-up of patients who underwent breast-conserving surgery.

METHODS

This thesis study was carried out before 2020 and since it is a retrospective clinical study, it was not necessary to take an ethics committee decision. However, the necessary permission was obtained from the hospital management to use the data. However, the necessary permission was obtained from the hospital management to use the data. A written informed consent form was approved by the each patients and necessary permissions were obtained for the use of their data. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

421 patients who were diagnosed with breast cancer and underwent BCS between December 1994 and May 2007 were retrospectively analyzed.

63 patients with a biopsied tissue diagnosis due to the presence of lesions categorized as BI-RADS 4 or 5 according to imaging findings were included in the study. The medical records of the patients included in the study (age, positive family history, time taken for the detection of lesion requiring biopsy in the follow-up after BCS) and radiological imaging findings were reviewed.

Postoperative Imaging Surveillance

After breast cancer surgery, all patients underwent follow-up examinations with imaging methods every 6 months for the first 2 years, and then annually.

Mammography examinations were performed with conventional mammography devices (Senograph 600T, General Electric Medical System; Flat SE, Metaltronica; Selenia, Hologic Inc.). All mammography examinations included images in two standard plans (mediolateral oblique and craniocaudal). Additional projections were used when necessary (lateral projection, roll graphy, spot compression, magnification).

Breast ultrasound examinations were performed with US machines (Schimatzu SDU-450, Hitachi EUB 6500, and GE Vivid 3 Pro) equipped with a matrix linear converter with a bandwidth of 5-18 MHz.

MRI examination was performed using a breast coil with a 1.5 Tesla MRI device (Signa excite HDx, General Electric, Milwaukee, WI, USA) using the following protocols: Fat suppressed TSE T1 and T2-weighted axial image, 3D FFE T1-weighted axial image, dynamic 3D FFE T1 and postcontrast 3D FFE T1-weighted axial image after 0.1 millimole/kg contrast injection. During the dynamic examination, 8 imaging (30 sec, 1 min, 1.40 min, 2.30 min, 3.30 min, 4.30 min, 5.30 min, 7 min) was performed for 7 minutes. MRI images were transferred digitally to the workstation (Advantage Windows, software version 4.4, GE Medical Systems) and the time signal intensity curves of the lesions were drawn.

Postoperative Imaging Interpretation

All imaging studies were interpreted according to the 4th edition of the Breast Imaging Reporting and Data System (BI-RADS) classification. BI-RADS category 4 or 5 was considered positive and tissue diagnosis was performed. For lesions classified as BI-RADS category 3, short-interval follow-up (6–12 months) was recommended. If the lesions were stable during the follow-up period, they were reduced to BI-RADS category 2. In case of any change, the lesions were upgraded to BI-RADS category 4 and biopsy was performed.

If a suspicious lesion was detected on MG or US, MG-guided or US-guided biopsy was performed. If a suspicious lesion was detected only on MRI, a second US was performed first. If there was a correlation on second-look US, US-guided biopsy was performed.

According to the tissue diagnosis, patients were divided into two groups as benign and malignant groups. For both groups, the presence of mass, microcalcification, focal asymmetric density and architectural distortion were evaluated on mammography. In BI-RADS mammography indication; skin and vascular calcifications, rough or popcorn type, round, rim, dystrophic, calcium milk, suture calcifications are typical benign calcifications; Amorphous, coarse heterogeneous, fine pleomorphic and fine pleomorphic branching microcalcification are stated as suspicious.[10] In our study microcalcifications were examined according to their morphology by dividing them into 3 groups (punctate, amorphous, pleomorphic). The distribution pattern of microcalcifications is also important in predicting malignancy; microcalcifications with linear and segmental distribution are the high-risk distribution pattern in terms of malignancy.[11] Therefore, microcalcifications were additionally examined by dividing them into 4 groups (regional, linear, segmental and cluster) according to their distribution regions. In US, the contour feature, shape, echo pattern, size, boundary feature and acoustic shadowing of the mass were noted. The type of contrast of the lesions was recorded on MRI.

Imaging findings were interpreted by two expert radiologists experienced in breast radiology.

Statistical Analysis

The analysis of the data was performed in SPSS for Windows 11.5 package program. Descriptive statistics and continuous variables were shown as mean ±

standard deviation, and categorical variables were shown as number of cases and (%).

Sensitivity, specificity, positive and negative predictive values and diagnostic accuracy levels were calculated to evaluate the diagnostic predictions of mammography, US and MRI indicators according to pathology.

The significance of the difference between the pathology groups in terms of age at diagnosis and mean biopsy time was evaluated with Mann-Whitney U test. Categorical variables were evaluated with Yates test or Fisher's Exact Chi-Square test. For $p<0.05$, the results were considered statistically significant.

RESULTS

The mean age of patients included in the study was 48.5±8.7 (range:28-73) years. The primary diagnoses of the patients before lumpectomy were infiltrative/invasive ductal carcinoma in 48 (76.2%), ductal carcinoma in-situ in 10 (15.8%), infiltrative/invasive lobular carcinoma in 2 (3.2%) and 2 (3.2%) patients were lobular carcinoma in-situ and one (1.6%) was a mixed invasive ductal+infiltrative lobular carcinoma. Postoperative radiotherapy was applied to all patients who underwent BCS, chemotherapy to 51 patients, and hormone therapy to 55 (87.3%) patients with hormone receptor positive. In imaging, mammography was performed in all 63 patients, ultrasonography was performed in 53 patients, and MRI examinations were performed in 22 patients.

The biopsy results of 63 patients with lesions classified as BI-RADS 4 or 5 were as follows: 22.2% (14/63) of patients had malign lesions (11 patients with infiltrative/invasive ductal carcinoma, 3 patients with ductal carcinoma in-situ) and 77.7% (49/63) of patients had benign lesions (16 patients with fat necrosis, 8 patients with fibrocystic changes, 8 patients with granulation tissue, 7 patients with sclerosing adenosis, 7 patients with atypical ductal hyperplasia, and 3 patients with fibroadenoma) (Figures 1 and 2).

Although there was a higher rate of family history in patients with malignancy (n=4/14, 28.6%) than in benign group (n=10/49 20.4%), there was no significant statistical difference between the groups. Similarly, there was no significant difference between two groups in terms of biopsy application time after BCS and age of diagnosis (Table 1).

Table 1. The relationship between the groups in terms of biopsy application time and age at diagnosis

	Benign (n=49)	Malignant (n=14)	P
Age	49.2±8.8	45.2±8.1	0.161
Family history			0.725
Yes	10 (20.4%)	4 (28.6%)	
No	39 (79.6%)	10 (71.4%)	
Postoperative biopsy time (month)	46.3±31.6	55.2±45.0	0.823

Architectural distortion, microcalcification, and asymmetric density were the most common pathologies detected in referral to biopsy on mammography (Table 2). The diagnostic accuracy measures of mammography are summarized in Table 3. While pleomorphic microcalcification had the highest specificity value (93.9%), sensitivity and positive predictive value (PPV) were found to be quite low.

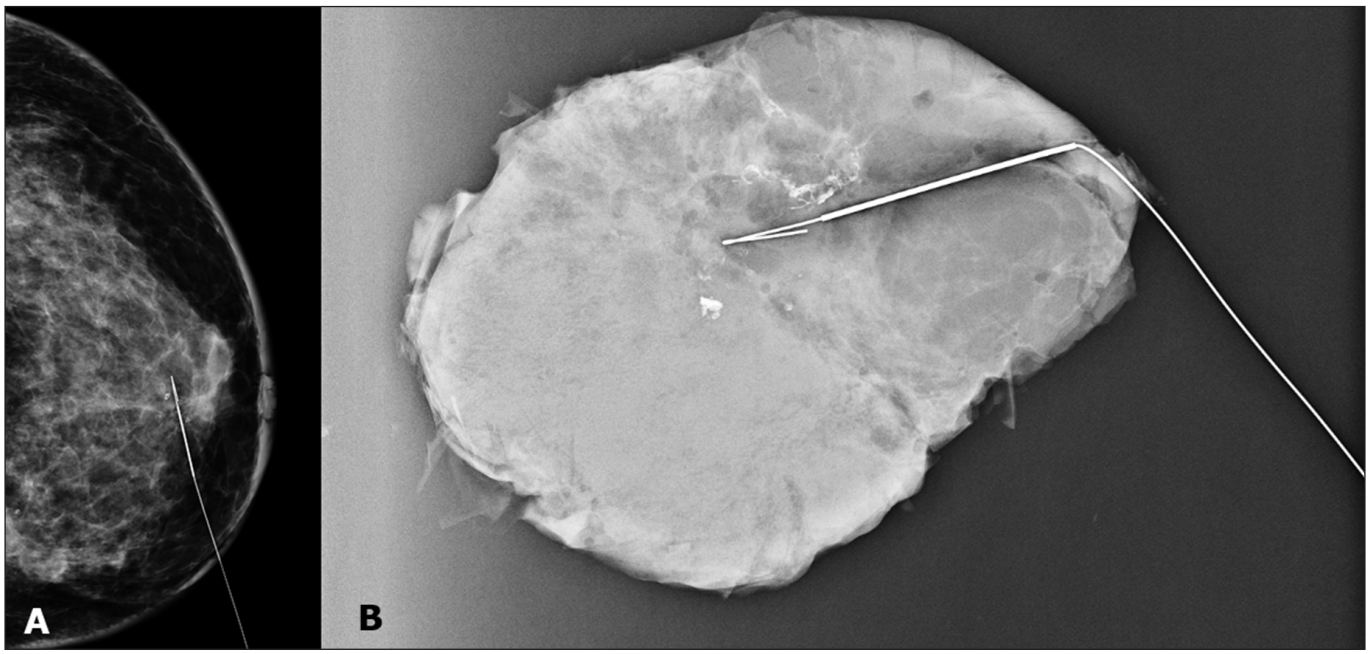

Figure 1. (A) Newly developed pleomorphic microcalcifications are observed in the lumpectomy area of a patient who underwent BCS for intraductal carcinoma. (B) The radiograph of the specimen. which was marked with wire localization. was consistent with intraductal carcinoma.

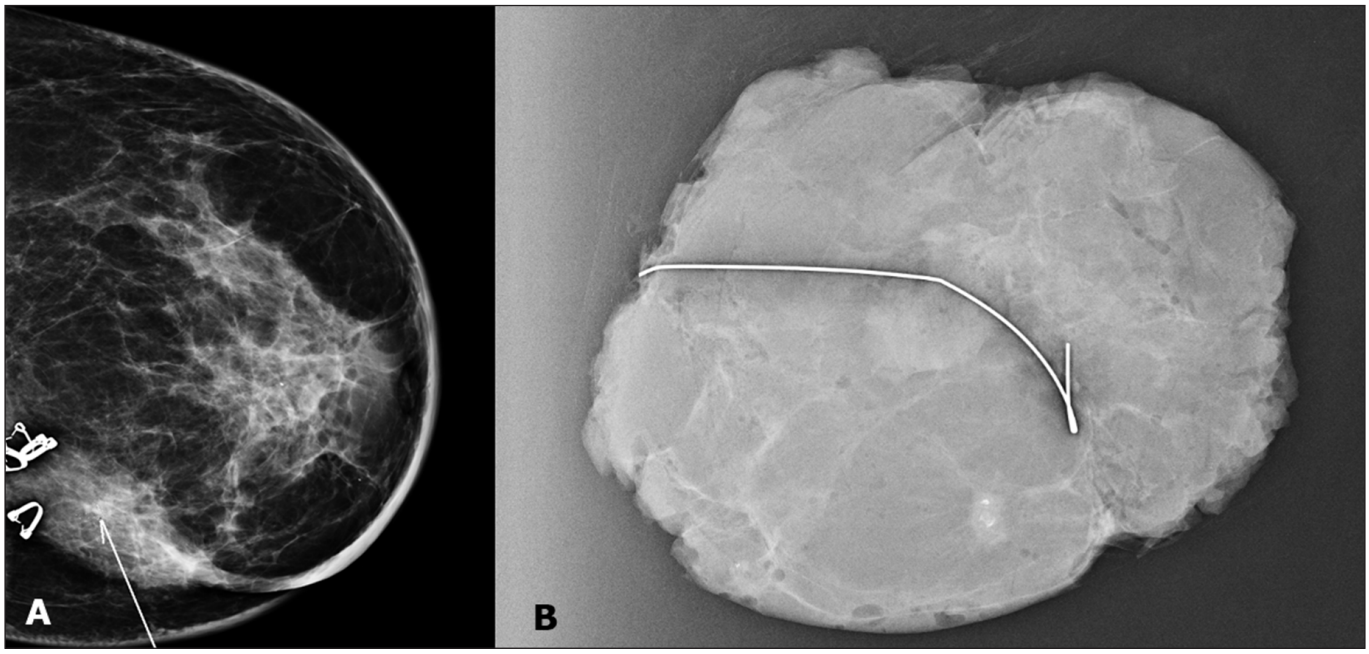

Figure 2. (A) Clustered punctate microcalcifications accompanied by asymmetrical density are observed in the lumpectomy area of a patient who underwent BCS for intraductal carcinoma. (B) The radiograph of the specimen removed by marking with wire localization was consistent with fat necrosis.

Table 2. Distribution of cases according to mammography findings

Variables	Benign (n=49)	Malignant (n=14)	p
Mass			0.011**
Yes	5 (10.2%)	6 (42.9%)	
No	44 (89.8%)	8 (57.1%)	
Microcalcification			0.110
Yes	21(42.9%)	2(14.3%)	
No	28 (57.1%)	12 (85.7%)	
Microcalcification morphology			0.122
Punctate	14 (28.6%)	1 (7.1%)	
Amorphous	4 (8.2%)	-	
Pleomorphic	3 (6.1%)	1 (7.1%)	
Microcalcification distribution			0.172
Local	3 (6.1%)	-	
Linear	2 (4.1%)	-	
Segmental	4 (8.2%)	-	
Cluster	12 (24.5%)	2 (14.3%)	
Focal asymmetric density			0.783
Yes	19 (38.8%)	6 (42.9%)	
No	30 (61.2%)	8 (57.1%)	
Structure distortion			0.298
Yes	17 (34.7%)	7 (50.0%)	
No	32 (65.3%)	7 (50.0%)	

In the comparison of the two groups according to the mammography findings, the mass finding was significantly higher in malignant cases (42.9%) than in benign cases (10.2%) (p=0.011). There was no statistically significant difference between the groups in terms of other mammography findings (Table 2).

The most common finding detected by ultrasound was the presence of a mass (n=53, 84.1%) In the comparison of both groups according to US findings, posterior acoustic shadowing was found to be significantly more common in malignant cases (46.2%) than in benign cases (15%) (p=0.049). No statistically significant difference was found between the two groups in terms of other US findings (Table 4). Diagnostic accuracy measures of US are summarized in Table 5. Posterior acoustic shadowing was found to have the highest specificity (85%), the highest PPV, and the highest accuracy.

MRI was performed as a further examination in 22 of 63 patients evaluated with mammography and US. When both groups were compared according to the contrast enhancement pattern of the lesion in MRI; Type 2-3 and type 3 contrast enhancement patterns were observed at

Table 3. Diagnostic performance levels of mammography findings according to pathology results

Findings	Sensitivity	Specificity	PPV	NPV	Accuracy
Mass	6/14 (42.9%)	44/49 (89.8%)	6/11 (54.5%)	44/52 (84.6%)	50/63 (79.4%)
Microcalcification1	2/14 (14.3%)	35/49 (71.4%)	2/16 (12.5%)	35/47 (74.5%)	37/63 (58.7%)
Microcalcification2	1/14 (7.1%)	46/49 (93.9%)	1/4 (25.0%)	46/59 (78.0%)	47/63 (74.6%)
Focal asymmetrical density	6/14 (42.9%)	30/49 (61.2%)	6/25 (24.0%)	30/38 (78.9%)	36/63 (57.1%)
Structural distortion	7/14 (50.0%)	32/49 (65.3%)	7/24 (29.2%)	32/39 (82.1%)	39/63 (61.9%)

** PPV: Positive Predictive Value, NPV: Negative Predictive Value, Microcalcification1: Microcalcification cluster or segmental distribution, Microcalcification2: Pleomorphic microcalcification

a higher rate in the malignant group than in the benign group (100%, 31.6%, respectively). However, no statistically significant difference was found between both groups (p=0.055) (Table 6). The contrast enhancement pattern in MRI was found to be the most sensitive finding in the detection of malignancy. However, the positive predictive value of malignant enhancement pattern in detecting malignancy was found to be low (Table 7).

Table 4. Distribution of cases in terms of ultrasonographic findings

	Benign (n=40)	Malignant (n=13)	P
Contour			0.667
Regular	6 (15%)	1 (7.7%)	
Irregular	34 (85.%)	12 (92.3%)	
Size			0.690
<1 cm	11 (27.5%)	4 (30.8%)	
≥1 cm	29 (72.5%)	9 (69.2%)	
Echo pattern			0.150
Homogeneous-hypoechoic	3(7.5%)	3 (23.1%)	
Heterogeneous-hypoechoic	37 (92.5%)	10 (76.9%)	
Posterior acoustic shadow			0.049**
Yes	6 (15%)	6 (46.2%)	
No	34 (85%)	7(53.8%)	
Shape			1.000
Regular	10 (25%)	3(23.1%)	
Irregular	30 (75%)	10 (76.9%)	

Table 5. Diagnostic performance levels of ultrasonography findings according to pathology results

Findings	Sensitivity	Specificity	PPV	NPV	Accuracy
Contour	12/13 (92.3%)	6/40 (15.0%)	12/46 (26.1%)	6/7 (85.7%)	18/53 (34.0%)
Size	9/13 (69.2%)	11/40 (27.5%)	9/38 (23.7%)	11/15 (73.3%)	20/53 (37.7%)
Echo pattern	10/13 (76.9%)	3/40 (7.5%)	10/47 (21.3%)	3/6 (50.0%)	13/53 (24.5%)
Posterior acoustic shadow	6/13 (46.2%)	34/40 (85.0%)	6/12 (50.0%)	34/41 (82.9%)	40/53 (75.5%)
Shape	10/13 (76.9%)	10/40 (25.0%)	10/40 (25.0%)	10/13 (76.9%)	20/53 (37.7%)

** PPV: Positive Predictive Value, NPV: Negative Predictive Value

Table 6. Distribution of cases in terms of MRI enhancement pattern

	Benign (n=19)	Malignant (n=3)	P
Enhancement pattern			0.055
Type 1-2/ type 2	13 (68.4%)	0 (0%)	
Type 2-3/ type 3	6 (31.6%)	3 (100%)	

In the classification of 63 lesions requiring biopsy according to the BI-RADS category, 6 lesions (9.5%) were BI-RADS 4A, 23 lesions (36.5%) BI-RADS 4B, 29 lesions (46.0%) BI-RADS 4C and 5 The lesion (7.9%) was evaluated as BI-RADS 5. Histopathological examination revealed that 11 (78.6%) of 14 lesions had malignant features and 23 (46.9%) of 49 benign lesions were in the BI-RADS 4C/5 category. When comparing between groups in terms of BI-RADS category, BIRADS 4A/4B lesions were higher in the benign group than in malignant patients (53.1% and 21.4%, respectively); BIRADS 4C/5 lesions were found to be higher in the malignant group than in benign patients (78.6% and 46.9%, respectively). However, no significant difference was found between the two groups in terms of BIRADS category (p=0.073) (Table 8).

Table 8. Distribution of cases in terms of BI-RADS categories

	Benign (n=49)	Malignant (n=14)	P
BI-RADS			0.073
4A/4B	26 (53.1%)	3 (21.4%)	
4C/5	23 (46.9%)	11 (78.6%)	

DISCUSSION

Most women diagnosed with early-stage breast cancer can be successfully treated with BCS. For this reason, the prevalence of use of BCS is gradually increasing. Our study is very important in terms of revealing the sensitivity of the findings of imaging methods used in the follow-up of patients with BCS and their predictive value for malignancy.

Recurrences that develop in or around the BCS bed after BCS are usually caused by failure to eradicate the primary tumor and occur within the first few years following treatment. Recurrences that develop long after surgery (10 years on average) are more likely to occur outside the BCS bed and probably indicate new metachronous cancer.[12] Ultrasonography and mammography are the basic imaging methods in BCS follow-up. Recurrences may or may not have mammographic features similar to the original lesion. Gunhan-Bilgen et al.[13] reported that 66% of recurrences had mammographic findings similar to those of primary tumors. Liberman et al.[14] in their series of 162 patients, they were reported that local recurrence was found in 13 of 20 patients (65%) with malignant mass on mammography performed after BCS. Similar results have been reported by different authors.[15,16] The findings of our study also reveal

Table 7. Diagnostic performance levels of MRI findings and BI-RADS categorization according to pathology results

	Number of cases	Sensitivity	Specificity	PPV	NPV	Accuracy
MRI						
Enhancement pattern	n (22)	3/3 (100%)	13/19 (68.4%)	3/9 (33.3%)	13/13 (100%)	16/22 (72.7%)
BI-RADS						
BI-RADS 4/5	n (63)	11/14 (78.6%)	26/49 (53.1%)	11/34 (32.4%)	26/29 (89.7%)	37/63 (58.7%)
Mass	n (63)	6/14 (42.9%)	44/49 (89.8%)	6/11 (54.5%)	44/52 (84.6%)	50/63 (79.4%)

** PPV: Positive Predictive Value, NPV: Negative Predictive Value

that the finding of a mass on mammography is the most significant parameter to predict the malignant pathology (PPV: 54.5%).

The presence of microcalcification has been reported as one of the most common morphologic criteria for biopsy in mammograms.[15,16] It is common to see new calcifications in the area where the tumor was removed after BCS. In previous studies, the predictive value of the presence of microcalcifications has been reported to be 25-35%.[17,18] Dershaw et al.[18] investigated the relationship between morphology of microcalcification and recurrence risk, reported that 68% of recurrent microcalcifications was linear, 77% of them was pleomorphic, 73% of them was cluster-forming, and 18% of them was segmental distribution. In our study, microcalcification was detected in 23 (36.5%) patients. Of these patients, 2 had malignant pathology and 21 had benign pathology. There was no significant difference between the groups in the presence and morphology of microcalcifications. According to the morphology and distribution characteristics of microcalcifications in predicting malignant pathology, PPVs were found to be 25.0% and 12.5%, respectively.

In patients who underwent BCS, mammography findings may resemble local recurrence and may hide the recurrence. For this reason, ultrasonography has been accepted as an additional imaging to mammography. Park et al.[19] in their study investigating the effectiveness of US and MG in detecting ipsilateral metachronous tumors in patients who underwent BCS, they found a similar effectiveness of US and mammography in detecting recurrence (84.2% vs. 85.7%; P=0.898, respectively). They also reported that the effectiveness of US and mammography in detecting recurrence when used together was higher than mammography. In ultrasonography, heterogeneous-hypoechoic echo pattern, indistinct contour, irregular shape and presence of posterior acoustic shadow have been reported as malignant criteria for breast mass.[20] In the ultrasonography study in which Hong et al.[21] examined 141 malignant breast masses, the PPV of the presence of posterior acoustic shadow was found to be 52.8%. Our findings reveal that the presence of posterior acoustic shadow has the highest PPV value in ultrasonography.

Due to the high sensitivity of MRI, the tendency to use it for diagnostic purposes before BCS has gradually increased. MRI is now used as the imaging of choice for surveillance evaluation of mammographically occult tumors, familial tumors, and tumor size and detection, especially in young women with dense breast tissue.[22] Additionally, in a recent randomized controlled multicenter study, the re-operation rate was compared between patients who underwent MRI in the preoperative period and patients who did not undergo MRI. The results of the study found that preoperative MRI significantly reduced the re-operation rate in women who underwent BCS.[23] Compared to traditional imaging methods such as MG and US after BCS, MRI is considered the most sensitive imaging method in distinguishing between postoperative scar and tumor recurrence.[24,25] Current data support MRI as a postoperative surveillance method with high diagnostic yield, sensitivity, and specificity for detecting recurrent cancer.[24,26-29] Gorechlad et al.[30] in their MRI study, they reported that the malignant enhancement pattern (types 2-3 and type 3) is an important finding in detecting recurrence after BCS, but the malignancy predictive value of the malignant enhancement pattern is low. In our study, when the biopsy results of 22 patients who underwent MRI were examined: In all 3 lesions with malignant histopathology, type 2-3 or type 3 contrast enhancement pattern (sensitivity:100%) was detected on MRI. Malignant histopathology (PPV:33.3%) was present in 3 of 9 lesions with a malignant enhancement pattern on MRI. A type 2-3/type 3 contrast enhancement pattern of MRI was observed in all 3 (100%) patients with malignant biopsy results and in 31.6% (6/19) of patients with benign biopsy results. However, no statistical difference was detected between the two groups in terms of malignant contrast enhancement. Our results are compatible with to the study of Gorechlad et al.[30] and show that the detection of malignant enhancement pattern on MRI in patients undergoing BCS is highly sensitive for recurrence, but the positive predictive value is low.

The purpose of surveillance in breast cancer survivors is to detect second breast cancers in the asymptomatic phase; this allows for interventions that increase chances of survival and can lead to improved quality of life. Careful clinical and imaging surveillance is required in patients undergoing BCS, as early detection of tumor recurrence will allow rapid treatment decisions that may affect the patient's prognosis.31 It is extremely important to know the sensitivity and positive predictive values of the findings in the imaging methods, since the imaging findings after treatment (BCS+RT) will differ from those of normal breast tissue.

Our study had several limitations. Its main limitation was the small sample size. Significant results can be obtained in different parameters in higher sampling groups. The second is the histopathological subtypes could not be evaluated due to the limited number of patients with malignant pathology results in our study. Thirdly, in cases that are considered benign, the absence of tissue diagnosis can be considered as a limitation. However, no progression was noted in the follow-up of these lesions. The last restriction is that the lesions were not examined by removing all of them by operation. A comprehensive

examination by evaluating the excisional biopsy results, dimensions, and histopathological subtypes of the lesions would provide more information.

CONCLUSIONS

Our study, in which we examined 63 patients who underwent biopsy and required tissue diagnosis, categorized as BI-RADS 4 or 5 according to imaging findings after BCS reveals that MRI is the imaging method with the highest sensitivity and mammography is the imaging method with the highest specificity in the follow-up after BCS. The presence of a mass on mammography and a posterior acoustic shadow on ultrasonography are the findings which have the highest positive predictive value for the detection of malignancy. Further studies are needed to better understand the diagnostic accuracy of imaging methods for screening breast lesions.

ETHICAL DECLARATIONS

Ethics Committee Approval

This thesis study was carried out before 2020 and since it is a retrospective clinical study, it was not necessary to take an ethics committee decision. However, the necessary permission was obtained from the hospital management to use the data.

Informed Consent

A written informed consent form was approved by the each patients and necessary permissions were obtained for the use of their data.

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 the health literacy in patients with stroke and relationship between health literacy and functional status on quality of life in patients with stroke

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ABSTRACT

Aims: This study was aimed to evaluate the health literacy in patients with stroke and relationship between health literacy and functional status on quality of life.

Methods: This cross-sectional study was studied on 50 participants with stroke (mean age: 59.4±10.6 years) and 50 healthy controls (mean age: 61.7±10.1 years). Clinical and demographic and characteristics were recorded. Health literacy levels of the participants were assessed with the Turkish version of European Health Literacy Scale (EHLS-TR), Motor development for hand, upper and lover extremity with Brunnstrom staging, evaluation of health-related quality of life with Notthingham Health Profile (NHP), pain with assessed with numeric rating scale (NRS).

Results: Age, gender, marital status, education, job and residential area were similar between the groups. The EHLS-TR scores were significantly lower, in patients with stroke compared to the control group (p=0.041δ). There was a statistically significant negative correlation between EHLS-TR scores, age and positive correlation with educational status. EHLS-TR scores were found to be higher in patients younger than 60 years old and university graduates. Health releated quality of life levels are similar between the groups. EHLS-TR scores were found to be statistically effective on Nottingham Health Profile (NHP) total scores (rho:-0,357 and p=0,011) and also physical activity sub domain and energy level sub domains. There was moderate negative correlation between the EHLS-TR scores and NHP energy sub domain (p=0.002, r:-0.436) and weak negative correlation between the EHLS-TR scores and NHP emotional, sleep, physical activity sub domains (r:-0.279 p=0.049, r:-0.344 p=0.015, r:-0.288 p=0.043). There was no correlation between the EHLS-TR scores and Brunnstrom staging, Functional ambulation and pain levels in patients with stroke.

Conclusion: Our study results suggest that healthy literacy level is lower in patients with stroke compared to healthy controls. In addition, healthy literacy is associated with age and education. Health literacy has been found to have an impact on quality of life and energy, emotional, sleep and physical activity subdomains of NHP. There was no association found between functional status on health literacy in this patient population.

Keywords: Stroke, health literacy, functional status, quality of life

INTRODUCTION

Stroke is one of the major causes of long-term neurological disability and functional disability. The reasons for this are that is common in the population and has gradually decreased in recent years it can be explained by mortality rates.[1-3] The consequences of stroke are usually complex and variable. This disease affects not only neurological and physical functions, but also in survivors it leads to addiction, cognitive and mental disorders in daily life activities.[4]

Health literacy is defined by World Health Organization (WHO) as individuals' health-related information defined as cognitive and social skills in which the individuals have the ability to reach, understand, and use information.[5] By the help of the high health literacy levels individuals can easily access, understand and evaluate the information and take the right steps for their health status. In the recent studies it has been shown that low or inadequate health literacy is associated with more severe health conditions and even earlier death.[6-8] For powerful stroke rehabilitation, health literacy among patients is very important. For stroke survivors, low health literacy could attribute to longer hospital stay, worse clinical health outcomes, and ineffective health education.

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Secondary prevention of stroke is crucial. Secondary stroke prevention includes managing modifiable risk factors such as smoking, dyslipidemia, hypertension unhealthy diet, and physical inactivity.[9] Hence, for the individual patient, the life after stroke may entail several lifestyle changes and new routines such as medication management, rehabilitation, and healthcare follow-ups including information and recommendations from healthcare professionals. Consequently, for people with stroke, the ability to understand and to use health information is important to prevent recurrent strokes and to regain functioning.

Improving the quality of life of patients with stroke is a priority not only for patients but also caregivers and clinicians. Improving the quality of life after stroke stands on the centre of the rehabilitation treatment. Quality of life takes into account the impact of a disease or condition and its treatments on the lives of individuals.[10] Quality of life is multidimensional. If we can understand the factors that can affect the quality of life in patients with stroke, we can produce solutions to improve these factors in rehabilitation.

The aim of this study is to evaluate the health literacy in patients with stroke and relationship between health literacy and functional status on quality of life in patients with stroke.

METHODS

This study was approved by Hitit University Medical Faculty Clinical Researches Ethics Committee (Date: 21.08.2023 Decision No: 2023-85) and written informed consent was obtained from each participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Participants

This cross-sectional study was studied on 50 patients who were diagnosed with stroke by an experienced neurologist and 50 healthy controls who were admitted to the outpatient clinic for health check-up including physical examination and hemogram measurements between July 2023 and September 2023.

Patients over 18 years old, who was diagnosed with stroke for the first time according to the criteria of the World Health Organization (WHO) who were at least 1 month after the event and would be able to understand simple verbal commands were enrolled. Those with concomitant neurological disease; other systemic diseases that affects the functional status such as severe cardiopulmonary diseases, joint contractions, amputations; severe hearing or visual impairments and mental and cognitive impairments that affects the communication were excluded.

Clinical and demographic characteristics were recorded. Age, sex, income level (minimum salary or over), educational level (elementary, secondary, or university/college) were noted. The health literacy levels of the participants were assessed with the Turkish version of European Health Literacy Scale (EHLS-TR), motor development for hand, upper and lover extremity with Brunnstrom staging, evaluation of health-related quality of life with Notthingham Health Profile (NHP), pain levels with numeric rating scale (NRS).

The health literacy level of patients were assessed with The EHLS-TR. There is 47 questions in this scale. And the total score is calculated with a formula from 0 to 50 (Formula=(arithmetic mean -1) ¥ 50/3). Health literacy is evaluated in four groups according to the total score. Excellent health literacy level: 50-42, sufficient health literacy level: 42-33, limited health literacy level: 33-25 and insufficient health literacy level: 25-0.[11]

Brunnstrom staging is a test that evaluates the motor development of hemiplegic patients. In this test, the recovery process of the hemiplegic patient is defined as 6 stages. At stage I there is no voluntary movement, flask phase and at stag VI there is isolated joint movement. The upper limb, lower limb and hand are evaluated separately. High brunnstrom stages indicate better motor development.[12]

The Turkish version of NHP was used in the evaluation of health-related quality of life. The NHP has sub-sections entitled physical mobility (pm), pain (p), sleep (sl), emotional reactions (em), social isolation (so) and energy (en) and distress (d). Each section is scored between 0-100.[13] Higher scores shows greater severity of health problems.

Pain was assessed with the numeric rating scale (NRS). Patient rated the pain on a numerical scale 0-10 (no pain-worst pain).[14]

Statistical Analysis

The data were evaluated in the statistical package program IBM SPSS Statistics Standard Concurrent User V 26 (IBM Corp., Armonk, New York, USA). Descriptive statistics were given as number of units (n), percentage (%), mean ± standard deviation, median and interquartile range values. Normal distribution of the data of numerical variables was evaluated with the Shapiro Wilk normality test. The homogeneity of variance of the groups was analyzed with the Levent test. Comparisons between two groups for numerical variables were made with independent samples t-test if the data showed a normal distribution, and with the Mann-Whitney U test if the data did not show a normal distribution. More than two group comparisons were made with Kruskal-Wallis analysis. Dunn-Bonferroni test was used in Kruskal

Wallis analysis. Chi-square analyzes (Pearson chi-square test, Continuity correction test, Fisher exact test) were used to compare groups with categorical variables. If the chi-square analysis results were found to be significant, subgroup analyzes were performed with a two-ratio Z test with Bonferroni correction. Relationships between numerical variables were evaluated with the Spearman correlation coefficient. The effect of health literacy on quality of life and functional status in stroke patients was evaluated by linear regression analysis. A value of $p<0.05$ was considered statistically significant.

RESULTS

Our study consists of a group of 50 patients diagnosed with stroke and a control group of 50 healthy people. Age, gender, marital status, education and job and residential area were similar between the groups (Table 1).

Table 1. Demographic characteristics of patient and control groups

	Patient Group (n=50)		Control group (n=50)		Test value	p value
Age(Mean± SD)	59.4±10.6		"		1.114	0.268¥
Gender (F/M)	23/27		24/26		0.040	0.841†
Married (n) Single (n)	42 8		41 9		0.001	>0.999‡
Stroke duration (month) (Mean± SD)	8.0 (12.5)		-			
Education	**n**	**%**	**n**	**%**		
Primary school Secondary school High school or higher	31 9 10	62.0 18.0 20.0	27 5 18	54.0 10.0 36.0	3.704	0.157†
Income level, n (%)	**n**	**%**	**n**	**%**		
Minimum salary > Minimum salary	25 25	50 50	21 29	42 58	0.731	0.694†
Job Worker Retired Housewife	 15 17 18	 30.0 34.0 36.0	 9 19 22	 18 38 44	2.011	0.366†
Residential Area Village District City	 5 8 37	 10.0 16.0 74.0	 10 4 36	 20.0 8.0 72.0	3.014	0.222†

n= Number, %: Column percent, Numerical data are given as mean±standard deviation or median (interquartile range) values. ¥: Independent samples t test, †: Pearson chi-square test, ‡: Continuity correction test

EHLS-TR scores of the patient group are statistically significantly lower than the control group. The number of participants with insufficient health literacy in the patient group (%58 n=29) is statistically significantly higher than in the control group (%28 n=14). According to the NHP results in terms of interference in quality of life, no significant difference was observed between the groups. When the subgroups were evaluated, it was seen that the impact was significant only in the energy category (Table 2).

Table 2. Comparison of the groups

	Groups		Test statistics	
	Patients n=50	Controls n=50	Test value	p value
Brunnstrom				
Upper extremity	4.82±1.67			
Hand	4.48±1.55			
Lower extremity	4.64±1.27			
FAS	4.06±0.84			
NRS	4.5 (4.2)			
EHLS-TR (score)	24.0 (14.3)	29.0 (12.9)	2.048	0.041δ
EHLS-TR classification (n,%)				
Insufficient	29 (58.0)a	14 (28.0)b		
Limited	9 (18.0)a	17 (34.0)a	9.839	0.020†
Sufficient	8 (16.0)a	10 (20.0)a		
Excellent	4 (8.0)a	9 (18.0)a		
NHP				
Pain	20.5 (51.5)	34.1 (60.3)	0.631	0.528δ
Emotional reactıons	33.3 (70.7)	15.7 (63.5)	1.257	0.259δ
Sleep	38.7 (77.6)	12.5 (55.9)	1.245	0.213δ
Social isolation	22.0 (48.1)	0.0 (41.3)	0.947	0.343δ
Physical activity	34.9 (67.0)	22.4 (50.1)	1.356	0.175δ
Energy level	81.6 (66.4)	24.0 (100.0)	2.820	0.005δ
Total	225.6 (298.1)	184.0 (271.6)	1.559	0.119δ

n= Number, %: Column percent, Numerical data are given as mean±standard deviation or median (interquartile range) values,†: Pearson chi-square test δ: Mann-Whitney U test, a and b superscripts indicate differences between groups at each rows. There is no statistically differences between groups with the same superscripts at each rows. FAS: Functional ambulation scale, NRS: numeric rating scale, NHP= Nottingham Health Profile, EHLS-TR: Turkish version of European Health Literacy Scale

In stroke group there was a statistically significant correlation between EHLS-TR scores, age and educational status. EHLS-TR scores were lower in patients over 60 years old than the younger ones (p=0.015). EHLS-TR scores are higher in the participant who are graduated from university than primary school graduates (p=0.009) (Table 3).

Table 3. Correlation Between EHLS-TR score demographic characteristics of patients with stroke

	EHLS-TR		Test statistics	
			Test value	p value
Groups of Age, n (%)			2.436	0.015δ
31-60	28.71	15.24		
>60	20.55	9.53		
Gender, n (%)			1.032	0.302δ
Male	22.68	12.06		
Female	24.10	16.66		
Job, n (%)			0.208	0.901η
Worker	24.37	9.93		
Retired	22.68	17.12		
Non worker	23.03	15.42		
Education Level, n (%)			9.468	0.009 η
Primary school	20.91a	8.50		
High school	31.19ab	24.93		
University	35.26b	18.69		
Marital Status, n (%)			0.754	0.458δ
Married	23.34	11.94		
Single	30.30	25.36		

Numerical data are given as median (interquartile range) values, δ: Mann-Whitney U test, η: Kruskal Wallis test, a and b superscripts indicate differences between. There is no statistically differences between groups with the same superscripts.

There was no statistically significant correlation between EHLS-TR scores and Brunnstrom staging, functional ambulation scores or pain levels. There was moderate negative correlation between the EHLS-TR scores and NHP energy sub domain and weak negative correlation between the EHLS-TR scores and NHP emotional, sleep, physical activity sub domains (Table 4).

Table 4. Correlation between EHLS-TR score and Brunnstrom, FAS, VAS, NHP in stroke patients

	EHLS-TR	
	rho	p
Brunnstrom		
Upper extremity	-0.014	0.924
Hand	-0.094	0.517
Lower extremity	0.059	0.682
FAS	0.104	0.471
NRS	-0.138	0.338
NHP		
Pain	-0.152	0.292
Emotinal reactıons	-0.279	0.049
Sleep	-0.344	0.015
Social isolation	-0.181	0.208
Physical activity	-0.288	0.043
Energy level	-0.436	0.002
Total	-0.357	0.011

rho: Spearman correlation coefficient, FAS: Functional ambulation scale, NRS: numeric rating scale, NHP= Nottingham Health Profile, EHLS-TR: Turkish version of European Health Literacy Scale

EHLS-TR scores were found to be statistically effective on Nottingham Health Profile (NHP) total scores and also physical activity sub domain and energy level sub domains. When the EHLS-TR score increases by one unit, there is a decrease of 0.955 points in NHP physical activity scores, 1.052 points in energy level scores and 4.163 points in total scores. There is no statistical effect of EHLS-TR scores on pain, emotional reactions, sleep and social isolation scores (Table 5).

DISCUSSION

In this study, health literacy level in stroke patients was found to be significantly lower than in the control group. The number of insufficient health literacy was detected significantly more in stroke patients. In our results there was a significant negative correlation between HL level and NHP emotional reactions, sleep, physical activity, energy and total scores, Stroke is a clinical syndrome characterized by focal loss of cerebral function and disruption of the complex internal circulation of the brain without any apparent cause other than vascular causes.15 Stroke ranks first in terms of frequency and importance among adult neurological diseases worldwide. Although recent studies have shown that the mortality rate has decreased, the neurological deficits it causes are still important causes of functional limitations. Stroke incidence increases with age, more common in men than women.[16,17] In our study, the number of male patients was found to be higher in stroke patients, this is probably small sample size of our study. Some studies have found a reduced risk of stroke in married people, while others have found nonsignificant differences.[18] However, there are studies that find that the risk of stroke increases in married people.[19] In our results, approximately 90% of stroke patients were married. Low levels of education and socioeconomic status have been identified as stroke risk factors. Since people with high socioeconomic status have a higher probability of accessing health resources, predisposing factors such as hypertension and diabetes can be controlled, and healthy eating habits in these people can also reduce the risk of stroke.[20,21] In our study, younger and university graduated stroke patients have higher health literacy levels but no difference was found in income levels, working status between stroke patients and the control group. In our study, 76% of stroke patients and 62% of healthy controls had insufficient or limited health literacy levels. Büyükşireci et al.[22] were found

Table 5. Evaluation of the effect of health literacy on quality of life and functional status in stroke patients with linear regression analysis

Dependent Variables	Regression Coefficients*							
	β0	β1	se for β1	zβ1	t	p	95.0% CI for β1	
							LB	UB
Brunnstrom								
Upper extremity	4.478	0.021	0.022	0.151	0.940	0.352	-0.023	0.065
Hand	4.533	0.017	0.019	0.137	0.895	0.375	-0.022	0.056
Lower extremity	4.455	0.016	0.017	0.151	0.944	0.350	-0.018	0.049
FAS	3.830	0.010	0.011	0.152	0.931	0.357	-0.012	0.033
NRS	4.875	-0.028	0.034	-0.135	-0.824	0.415	-0.097	0.041
NHP								
Pain	48.188	-0.621	0.439	-0.231	-1.415	0.164	-1.504	0.263
Emotinal reactıons	61.644	-0.623	0.479	-0.210	-1.301	0.200	-1.588	0.342
Sleep	63.329	-0.524	0.436	-0.174	-1.202	0.236	-1.402	0.354
Social isolation	43.261	-0.027	0.444	-0.009	-0.061	0.952	-0.922	0.868
Physical activity	63.674	-0.955	0.406	-0.362	-2.352	0.023	-1.772	-0.137
Energy level	104.260	-1.052	0.465	-0.323	-2.259	0.029	-1.989	-0.114
Total	388.734	-4.163	1.986	-0.308	-2.096	0.042	-8.163	-0.162

*Adjusted for age groups and education levels, β0: Constant, β1: Non-standardized regression coefficient, zβ1: Standardized regression coefficient, CI: Confidence interval, LB: Lower bound, UB: Upper bound, FAS: Functional ambulation scale, NRS: numeric rating scale, NHP= Nottingham Health Profile.

higher health literacy levels in patients with fibromialgia in Turkish population. Compared to this study, patients with stroke had a lower health literacy level. In our study patients are older and have more comorbit diseases this may be affect the health literacy levels.

Risk factors for stroke are divided into two groups: modifiable and non-modifiable risk factors. Modifiable risk factors include hypertension, diabetes, unhealthy diet, low socioeconomic and educational level, lack of physical activity, smoking and alcohol use.[23,24] Health literacy involves obtaining, understanding, and using health information to make appropriate health decisions and follow treatment instructions.[25] Although there is no clear study explaining the relationship between stroke and HL, reducing certain modifiable risk factors with the level of HL may establish a strong link between stroke and HL.[26] Additionally, high HL levels are important for successful stroke management.[27] In a study by Pien et al.[28] low HL levels were found to be higher in patients with a history of stroke. Jeong et al.[29] showed that HL levels are an important factor affecting health status in stroke patients. Sanders et al.[30] showed that 59% of post-stroke patients had inadequate or marginal health literacy. In our results, HL level was found to be significantly lower in stroke patients. In addition, the number of insufficient HL was found to be significantly higher in stroke patients than in the control group.

The social, physical and psychological consequences of stroke cause social and economic burden all over the world. It is considered a global health problem due to impaired quality of life.[31] Health literacy is an important factor in stroke prevention, management and rehabilitation. The American Heart Association clearly recommends that patients with a history of stroke learn the risk factors for this disease, warning signs, emergency medical aid systems, and information through post-treatment follow-up education.[32] In the study of Flink et al.[33] a close relationship was observed between post-stroke health literacy level and quality of life 12 months later. In a study conducted by Pien et al.[28] in 7702 patients, a negative relationship was found between low HL level and quality of life in patients with a history of stroke. In a cross-sectional study in elderly the level of health literacy affects the level of quality of life.[34] According to our study results, a negative relationship was found between HL levels and NHP total scores. Regardless of the stroke patients functional status as health literacy increases quality of life increases. This is important because even if the patients functional status does not improve, improving the health literacy will improve quality of life.

Few studies have assessed the association between health literacy and quality of life after stroke. As our knowledge this is the first study evaluating the health literacy level and association between health literacy and functional status or quality of life in patients with stroke in Turkish population.

In patients with stroke it is important to follow up the rehabilitation program not only for the improvement of the functional status but also adaptation to the social life and mental health. High health literacy levels helps to understand the importance of treatment strategies, rehabilitation programmes and improving self-care behaviours. As the level of health literacy increases, so does the level of quality of life. It is recommended to design policies that will help to increase the health literacy of the patients with stroke.

Limitations

In this study we had a relatively small sample and the majority of the patients have mild to moderate stroke. Persons who could not provide informed consent, because of severe aphasia and/or cognitive impairment was excluded from the study. More studies needed to explore associations between health literacy and relevant clinical outcomes in people with more severe symptoms after stroke. In the future well designed larger studies help us to firm a conclusion after stroke to explore the underlying reasons for these associations and other plausible variables of importance.

Sample Size

According to the results obtained from the power analysis conducted with the G* Power program[35] based on the mean±standard deviation values of the health literacy level scores obtained from the study to Huang et al.[36] the sample size was calculated as 50 patients in each group.

CONCLUSION

Our study results suggest that healthy literacy level is lower in patients with stroke compared to healthy controls. In addition, healthy literacy is associated with age, education and quality of life in store patients. There was no association between functional status and health literacy in stroke patients.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Hitit University Medical Faculty Clinical Researchs Ethics Committee (Date: 21.08.2023 Decision No: 2023-85).

Informed Consent

Written informed consent was obtained from all participants who participated in this 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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The evaluation of potential toxic metal levels of various drugs used in children

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ABSTRACT

Aims: Medicines have been widely used in recent years to support the immune system. Exposure to toxic metals can occur at different stages, such as raw material procurement, production, and packaging of drugs designed to support the immune system. This exposure can lead to serious health problems. In this study, the aim was to determine the levels of toxic metals in drugs used to support the immune system in children.

Methods: Ten drug samples, available in pharmacies and intended for strengthening the immune system in children, were collected. Preliminary processes were conducted for the ICP-MS analysis of these collected drugs. Following the initial preparation, levels of Arsenic (As), Copper (Cu), Zinc (Zn), Manganese (Mn), Selenium (Se), Chromium (Cr), Mercury (Hg), Lead (Pb), Cadmium (Cd), Tin (Sn), Cobalt (Co), Aluminum (Al), Molybdenum (Mo), Antimony (Sb), Nickel (Ni) were determined using an ICP-MS device.

Results: In our study, we evaluated the levels of toxic metals in drugs used to support the immune system. The average levels of Cr, Zn, As, Se, Cd, and Sn in the samples were found to exceed the limits set by international organizations. However, the average levels of Al, Cu, and Hg were very close to these limit levels. On the other hand, the levels of Mo, Sb, and Pb in the analyzed products were well below the established limits.

Conclusion: The levels of toxic metals in immune-supporting drugs can lead to toxicity when the results of the study are evaluated. To inform consumers and safeguard their health, it would be appropriate for manufacturers to include information about the daily intake limits set by international organizations for toxic metals and trace elements. Conducting toxicological tests, especially during these inspections, will greatly contribute to protecting the health of children and fostering the development of healthy generations.

Keywords: Drug, child, toxic metals, immune system

INTRODUCTION

To support the immune system, it is used in immune-supportive drugs and food supplements. The licensing process for food supplements is carried out by the Ministry of Agriculture and Forestry.[1] As with other drugs, the licensing process for drugs that support the immune system is carried out by the Turkish Medicines and Medical Devices Agency. The legal definition has been established as follows: "Pharmaceutical product for human use (medicine): A substance or combination of substances that are presented as having therapeutic or preventive properties for human disease, or that are used or administered to humans for the purpose of correcting, improving, or changing physiological functions or medical diagnosis by showing pharmacological, immunological, or metabolic effects".[2]

For a product to be licensed as a drug, it must pass numerous tests. Additionally, advertising of a drug-licensed product is prohibited.[3] From this perspective, it is quite natural that food supplements, licensed as immune system supporters and easily advertised, are widely used by society. However, the use of drugs that support the immune system by physicians for pediatric patients is also quite high. In the USA, 60% of the public uses the 55,000 immune system supplements available in the market. About 50% of people in Europe and 71% in Canada use natural herbal products.[4–6] In another study, it was reported that children constituted 9% of the immune system supporters consumed in the United States. About one-third of infants, children, and adolescents in the United States use immunosuppressants.[7,8] It consists

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of vitamins and vitamin-mineral complexes, and it is among the most commonly used immune system supporters in Turkiye.[9] Immune system boosters are products marketed to protect or improve health, and they can also be used to reduce disease symptoms or treat diseases.[10] However, numerous studies demonstrate that immunosuppressants can lead to both acute and chronic toxicity.[11] Cases of poisoning with toxic metals found in medicinal plants used as immune system boosters have been reported in the USA, Europe, and Asia.[12–15]

In fact, some of the metals evaluated are essential for the body and are included in products that support the immune system. However, these metals are assessed due to the potential harm caused by their high levels. ATSDR, a federal public health agency under the U.S. Department of Health and Human Services, aims to protect the public from health problems that may arise from exposure to natural and man-made hazardous materials. Additionally, ATSDR conducts research on the health effects of hazardous waste sites and provides actionable guidance to address environmental health threats.[16] USP is an independent, scientific, non-profit organization focused on building trust in the supply of safe and quality medicines. It was established to strengthen the global supply chain, ensuring that medicines, which people trust for their health, are ready when needed and working as expected.[17] The Turkish Food Codex has been prepared by the Ministry of Agriculture and Forestry to ensure consumer and human health, protect consumer rights, ensure justice in food sales, and promote environmental protection where appropriate.[18]

The increasing environmental pollution resulting from industrialization and urbanization, along with soil pollution, has reached levels that can pose a danger to living things. Environmental and soil pollution can cause contamination in food sources through the food chain. This situation has the potential to create significant health problems for people.[19]

One of the most significant threats to human health is toxic metals.[19] oxic metals can enter the human body through various pathways, including food, respiratory exposure, or skin contact. They are known to cause adverse health effects and, in severe cases, can lead to death.[20] It is observed that human activities have a more significant impact than natural cycles in the spread of toxic metals into the environment. These metals, transmitted to the environment in various ways, can further spread to grains, animals fed with contaminated grass, milk and meat, fish caught from polluted waters, or tools and equipment used during food production.[19] With the increase in industrialization, the risk of drug contamination with toxic metals is rising day by day.

Due to the COVID-19 pandemic, drugs with antimicrobial and immunomodulatory activities are considered promising therapeutic support for preventing viral spread. This has led to physicians prescribing these drugs, which are frequently used by parents to protect the health of their children.[21] There is a possibility that our children may experience health problems due to the presence of toxic metals in immunosuppressive drugs used to protect their health. This situation emerges as a significant problem that can threaten public health.

This study was conducted to determine the levels of toxic metals, which can pose serious threats to human health, especially in drugs used to support the immune system in children. The aim is to provide information about the residue status of these drugs.

METHODS

Supply of Immune System-Supporting Drugs

Immune system-supporting drugs from commercial companies were obtained from pharmacies in Yozgat. These products were numbered, and subsequent analysis and evaluation processes were conducted based on these numbers. Our study did not receive ethical committee approval since it does not fall within the scope of "Regulation on Clinical Research of Medicinal and Biological Products," "Guideline on Good Clinical Practice," and "Yozgat Bozok University Clinical Research Ethics Committee Regulation."

Preliminary Preparation of Samples

The preliminary preparation of the samples was carried out by the method developed by Türksoy et al.[22] in the Yozgat Bozok University Faculty of Medicine Research Laboratory. The samples were dried in a polystyrene petri dish in an incubator at 75°C for 24 hours. The samples collected from the incubator were weighed to determine the amount of dry matter. Afterwards, the samples were placed in 50 ml polypropylene tubes. The preparation of samples was performed by treating the samples with 2 ml of hydrogen peroxide (H_2O_2) and 7 ml of suprapur nitric acid (HNO_3). The prepared samples were filtered and taken into 15 ml polypropylene tubes for analysis by inductively coupled plasma mass spectrometry (ICP-MS). The prepared samples were stored at 4°C until the date of ICP-MS analysis.[22]

Determination of Toxic Metal Levels in Drugs Used to Support The Immune System With ICP-MS

ICP-MS (Thermo Scientific, ICAPQc, USA) system was used to determine the levels of Arsenic (As), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se), chromium (Cr), mercury (Hg), lead (Pb), cadmium (Cd), tin (Sn), cobalt (Co), aluminum (Al), molybdenum

(Mo), antimony (Sb), nickel (Ni) in the samples. For this, the method developed by Türksoy et al.[22] was used. The operating parameters were set as follows: RF power 1550 W, nebulizer gas flow 0.90 L,/min, nebulizer pressure 3.08 bar, plasma gas flow 0.80 L/min, dwell tie 0.01, spray chamber temperature 3.01. The probe of sampler was washed between injections for rinsing. Rinsing of the probe firstly washed 30s with ultrapure water, then 45s with 2% HNO_3 , followed by washing 45s with ultrapure water. A total of 11 point calibration curves (quanitative mode linear calibration; R^2>0.99 with interval of calibration 0.5-1000 µg/g) were drawn for each toxic metal and the results were evaluated according to these calibration curves.

Statistical Analysis

Data analysis was performed using the IBM SPSS 23.0 package program. Parametric tests were used for ordered data and non-parametric tests were used for non-ordered data. Descriptive statistics were shown with mean, standard error, and minimum-maximum values. Evaluation of data in terms of toxicity was done with reference values of Turkish Food Codex Contaminants Regulation, Agency for toxic substances and disease registry (ATSDR) and United States Pharmacopeia (USP).[16,17,23] The Kolmogorov-Smirnov test was used to determine whether the data were distributed normally. Spearman Correlation Analysis was applied in order to determine the relationship between the data. p<0.05 was considered significant.

RESULTS

In our study, we evaluated the toxic metal levels in 10 drugs used to support the immune system. Arsenic (As) was detected at 0.237 µg/g in one of our samples, with a mean level of 0.053 µg/g. The levels of Chromium (Cr) and Cobalt (Co) exceeded the daily intake limits determined by ATSDR in 10% of the samples, while Mercury (Hg) exceeded the limit in 20%. Zinc (Zn), Arsenic (As), Selenium (Se), and Cadmium (Cd) exceeded the daily intake limits in 100% of the samples in our study.[16] The toxic metal levels detected in the immune system-supporting drugs, the daily allowable intake limits accepted by various organizations, and the maximum toxic metal levels that should be found in immune system supporters are presented in **Table 1**.

The levels of toxic metals detected as a result of the analysis of drugs used to support the immune system are given in **Figure 1**.

Table 1. Toxic metal levels in drugs used to support the immune system

	N	Mean (µg/g)	Standard error	Minimum	Maximum	Daily allowable limits (µg/day)	Accepted limits µg/g
Al	10	0.037	0.042	0.001	0.119	1λ	
Cr	10	0.004	0.002	0.003	0.009	0.0005λ 150*	
Mn	10	0.189	0.155	0.126	0.630		1500**
Co	10	0.014	0.008	0.009	0.035	0.03λ	
Ni	10	0.002	0.002	0.002	0.007		
Cu	10	0.010	0.004	0.007	0.018	0.02λ 1000* 300†	100**
Zn	10	0.626	0.192	0.377	1.04	0.3λ 15000* 6000†	1500**
As	10	0.053	0.067	0.007	0.237	0.005λ 1.5* 10†	0.020* 0.15**
Se	10	0.041	0.028	0.021	0.110	0.005λ 250*	25**
Mo	10	0.003	0.003	0.002	0.011	0.06λ 100*	10**
Cd	10	0.005	0.004	0.004	0.016	0.0005λ 25* 6†	1* 2.5**
Sn	10	0.003	0.002	0.001	0.006	0.0003λ	50*
Sb	10	0.001	0.0004	0.001	0.002	1λ	
Hg	10	0.001	0.001	0.0004	0.003	0.002λ 15* 3.42†	0.10* 1.5**
Pb	10	0.034	0.030	0.002	0.073	5* 21.4†	3* 0.5**

* Turkish Food Codex Contaminants Regulation.[47]
** Maximum metal levels accepted in Tablets according to the US Pharmacopoeia (USP) (µg/g).[48]
⁕ Allowable daily exposure according to the US Pharmacopeia (USP) (µg/day).[48]
† WHO/FAO: Allowed daily intake calculated using 10% for 60 kg body weight and dietary supplements (µg/day).[48]
λ ATSDR (minimal risk seviyesi) göre izin verilen günlük maruziyet miktarı (µg/g /day).[49]

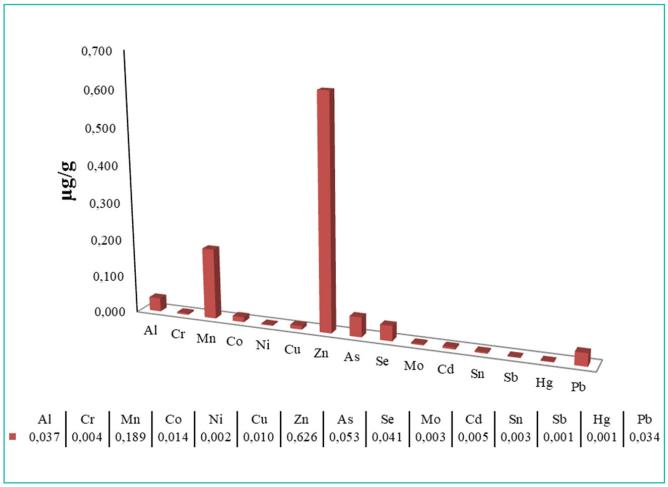

Figure 1. Toxic metal levels in immune system supplements sold as drugs

As a result of our study, the mean and standard error values of the Al, Cr, Sn, Co, Zn, Mn, Cu and Se levels detected in the drugs used to support the immune system, together with the values determined to be more or less than the mean values, are given in Figure 2. Excess values of these metals were detected in 1 sample each for Co, Mn and Cu, and 2 samples each for Cr and Se in the studied immune system supporters.

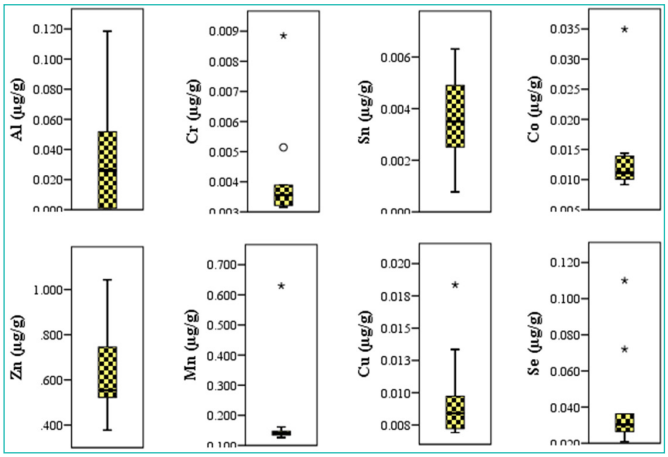

Figure 2. Aluminum (Al), Chromium (Cr), Tin (Sn), Cobalt (Co), Zinc (Zn), Manganese (Mn), Copper (Cu) and Selenium (Se) values in immune system supporting products sold as drugs . "*" and "o" indicate extremely high values and "⊤" indicates standard error. "▩" indicates immune system boosters sold as drugs.

The mean and standard error values of As, Cd, Hg, Pb, Sb, Mo and Ni levels detected in drugs used to support the immune system, as well as values that were determined to be more or less than the mean values are given in Figure 3. Levels of 1 each for As, Cd, Sb, Mo and Ni and 2 samples for Hg were well above the average values.

It was determined that the limit set by ATSDR was exceeded in 10 samples for Zn, Se, Cd, and As, 2 samples for Hg, and 1 sample for Cr and Co samples exceeding the limits set for Zn, Se, Cr, Cd, As, Hg and Co are given in Figure 4.

The differences between toxic metal levels from immune system boosters are described in Table 2. The positive correlations were found between Cr and Mn, Co, Cu, Se, and negative correlation was found between Cr and Pb (respectively; r=0.721; r=0.697; r=0.648; r=0.661; r=-0.661; p<0.01). On the other hand positive correlations were found with Mn and Se, Mn and Mo, Co and Ni, Co and Hg (respectively; r=0.867; r=0.733; r=0.794; p<0.01; r=0.648; p<0.05). However a positive correlation was too detected between Ni and Sn, Ni and Hg, Mo and Cd (respectively; r=0.721; r=0.661; r=0.733; p<0.05).

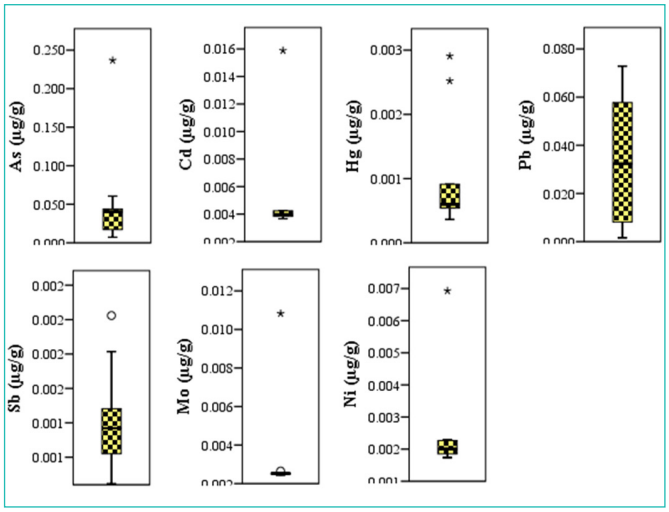

Figure 3. Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb), Antimony (Sb), Molybdenum (Mo) and Nickel (Ni) values in immune system support products sold as drugs. "*" and "o" indicate extremely high values and "⊤" indicates standard error. "▩" indicates immune system boosters sold as drugs.

Figure 4. Samples exceeding the specified limit for Zinc (Zn), Selenium (Se), Chromium (Cr), Cobalt (Co), Mercury (Hg), Cadmium (Cd) and Arsenic (As). Limit: Allowed daily exposure amount (µg/g/day) according to minimal risk level of ATSDR

Table 2. Relationship between toxic metals in immune system supplements sold as drugs

	Cr (µg/L)	Mn (µg/L)	Co (µg/L)	Ni (µg/L)	Cu (µg/L)	Zn (µg/L)	As (µg/L)	Se (µg/L)	Mo (µg/L)	Cd (µg/L)	Sn (µg/L)	Sb (µg/L)	Hg (µg/L)	Pb (µg/L)
Al (µg/L)	-0.129	-0.276	0.104	0.276	-0.399	-0.62	-0.534	0.031	-0.08	-0.264	-0.043	0.092	-0.252	-0.141
Cr (µg/L)	1.000	0.721*	0.697*	0.37	0.648*	0.103	0.03	0.661*	0.358	0.212	0.127	-0.358	0.406	-0.661*
Mn (µg/L)		1.000	0.467	0.224	0.43	0.248	-0.018	0.867**	0.733*	0.43	-0.152	-0.442	0.406	-0.539
Co (µg/L)			1.000	0.794**	0.515	-0.067	-0.03	0.6	0.103	-0.285	0.564	-0.115	0.648*	-0.261
Ni (µg/L)				1.000	0.406	0.018	0.127	0.515	0.03	-0.188	0.721*	-0.03	0.661*	0.042
Cu (µg/L)					1.000	0.503	0.103	0.515	-0.103	0.103	0.394	-0.394	0.37	0.018
Zn (µg/L)						1.000	0.345	0.273	0.224	0.442	-0.091	-0.261	-0.006	0.091
As (µg/L)							1.000	-0.079	0.091	0.285	0.442	-0.236	0.188	0.273
Se (µg/L)								1.000	0.6	0.309	0.042	-0.588	0.297	-0.345
Mo (µg/L)									1.000	0.733*	-0.479	-0.382	0.103	-0.612
Cd (µg/L)										1.000	-0.479	-0.515	-0.127	-0.345
Sn (µg/L)											1.000	0.055	0.576	0.491
Sb (µg/L)												1.000	0.309	-0.018
Hg (µg/L)													1.000	-0.103

DISCUSSION

In this study, we evaluated the immune-supporting drugs that are most commonly used in the market. Drugs containing toxic metals, organic solvents, and various toxins can cause damage to many systems, especially the nervous system.[24] When the level of heavy metals in our body exceeds permissible limits, it can lead to serious health problems. Elevated levels of heavy metals such as Pb, Cr, As, Hg, Ni, and Cd beyond the permissible limits can cause hepatotoxicity.[25] In their study (Gravey et al.[11]), stated that there are toxic metals in medicinal plants used as immune system supporters and this will adversely affect people's health. In another study (Dunbabin et al.[14]), reported that lead poisoning occurred due to Indian herbal medicines used as immune system supporters.

In our study, the mean value of As in the samples was determined to be 0.053 µg/g, which significantly exceeded the level accepted in the Turkish Food Codex Contaminants Regulation of 0.020 µg/g.[18] However, the As level detected in all samples exceeded the limits set by the ATSDR.[16] In a study of acute As poisoning in swine, symptoms such as vomiting, diarrhea, colic, dehydration, collapse, convulsions, and death were observed within a time period ranging from hours to days. This study revealed a one-tenth difference between the administered dose and the concentration of As in liver and kidney tissue.[26]

Cadmium is a heavy metal that lacks physiological function and is generally considered toxic. The biological half-life of Cd in humans is approximately 20-30 years. Cd exhibits various toxic effects, including nephrotoxicity, carcinogenicity, teratogenicity, as well as endocrine and reproductive toxicities. At the cellular level, cadmium influences cell proliferation, differentiation, apoptosis, and other cellular activities.[27] One of the negative effects of Cd exposure in children is osteoporosis. Due to the limited excretion of Cd, children may experience the accumulation of this metal in their bodies. Afterward, osteoporosis and an increased risk of fractures emerge when the child becomes an adult.[28] Sherief et al.[29] associated Cd exposure with pediatric cancer in their study. When cadmium levels in nails, hair, urine and serum of cancer patients and healthy people were measured by Atomic Using an Absorption Spectrophotometer, it was determined that children with cancer had higher cadmium levels than children without cancer. In our study, it was found that the level of Cd exceeded the limit set by ATSDR in all samples. When the results of our study and the existing literature are evaluated together, it suggests that parents using immune system supporters to protect their children's health may inadvertently expose them to the risk of osteoporosis and even cancer.

Hg is a toxic heavy metal commonly found in nature. It provides no physiological benefit to either children or adults. Hg has teratogenic, carcinogenic, and mutagenic effects, as well as cardio-, nephro-, and immunotoxicity.[30] Hg accumulates in various parts of the human body; therefore, the range of symptoms caused by Hg is quite wide. These symptoms include fatigue, anxiety, depression, paresthesias, weight loss, memory loss, and difficulty concentrating.[31] Prenatal Hg exposure is associated with decreased IQ scores and impaired performance on tests of memory, attention, language, and spatial cognition.[30] In a study conducted in the USA, it was stated that industrial Hg exposure decreased the IQs of 300.000-600.000 US children.[32] In our study, the sample rate above the limit value set by ATSDR for Hg was determined as 20%. Considering the mutagenic and carcinogenic effects observed in children after Hg exposure, it can be concluded that the use of immune system supporters may potentially lead to such toxic symptoms in children in the future.

Pb enters the human body through the food chain, including food and water. It accumulates in the kidneys, liver, brain, bones, and other organs.[33] Oxidative stress and inflammation occurring after Pb exposure can cause cell damage.[34,35] Pb exposure increases the risk of developing neurodegenerative diseases especially in childhood.[36] The Scientific Committee on Neurotoxicology and Psychophysiology and The Scientific Committee on The Toxicology of Metals of The International Commission on Occupational Health in 2006 recommended that blood lead level should be below 5 µg/dl in children.[37] In our study, the Pb value was well below the level accepted in the Turkish Food Codex Contaminants Regulation, at 3 µg/g.

Although Cr is an essential metal for the human body, it is also recognized as a potent human carcinogen by the International Agency for Research on Cancer (IARC). There are epidemiological studies of Cr-induced cancer development among industrially exposed workers.38 In one of the samples in our study, Cr values were found above the limit set by ATSDR. This situation can be considered as an indication that those who use these products to support the immune system may face cancer risk in the future.

Se is an essential trace element with both beneficial and adverse health consequences as a component of major metabolic pathways, including the antioxidant defense system, DNA synthesis, and immunological functions. Se deficiency has been associated with many diseases such as cancer, immune deficiency, hormone synthesis disorders, and neurodegenerative diseases including Alzheimer's and Parkinson's disease.[39–42] However, selenium is toxic in high concentration. Se toxicity can be classified to acute or chronic. Acute poisoning typically involves a single dose that produces symptoms within minutes, while chronic poisoning involves smaller doses given repeatedly that produce symptoms that become evident within days or longer.[43] In a study, Gardiner stated that chronic Se exposure causes Se accumulation in the liver and kidneys of sheep, and as a result of the 72-week study, 5 out of 9 sheep died.[44] The Se level detected in all of the samples was determined above the limit set by ATSDR.

Zn is an essential trace element that the body uses in many ways. Zn functions as a component of 200-300 enzymes to assist the most important metabolic pathways in the body responsible for structural, catalytic and biochemical functions. It is also thought to have an important role in cell proliferation in the body and in Zn hemostasis of the thyroid·45 Zn is included in the composition of many immune-supporting drugs and food supplements. Zn toxicity has been rarely reported in childhood. As a result of excessive Zn intake, nausea, vomiting, loss of appetite, diarrhea and headache may occur.[46] In our study, it was determined that the limit value set by ATSDR for Zn was exceeded in all of the samples.

CONCLUSION

The aim of our study is to unveil the status of toxic metal levels in immune-supporting drugs, the usage of which has increased in recent years, particularly with the pandemic. It's important to note that our goal is not to disparage immune system-supporting drugs or the industry. Our findings indicate that those who use these products for health protection may be exposed to varying levels of toxic metals, as we have detected certain rates of these metals and trace elements that can lead to toxicity if consumed excessively. To inform consumers, we suggest that manufacturers consider adding the daily intake limits set by international organizations for metals and the metal levels in their products to product labels (prospectus). However, we emphasize the need for more effective legal regulation and supervision in the supply of raw materials, production, and marketing of immune system-supportive drugs. This approach is essential to prevent potential harm to individuals who use these products for health protection. Conducting toxicological tests, especially during these inspections, will significantly contribute to safeguarding the health of children and fostering the development of healthy generations

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethics committee approval is not required for this study.

Informed Consent

Informed Consent is not required for this 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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Latent tuberculosis infection in psoriasis patients

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ABSTRACT

Aims: Psoriasis is a chronic, inflammatory, and life-long skin disease. Patients may need to change the treatment regimen by time due to the course of the disease. According to the guidelines, patients should be screened for latent tuberculosis infection (LTBI) before starting treatment with biological agents. We aimed to evaluate the associations between positive interferon-gamma release assay (IGRA) tests, the chest CT findings and inflammatory blood markers of the psoriasis patients who have undergone screening for LTBI before starting systemic treatment with biological agents or conventional options.

Methods: The electronic medical records, Chest CT reports and blood tests of 123 consecutive patients with a diagnosis of psoriasis who were candidates for systemic treatment (methotrexate, cyclosporin and biological agents) and screened for LTBI were examined.

Results: The mean age of the patients was 49.24 and 64 (52%) of them were males. 37(30%) had a family history of tuberculosis 103(83%) of them had BCG vaccination scars. 59% had radiological features on their Chest CT scans. 28% of the patients had positive Quantiferon test results. When compared to the Quantiferon negative group, there was no difference between the two groups according to demographic characteristics, comorbidities, family history of tuberculosis, BCG vaccination status, smoking habits, occupation, and qualification details ($p>0.05$). The values of WBC, neutrophils, lymphocyte, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, systemic inflammation index and erythrocyte sedimentation rates were found statistically higher in the patients with positive Quantiferon test ($p<0.05$).

Conclusion: The patients with psoriasis requiring systemic treatment and having positive IGRA test results have increased levels of inflammation. Psoriasis and LTBI might have a synergistic action in the inflammatory response which necessitates further studies to find out the associations between these two entities.

Keywords: Latent TB, psoriasis, quantiferon, lung, chest CT

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease with a complex pathogenesis, that may cause an impairment in a patient's quality of life.[1] The disease is most likely as old as mankind. Hippocrates used the word "psora" for the itchy lesions on the skin.[2] During the Roman Empire, Galen was the first physician who use the term "psoriasis".[2] D. Turner described the disease and tried to apply some treatment modalities containing mercury.[3]

Today, conventional systemic treatment with acitretin, methotrexate, cyclosporine, and biologics are the options for therapy in moderate to severe psoriasis.1 Biological agents are a new era in treating psoriasis patients, bringing a marked improvement in managing the disease. However, as the other systemic treatment agents do biologics also include the risk of *Mycobacterium tuberculosis (M. tuberculosis)* infection reactivation as a severe complication, which leads to problems in a high-burden country for tuberculosis.[3]

Mycobacterium tuberculosis is the infectious disease that causes tuberculosis (TB). The TB etiologic agent most likely existed before humans evolved on Earth.[4] It evolved into an uncommon endemic illness in humans around the time that they started to establish villages and advance agriculture. Robert Koch discovered the microscopic cause only in 1882.[4] According to the Global Tuberculosis Report, about a quarter of the world's population is infected with MTB.[5] In Turkiye, with the efforts of the National Tuberculosis Control Program, the number of TB patients decreased from 20,535 in 2005 to 10,600 in 2020.[6]

A considerable portion of the population, particularly in areas with high endemicity, has latent tubercle bacilli, which act as a reservoir for active tuberculosis depending on the host conditions.[7] *M. tuberculosis* exposure is a frequent cause of latent TB infection

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(LTBI), which has a 5-10% lifetime probability of evolving into active TB, with most TB cases occurring within the first two years of infection.[7,8] The diagnosis and treatment of *M. tuberculosis*-infected individuals who would otherwise be at a high risk of acquiring and transmitting active disease is crucial for the complete eradication of tuberculosis according to the End TB strategy that was adopted by the World Health Assembly.[7]

Therefore, it is a firm requirement for patients to be screened for LTBI who are candidates for either biological agents or conventional therapeutic options. According to WHO guidelines, LTBI is characterized as a condition of sustained immunological response to *M. tuberculosis* antigen stimulation without signs of clinically manifested active TB.[9] The diagnosis of LTBI is currently made using either a positive interferon-gamma release assay (IGRA) or a reactive tuberculin skin test (TST). The activity of the infectious focus or the likelihood of developing into active TB is not determined by any diagnostic test.[7] Quantiferon-TB Gold Plus (QFT-Plus) is a T-cell interferon-gamma release assay test, which is one of the diagnostic tools used for LTBI and provides some practical advantages in a population that has received bacilli Calmette-Guerin (BCG) vaccinations.[10] IGRAs are not utilized to diagnose active tuberculosis because they are unable to differentiate between LTBI and active TB.[7] The assessments are based on patient and household anamnesis of past history of TB infection, chest X-rays, and chest computed tomography (CT) findings in some cases where required, as it is crucial to assess the presence of previous exposure to TB infection prior to treatment with methotrexate, cyclosporin, and biological agents.

The objective of our study was to investigate the associations between IGRA tests, the chest CT findings and inflammatory blood markers of psoriasis patients who have undergone screening for LTBI before starting systemic treatment with either biological therapy or other conventional agents.

METHODS

The study was conducted in a single center after the Health Science University Şişli Hamidiye Etfal Training and Research Hospital Clinical Researches Ethics Committee approved it (Date: 21.02.2023, Decision No: 2248). The medical records of 123 consecutive patients with a diagnosis of psoriasis who were candidates for systemic treatment (methotrexate, cyclosporin and biological agents) and consulted for evaluating LTBI in the Chest Diseases Outpatient Department between 2019 and 2023 were retrospectively examined. Patients were referred from the department of dermatology and the diagnosis of plaque psoriasis was based on clinical symptoms and histopathological evaluations. The psoriasis area severity index (PASI) score was utilized for evaluating the severity of psoriasis.

The patients who had QFT-Plus tests resulting in negative or positive but not indeterminate, who had blood tests (complete blood count and blood biochemistry) during admission, and who have undergone Chest CT for some purpose in the last four weeks prior to the study period were enrolled. Exclusion criteria were as follows: patients <18 years old, having a history of tuberculosis, autoimmune diseases, malignancies, active infections in the last two weeks, and administration of systemic corticosteroids prior to the study were not included. Patients having confirmed diagnosis of active TB with sputum smear/culture were exempted from the study. All the patients were provided with written consent forms. The study was performed in line with the criteria of the Declaration of Helsinki.

Systemic inflammation index (SII) is developed for assessing chronic inflammation which reflects increased counts of blood neutrophils and platelets and decreased counts of blood lymphocytes. SII= platelet counts X neutrophil counts / lymphocyte counts. The inflammatory markers were calculated as follows: The neutrophil-to-lymphocyte ratio (NLR)=neutrophil counts/lymphocyte counts, the platelet to lymphocyte ratio (PLR)=platelet counts/lymphocyte counts.

The demographic characteristics, comorbidities, family history of tuberculosis, BCG vaccination status, smoking habits, occupation, and qualification details of the patients were all recorded. Psoriasis area and severity index (PASI), the scoring system defined for psoriasis patients has been noted for each of the patients. The findings on Chest CT were reported by an experienced thoracic radiologist and categorized as; fibrotic irregular lines, nodules uncalcified or calcified, fibrotic consolidation, mediastinal or hilar lymphadenopathy, pleural thickening, volume loss, and bronchiectasis. Also, the localization of the lesions on CT was examined.

The commercial name of the IGRA test used in the study was Quantiferon-TB Gold Plus (Qiagen, Germany).

Statistical Analysis

Statistical analysis of the data was performed in IBM SPSS Version 26 program. Pearson Chi-Square and Fisher's Exact tests were used to compare categorical variables between groups, and Mann Whitney U statistical analyzes were used to compare between two groups since continuous variables are not normally distributed (Kolmogorov Smirnov $p<0.05$). Variables affecting the positivity of Quantiferon test were evaluated with logistic regression analysis. $p<0.05$ was considered statistically significant.

RESULTS

As shown in Table 1, according to PASI scores 78% of the patients had mild to moderate psoriasis. The mean age of the participants was 49.24 and 52% were males. 40% had graduated from university, 53% had a normal income standard and 52% of the patients were married.

Current smokers presented 37% of the study group, whereas 18% had physician-diagnosed asthma and 0.6% had COPD. Twenty-six percent of the patients had cardiovascular disease, and seventeen percent had diabetes.

30% of the participants had a family history of TB and 83% of them had BCG vaccination scars.

When we examined the CT scans, radiological findings were reported in 58% of the patients. The most common CT findings were fibrotic irregular lines (48%), followed by calcified nodules (31%), fibrotic consolidation and pleural thickening (12%, 13%). Besides, right upper lobe localization was found to be dominant.

We divided the patients into two groups based on whether they had a positive or negative Quantiferon test, as 28% of the individuals had a positive result. Upon comparing the sociodemographic factors of the groups, the only variable that was determined to be statistically significantly different was income status ($p<0.05$). There were no differences noted between the groups according to disease severity, age, gender, BMI, educational or marital status, smoking habits, presence of comorbidity, family history of TB and chest CT findings ($p>0.05$). The patients whose Quantiferon test resulted positive, had significantly higher values of white blood cell count, neutrophils, lymphocytes, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, systemic inflammation index, and erythrocyte sedimentation rates (ESR) ($p<0.05$) (Table 2).

The effects of WBC, neutrophil, lymphocyte, NLR, and SII variables on Quantiferon test positivity were shown to be statistically significant ($p\sim<0.05$) in the logistic regression analysis. It was determined that the presence of radiological findings on the chest CT scan or comorbidities were not statistically significant on Quantiferon test positivity ($p>0.005$). (Table 3).

Table 1: Comparison of sociodemographic variables and Chest CT findings according to Quantiferon test

	Quantiferon negative		Quantiferon positive		X2	p
	n	%	n	%		
PASI scores						
Mild	23	26.1	8	22.9	0.701	0.704
Moderate	51	58	23	65.7		
Severe	14	15.9	4	11.4		
Gender						
Female	46	52.3	13	37.1	2.297	0.130
Male	42	47.7	22	62.9		
Marital status						
Married	46	52.3	19	54.3	0.041	0.840
Single	42	47.7	16	45.7		
Education						
University graduate	33	37.5	17	48.6	1.272	0.259
High school and lower	55	62.5	18	51.4		
Income status						
Normal standard	41	46.6	25	71.4	6.212	0.013
Lower income	47	53.4	10	28.6		
Smoking status						
Former smoker	30	34.1	10	28.6	2.716	0.257
Current smoker	29	33	17	48.6		
Never smoker	29	33	8	22.9		
Presence of comorbidity						
+	27	30.7	7	20	1.428	0.232
-	61	69.3	28	80		
Asthma	19	21.6	4	11.4	1.701	0.192
COPD	8	9.1	0	0	3.403	0.104
Allergic rhinitis	0	0	3	8.6	7.731	0.022
Diabetes	16	18	5	14	1.810	0.189
Cardiovascular disease	24	27	8	22	1.911	0,103
BCG scar						
>1	6	6,8	1	2,9	1.116	0.655
+	69	78.4	27	77.1		
-	13	14.8	7	20		
Family history of tuberculosis						
+	27	30.7	10	28.6	0.053	0.818
-	61	69.3	25	71.4		
Localization of chest CT findings						
Left lower lobe	3	6.3	0	0	2.978	0.569
Right lower lobe	3	6.3	0	0		
Bilateral	9	18.8	7	29.2		
Left upper lobe	6	12.5	3	12.5		
Right upper lobe	27	56.3	14	58.3		
Presence of chest CT findings						
+	48	54.5	24	68.6	2.03	0.154
-	40	45.5	11	31.4		
Fibrotic irregular lines	24	27.3	11	31.4	0.212	0.645
Uncalcified nodules	1	1.1	2	5.7	2.205	0.195
Calcified nodules	18	20.5	5	14.3	0.627	0.429
Fibrotic consolidation	5	5.7	4	11.4	1.219	0.272
Mediastinal lymphadenopathy	1	1.1	2	5.7	2.205	0.195
Pleural thickening	5	5.7	5	14.3	2.482	0.146
Volume loss + Bronchiectasis	2	2.3	2	5.7	0.943	0.320

Pearson Chi Square, Fisher's Exact test, CT: Computerized Tomography, BCG: Bacille Calmette-Guerin, COPD: Chronic Obstructive Pulmonary Disease

Table 2: Comparison of laboratory findings according to Quantiferon test

	Quantiferon negative Mean±SD	Quantiferon positive Mean±SD	Z / t	p
Age	49.48±9.61	48.63±10.65	-0.087	0.931
BMI	24.83±1.89	25.2±1.73	-1.492	0.136
PASI scores	8.03±3.55	7.93± 4.39	-0.526	0.599
WBC	7.38±1.63	8.72±1.97	-3.871	<0.001
Neutrophil	4.62±1.36	6.35±1.59	-6.046	<0.001
Lymphocyte	2.49±0.7	2.04±0.55	-3.574	<0.001
Platelet	286.49±62.02	262.97±63.15	1.888	0.061
NLR	1.97±0.69	3.22±0.82	-8.550	<0.001
PLR	124.99±47.58	138.64±50.25	-1.412	0.160
RDW	12.59±1.05	12.59±0.98	-0.180	0.857
SII	562.05±233.71	839.37±270.04	-5.333	<0.001
AST	20.61±11.48	18.49±6.98	-0.306	0.760
ALT	20.5±8.62	19.34±5.32	-0.096	0.924
CRP	5.39±7.5	6.12±9.03	-0.572	0.567
Hemoglobin	14.4±1.48	14.55±1.65	-0.575	0.565
ESR	24.35±8.77	27.89±10.03	-2.108	0.035

Independent sample t test, Mann Whitney U analysis, BMI: Body mass index, WBC: White blood cell count, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, RDW: Red cell distribution width, SII: Systemic inflammation index, AST: Aspartate aminotransferase ALT: Alanine aminotransferase, CRP: C-reactive protein ESR: Erythrocyte sedimentation rate.

Table 3: Logistic regression analysis for the effect of laboratory values on Quantiferon positivity

	B	S.E.	Wald	p	Exp (B)	95% C.I.	
Presence of CT finding	0.598	0.422	2.003	0.157	1.818	0.795	4.161
Presence of comorbidities	0.799	0.501	3.012	0.611	1.455	0.887	7.223
WBC	0.425	0.122	12.037	0.001	1.529	1.203	1.943
Neutrophil	0.810	0.173	21.816	0.000	2.247	1.600	3.156
Lymphocyte	-1.181	0.372	10.075	0.002	0.307	0.148	0.636
Platelet	-0.006	0.003	3.430	0.064	0.994	0.987	1.000
NLR	2.257	0.424	28.333	0.000	9.550	4.161	21.921
PLR	0.006	0.004	1.932	0.165	1.006	0.998	1.014
RDW	0.003	0.195	0.000	0.989	1.003	0.685	1.468
SII	0.004	0.001	20.663	0.000	1.004	1.002	1.006
CRP	0.011	0.024	0.216	0.642	1.011	0.965	1.060
ESR	0.040	0.022	3.517	0.061	1.041	0.998	1.086

S.E: Standard error, C.I.: Confidence Interval, , WBC: White blood cell count, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, RDW: Red cell distribution width, SII: Systemic inflammation index, CRP: C-reactive protein ESR: Erythrocyte sedimentation rate CT: Computerized Tomography.

DISCUSSION

Patients with psoriasis who were evaluated for LTBI and had no evidence of infection or respiratory symptoms were included in this study. The Quantiferon test, a screening tool for LTBI, was positive in 28% of the study group. A family history of tuberculosis was present in 30% of the individuals, and 16% of them showed no scars from the BCG immunization. It's interesting to note that 59% of the patients exhibited radiological abnormalities on their CT scans, which could indicate a history of tuberculosis infection and need to be considered when these individuals are being monitored.

Psoriasis is a complex disease and given the rapid developments in this field, more frequent updates to psoriasis treatment recommendations are becoming more crucial.[1] The novel therapies allow for significant symptom reduction and quality of life improvement. The treatment recommendations might not be applicable in all cases because psoriasis is a complicated condition with numerous co-morbidities.[11] The basic treatment algorithms include patient preference, disease severity, comorbidities, and specific conditions that should be considered particularly.[11] Due to the risk of LTBI, screening before starting biological agents may be challenging in psoriasis patients who are living in a country with a high prevalence of TB.[9]

According to the guidelines, once biologic therapy has begun, all patients should undergo an annual TB screening.[12] Prophylactic treatment should be started two months before the commencement of biological agents in latent TB patients.[12] LTBI contributes to the continuation of the disease cycle at the population level as a reservoir for new diseases and continuous Mtb transmission within communities.[12]

The conventional description of LTBI is, measurably elevated immunological sensitivity to Mtb in the absence of symptoms of the disease, such as fever, chills, night sweats, weight loss, cough, hemoptysis, or a newly generating finding on a chest radiograph.[13] The duration and activity of the latent focus varies from person to person based on timing, and host- and pathogen-specific characteristics, which are not addressed by this confounding definition.14 The course of LTBI is influenced by host variables such age and immunological state.[14]

In our study, when compared to the Quantiferon negative group, patients with Quantiferon positive tests did not differ in demographic characteristics, family history of TB or chest CT findings. In contrast, their inflammatory blood parameters were found to be higher. The biology of LTBI has remained a poorly understood area in TB research. In a previous study the researchers observed a correlation between the cellular activity in the thoracic lymph nodes shown with the positron emission tomography and IGRA responses in the patients with LTBI which suggested the immune response to *M. tuberculosis* infection.[15] The tuberculosis antigen-stimulated interferon release assay detects sensitization to mycobacterial antigens, but it does not distinguish between latent infection that has resolved and infection that is still present.[16]

Inflammation plays an important role in LTBI and prior to any clinical symptoms, an increase in the host biomarkers may be valuable as the first sign of infection or inflammation in the body. In research conducted in

Nigeria, while the values of WBC in the LTBI group approximated the normal range, ESR and CRP levels were found to be higher in these patients than in the healthy group.[17] In our results, not the CRP levels, but white blood cell count, and ESR were found to be higher in the psoriasis patients with positive Quantiferon test than in the negative ones. In a Japanese study comparing blood markers in LTBI and active TB patients, WBC and lymphocyte counts were higher in LTBI patients whereas neutrophil, platelet counts, and CRP levels were higher in patients with sputum-positive active tuberculosis.[18]

In our study, 28.6% of the patients had a family history of TB and 68.6% of the subjects in the positive Quantiferon test group had radiological findings in Chest CT, but this displayed no effect on regression analysis. Besides, no participant had a prior history of TB or contact with a person with TB, so, the CT findings were incidental. Chest CT is an important imaging modality in detecting LTBI for candidates scheduled for systemic treatment, particularly with immunomodulatory biologic drugs in daily practice.[19] On the other hand, as commented on the previous reports, due to the risk of radiation exposure, CT should be performed only in specific subgroups when the diagnostic indications are present, or disagreement occurs in clinical examinations.[19] Therefore, when screening patients for LTBI, the radiological findings should be evaluated more cautiously since these might be suggestive of an old TB rather than a latent infection in a country with a high prevalence of TB.

Our data, which showed an elevated inflammatory status, were consistent with our hypothesis that psoriasis and LTBI would have a synergistic effect in the inflammatory response. This increased inflammatory process was accordingly reflected by an increase in other parameters such as neutrophil-to-lymphocyte ratio, and systemic inflammation index.[20] It has been shown that the systemic immune inflammation index (SII), a unique blood cell index that provides an opinion about ongoing inflammation, is a simple and useful prognostic factor for diseases with an inflammatory etiology.[21] Furthermore, WBC subtypes that are well-known as systemic, non-specific cellular indicators of general inflammation include neutrophils, lymphocytes, and monocytes. In recent years, clinical practice has increasingly used inflammation-based indices, such as the NLR and PLR, as assessment tools for disease activity of many forms of inflammatory illnesses.

It is currently widely acknowledged that psoriasis is an immune-mediated inflammatory disease, with the primary cause being an abnormal immune response in the skin.11 In a recent study, SII, NLR, and WBC were found to be higher in the patients' group which comprised the patients mostly with the mildly severe psoriasis.[22] Comparably, a different study that examined the SII in psoriasis patients found that it was higher than in the group of healthy controls.[23] Although several pathophysiological factors lead to psoriasis, immune-mediated processes involving T lymphocytes, dendritic cells, and other cytokines that result in a markedly inflammatory process are the primary culprits.1 Physicians need to measure inflammation for developing more effective treatment plans.

Limitationsww

Our limitations in the study were the retrospective design, small sample size and the lack of other clinically assayed biomarkers such as albumin, LDH, IL-2, and tumor necrosis factor-alpha for more accurate evaluation of inflammation and LTBI in psoriasis patients. Since there is still no gold standard for the diagnosis of LTBI and the number of cases with LTBI is on the rise due to the screening procedure of patients treated with biological agents by IGRAs, larger studies combining radiologic procedures and available biomarkers are needed to expand the knowledge in this field.

CONCLUSION

Psoriasis is a systemic inflammatory disease. Inflammation levels are higher in the psoriasis patients in this study who need systemic treatment, are being screened for LTBI, and have positive IGRA test results. Future studies may help to define new parameters to assess the inflammatory status of these patients in clinical judgment for new therapeutic options.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Health Science University Şişli Hamidiye Etfal Training and Research Hospital Clinical Researches Ethics Committee (Date: 21.02.2023, Decision No: 2248).

Informed Consent

All patients signed an 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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The prevalence of hypogonadism in male patients with type 2 diabetes mellitus and clinically relevant factors

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ABSTRACT

Aims: Hypogonadism has been reported at high rates in male patients with type 2 diabetes mellitus (T2DM). However, the origin of male hypogonadism in patients with T2DM is poorly known. The aim of this study was to determine the prevalence of hypogonadism and to investigate the potential impact of certain clinical and biochemical variables on hypogonadism in patients with T2DM.

Methods: The study included a total of 513 consecutive males (aged 30 - 60 years) with T2DM who presented at the endocrinology outpatient clinic. The demographic and clinical characteristics of the patients were recorded. Biochemical parameters, total testosterone (TT), gonadotrophins, prolactin, serum lipids, and hemoglobin A1c (HbA1c) were measured. Correlations between metabolic and clinical conditions and T levels were analyzed.

Results: The mean age of the study population was 45.5±12.6 years. Hypogonadism was present in 122 (23.7%) patients, of which 24 (23.3%) were determined with primary hypogonadism. Compared with participants with normal testosterone, those with hypogonadism had lower estimated glomerular filtration rate (eGFR), and the liver function test results, HbA1c and triglycerides levels, and duration of diabetes were higher. Correlation analyses showed that TT was negatively correlated with body mass index (BMI), waist circumference, age, fasting blood glucose, HbA1c, uric acid and triglycerides, and positively correlated with eGFR and high density lipoprotein cholesterol (HDL-C). Multivariate logistic regression analysis revealed that BMI, age, diabetes course, hypertrglyceridemia, hyperuricemia and eGFR <60 ml/min/1.73 m² are independent risk factors for hypogonadism in male patients with type 2 diabetes.

Conclusion: The current study results demonstrated that the prevalence of hypogonadism is higher in men with type 2 diabetes than in the general population and age, diabetes duration, BMI, triglycerides and uric elevation are independent risk factors.

Keywords: Diabetes, male hypogonadism, testosterone

INTRODUCTION

Approximately 537 million people worldwide are affected by diabetes mellitus (DM), which is a chronic, progressive, metabolic disease that can cause complications in several organ systems.[1,2] Male hypogonadism is one of these complications and the frequency of this has been reported to vary between 20% and 40% in previous studies.[3-5] Male hypogonadism is a disorder in which testosterone (T) deficiency is clinically characterized and biochemically confirmed.[5] The Endocrine Society recommends routine T measurement in males with Type 2 DM (T2DM).[6] However, despite being frequently seen, it can be said that male hypogonadism is often overlooked.[7] Diseases such as dyslipidemia, obesity, and metabolic syndrome, which often accompany T2DM, can also cause hypogonadism.[5,8] The fact that hypogonadism in T2DM is mostly in the form of hypogonadotropic hypogonadism suggests that disruption of the hypothalamic-pituitary axis plays a fundamental role in the pathogenesis.[9] However, the underlying mechanism of hypogonadism that develops associated with diabetes, and which correlations of diabetes are associated with hypogonadism are not clear. When it is considered that the incidence of diabetes is continously increasing and a significant proportion of patients are of reproductive age, there can be seen to be a significant problem. The aim of this study was to determine the frequency of hypogonadism in males with T2DM, and to evaluate the risk factors by examining the correlations of biochemical and clinical conditions with serum T levels.

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METHODS

Approval for this retrospective study was granted by the Clinical Researches Ethics Committee of Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital (Date: 17.05.2021, Decision No: 111/11). The study was conducted in Kilis Prof. Dr. Alâeddin Yavaşça State Hospital and in accordance with the Declaration of Helsinki. The study included 513 consecutive male patients, aged 30-60 years, who presented at the Endocrinology Outpatient Clinic between January 2023 and July 2023 with a diagnosis of T2DM. Patients were excluded from the study if they had Type 1 DM, hypopituitarism, end-stage renal failure, cirrhosis of the liver, chronic alcoholism, or malignancy.

All the patients included in the study met the diagnostic criteria for diabetes defined by the World Health Organisation (WHO) in 1999.[10,11] The diagnosis of hypogonadism was defined as total testosterone (TT) level <240 ng/dl or a TT level of <300 ng/dl in the presence of signs or symptoms.6 TT measurements were taken before 10.00 a.m. in a fasting condition. Low T levels were confirmed with a second measurement, in accordance with the recommendation of the Endocrine Society.[6] The body mass index (BMI) values of the patient, waist circumference, duration of diabetes, smoking status, the presence of retinopathy, neuropathy, or nephropathy, and macrovascular complications related to diabetes were recorded from the medical records. Blood samples were taken in the morning after overnight fasting, and the values were recorded of fasting blood glucose, glycosylated hemoglobin (HbA1c), serum lipids, follicle-stimulating hormone (FSH), luteinising hormone (LH), prolactin, liver transaminases, creatinine, estimated glomerular filtration rate (eGFR), and serum uric acid. FSH or LH ≥10 IU/L was defined as primary hypogonadism, and FSH or LH <10 IU/L as secondary (central) hypogonadism. The normal reference range for uric acid is 2.6-6 mg/dl, and values >6 mg/dl are considered hyperuricemia. The normal reference ranges for liver transaminases AST (Aspartate transaminase): 5-34 U/L and ALT (alanine aminotransferase): 0-55 U/L. Values above this range were considered as elevated liver transaminases.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS 28.0 software. Continuous data were reported as mean±standard deviation values, and categorical data as number and percentage. Normality of distribution of the data was tested with the Kolmogorov-Smirnov test. Categorical variables were analyzed using the Chi-square test or Fisher's Exact test. The Student's t-test was applied to continuous variables showing normal distribution, and the Mann Whitney U-test was used when distribution was not normal. Correlations between the data were examined using Pearson and Spearman correlation analyses. Multivariate logistic regression analysis was performed to determine the risk factors associated with hypogonadism. Results were shown as odds ratio (OR) and 95% confidence interval (CI). A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Hypogonadism was determined in 122 (23.7%) of the 513 patients evaluated. Secondary hypogonadism was seen in 98 (80.3%) of the patients with hypogonadism. When the patients with and without hypogonadism were compared, higher values were determined in the patient group with hypogonadism (Group 1) than in the patient group without hypogonadism (Group 2) in respect of age (51.2±8.9 vs. 44.3±11.2 years, $p<0.001$), duration of diabetes (11.4±4.5 vs. 7.6±4.2 years, $p<0.001$), BMI (31.5±3.8 vs. 26.4±4.1, $p<0.001$), fasting blood glucose (198±21.3 vs. 143±17.6, $p=0.014$), HbA1c (9.4±1.3 vs. 8.2±1.1, $p=0.027$), and triglycerides (291±71.7 vs. 216±53.3, $p=0.02$). The levels of high-density lipoprotein (HDL) cholesterol (38.9±4.5 vs. 47.1±5.6, $p=0.043$) and eGFR (66.1±22.3 vs. 75±21.2, $p=0.002$) were determined to be lower in Group 1 (Table 1). Elevations in hypertension, hyperuricemia, and liver tests were seen at a higher rate in the patients with hypogonadism ($p=0.03$, $p<0.001$, $p=0.002$, respectively). The gonadal hormone levels in the study population are shown in Table 2. In addition to TT, the LH (3.5±0.8 vs. 5.1±1.1, $p=0.034$) and FSH (2.7±0.9 vs. 5.9±1.6, $p=0.014$) levels were significantly lower in the patients with hypogonadism compared to those without.

Table 1. Comparisons of general data in the two groups

	Low TT group (n=102)	Normal TT group (n=411)	p value
Age (years)	51.2±8.9	44.3±11.2	<.001
Duration of diabetes (years)	11.4±4.5	7.6±4.2	<.001
Waist circumference (cm)	106.7±9.1	101.2±10	0.569
Body mass index (kg/m²)	31.5±3.8	26.4±4.1	0.034
Fasting blood glucose (mg/dl)	198±21.3	143±17.6	0.014
HbA1c (%)	9.4±1.3	8.2±1.8	0.027
Triglycerides (mg/dl)	291±71.7	216±53.3	0.02
Total cholesterol (mg/dl)	256±66.3	234±56.2	0.78
LDL-C (mg/dl)	153±31.1	137±24.3	0.11
HDL-C (mg/dl)	38.9±4.5	47.1±5.6	0.043
eGFR (ml/min/1.73 m²)	66.1±22.3	75±21.2	0.002
Elevated liver transaminases (%)	22	14.5	0.07
Hyperuricemia (%)	31.3	19.8	<.001
Microalbuminuria (%)	14.5	12.9	0.65
Diabetic retinopathy (%)	21.2	19.3	0.73
Diabetic neuropathy (%)	36.7	31.4	0.69
Hypertension (%)	41.6	33.8	0.03
Ischemic heart disease (%)	21.7	18.8	0.16
Smokers	25.5	27.6	0.89

Results are expressed as mean±SD values or prevalence (%).

Table 2. Comparisons of sex hormones in two groups

	Low TT group (n=102)	Normal TT group (n=411)	p value
Total testosteron (ng/dl)	251.2±38.9	444.3±56.2	<.001
Prolactin (ng/ml)	24.7±3.1	21.8±1.6	0.569
Luteinizing hormone (IU/ml)	3.5±0.8	5.1±1.1	0.034
Follicle-stimulating hormone (IU/ml)	2.7±0.9	5.9±1.6	0.014

Data are presented as mean±SD values in each group

The results of the correlation analyses showed a negative correlation of the TT levels with age (r=-0.223, p<.001), BMI (r=-0.209, p=0.004), fasting blood glucose (r=-0.063, p=0.242), HbA1c (r=-0.286, p=0.013), uric acid (r=-0.291, p=<0.001), and triglycerides (r=-0.086, p=0.506), and a positive correlation with eGFR (r=0.156, p=0.02) and HDL cholesterol (r=0.071, p=0.061) (Table 3).

Table 3. The correlations of serum TT with the clinical and biochemical parameters

	r	p
Age (years)	-0.223	<.001
Duration of diabetes (years)	-0.211	<.001
Waist circumference (cm)	-0.071	0.177
Body mass index (kg/m²)	-0.209	0.04
Fasting blood glucose (mg/dl)	-0.063	0.242
HbA1c (%)	-0.286	0.013
Triglycerides (mg/dl)	-0.086	0.506
HDL-C (mg/dl)	0.071	0.061
GFR (mL/min/1.73 m²)	0.156	0.02
Uric acid (mg/dl)	-0.291	<.001

According to the results of the multivariate logistic regression analyses, the variables of age (ref: <50 years) (OR:2.83, CI 95%: 1.838-3.611, p<0.001), duration of diabetes (ref: <10 years) (OR:2.79, CI 95%: 2.131-3.237, p<0.001), BMI (ref: <30 kg/m²) (OR:1.427, CI 95%: 1.119-2.011, p=0.034), HbA1c (ref: <10%) (OR:2.122, CI 95%: 1.875-2.651, p=0.010), eGFR (ref: >60 ml/min/1.73 m²) (OR: 3.455, CI 95%: 2.887-4.011, p<0.001), hypertriglyceridemia (OR: 1.643, CI 95%: 1.111-2.430, p=0.018), and hyperuricemia (OR:3.182, CI 95%:2.981-4.211, p<0.001) were determined to be independent risk factors for hypogonadism (Table 4).

Table 4. Multivariate logistic regression analysis of the relationship between hypogonadism and clinical and biochemical variables

Variables	Odds ratio (95% CI)	p value
Age (ref:<50 years)	2.83 (1.838-3.611)	<.001
Duration of diabetes (ref: <10 years)	2.79 (2.131-3.237)	<.001
Body mass index (ref: < 30 kg/m²)	1.427 (1.119-2.011)	0.034
Hba1c (ref: <10%)	2.122 (1.875-2.651)	0.010
Hypertriglyceridemia	1.643 (1.111-2.430)	0.018
Hyperuricemia	3.182 (2.981-4.211)	<.001
eGFR (ref: >60 ml/min/1.73 m²)	3.455 (2.887-4.011)	<.001

CI: Confidence interval

DISCUSSION

In this study, which examined the frequency and risk factors of hypogonadism in males with T2DM, the frequency of hypogonadism was seen to be consistent with the literature.[4,5,10] Males with T2DM are known to be at greater risk of hypogonadism than those without diabetes, regardless of the metabolic status.[13] The results of the current study showed that age, duration of diabetes, BMI, poor glycemic control, eGFR <60 ml/min/1.73 m², hypertriglyceridemia, and hyperuricemia were independent risk factors for hypogonadism.

A decrease in T levels is associated with ageing, even in healthy males.[14,15] The effect of ageing on T is due to increased visceral obesity, drug use, and an unhealthy lifestyle.[16,17] In a study of diabetic patients aged >60 years, Harman SM et al.[18] reported that no increase was seen in the frequency of hypogonadism compared to those without diabetes, and there was an increase in the frequency of hypogonadism at an earlier age. Al Hayek AA et al.[5] reached similar results. In the current study, the inclusion of diabetic patients aged 30-60 years seemed rational, and a negative correlation was determined between TT levels and age. This result showed that increasing age up to 60 years was associated with the development of hypogonadism in males with T2DM. This correlation was confirmed by the 2.83-fold increased risk of hypogonadism in males with T2DM aged >45 years.

Type 2 DM, which is traditionally known as a disease of middle age and old age, is increasingly occurring at younger ages.[1,19] This means that patients are living with diabetes for a longer period. In literature, there has been shown to be an increase in both microvascular and macrovascular complications as the duration of diabetes becomes prolonged.[20,21] In a study conducted in 2004, it was reported that hypogonadotropic hypogonadism was frequently seen in males with diabetes, and this was independent of glycemic control and the duration of diabetes.[22] However, the current study results showed a 2.79-fold increase in the risk of hypogonadism when the duration of diabetes exceeded 10 years. This could be a natural result of a longer disease duration as age increases. In the current study, no correlation was determined between hypogonadism and microvascular and macrovascular complications associated with diabetes. This situation is an indirect sign that insulin resistance, increased adipose tissue, and metabolic syndrome are at the forefront rather than vascular problems in the pathogenesis of hypogonadism in males with T2DM.

Insufficient glycemic control is a significant risk factor for the progression of diabetes and complications in T2DM patients.[23] In the current study, there was a negative correlation between HbA1c and TT, and the risk of

hypogonadism was seen to be increased 2.12-fold in patients with HbA1c >10%. Advanced age, the duration of diabetes and dyslipidemia are known to play a role in poor glycemic control.[9] At the same time, these variables are independent risk factors for male hypogonadism.[24] Even so, hypogonadotropic hypogonadism is seen comparatively less in Type 1 DM than in T2DM, which basically shows that the underlying condition is due to insulin resistance.[25] In one way, poor glycemic control in patients with T2DM reflects the severity of insulin resistance, and in this respect, it is expected that poor glycemic control is a risk factor for hypogonadism in T2DM.

Hypertriglyceridemia, which is a component of metabolic syndrome, is known to be associated with hypogonadism.[26] By impairing intracellular signalling of insulin, the fat mass and excess triglycerides cause insulin resistance.[27] This also causes hypogonadism by decreasing LH expression with mechanisms such as an increase in estradiol, leptin, and cytokines.[28-31] In the current study, hypertriglyceridemia and BMI >30 kg/m² were seen to be independent risk factors for hypogonadism. A possible explanation of the significant effect of BMI on hypogonadism in males with T2DM is that the conversion of testosterone to oestrogen by aromatase can lead to a decrease in testosterone levels.[32]

Another important result of the current study was the increased risk of hypogonadism in males with T2DM with chronic renal disease (eGFR <60 nl/min/1.73m²). This finding was similar to the results shown for the first time by Herrero A et al.[33] but in contrast, no correlation was determined between hypogonadism and the presence of microalbuminuria, which is a microvascular complication of diabetes. There are similar results in previous studies.[5]

Another noticeable result of the current study was the determination of a negative correlation between T and serum uric acid level, and in males with T2DM, hyperuricemia is an independent risk factor for hypogonadism. T2DM may have a direct effect on the oxidisation of purine nucleotides and this causes an increase in uric acid levels.[34] Hyperinsulinemia can lead to hyperuricemia by increasing the xanthine oxidase synthesis rate.[34] Previous studies have shown a strong correlation of hyperuricemia with insulin resistance, metabolic syndrome, increased BMI, and increased visceral adipose tissue.[35,36] That the complex and multidirectional correlation of uric acid with other risk factors of hypogonadism is a risk factor for hypogonadism was seen to be in parallel with previous studies.

Limitations

There were some limitations to this study, primarily the retrospective design. A second limitation was that the results were the data from single centre, and as they had a regional characteristic do not have sufficient power for definitive clinical recommendations. Therefore, the findings should be confirmed with further, prospective, multicentre studies. Another limitation was the absence of free testosterone and sex hormone binding globulin (SHBG) results. Finally hypogonadism in this study was based only on the testosterone level, and a validated international scale was not used to question symptoms.

CONCLUSION

The prevalence of hypogonadism is higher in males with T2DM than in the general population. There was determined to be a significant correlation between hypogonadism and age, duration of diabetes, BMI, chronic kidney disease, hypertriglyceridemia and hyperuricemia. Just as for other typical complications of diabetes, screening for hypogonadism, which is often seen and is associated with many variable risk factors, can be recommended while patients are still at the asymptomatic stage and the measurement of T levels should be performed in all males with T2DM.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Clinical Researches Ethics Committee of Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital (Date: 17.05.2021, Decision no: 111/11).

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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Bibliometric analysis of publications on kinesiophobia in orthopedics between 1970 - 2023

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ABSTRACT

Aims: This bibliometric study aimed to examine the hotspots and frontiers of kinesiophobia research in orthopedics and to assess the overall scientific output of the field.

Methods: The Web of Science Core Collection was mined for articles on kinesiophobia that were published between 1970 and September 2023. Using common bibliometric indicators, Vosviewer was used to examine the number of publications, countries, institutions, journals, authors, cited references, and keywords.

Results: The results of a bibliometric analysis focused on the body of knowledge on kinesiophobia. The study analyzed 2,035 articles from 75 different countries and identified important trends and groundbreaking research. In particular, there has been a steady increase in recent years, with publications increasing significantly between 2001 and 2012. The highest contribution came from the United States and the most cited articles addressed clinical recommendations for low back pain. Both the Florida State University System and the University of Florida made significant contributions. With a total of 51,443 citations, this study provides a comprehensive overview to help guide future research in orthopaedics and related fields.

Conclusion: The results of this bibliometric study give an overview of the state and trends in clinical research on kinesiophobia and may be used by researchers to pinpoint hot themes and consider fresh lines of inquiry.

Keywords: Kinesiophobia, bibliometric analysis, publications

INTRODUCTION

There are several conceptual definitions for fear in relation to pain, but the most prevalent ones include pain-related fear, fear-avoidance beliefs, fear of movement, and kinesiophobia.[1] Kinesiophobia is the fear of excessive, irrational and disabling physical movement and activity, manifested by the fear of a painful injury or re-injury.[2,3] It is connected to the degree of pain in those with chronic pain.[4] Also, fear of pain is thought to be a powerful psychological predictor of both chronic pain and impairment.[5] Pain perceptions and reactions to physical exercise have been shown to be significantly influenced by psychological variables, such as pain catastrophizing and kinesiophobia.[6,7] According to a recent study, orthopedic trauma patients may experience kinesiophobia at a rate of up to 52.8%.[8] After orthopedic surgery, rehabilitation is hampered by the psycho-cognitive aspect of kinesiophobia. There is no proof that kinesiophobia affects a patient's ability to function in the short term after having orthopedic surgery.[9]

The bibliometric approach is a quantitative statistical analysis tool used to evaluate and track developments in research. We can swiftly identify the characteristics of literature, study and comprehend the growth process, and identify the research hotspots in research topics using the bibliometrics method. Currently, bibliometric analysis is used extensively in many different domains.[10-14]

In recent years, there have been many publications examining the available research evidence of kinesiophobia. However, the publication characteristics of kinesiophobia research have only been briefly summarized in one publication.[15] Therefore, it is crucial to ascertain the current state of kinesiophobia as a whole in order to serve as a reference for future research. The Vosviewer software was used in this study to conduct a bibliometric analysis of articles on kinesiophobia covering the period from 1970 to September 2023. The analysis produced a thorough summary of accomplishments, new trends, and research hotspots in this field. The study, which concentrated on orthopedics and related fields, sought to provide a review

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of the literature to support future research, particularly in devising treatment methods for people with kinesiophobia. The study provided insights that informed and guided subsequent research into successful interventions for patients experiencing kinesiophobia in orthopedic contexts by summarizing the landscape of kinesiophobia literature.

METHODS

Ethics

As it is not a human or animal study there is no need for ethical approval. All procedures were carried out in accordance with the ethical rules and the principles.

Data Collection

Literature databases are used in the bibliometric analyses. Databases like Scopus, Web of Science, Pubmed, Cochrane Library, and etc., are currently used extensively. The Web of Science (WoS) database (https://www.webofscience.com/wos/woscc/basic-search) includes these, as well as sizable, multidisciplinary, high-impact, global, and thorough academic journals. Evidence has been demonstrated that when Vosviewer[16] is used for visual analysis in which the most powerful and easy to understand visualization tool for depicting the relations between related aspects and the WoS database produces a greater knowledge map effect.

The WoS database is a rational and efficient choice for our study's data source. In particular, the data were gathered from the SCI-EXPANDED, and Emerging Sources Citation Index (ESCI) databases in the Web of Science Core Collection (WoSCC). We searched the literature retrieved from WoSCC on a single day, October 5, 2023, to prevent bias due to the daily database changes.

Inclusion Criteria

The search technique included the keywords: pain catastrophizing OR Kinesiophobia (Topic) OR fear of movement (Topic) OR fear-avoidance beliefs (Topic) OR pain-related fear (Topic) and the literature type was restricted to "ARTICLE." The research area selected as orthopedics and related fields [(Orthopedics or Rehabilitation or Sport Sciences (Web of Science Categories)]. We extracted English-language articles from publications between 1970 and October 30, 2023, and we evaluated the results for relevancy.

Exclusion Criteria

Only the “Articles in English Language” Documents were accepted. Remainder documents was excluded.

A total number of references was then gathered. We recorded the document's information as complete records and cited sources as plain text. Data must first be prepared before being imported into the Vosviewer. Four folders were made in the newly constructed folder: input, output, data, and project. The file was previously exported in WoSCC to input and saved with the name "download _ **.txt" in a Vosviewer-compatible format.

Analysis Tool

The scientific mapping tool VOSviewer was created by Van Eck and Waltman,[16] We used VOSviewer (version 1.6.19) for our bibliometric analysis. The superior network and cluster analysis visualization capabilities of VOSviewer led to its selection. Diagrams of institutional collaboration, journal co-citation, keyword co-occurrence, author collaboration, author co- citation, and literature co-citation were made easier by the tool. These analyses were essential for figuring out intricate relationships in academic literature.

Also Microsoft Excel was used to examine and decipher publication patterns across countries, institutions, journals, and authors for the distribution component. Excel's ability to handle and analyze bibliometric data systematically was made possible by its computational prowess. This combined strategy, which used Excel for thorough distribution analyses and VOSviewer for nuanced visualization, provided a thorough understanding of the scholarly environment under study. The strengths of VOSviewer and Microsoft Excel were combined in our methodology to produce a thorough and perceptive bibliometric analysis of institutional, authorship, and publication dynamics in the selected research domain.

RESULTS

General Information

A total of 2,035 academic articles on kinesiophobia in orthopedics were written by a total of 6,742 authors, who represented 2,377 affiliations and 75 different countries.

Our thorough bibliometric analysis revealed a total of 2,035 academic articles between 1970 and 2023, which together accumulated a significant 51,443 citations. An important metric for evaluating the scholarly impact of individual works is the average number of citations per item, which for this dataset was 25.27. Additionally, the h-index, a reliable measure of academic productivity and impact, reached a significant value of 98. This indicated that 98 works in the dataset had at least 98 citations each, demonstrating the breadth and impact of the body of literature that had been compiled over the time period under consideration.

Notable trends in scholarly output were revealed by comparing the distribution of publications over various time series. The division of time into the three categories of before 2000, between 2001 and 2012, and between 2013 and 2023 provided insights into the development of research productivity. There were few contributions made before 2000, making up just 0.197% of the entire dataset. The following decade, from 2001 to 2012, saw

a sharp increase in scholarly activity, accounting for 58.7% of all publications. Together, the years 2023, 2022, 2021, and 2020 contributed 35.32% of the total, demonstrating a consistent and significant output in recent years. Notably, the publication numbers showed a declining trend from 2013 to 2019, contributing a total of 35.83%. 1994 and 1995 were the first years mentioned in the dataset, with 2 and 1 publications, respectively. Recent years have seen an increase in the number of publications, with 2020 having the most with 201 publications, followed by 2021 with 200 publications and 199 publications 2022. The highest publication numbers were thus concentrated in the most recent years, particularly in 2020, 2021, and 2022, even though the dataset started in the mid-1990s. Figure 1 shows trends in kinesiophobia publications and citations.

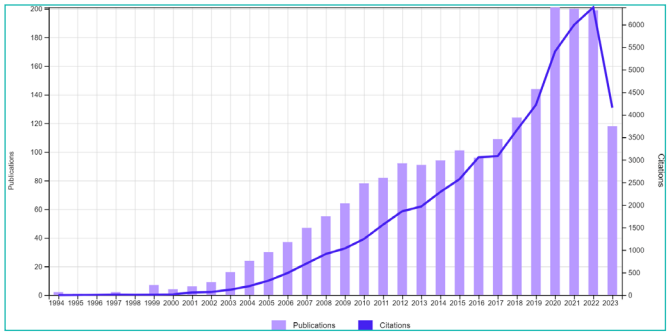

Figure 1. The trends in kinesiophobia publications and citations

Top Publishing Countries, and Affiliations

The field received contributions from 76 countries, with the United States providing the majority (28.138%) of the 2,035 records. The Netherlands (8.76%), England (8.71%), Australia (10.33%), and Canada (8.02%) were close behind. Sweden, Belgium, Brazil, Spain, and Norway were additional significant contributors. Contributions from the People's Republic of China, New Zealand, France, Ireland, Scotland, Switzerland, Denmark, Italy, Japan, Iran, Turkiye, Ireland, and the United Kingdom were also significant.

The top publishing organisations were listed in Table 1. According to affiliations, the top publishing countries represented a variety of regions. With 3.587% of the total number of records, the State University System of Florida was in the lead, followed by the University of Florida at 3.145%. The list also prominently included European universities like Maastricht University, Karolinska Institutet, University of Oslo, University of Sydney, and Vrije Universiteit Amsterdam. Among the notable contributors were the Pennsylvania Commonwealth System of Higher Education PCSHE, the University System of Ohio, and the University of Gothenburg. Vrije Universiteit Brussel, Ghent University, and the University of Southern Denmark were all included in the international presence. The list showcased the interconnectedness of the academic landscape by including institutions from Australia, the United States, Europe, and beyond, reflecting a global collaboration in research.

Table 1. Top publishing organisations

Affiliations	Record count	% of 2.035
State University System of Florida	73	3.587
University of Florida	64	3.145
Maastricht University	58	2.850
Karolinska Institutet	55	2.703
University of Oslo	53	2.604
University of Sydney	53	2.604
Vrije Universiteit Amsterdam	52	2.555
University System of Ohio	43	2.113
University of Gothenburg	42	2.064
Pennsylvania Commonwealth System of Higher Education Pcshe	41	2.015
Vrije Universiteit Brussel	40	1.966
University of Queensland	39	1.916
Ghent University	38	1.867
University of Southern Denmark	36	1.769
Curtin University	35	1.720
University of Pittsburgh	34	1.671
University of Antwerp	32	1.572
University of Kentucky	30	1.474
University of Utah	30	1.474
Utah System of Higher Education	30	1.474
Linkoping University	29	1.425
University of Groningen	29	1.425
University of Texas System	29	1.425
Harvard University	28	1.376
Ku Leuven	28	1.376

Showing 25 out of 2.377 entries; 14 record(s) (0.688%) do not contain data in the field being analyzed

Top Cited Articles

Table 2 provides a concise overview of articles on kinesiophobia with a focus on three important studies. Clinical practice guidelines for low back pain are covered in the first article, which was written by Delitto et al. and published in the Journal of Orthopaedic & Sports Physical Therapy in 2012. It has received 571 citations overall, or an average of 47.58 per year. With 496 citations, or an average of 22.55 per year, the second study by Dite and Temple (2002) published in the Archives of Physical Medicine and Rehabilitation introduces a clinical test for identifying multiple falling incidents in elderly individuals. With 452 citations and an average of 20.55 per citation, Flynn et al. .'s 2002 study published in SPINE presents a clinical prediction rule for classifying low back pain patients with short-term improvement through spinal manipulation.

Citation Topics Micro

Table 2. The summary of the kinesiophobia articles that were most frequently cited

Title	Authors	Source Title	Publication Year	DOI	Total Citations	Average citation per year
Low Back Pain Clinical Practice Guidelines Linked to the International Classification of Functioning, Disability, and Health from the Orthopaedic Section of the American Physical Therapy Association	Delitto, et al.	Journal of Orthopaedic & Sports Physical Therapy	2012	10.2519/ jospt.2012.42.4.A1	571	47.58
A clinical test of stepping and change of direction to identify multiple falling older adults	Dite and Temple	Archives of Physical Medicine and Rehabilitation	2002	10.1053/ apmr.2002.35469	496	22.55
A clinical prediction rule for classifying patients with low back pain who demonstrate short-term improvement with spinal manipulation	Flynn, et al.	Spine	2002	10.1097/00007632-200212150-00021	452	20.55
Falls in individuals with stroke	Weerdesteyn, et al.	Journal of Rehabilitation Research and Development	2008	10.1682/ JRRD.2007.09.0145	437	27.31
Impact of Psychological Factors in the Experience of Pain	Linton, et al.	Physical Therapy	2011	10.2522/ptj.20100330	429	33
Fear of re-injury: a hindrance for returning to sports after anterior cruciate ligament reconstruction	Kvist, et al.	Knee Surgery Sports Traumatology Arthroscopy	2005	10.1007/s00167-004-0591-8	426	22.42
Neck pain: Clinical practice guidelines linked to the international classification of functioning, disability, and health from the orthopaedic section of the American physical therapy association	Childs, et al.	Journal of Orthopaedic & Sports Physical Therapy	2008	10.2519/jospt.2008.0303	415	25.94
Preliminary development of a clinical prediction rule for determining which patients with low back pain will respond to a stabilization exercise program	Hicks, et al.	Archives of Physical Medicine and Rehabilitation	2005	10.1016/j. apmr.2005.03.033	408	21.47
Information and advice to patients with back pain can have a positive effect - A randomized controlled trial of a novel educational booklet in primary care	Burton, et al.	Spine	1999	10.1097/00007632-199912010-00010	407	16.28
Randomized clinical trial of lumbar instrumented fusion and cognitive intervention and exercises in patients with chronic low back pain and disc degeneration	Brox, et al.	Spine	2003		395	18.81
The Effect of Neuroscience Education on Pain, Disability, Anxiety, and Stress in Chronic Musculoskeletal Pain	Louw, et al.	Archives of Physical Medicine and Rehabilitation	2011	10.1016/j. apmr.2011.07.198	392	30.15
Evaluating Common Outcomes for Measuring Treatment Success for Chronic Low Back Pain	Chapman, et al.	Spine	2011	10.1097/ BRS.0b013e31822ef74d	349	26.85
Course and prognostic factors for neck pain in whiplash- associated disorders (WAD) -: Results of the bone and joint decade 2000- 2010 task force on neck pain and its associated disorders	Carroll, et al.	Spine	2008	10.1097/ BRS.0b013e3181643eb8	339	21.19
Psychometric properties of the Tampa Scale for kinesiophobia and the fear- avoidance beliefs questionnaire in acute low back pain	Swinkels-Meewisse, et al.	Manual Therapy	2003	10.1054/ math.2002.0484	339	16.14
Identifying subgroups of patients with acute/subacute nonspecific low back pain - Results of a randomized clinical trial	Brennan, et al.	Spine	2006	10.1097/01. brs.0000202807.72292.a8	334	18.56

The dataset for "citation topics micro" displayed a wide range of topics, including 95 entry points, totaling 2,035 records. The majority of the records (61.93%) were about low back pain, which was followed by records about anterior cruciate ligament, falls, shoulder, and intervertebral disc. Notably, the analyzed field contained no data for 1.82% of the entries. The compilation provided a thorough look at the distribution of research emphasis within the micro context of citations, ranging from more general topics like sports psychology and cancer survivors to orthopedic concerns like Achilles tendon and osteoarthritis.

Top Publishing Journals

According to record counts and percentages of the 2,035 records, the top publishing journals on this topic were as follows: BMC Musculoskeletal Disorders was in first place with 7.961%, and Spine was close behind with 7.813%. Physiotherapy Theory and Practice (3.147%), Disability and Rehabilitation (3.980%), European Spine Journal (3.980%), Journal of Orthopaedic Sports Physical Therapy (3.735%), and Physical Therapy (5.160%) were some of the other well-known journals. Significant literature contributions were also made by Archives of Physical Medicine and Rehabilitation, Musculoskeletal Science and Practice, and Journal of Rehabilitation Medicine (Table 3). The list highlighted a wide variety of journals, highlighting the fact that research on musculoskeletal disorders, rehabilitation, and related topics is multidisciplinary.

Table 3. Top publishing journals on kinesiophobia

Publication journals	Record Count	% of 2.035
BMC Musculoskeletal Disorders	162	7.961
Spine	159	7.813
Physical Therapy	105	5.160
Disability and Rehabilitation	81	3.980
European Spine Journal	81	3.980
Journal of Orthopaedic Sports Physical Therapy	76	3.735
Physiotherapy Theory and Practice	62	3.047
Archives of Physical Medicine and Rehabilitation	59	2.899
Musculoskeletal Science and Practice	55	2.703
Journal of Rehabilitation Medicine	50	2.457
Spine Journal	45	2.211
Manual Therapy	43	2.113
Journal of Back and Musculoskeletal Rehabilitation	42	2.064
Journal of Manipulative and Physiological Therapeutics	38	1.867
Clinical Rehabilitation	35	1.720
Gait Posture	34	1.671
Journal of Manual Manipulative Therapy	29	1.425
Journal of Bodywork and Movement Therapies	28	1.376
Journal of Sport Rehabilitation	28	1.376
Physical Therapy In Sport	26	1.278
PMR	26	1.278
Physiotherapy Research International	24	1.179
European Journal of Physical and Rehabilitation Medicine	23	1.130
Knee Surgery Sports Traumatology Arthroscopy	22	1.081
Brazilian Journal of Physical Therapy	18	0.885

Showing 25 out of 195 entries

Keyword Analysis

There were 3,116 total keywords in the Vosviewer keyword analysis, 276 of which appeared more than five times. The most popular search terms, along with their respective frequency and total link power, were as follows: With 358 occurrences and an overall link strength of 849, "low back pain" was the most popular keyword. The term "rehabilitation" came in second with 186 occurrences and a link strength of 472 while the term "kinesiophobia" showed up 173 times and a link strength of 428. Other significant terms included "chronic pain" (127 occurrences, 334 link strength), "disability" (124 occurrences, 364 link strength), and "pain" (122 occurrences, 346 link strength). The focus and connections between these themes in the research literature are shown by the prominence of terms like "neck pain," "chronic low back pain," "exercise," "physiotherapy," "back pain," "physical therapy," "fear," "fear of movement," and "physical activity" in the keyword analysis. Figure 2 shows the keyword analysis with Vosviewer.

Bibliographic Coupling Analysis Among Countries

Figure 3 shows the bibliographic coupling analysis among countries.

In total, 42 of the 76 publishing countries contributed at least 5 articles each. The outcomes of the analysis of bibliographic coupling with Vosviewer are as follows: With 576 documents, 19,184 citations, and a total link strength of 584,097, the United States took the lead. With 209 documents (5,679 citations, 238,145 link strength), 177 documents (4,806 citations, 201,064 link strength), and 177 documents (5,302 citations, 235,012 link strength), respectively, Australia, England, and the Netherlands were also significant contributors. Following closely were Brazil, Canada, Sweden, Belgium, and Belgium, all of which significantly improved the state of research. The analysis sheds light on how research efforts in the field are distributed globally, highlighting the extensive international cooperation and knowledge exchange.

In North America, the United States took the lead among the continents, making a significant contribution with 576 documents, 19,184 citations, and a total link strength of 584,097. Furthermore, Canada contributed significantly with 163 documents, 3,834 citations, and 198,873 link strength. With 209 documents, 5,679 citations, and a total link strength of 238,145, Australia stood out in Oceania. Several European countries made significant contributions, including Belgium (108 documents, 2,961 citations, 146,034 link strength), Sweden (143 documents, 5,484 citations, 191,448 link strength), the Netherlands (177 documents, 5,302 citations, 235,012 link strength), and England (177 documents, 4,806

citations, 201,064 link strength). Brazil provided 106 documents, 1,080 citations, and 89,437 link strength to represent South America. Turkiye, Germany, and Iran made notable contributions in Asia. The global distribution of research efforts on the subject is thoroughly outlined by this bibliographic coupling analysis with

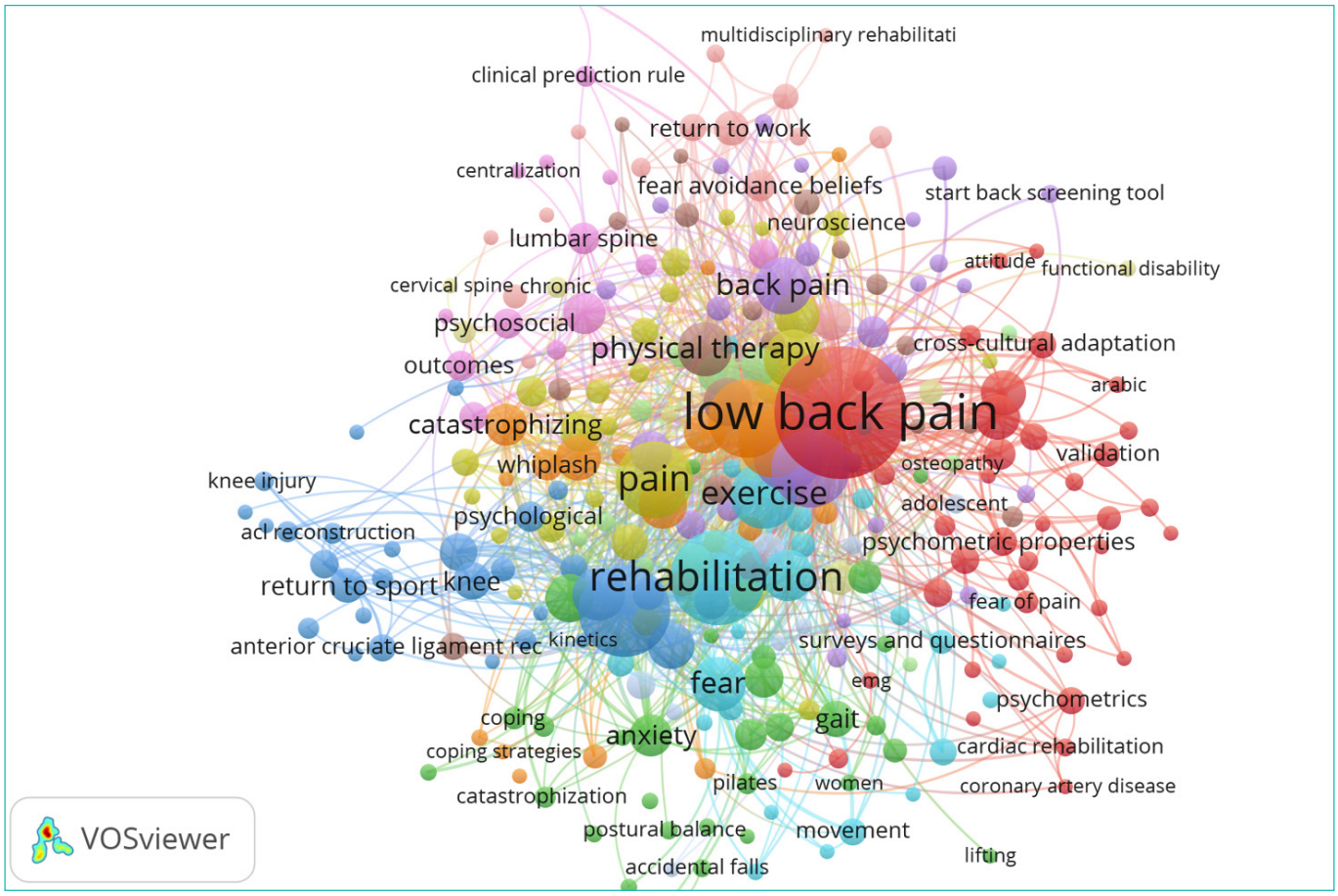

Figure 2. Keyword analysis

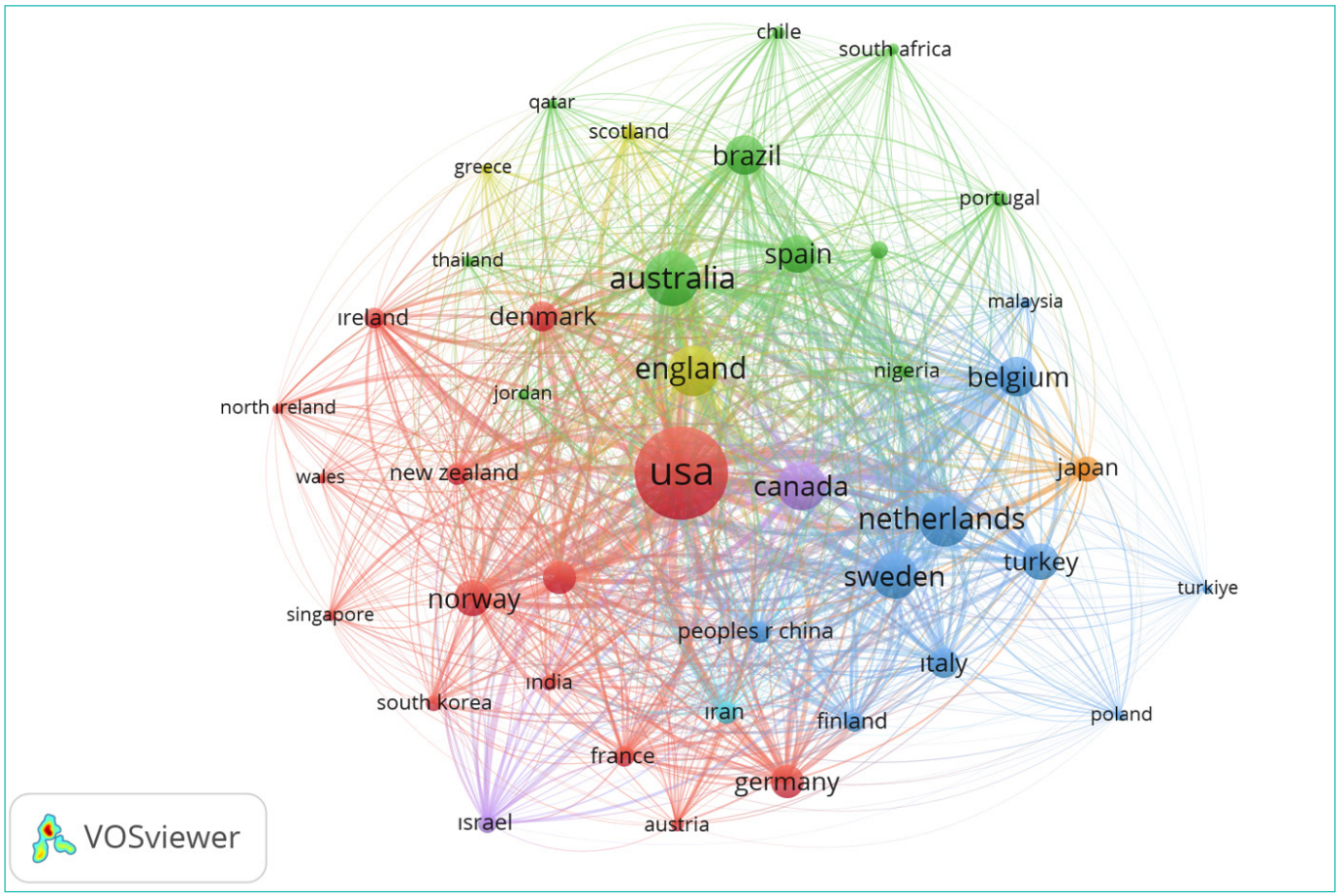

Figure 3. Bibliographic coupling between countries

Vosviewer, which emphasizes the cooperative nature of scientific exploration across continents.

Bibliographic Coupling Analysis Among Organisations

Figure 4 shows the bibliographic coupling analysis among organisations.

The bibliographic coupling analysis with Vosviewer showed that 249 of the 2,518 publishing companies made noteworthy contributions with at least 5 articles each. With 64 documents, 2,814 citations, and a significant total link strength of 183,088 the University of Florida emerged as a notable leader. Maastricht University and Karolinska Institutet came in second and third, respectively, with 54 documents (1,411 citations, 97,015 link strength), and 49 documents (1,411 citations, 111,668 link strength), demonstrating their influence. University of Sydney, University of Oslo, University of Queensland, University of Ghent, and University of Gothenburg are just a few more noteworthy institutions. With 33 documents, 2,730 citations, and 66,194 link strength, the University of Pittsburgh stood out and demonstrated its significant impact on the research landscape. Furthermore, 33 documents from Vrije Universiteit Amsterdam and Vrije Universiteit Brussel, with 926 citations and 49,746 link strength and 921 citations and 66,603 link strength, respectively, were also contributed. These findings demonstrate the variety of international organizations actively advancing the field's scholarly conversation.

DISCUSSION

The use of Vosviewer software to conduct a bibliometric analysis of articles on kinesiophobia from September 2023 to 1970 gave important insights into the state of research in this field. Key findings are highlighted in this discussion section, along with general information, noteworthy trends, top publishing countries and affiliations, most-cited articles, citation topics, top publishing journals, keyword analysis, and analyses of the bibliographic couplings between various nations and organizations.

The study by Luo et al.[15] used bibliometric techniques to evaluate the global scientific output on pain catastrophizing and to pinpoint hotspots and frontiers from 2010 to 2020. Through the use of CiteSpace, they extracted publications from the WoSCC and examined various bibliometric indicators. Their analysis of 1,576 publications showed a steady rise in annual publications. In terms of publication and citation frequency, the pain journal led productivity, and the University of Washington in particular dominated in the United States. The current bibliometric analysis included a sizable dataset of 2,035 academic articles on kinesiophobia in orthopedics written by 6,742 authors with 2,377 affiliations and contributors from 75 different nations. A thorough analysis of the scholarly output and teamwork within the field was made possible by the dataset's sizeable scope.

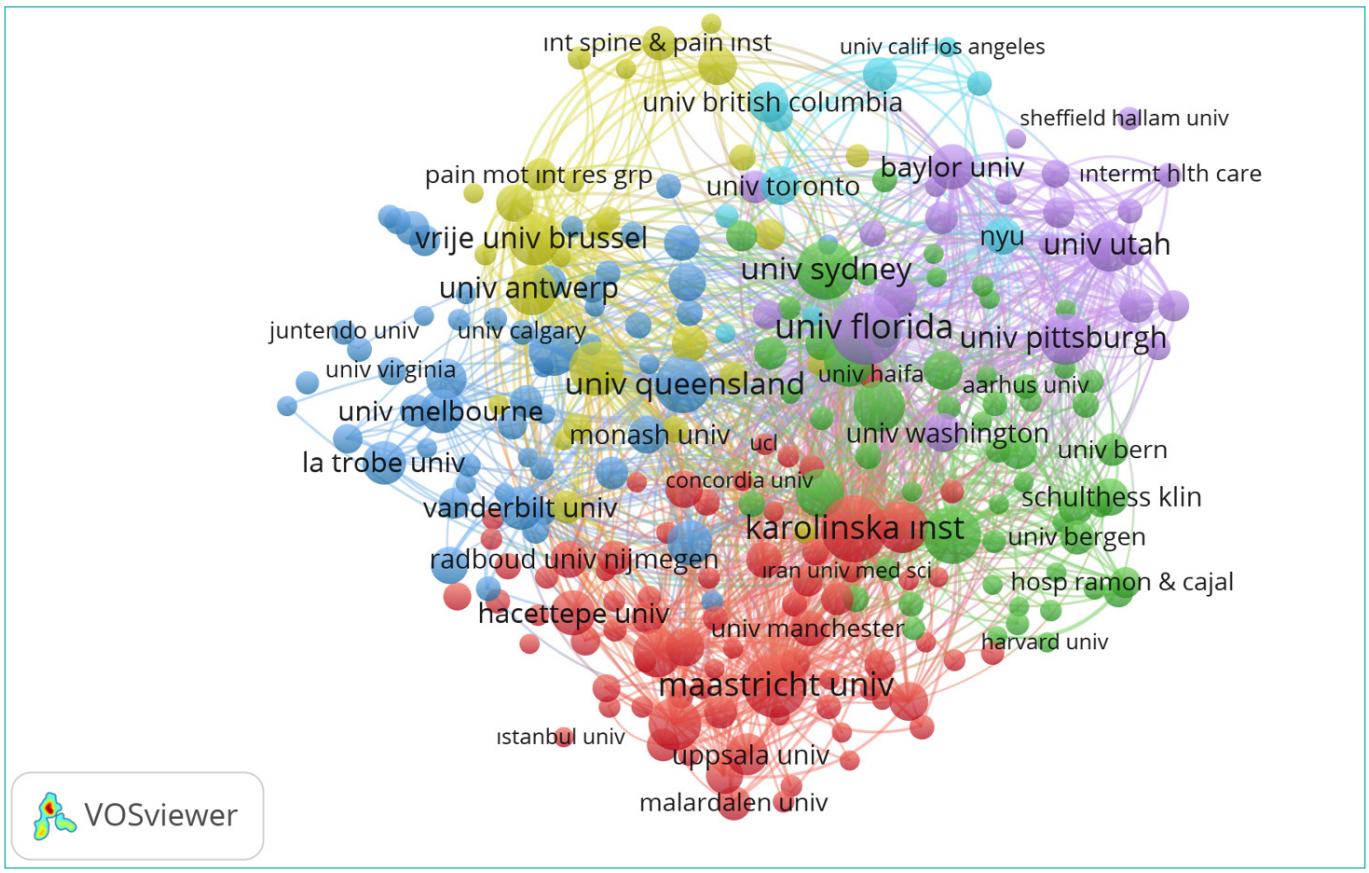

Figure 4. The bibliographic coupling analysis among organisations

The analysis showed that the 2,035 articles had a significant total of 51,443 citations, with an average of 25.27 citations per article. The depth and significance of the kinesiophobia literature are reflected in the high h-index of 98, which indicated a significant number of highly cited works. Indicating a noticeable increase in publications from 2001 to 2012 and a consistent and significant output in more recent years, particularly in 2020, 2021, and 2022, trends in scholarly output were identified over a variety of time periods.

There were both noticeable differences and similarities between the findings of the current study on kinesiophobia and those reported by Luo et al.[15] in their study on pain catastrophizing. The United States' status as a significant research power in both fields was recognized, reflecting the country's significant contributions to the literature on kinesiophobia and pain catastrophizing. Both studies emphasized the value of international cooperation and the requirement for greater collaboration between nations and institutions in order to improve knowledge exchange. The landscape of collaborative work revealed differences. While Luo et al.[15] noted limited international cooperation in pain catastrophizing research, the current study on kinesiophobia highlighted more extensive collaboration, involving contributions from 76 countries. As a result of numerous institutions from various geographical areas taking part in the kinesiophobia research, the academic landscape's interconnectedness was clear. The State University System of Florida, University of Florida, Maastricht University, Karolinska Institutet, Vrije Universiteit Brussel, and the University of Southern Denmark were all recognized as major contributors to kinesiophobia literature in the current study. The University of Washington was the top publishing institution in the Luo et al. study.[15] In the current study, the analyses of the bibliographic coupling between countries and organizations shed light on joint research initiatives. The United States emerged as a significant contributor in both instances, highlighting its pioneering work in advancing kinesiophobia research. Significant contributions from Australia, England, the Netherlands, and numerous international organizations were also highlighted in the analyses, highlighting a global network of collaboration.

Overall, Luo et al.[15] study and the current study shed light on the global research landscape in their respective fields, but the distinct dynamics of each field of study—pain catastrophizing 15 and kinesiophobia—were highlighted by differences in international collaboration and institutional contributions.

The "citation topics micro" analysis showed a wide range of topics, with low back pain receiving a disproportionate amount of attention (61.93% of records). This thorough explanation offered a nuanced understanding of the research focuses within the literature on kinesiophobia, covering various topics like sports psychology and orthopedic issues.

Researchers can assess the standing and influence of particular publications within their field by knowing the best journals to publish in. Additionally, it assists in evaluating the methods of research dissemination by highlighting the venues that draw significant contributions. Understanding the significance of particular journals is crucial for putting research into context and identifying scholarly communication trends. Additionally, by using this data, it is possible to select pertinent sources for literature reviews, extract data, and make sure that the bibliometric analysis is based on a thorough knowledge of the academic ecosystem.[17-20] In the forefront of the kinesiophobia literature, BMC Musculoskeletal Disorders and Spine stand out as significant contributors, underscoring their importance in influencing the conversation about musculoskeletal health. With their large readerships and strict editorial standards, these journals stand for the authority and significance of the research they publish. Their placement at the top of the list highlights the significant influence they have on the dissemination of information about kinesiophobia-related topics. Furthermore, the inclusion of prestigious journals like Physiotherapy Theory and Practice, Disability and Rehabilitation, and European Spine Journal demonstrates the diversity of sources that enrich the literature on kinesiophobia. This eclectic collection of journals, which includes topics like physiotherapy, rehabilitation, and spinal health, reflects the multidisciplinary nature of research on kinesiophobia. The existence of these journals not only widens the scope of the literature but also represents interdisciplinary cooperation in tackling the complex issues related to kinesiophobia.

The discovery of "burst" keywords, according to Luo et al.[15] reflects current research areas, and the development of keywords in papers that catastrophize pain sheds light on the research's past. As an illustration, the terms "adjustment," "dimension," and "confirmatory factor analysis" all showed strong citation bursts in the earlier stages, indicating a focus on emotional psychological adjustment and the reliability and validity testing of pain catastrophizing scales. Notably, the term "total hip" became a popular search term in 2016, indicating a persistent pattern in orthopedic studies on pain catastrophizing. In contrast, the current study on kinesiophobia showed a wide range of topics in the "citation topics micro" analysis, with low back pain (61.93% of records) receiving disproportionate attention. This nuanced understanding touched on a number of topics, including orthopedic

problems and sports psychology. "Low back pain" was the keyword with the highest popularity according to the Vosviewer keyword analysis, with 358 occurrences and an 849 link strength. With terms like "adjustment" and "confirmatory factor analysis" indicating early research foci, Luo et al.'s study[15] contrasted and displayed a different emphasis. Both studies demonstrate the dynamic nature of research in their respective fields, but the emphasis on particular keywords and research areas varies, highlighting the divergent trajectories of the literature on pain catastrophizing and kinesiophobia.

Limitations

The Web of Science Core Collection was used as the only data source, which could lead to biases in the bibliometric study on kinesiophobia in orthopedics. Despite being a comprehensive database, the Web of Science might not contain all pertinent articles since some might be indexed in other databases not used in this study. Furthermore, the search was limited to articles written in English, potentially omitting valuable contributions written in other languages. The study's concentration on orthopedics might make it less applicable to other medical specialties. Kinesiophobia can be researched and treated in a variety of medical settings, so limiting the analysis to orthopedics may leave out crucial information from related disciplines. Additionally, the selection of keywords and analytical tools may affect how "hotspots" and "frontiers" are identified in kinesiophobia research. The accuracy of these tools and the consistency of keyword usage over time are prerequisites for the interpretation of trends and patterns in the literature.

Last but not least, the study gives a brief overview of the literature up to September 2023. Since the field is dynamic, new research is constantly being produced. After this cutoff date, changes in the research landscape are not captured, and the study's findings could be out of date as the field develops.

CONCLUSION

The thorough bibliometric analysis presented in this study offers a thorough overview of the literature on kinesiophobia, taking into account scholarly impact, trends, prominent authors, and thematic emphases. These results not only deepen our understanding of the state of kinesiophobia research, but they also provide important information for guiding future research and treatment in orthopedics and related fields. The collaborative nature of the research, as shown by the contributions from various nations and organizations, emphasizes the importance of the scientific study of kinesiophobia on a global scale and how interconnected it is.

ETHICAL DECLARATIONS

Ethics Committee Approval

As it is not a human or animal study there is no need for ethical approval.

Informed Consent

As it is not a human or animal study there is no need for 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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Predictive value of cervical length in placenta previa totalis: a single-center experience

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ABSTRACT

Aims: In our study, we aimed to scientifically assess the utility of measuring cervical length in predicting the risk of postpartum hemorrhage and the necessity for emergency preterm cesarean delivery in women diagnosed with placenta previa totalis.

Methods: We conducted a retrospective study at a single medical center, comprising 48 pregnant women diagnosed with placenta previa totalis. Cervical length was precisely measured through transvaginal ultrasound. Patients were categorized based on cervical length (CL): CL >30mm, CL 25-30 mm, and CL <25 mm. Primary outcomes included preterm birth, postpartum hemorrhage, and emergency cesarean section.

Results: Women with CL <25 mm exhibited a notably elevated risk of postpartum hemorrhage (60%) and emergency cesarean section (80%). While preterm birth rates displayed variations among groups, statistical significance remained elusive. APGAR scores demonstrated consistency across cervical length categories.

Conclusion: Evaluation of cervical length holds promise as a valuable tool in the comprehensive management of placenta previa totalis. Women presenting with a cervical length less than 25 mm may warrant intensified monitoring and targeted interventions to mitigate adverse perinatal outcomes. Further research endeavors are imperative to corroborate these findings and advance the care provided for these intricate pregnancies.

Keywords: Placenta previa, hemorrhage, preterm birth, ultrasonography

INTRODUCTION

Placenta previa totalis, characterized by complete coverage of the cervical os by the placenta, presents a formidable challenge within obstetrics due to its significant association with adverse perinatal outcomes.[1-3] It exhibits an approximate incidence rate of 1% among all pregnancies[4,5], and its prevalence seems to be on the ascent, possibly attributed to escalating rates of cesarean sections and the increasing age of expectant mothers.[6,7] Despite advances in management and monitoring, the accurate prediction and prevention of unfavorable outcomes in these cases continue to be intricate endeavors.

Cervical length, a well-established metric in obstetrics, has surfaced as a potential pivotal factor in the comprehension and management of pregnancies complicated by placenta previa totalis.[8-12] Extensively scrutinized for its prognostic capacity regarding preterm birth risk across diverse obstetric scenarios,[13] cervical length has gained pronounced relevance in this specific context. An investigation by Ghi et al.[14] manifested that women afflicted with placenta previa totalis, bearing a cervical length measuring less than 31 mm, exhibited a staggering 16-fold greater likelihood of delivering preterm before 34 weeks of gestation when contrasted with those possessing a cervical length of 31 mm or greater.

Notwithstanding this burgeoning fascination with cervical length as a predictive determinant, its precise role and impact in the realm of placenta previa totalis cases endure as enigmatic facets. Within the confines of this research article, our primary objective is the exhaustive exploration of the intricate interplay between cervical length and perinatal outcomes in patients bearing the diagnosis of placenta previa totalis. Our exclusive focus on cervical length is aimed at illuminating its predictive efficacy, prospective complications, and ramifications for clinical management.

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This study is imbued with the aspiration of furnishing a profound comprehension of the role of cervical length in pregnancies marred by placenta previa totalis. Our findings have the potential to proffer invaluable insights to clinicians, thereby facilitating risk assessment, early intervention, and the formulation of bespoke management strategies for expectant mothers grappling with this multifaceted obstetric challenge. Ultimately, our overarching objective resides in the enhancement of care and the amelioration of outcomes for both mothers and neonates ensnared within the labyrinthine confines of these taxing pregnancies.

METHODS

This study was conducted in accordance with the Helsinki Declaration and received approval from Haseki Training and Research Hospital Clinical Researches Ethics Committee. (Date: 20.09.2023, Decision No: 162-2023). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Participants

The study included pregnant women admitted to our clinic between January 2021 and August 2023, diagnosed with placenta previa totalis with a gestational age between 33-37 weeks. Only singleton pregnancies were considered.

Exclusion criteria encompassed multiple pregnancies, placenta accreta, active genital tract infections, a history of preterm delivery, prior cervical surgeries, severe medical conditions potentially impacting pregnancy outcomes, and fetal anomalies.

Clinical data such as patients' age, parity, presence of antepartum bleeding (APH), type of caesarean section (emergency or elective), gestational age, birth weight, and Apgar scores were retrieved from patients' medical records. Obstetric outcomes were extracted from hospital maternity records.

Perinatal Outcome Assessment

Perinatal outcomes were assessed at the time of delivery. The primary outcome of interest was preterm birth, defined as delivery occurring before 37 weeks of gestation. Secondary outcomes included rates of emergency cesarean section, postpartum hemorrhage, and neonatal complications.

Measurement of Cervical Length (CL)

Cervical Length (CL) was meticulously measured through transvaginal ultrasound examinations using a Voluson E6 ultrasound system (GE Medical Systems, Milwaukee, WI, USA) equipped with a 2-8 MHz probe. All cervical measurements were consistently performed by the same experienced fetal medicine consultant.

For CL measurement, we recorded the distance from the internal os to the most distal edge of the cervix. Cervical evaluation adhered to a strict and uniform protocol to ensure precision. Prior to the examination, participants were instructed to empty their bladder to optimize ultrasound imaging clarity. A true sagittal plane was obtained during ultrasound to visualize the full cervical canal length. CL was measured three times, and the shortest measurement among the three was recorded for each participant. This standardized approach minimized variability and bolstered the data's reliability, enhancing the study's robustness.

Statistical Analyses

To evaluate the normality of continuous variables, appropriate normality tests were conducted. Non-normally distributed data were described using median and interquartile range (25^{th}-75^{th} percentile), while normally distributed continuous data were presented as mean and standard deviation. Categorical variables were compared using the Chi-square test or Fisher's exact test, depending on the data's nature. Statistical significance was defined as $p \leq 0.05$. All statistical analyses were carried out using IBM SPSS software, version 21 (IBM, US).

RESULTS

Study Population

Initially, a total of 63 asymptomatic women diagnosed with placenta previa totalis were retrospectively enrolled for this study. However, after applying stringent exclusion criteria, four cases were excluded due to the presence of placenta accreta, while ten cases were excluded for various reasons including a history of previous cervical surgeries (1 case), multiple pregnancies (2 cases), a history of preterm delivery (3 cases), maternal comorbidities such as preeclampsia (4 cases), and fetal anomalies (1 case). This meticulous screening and exclusion process culminated in a final study population of 48 women. A comprehensive summary of patient data and pregnancy outcomes for this ultimate cohort is meticulously presented in **Table 1**, and **Figure**.

Table 1. Patient characteristics and pregnancy outcome

Characteristics/outcome	n(%) or mean±SD (n=48)
Maternal age	34.2±4.5
Parity	
Nulliparous	13 (27)
Multiparous	35 (72.9)
Previous Cesarean section	
0	9 (18,7)
1	7 (14,5)
>1	32 (66,6)
Gestational age at diagnosis (week)	30.3±3.2
Cervical length (mm)	
>30	31 (64.6)
25-30	12 (25)
<25	5 (10.4)
Blood transfusion	17 (35.4)
Gestational age at delivery (weeks)	35.4±2.5
Birth weight (g)	2938.5±330.6
APGAR score	
>7	41 (85.4)
<7	7 (14.5)

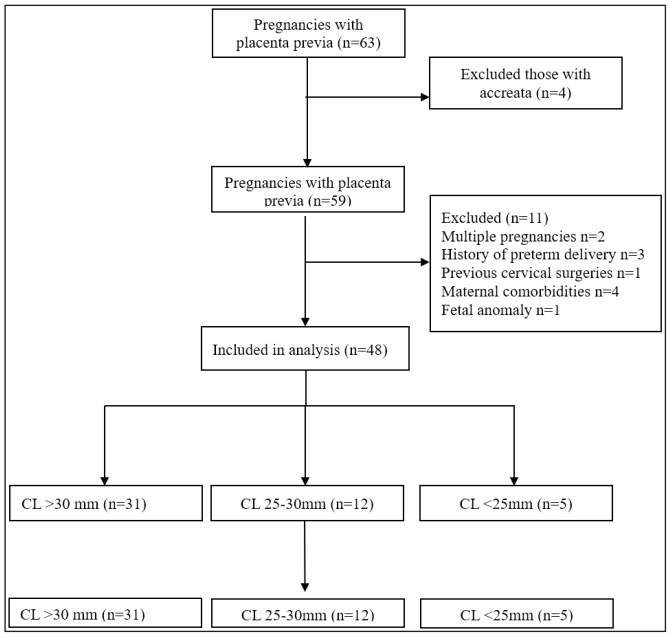

Figure. Flow diagram of the cohort

Demographics and Obstetric Characteristics

Within this definitive study population, 13 women (27%) were nulliparous. The mean gestational age at the time of transvaginal ultrasound assessment was 30.3±3.2 weeks. In all instances, the mode of delivery selected was cesarean section, and it was executed at a mean gestational age of 35.4±2.5 weeks. Neonates in this cohort exhibited a mean birth weight of 2938.5±330.6 g. Pertaining to cervical length measurements, 31 women (64.6%) demonstrated cervical lengths exceeding 30 mm, 12 women (25%) exhibited cervical lengths ranging between 25 mm and 30 mm, and 5 women (10.4%) displayed cervical lengths less than 25 mm. Furthermore, 17 cases (35.4%) necessitated blood transfusion during the course of their clinical management.

Outcome of Pregnancy According to Cervical Length

In addition to the abovementioned demographic and obstetric characteristics, Table 2 provides an all-encompassing overview of pregnancy outcomes meticulously categorized based on cervical length (CL) measurements. Participants were meticulously stratified into three distinct cervical length categories: those harboring CL >30 mm, CL 25-30 mm, and CL <25 mm.

Table 2. Outcome of pregnancy according to cervical length

Outcomes	CL >30 mm n=31 (%)	CL 25-30 mm n=12 (%)	CL <25 mm n=5 (%)	p
Postpartum haemorrhage	4 (12.9)	6 (50)	3 (60)	<0.05
Emergency cesarean section	7 (22.5)	6 (50)	4 (80)	<0.05
Preterm birth	8 (25.8)	5 (41.6)	5 (100)	0.3
APGAR score (5 min)				
>7	29 (93.5)	9 (75)	3 (60)	0.07
<7	2 (6.5)	3 (25)	2 (40)	0.07

Postpartum hemorrhage rates exhibited notable variations among the cervical length groups (p <0.05). Notably, among women with CL>30 mm, merely 12.9% encountered postpartum hemorrhage. In stark contrast, this rate significantly escalated to 50% in the CL 25-30 mm group and surged further to 60% in the CL<25 mm group.

Statistical analysis unveiled a noteworthy difference in the incidence of emergency cesarean sections across the cervical length categories (p<0.05). Specifically, within the CL>30 mm group, 22.5% underwent emergency cesarean sections. This rate surged to 50% in the CL 25-30 mm group and peaked at 80% in the CL<25 mm group.

Although numerical disparities in preterm birth rates were observable among the groups, statistical significance remained elusive (p=0.3). Concretely, preterm birth rates stood at 25.8% in the CL >30 mm group, 41.6% in the CL 25-30 mm group, and 100% in the CL<25 mm group.

APGAR scores, while generally consistent across the cervical length groups, warrant attention. Notably, the CL <25 mm group displayed a lower percentage of neonates with APGAR scores >7 (60%) and a higher percentage with scores <7 (40%) in comparison to the other groups.

These findings underscore the profound associations between cervical length and specific perinatal outcomes in placenta previa totalis cases, shedding light on the intricate nuances of clinical management and risk assessment.

DISCUSSION

Our findings distinctly revealed that women with a cervical length measuring less than 25 mm faced a significantly elevated risk of postpartum hemorrhage and emergency cesarean section. These outcomes align with the results of previous investigations, which consistently demonstrated that a shorter cervical length in women with placenta previa totalis is associated with an increased susceptibility to adverse perinatal outcomes, including preterm birth and low birth weight infants.[14-17]

The precise mechanistic link between a shorter cervical length and the heightened risk of adverse perinatal outcomes in placenta previa totalis cases remains not entirely elucidated. Nonetheless, it is postulated that a shorter cervical length may render the cervix more susceptible to dilatation and effacement, potentially culminating in preterm birth or necessitating emergency cesarean delivery. Additionally, a shorter cervical length may pose challenges in managing postpartum hemorrhage, potentially complicating efforts to control bleeding after delivery.[18]

In a study conducted by Zaitoun et al.[19] which focused on cases of placenta previa totalis, the research highlights the potential value of measuring cervical length in predicting the likelihood of APH and underscores the importance of early emergency cesarean delivery. The results indicate that individuals with cervical lengths equal to or less than 30 mm frequently face a substantially increased risk of severe APH, often requiring urgent medical intervention. In another study, Stafford et al.[20] observed that women with placenta previa totalis and a cervical length of 30 mm or less had a threefold higher likelihood of experiencing preterm birth compared to those with longer cervixes. Additionally, among women with cervical lengths of 30 mm or less, the risk of hospitalization prior to delivery due to vaginal bleeding was more than twice as high as the risk of delivery due to bleeding.

The increased risk of preterm birth and hemorrhage among women with placenta previa totalis and a shortened cervix could be attributed to an elevated likelihood of spontaneous preterm labor. Notably, sonographically detected cervical shortening consistently serves as a predictive indicator of earlier labor initiation. In the context of placenta previa totalis, even minor cervical shortening might precipitate premature labor initiation and the potential detachment of the placenta from its low insertion site. Our study highlights that, in women with placenta previa totalis, the risk of minor APH may not substantially differ with cervical length. However, even slight cervical shortening may portend earlier placental detachment with the potential for significant hemorrhage.

Limitations

We acknowledge that our study possesses several limitations, primarily due to its retrospective nature and the fact that it was conducted at a single medical center. These factors may potentially restrict the applicability of our findings to broader patient populations. Furthermore, the relatively modest sample size in our study constitutes another limitation, which may have reduced the statistical power and rendered it more challenging to discern statistically significant differences among the cervical length groups.

However, it is worth highlighting a strength of our study, which lies in the consistent care provided by the same team throughout all procedures, including delivery. This uniformity ensured accuracy and reliability in data collection, and all fetal evaluations and follow-ups were conducted by a specialized perinatology team, thereby further enhancing the precision of our findings.

CONCLUSION

Our findings hold substantial clinical implications for the management of women diagnosed with placenta previa totalis. Specifically, women with a cervical length measuring less than 25 mm necessitate vigilant monitoring and may benefit from more proactive interventions to mitigate the risk of adverse perinatal outcomes. This pursuit of knowledge will ultimately lead to enhanced care and improved outcomes for women facing the complex challenges of placenta previa totalis during pregnancy.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Haseki Training and Research Hospital Clinical Researches Ethics Committee (Date: 20.09.2023, Decision No: 162-2023).

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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Comparison of early warning and sepsis scores for mortality prediction in patients with suspected infection admitted to medical intensive care units

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ABSTRACT

Aims: To compare the mortality prediction efficiency of the Modified Early Warning Score (MEWS), Systemic Inflammatory Response Syndrome (SIRS), Sepsis Related Organ Failure Assessment (SOFA), and Quick Sepsis Related Organ Failure Assessment (qSOFA) calculated within 48 hours before ICU admission.

Methods: A prospective, noninterventional, observational cohort study enrolled adult patients admitted to medical intensive care units (ICU) with suspected infection in a tertiary care medical center. MEWS SIRS, SOFA, and qSOFA scores were calculated at four different time points: 48, 24, and 8 hours before and at the time of the ICU admission (0. hour). The scores were analyzed for hospital mortality.

Results: A total of 120 patients were included. The median age was 68 (IQR 59.8-79) years, and 44.2% of patients were male. Of the study population, 75.8% were admitted to the medical ICU from the emergency department, while the remaining were from the medical wards. Considering the scores observed 48 hours before ICU admission, Odds Ratio (OR) of SIRS≥2 and SOFA≥2 showed a value of 7.6 (95% CI: 1.5-38.0) and 13.2 (95% CI: 2.3-74.3), respectively, while no increase in risk was observed for MEWS and the qSOFA score. Receiver operating characteristic analysis (ROC) performed with the highest scores observed at any time within 48 hours before ICU admission (ICU admission values were omitted) regarding hospital mortality yielded area under the curve (AUC) values (95% CI) of 0.80 (0.72-0.89) for SOFA, 0.66 (0.54-0.76) for MEWS, 0.63 (0.51-0.74) for qSOFA, and 0.61 (0.49-0.73) for SIRS. SOFA had the highest sensitivity of 92.6% (82.7-100.0), whereas qSOFA had the highest specificity of 63.0% (49.1-77.0) for hospital mortality.

Conclusion: SOFA score is the most sensitive scoring system to predict hospital mortality in patients admitted to the medical ICU with suspected infection compared to MEWS, SIRS, and qSOFA. Nevertheless, the sepsis and early warning scores should be combined in clinical practice whenever possible.

Keywords: Early warning score, qSOFA, critical care, sepsis, SOFA, systemic inflammatory response syndrome

The study has been derived from the Internal Medicine graduation thesis of Batuhan Başpınar, MD.

INTRODUCTION

Sepsis is a common health problem that causes high morbidity and mortality.[1,2] Increased health care expenditures are also a priority concern.[3] Therefore, it is crucial to detect sepsis early and prevent further complications. Clinical scoring systems were employed for this purpose, such as the systemic inflammatory response syndrome (SIRS), the sepsis-related organ failure assessment (SOFA), the quick sepsis-related organ failure assessment (qSOFA), and the modified early warning score (MEWS).[4-10] SIRS is the first clinical scoring system developed to predict sepsis mortality. Due to the low specificity attributed to SIRS, SOFA and qSOFA scores were introduced in clinical practice. Besides the sepsis scores, early warning scores were used to detect deteriorating patients. While National Early Warning Score (NEWS) is most widely used, MEWS is employed for early warning determination in our hospital.[6] Although last consensus guidelines suggested a combination of these scoring systems,[11] establishment of a standard in the use of scoring systems is still an issue.

Several studies have evaluated the early diagnostic value and predictive power of MEWS, SIRS, SOFA, and qSOFA scores and compared them in pairs and triads.[5-7,12-22]

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The aim of our study was to compare the mortality prediction of MEWS, SIRS, SOFA, and qSOFA scores calculated at different time periods 48 hours before ICU admission of patients with suspected infection.

METHODS

This study was approved by the Hacettepe University Scientific Researches Ethics Committee (Date: 19.12.2017, Decision No: GO17/948-11). Informed Consent was obtained from the patients or the legal guardians of the patients who could not give informed consent. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

A prospective observational cohort study was conducted in patients with suspected infection admitted to medical intensive care units of tertiary care university hospitals between January 1, 2018, and May 31, 2018. The medical ICUs consisted of a 3rd-level medical ICU with 9 beds, a 3rd-level medical oncology ICU with 6 beds, and a medical acute care unit operated as a 1st-level medical ICU with a capacity of 10 beds. Admission to the medical ICU was through the medical wards or the emergency department (ED). Patients who met the criteria for suspected infection defined below within 48 hours before admission to the ICU were recruited. Patients younger than 18 years, patients admitted directly to the ICU from another hospital, postoperative patients, patients transferred to another medical center, patients who refused to participate in the study, patients hospitalized within 28 days before ICU admission, patients receiving prophylactic antimicrobials, and patients without suspected infection were excluded from the study.

Data Collection

All patients admitted to the medical ICUs were screened for eligibility at the time of admission. Demographic data of the patients who met the enrollment criteria such as age, sex, body mass index (BMI), comorbidities, along with the length of hospital stay before ICU admission, department information where patients were admitted to the ICU were collected from printed or electronic patient file at the time of ICU admission. The Charlson Comorbidity Index (CCI), APACHE-II Scores, and early warning and sepsis scores (MEWS, SIRS, SOFA, qSOFA) were calculated during ICU admission. Early warning and sepsis scores from three different time periods before ICU admission, defined below, were calculated retrospectively from the printed and electronic patient files. Patients were followed for information on the total length of hospital stay (LOS) and the occurrence of mortality. Patient identity was not disclosed during data collection.

Definitions, Outcomes

Suspected infection is defined as suspicion of a physical examination, ordering of a culture of body fluids, radiologic examination, or empiric/preemptive antimicrobial treatment of a clinical infection.[14] Antimicrobial use is defined as oral or parenteral medications used to treat bacterial, fungal, or viral infections. MEWS the SIRS, SOFA, and qSOFA scores were calculated at four different time points: 50-46 hours (-48h), 24 hours (-24h), and 8 hours (-8h) before ICU admission and at ICU admission (0h). the 0-hour (0h) period included the first 2 hours after admission to the ICU. Accordingly, the -48-hour period included the time between the 50th and 46th hours, the -24-hour period included the time between the 26th and 22nd hours, and the -8-hour period included the time between the 10th and 6th hours. The following values were accepted as cut-off values for scoring systems: MEWS ≥3 or a parameter of MEWS ≥2, SIRS ≥2, SOFA score ≥2, qSOFA score ≥2. The primary end point of the study was in-hospital mortality.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) ver. 25.0 (SPSS, IBM, Armonk, New York, USA). Numbers and percentages were reported for categorical data. For normally distributed continuous variables, mean and standard deviation (SD) were used; for nonnormally distributed continuous variables, median and interquartile range were used. Pairwise comparison regarding hospital mortality was performed with the chi-square test for categorical variables, Student's T test for normally distributed continuous variables, and Mann-Whitney U test for nonnormally distributed continuous variables. A p value less than 0.05 was accepted as statistical significance. The effectiveness of the score for predicting mortality was evaluated with logistic regression to calculate odds ratios and with C-index and COX regression analyses for hazard ratios. Age, sex, BMI, and the department in which patients were admitted to the ICU were identified as confounders, and regression analyses were performed for each factor. Odds ratios and 95% confidence intervals were reported as results of logistic regression analysis, and hazard ratios were reported as results of COX regression. Receiver operating characteristic analysis (ROC) was performed to evaluate the efficacy, sensitivity, and specificity of the scores calculated in different time periods. The c-index value was reported as the result of the ROC analysis.

RESULTS

A total of 149 patients were enrolled in the study. Statistical analysis was carried out with 120 patients after excluding twenty-nine patients (Figure 1). Baseline patient characteristics and length of hospital stay are presented in Table 1. Survivor and non-survivor groups had similar age, gender, and BMI values according to hospital mortality (p>0.05). Although the length of ICU stay was the same, the median time before ICU admission and the total length of hospital stay (LOS) were longer in non-survivors. Most of the study population (n=91, 75.8%) of patients were admitted from ED, while 29 (24.2%) were from medical wards. The Charlson comorbidity index (CCI) was the same in both groups, and hypertension was the most seen comorbidity in the whole population. The mean APACHE II values were significantly higher in non-survivors (26.8, SD±8.1) than in survivors (16.2, SD±5.7, p<0.001). The highest values of MEWS, SIRS, SOFA, and qSOFA scores were significantly higher in non-survivors compared to survivors (p<0.001, p=0.007, p<0.001, and p<0.001, respectively).

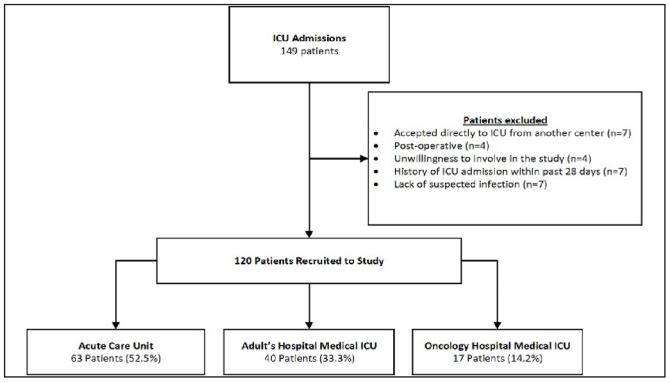

Figure 1. Flowchart of enrollment in the presented study

In-hospital mortality was observed in 33 (27.5%) patients, of which 14 (15.4%) were admitted from ED (n=91), whereas 19 (65.5%) of the patients were admitted from medical wards (n=29). The patients who admitted from other medical wards had a high mortality rate compared to ED (p<0.001). Moreover, the hospital mortality rate was higher in patients with steroid usage (83.3%), chronic liver disease (75.0%), cancer (53.1%), and non-steroidal anti-inflammatory drug usage (50.0%).

Table 1. Patient characteristics evaluated with respect to hospital mortality

	All Patients	Non-survivors	Survivors	p
	N=120	n=33	n=87	
Age, median (IQR), years	68.0 (59.8-79.0)	67.0 (61.0-78.0)	68.0 (59.0-79.0)	0.94
Male sex, No. (%)	53 (44.2)	15 (45.5)	38 (43.7)	1.00
BMI, mean (SD), kg/cm2	27.4 (6.3)	26.7 (5.9)	27.7 (6.5)	0.26
Length of stay before ICU admission, median (IQR), days	2.0 (1.0-5.0)	7.0 (2.0-18.5)	2.0 (1.0-3.0)	<0.001
Length of ICU stay, median (IQR), days	10.0 (6.0-168)	11.0 (6.5-23.5)	10.0 (6.0-14.0)	0.44
Length of hospital stay, median (IQR), days	17.0 (11.0-28.0)	25.0 (15.0-40.0)	16 (10.0-23.0)	0.003
Location prior to ICU				<0.001
Emergency	91 (75.8)	14 (42.4)	77 (88.5)	
Ward	29 (24.2)	19 (57.6)	10 (11.5)	
Charlson comorbidity index, mean (SD)	5.6 (2.8)	6.0 (3.0)	5.5 (2.8)	0.67
Comorbidity, No. (%)				
Hypertension	69 (57.5)	17 (51.5)	52 (59.8)	
COPD	47 (39.2)	5 (15.2)	42 (48.3)	
Diabetes Mellitus	42 (35.0)	9 (27.3)	33 (37.9)	
Coronary Artery Disease	38 (31.7)	5 (15.2)	33 (37.9)	
Malignancy	32 (26.7)	17 (51.5)	15 (17.2)	
Heart Failure	29 (24.2)	3 (9.1)	26 (29.9)	
CKD	24 (20.0)	3 (9.1)	21 (24.1)	
CVD	11 (9.2)	1 (3.0)	10 (11.5)	
Chronic Liver Disease	8 (6.7)	6 (18.2)	2 (2.3)	
Rheumatologic Disease	6 (5.0)	1 (3.0)	5 (5.7)	
Steroid Usage	6 (5.0)	5 (15.2)	1 (1.1)	
NSAID Usage	6 (5.0)	3 (9.1)	3 (3.4)	
APACHE II score, mean (SD)	19.1 (8.0)	26.8 (8.1)	16.2 (5.7)	<0.001
Highest score 48 hrs prior, mean (SD)				
MEWS	5.0 (2.3)	6.6 (2.5)	4.5 (1.9)	<0.001
SIRS	2.6 (0.8)	2.9 (0.8)	2.4 (0.8)	0.007
SOFA	4.8 (3.7)	8.0 (3.8)	3.6 (2.9)	<0.001
qSOFA	1.9 (0.7)	2.3 (0.6)	1.8 (0.7)	<0.001

CKD: Chronic Kidney Disease, COPD: Chronic Obstructive Pulmonary Disease, CVD: Cardiovascular Disease, ICU: Intensive Care Unit, IQR: Interquartile Range, MEWS: Modified Early Warning Score, NSAID: Non-Steroidal Anti-inflammatory Drug, No.: Number, qSOFA: Quick Sepsis-Related Organ Failure Assessment, SD: Standard Deviation, SIRS: Systemic Inflammatory Response Syndrome, SOFA: Sepsis-Related Organ Failure Assessment

Logistic regression analysis of score cut-off values observed in different time periods was performed regarding hospital mortality concerning age, sex, BMI, and unit from which patients accepted to medical ICUs (Table 2). At the -48h period, values greater than SOFA and SIRS cut-off were associated with increased mortality (OR 13.2 and 7.6, respectively). However, SOFA and qSOFA scores were associated with increased mortality at the -24h period (OR: 14.2 and 2.9 respectively), the -8h period (OR: 18.3 and 3.9 respectively), and the 0h period (OR: 10.2 and 4.8 respectively). COX regression analysis was performed with the highest score values calculated before ICU admission (ICU admission values were omitted) and given in Table 3. In the univariate and multivariate analysis, SOFA score was the only score that correlated with increased hospital mortality (OR: 1.2, p=0.01 and OR: 1.1, p=0.04, respectively). No mortality risk increment was found with MEWS, SIRS, and qSOFA scores.

Table 2. Logistic regression analysis * of MEWS, SIRS, SOFA and qSOFA score cut-off positivity observed in different time periods with respect to hospital mortality.

	Non-survivors	Survivors	OR	p
-48h, mean (SD)	n=27	n=46		
MEWS	21 (77.8)	40 (87.0)		0.61
SIRS	23 (85.2)	31 (67.4)	7.6 (1.5-38.0)	0.01
SOFA	25 (92.6)	26 (56.5)	13.2 (2.3-74.3)	0.004
qSOFA	15 (55.6)	29 (63.0)		0.19
-24h, mean (SD)	n=31	n=67		
MEWS	29 (93.5)	63 (94.0)		0.27
SIRS	25 (80.6)	50 (74.6)		0.20
SOFA	29 (93.5)	41 (61.2)	14.2 (2.5-80.6)	0.003
qSOFA	15 (48.4)	24 (35.8)	2.9 (1.0-8.4)	0.05
-8h, mean (SD)	n=33	n=84		
MEWS	30 (90.9)	74 (88.1)		0.08
SIRS	26 (78.8)	59 (70.2)		0.41
SOFA	32 (97.0)	52 (61.9)	18.3 (2.2-151.1)	0.01
qSOFA	19 (57.6)	30 (35.7)	3.9 (1.4-11.0)	0.01
0h, mean (SD)	n=33	n=87		
MEWS	33 (100.0)	85 (97.7)		NA
SIRS	30 (90.9)	60 (69.0)		0.09
SOFA	32 (97.0)	60 (69.0)	10.2 (1.3-83.5)	0.03
qSOFA	25 (75.8)	27 (31.0)	4.8 (1.8-12.8)	0.002

* Adjusted for age, sex, BMI, and admission unit. MEWS: Modified Early Warning Score, qSOFA: Quick Sepsis-Related Organ Failure Assessment, SD: Standard Deviation, SIRS: Systemic Inflammatory Response Syndrome, SOFA: Sepsis-Related Organ Failure Assessment

Table 3. COX regression analysis of highest MEWS, SIRS, SOFA and qSOFA scores calculated before ICU admission (omitting ICU admission values) with respect to hospital mortality

	HR	CI (%95)	p	HR*	CI* (%95)	p*
MEWS	1.1	0.9-1.2	0.41	1.1	0.9-1.2	0.34
SIRS	1.2	0.8-1.8	0.30	1.3	0.9-2.0	0.16
SOFA	1.2	1.0-1.3	0.01	1.1	1.0-1.3	0.04
qSOFA	1.3	0.8-2.0	0.24	1.3	0.9-2.0	0.23

*Corrected in respect of age, sex, BMI, and admission unit. CI: Confidence Interval, MEWS: Modified Early Warning Score, OR: Odds Ratio, qSOFA: Quick Sepsis-Related Organ Failure Assessment, SIRS: Systemic Inflammatory Response Syndrome, SOFA: Sepsis-Related Organ Failure Assessment

ROC analysis with the highest score values calculated before ICU admission (ICU admission values were omitted) is given in Figure 2. Observed AUROC values were 0.80 (95% CI: 0.72-0.89; p<0.001) for SOFA, 0.65 (95% CI: 0.54-0.76; p=0.01) for MEWS, 0.63 (95% CI: 0.51-0.74; p=0.04) for qSOFA and 0.61 (95% CI: 0.49-0.73; p=0.07) for SIRS.

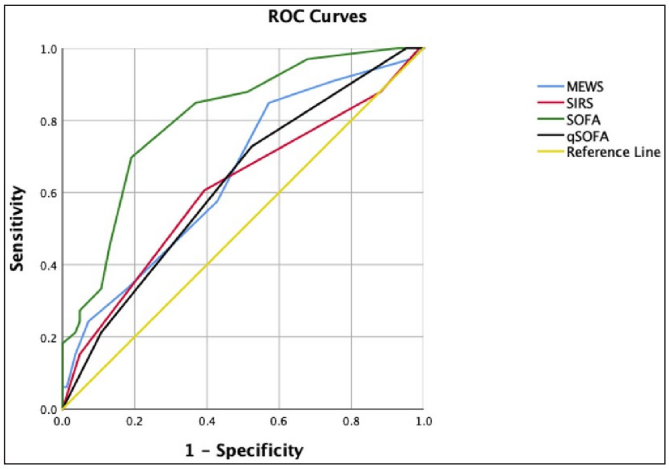

Figure 2. ROC Analysis of Highest MEWS, SIRS, SOFA and qSOFA Score Values Calculated Before ICU Admission in Respect to Hospital Mortality. Observed AUROC values are 0.80 (%95 CI: 0.72-0.89; p<0.001) for SOFA, 0.65 (%95 CI: 0.54-0.76; p=0.01) for MEWS, 0.63 (%95 CI: 0.51-0.74; p=0.04) for qSOFA and 0.61 (%95 CI: 0.49-0.73; p=0.07) for SIRS.

Sensitivity and specificity analysis of MEWS, SIRS, SOFA, and qSOFA score cut-off values are shown in Table 4. At all periods, specificity was highest in the qSOFA score. Sensitivity, positive predictive (PPV), and negative predictive values (NPV) were highest in SOFA score at -48h. At -24h, sensitivity was highest in MEWS and SOFA scores (93.5%), and NPV was highest in SOFA scores solely. Positive predictive values (PPV) were observed similarly between all four scores. -8h score characteristics were similar to -48h as SOFA had the highest sensitivity, PPV and NPV. At 0h, the MEWS score had 100% sensitivity and NPV, followed by the SOFA score (97.0% and 96.4%, respectively). Specificity and PPV were observed to be the highest in qSOFA scores at 0h.

Table 4. Sensitivity and specificity analysis of MEWS, SIRS, SOFA and qSOFA score cut-off values calculated in different time periods with respect to hospital mortality

	MEWS	SIRS	SOFA	qSOFA
- 48h, % (CI %95)				
Sensitivity	77.8 (62.1-93.5)	85.2 (71.8-98.6)	92.6 (82.7-100.0)	44.4 (25.7-63.1)
Specificity	13.0 (3.3-22.7)	32.6 (19.1-46.2)	43.5 (20.2-57.8)	63.0 (49.1-77.0)
Positive predictive value	34.4 (22.5-46.3)	36.0 (23.2-48.8)	49.0 (35.3-62.7)	41.4 (23.5-59.3)
Negative predictive value	50.0 (21.7-78.3)	79.0 (60.7-97.3)	90.9 (78.9-100.0)	65.9 (51.9-79.9)
- 24h, % (CI %95)				
Sensitivity	93.5 (84.8-100.0)	80.6 (66.7-94.5)	93.5 (84.8-100.0)	48.4 (30.8-66.0)
Specificity	6.0 (0.3-11.7)	25.4 (15.0-35.8)	38.8 (27.1-50.5)	64.2 (52.7-75.7)
Positive predictive value	31.5 (22.0-41.0)	33.3 (22.6-44.0)	41.4 (29.9-52.9)	38.5 (23.2-53.8)
Negative predictive value	66.7 (29.0-100.0)	74.0 (56.1-91.9)	92.9 (83.4-100.0)	72.9 (61.6-84.2)
- 8h, % (CI %95)				
Sensitivity	90.9 (81.1-100.0)	78.8 (64.9-92.8)	97.0 (91.2-100.0)	57.6 (40.7-74.5)
Specificity	11.9 (5.0-18.8)	29.8 (20.0-39.6)	38.1 (27.7-48.5)	64.3 (54.1-74.6)
Positive predictive value	28.9 (20.2-37.6)	30.6 (20.8-40.4)	38.1 (27.7-48.5)	38.8 (25.2-52.4)
Negative predictive value	76.9 (54.0-99.8)	78.1 (63.8-92.4)	97.0 (91.2-100.0)	79.4 (69.8-89.0)
0h, % (CI %95)				
Sensitivity	100.0	90.9 (81.1-100.0)	97.0 (91.2-100.0)	75.8 (61.2-90.4)
Specificity	2.9 (0.0-6.4)	31.0 (21.3-40.7)	31.0 (21.3-40.7)	69.0 (59.3-78.7)
Positive predictive value	28.0 (19.9-36.1)	33.3 (23.6-43.0)	34.8 (25.1-44.5)	48.1 (34.5-61.7)
Negative predictive value	100.0	90.0 (79.3-100.0)	96.4 (89.5-100.0)	88.2 (80.5-95.9)

Cut-off values for scores= MEWS total score ≥ 3 or one parameter ≥ 2, SIRS ≥ 2, SOFA ≥ 2, qSOFA ≥ 2, CI: Confidence Interval, MEWS: Modified Early Warning Score, OR: Odds Ratio, qSOFA: Quick Sepsis-Related Organ Failure Assessment, SIRS: Systemic Inflammatory Response Syndrome, SOFA: Sepsis-Related Organ Failure Assessment

DISCUSSION

In this study, MEWS, SIRS, SOFA, and qSOFA scores were compared regarding hospital mortality prediction among ED and ward patients who required ICU admission with suspected infection. It provides valuable contributions to the literature, as four frequently used early warning and sepsis scores were compared prospectively in the same cohort in the 48-hour period before ICU admission. SOFA at 48 hours prior to ICU admission was the most effective score compared to MEWS, SIRS and qSOFA, which were significantly associated with increased mortality (OR: 13.2, p=0.004) with 92.6% sensitivity. Analysis performed by omitting admission values revealed an AUROC value of 0.80 for SOFA in predicting hospital mortality (p<0.001).

SOFA score was employed to demonstrate organ dysfunction and placed in sepsis definition with Sepsis-3 criteria.[7] In our study, SOFA score had the highest sensitivity and NPV before ICU admission. Its PPV was also the highest in 48h and 24h periods compared to other scores. Thus, besides its diagnostic role, these features make SOFA score a valuable tool for predicting prognosis, especially mortality, in patients with suspected infection admitted to ICU. This superiority of SOFA score over MEWS, SIRS, and qSOFA was compatible with the literature in which AUROC values regarding hospital mortality were reported up to 0.91, 0.70, 0.72, and 0.77, respectively.[12-21,23-28] Despite the high sensitivity, SOFA score had moderate specificity in predicting mortality, which raised doubts about the accuracy of using SOFA in the definition of sepsis. Nevertheless, these concerns should be evaluated within the framework of consensus based on sepsis pathophysiology, not such analysis based on mortality.

In the presented study, qSOFA score cut-off specificity was highest, while sensitivity was lowest in mortality prediction for all periods. These findings are supported by the study conducted with 184 patients admitted to the ED with suspected infection by Garbero et al.[16] that demonstrated sensitivity and specificity values of 56.8% and 74.2% for qSOFA and 93.7% and 25.9% for SOFA. Kim et al.[18] demonstrated sensitivity and specificity values of 61.9% and 58.1% for qSOFA and 99.1% and 4.2% for SOFA among 928 patients with sepsis diagnosis. Similarly, Abdullah et al.[27] observed higher specificity values in qSOFA than SOFA (92.4% and 67.3%, respectively), whereas SOFA had higher sensitivity than qSOFA (61.4% and 19.6%, respectively). Data that is contrary to the usage of qSOFA score as a bedside screening tool to detect patients with suspected sepsis can further be exemplified.[29-32]

Moreover, in the presented study, SIRS had significantly higher sensitivity than qSOFA (85.2% and 44.4%, respectively) even 48 hours before ICU admission. This finding is similar to previous studies that reported up to 60% and 24% sensitivity for SIRS and qSOFA, respectively.[18,32-36] In this regard, it seems that qSOFA and SIRS are insufficient for screening patients with suspected infection who may have a poor prognosis, as argued by previous studies.[29,31,37] Liu et al.[29] recommended the combined use of SIRS and qSOFA to increase screening power.

When compared to MEWS, qSOFA had similar AUROC values with the highest score values observed before ICU admission within the current study (0.65 and 0.63, respectively). In the study of Khwannimit et al.[17] with 1589 patients diagnosed with sepsis, no difference was found between MEWS and qSOFA in terms of AUROC values (0.86 and 0.85, respectively), although the values were higher than our study. Likewise, similar AUROC values for MEWS and qSOFA was reported in several studies in the literature.[35,38-40] Although qSOFA was associated with increased mortality while MEWS was not in logistic regression analysis, sensitivity was higher in MEWS. Due to higher sensitivity, MEWS seems more helpful in detecting deteriorating patients with infection. However, as recent Surviving Sepsis Campaign-2021 guidelines stated, combined use of the prognostic scores could lead the clinicians to more appropriate predictions for deterioration.[11]

Limitations

Main limitation of our study is being a single center study with limited number of patients and short study course. Our patient cohort, though modest in size, was determined based on the specific criteria of our study's focus. Factors such as patient availability, consent, and stringent inclusion criteria played a significant role in shaping our recruitment process. We acknowledge the potential impact of a larger sample size on our results. However, our study offers important preliminary findings and serves as a catalyst for future research in this domain. The five-month study period was meticulously chosen based on expected incidence rates and resource availability. This timeframe was deemed optimal for achieving meaningful data collection within our logistical framework. We did not conduct a formal power analysis as the study aimed at generating hypotheses, due to the heterogeneity of our ICU patient population and the diverse nature of the scoring systems precluding an effect size to base our calculations on. All-cause mortality was accepted as an outcome rather than sepsis-related mortality. This situation limits comparability to studies conducted with sepsis-related scores. Our study population included selected patients due to limited capacity, patient refusals are possible. The definition of suspected infection in our study is broader than the other similar studies ant it may interfere with our findings since the diagnostic exclusion of infection may occur after ICU admission. Finally, not all patients had hospital admissions at least 48 hours before ICU admission. Therefore, analysis omitted the ICU admission values was performed with fewer patients than the total cohort number.

CONCLUSION

SOFA score is a good screening tool to identify patients with suspected infection who may have worse prognosis. The effectiveness of qSOFA score as a screening tool for sepsis suspicion remains controversial as a result of this study. MEWS and SIRS score can predict hospital mortality 48 hours early from ICU admission, and its abandonment with sepsis-3 criteria remains controversial. Thus, the combination of the scoring systems seems to be wise as recommended by Surviving Sepsis Campaign-2021 guidelines.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Hacettepe University Scientific Researches Ethics Committee (Date: 19.12.2017, Decision No: GO17/948-11).

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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Evaluation of artificial neural network and adaptive-network-based fuzzy inference system for ovarian and lung cancer prediction

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ABSTRACT

Aims: Every year, a significant number of individuals lose their lives due to cancer or undergo challenging treatments. Indeed, the development of an effective cancer prediction method holds great importance in the field of healthcare.

Methods: Machine learning methods have played a significant role in advancing cancer prediction models. In this context, this study focuses on exploring the potential of two machine learning methods: Artificial neural network (ANN) and adaptive-network-based fuzzy inference system (ANFIS) for cancer prediction. In this study, two different types of cancer, ovarian cancer and lung cancer, are taken into consideration. For the prediction of ovarian cancer, three specific biomarkers, namely human epididymis protein 4 (HE4), carbohydrate antigen 125 (CA-125), and carcinoembryonic antigen (CEA), are used to develop a prediction model. For the prediction of lung cancer, six different variables are utilized in the development of both the ANN and ANFIS methods.

Results: The findings demonstrated that the proposed methods had an accuracy rate of at least 93.9% in predicting ovarian cancer. With an accuracy rate of at least 89%, the proposed methods predicted lung cancer. Also, the proposed ANN method outperforms the ANFIS method in terms of predictive accuracy for both ovarian cancer and lung cancer.

Conclusion: This study suggests that the ANN method provides more reliable and accurate predictions for these specific cancer types based on the chosen variables or biomarkers. This study highlights the potential of machine learning methods, particularly ANN, in improving cancer prediction models and aiding in the early detection and effective management of ovarian and lung cancers.

Keywords: ANN, ANFIS, cancer prediction

INTRODUCTION

Cancer is a group of diseases in which abnormal cells multiply uncontrollably anywhere in the body. There are numerous forms of cancer that can affect different organs and tissues in the body.[1] Lung cancer remains the leading cause of cancer-related deaths globally. It affects both men and women and is responsible for a significant number of deaths each year. It is often diagnosed at advanced stages, making it more challenging to treat effectively.[2] Additionally, ovarian cancer (OC) is the sixth most common gynecological malignancy, with an increasing incidence rate with age and postmenopausal status.[3] Although it is not as common as some other cancers, ovarian cancer has a high mortality rate due to the challenges associated with early detection. Carbohydrate Antigen 125 (CA-125) is a primary biomarker used in the diagnosis of ovarian cancer and assessing treatment effectiveness. One of the primary biomarkers for diagnosing OC recurrence and evaluating treatment efficacy is the CA-125 test, which is now regarded as an important component in assessing patients with adnexal masses.[3,4] Therefore, CA-125 has been approved for use as a tool for detecting residual ovarian cancer in patients who have completed first-line therapy and are undergoing diagnostic second-look procedures.[5]

In addition to CA-125, Human Epididymis Protein 4 (HE4) is most widely used as a diagnostic and prognostic biomarker for ovarian malignancies.[2] HE4 is a protein that is overexpressed in ovarian cancer cells and is particularly useful in distinguishing between benign and malignant ovarian tumors. Drapkin et al.[6] characterized the HE4 gene product in benign and malignant tissues in order to identify features that will aid in further clinical follow-

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up as an ovarian cancer biomarker. It can be measured through blood tests and has shown promising results in improving the accuracy of ovarian cancer diagnosis, assessing prognosis, and monitoring treatment response.

Sørensen and Mosgaard[7] investigated whether the Cancer Antigen 125 (CA-125) together with the Tumor Marker Carcinoembryonic Antigen (CEA) could distinguish between malignant ovarian and malignant non-ovarian disease. The findings of the study supported the idea that, in addition to the Risk of Malignancy Index (RMI), the CA-125/CEA test should be used as a criterion for further evaluation in patients referred to the hospital with an undetected pelvic tumor. The study conducted by Li et al.[5] investigated the diagnostic accuracy and performance of the Risk of Ovarian Malignancy Algorithm (ROMA) as compared to the individual tumor markers HE4 and CA-125 in the prediction of epithelial ovarian cancer. The findings of the study revealed that for epithelial ovarian cancer and OC prediction, CA-125 has a higher diagnostic accuracy than HE4.

The study conducted by Ferraro et al.[3] aimed to evaluate the diagnostic value of HE4 and CA-125 levels for the diagnosis of OC. The levels of HE4 and CA-125 in the blood samples of patients with suspicious pelvic masses were assessed. They compared the diagnostic performance of HE4 and CA-125 individually and in combination. Zhen et al.[4] conducted a meta-analysis of the available information on the diagnostic accuracy of HE4 and CA-125. The results of the study showed that HE4 and CA-125 could be helpful biomarkers for OC diagnosis, with HE4 having a higher diagnostic accuracy than CA-125 in separating OC from other benign gynecological diseases. Zhu et al.[8] investigated how the HE4 protein affects malignant biological behaviors and how its gene expression profile alters in response to HE4 in ovarian cancer cells. Bolstad et al.[9] determined that HE4 levels are related to their age and smoking status in healthy individuals. Ribeiro et al.[10] found that recombinant HE4 increases matriptase activity in a dose-dependent manner, demonstrating for the first time that HE4 can stimulate the activity of at least one serine protease. Kumbasar et al.[2] suggested that HE4 could be used as a biomarker in the diagnosis of non-small cell lung cancer. Bashizadeh-Fakhar et al.[11] presented the efficacy of ROMA, CA-125, and CEA as predictors of peritoneal spread in the early diagnosis of low-grade serous ovarian cancer. Dochez et al.[12] explored the predictive abilities of CA-125, HE4, the RMI, and ROMA algorithms for ovarian cancer in women. Additionally, in women with a presumed benign ovarian tumor, a combination of increased CA-125 and HE4 appeared to be a good diagnostic tool for confirming ovarian cancer, and it can be utilized in conjunction with individual markers. Dai, Hu, and Ding[13] assessed the overall diagnostic significance of HE4 in combination with CA-125 in OC patients.

In this paper, OC prediction was evaluated considering HE4, CA-125, and CEA using ANFIS and ANN methods. Furthermore, ANFIS and ANN methods were developed considering six different variables: smoking, anxiety, peer pressure, alcohol, coughing, and chest pain for lung cancer prediction. Accurate cancer predictions are of paramount importance for improving patient outcomes in numerous cancer types characterized by high aggressiveness and low median survival rates. Over the years, advancements in statistics and computer engineering have motivated scientists to harness computational methods for disease prognosis. Such research has demonstrated significantly higher accuracy compared to empirical predictions. Notably, the integration of artificial intelligence (AI) into clinical cancer research in recent years has further elevated the accuracy of cancer prediction.[14] Lu et al.[15] employed decision trees, ROMA, and logistic regression to classify ovarian cancer and benign ovarian tumors. Kappen and Neijt[16] highlighted the potential of ANNs to predict patient survival at least as effectively as Cox's technique while enabling the discovery of prognostic factors. Furthermore, it was demonstrated that, utilizing the ANN, prognostic factors may be easily discovered. Floyd et al.[17] developed an ANN using radiologic findings as inputs to predict biopsy results, outperforming radiologists in diagnostic accuracy for cases assigned to biopsy.

When comparing the network output to the radiologists' categorical judgment for cases assigned to biopsy, the ANN shows much superior diagnostic performance than the radiologists. Burke et al.[18] conducted a comparative study between the Tumour, Node, and Metastasis (TNM) staging system and the ANNs, revealing improved accuracy when incorporating commonly obtained demographic and anatomic information into the TNM variables. Kim and Cho[19] utilized evolutionary ANNs to classify tumor classification based on microarray gene expression data, incorporating dimension reduction and information gain methods. Saritas[20] employed ANNs to predict the severity of a mammographic breast tissue masses. Ecke et al.[21] adopted systematic sextant patterns for prostate biopsies, with prostate volume being a crucial variable in their ANN model, demonstrating its potential for routine biopsy decision-making. Enshaei et al.[22] analyzed multiple parameters for ovarian cancer using three algorithms: decision tree, ANNs, and Bayesian network.

Hambali and Gbolagade[23] utilized the Synthetic Minority Oversampling Technique (SMOTE) to address imbalanced datasets, developing a hybrid SMOTE and ANN technique for diagnosing ovarian cancer. Hart et

al.[24] leveraged personal health information to construct a multi-parameterized ANN for predicting lung cancer risk. Charati et al.[25] employed ANNs to estimate survival rates for gastric cancer patients and identify influential factors. Nejatzadeh et al.[26] collected relevant data to create an ANN based prediction model for laryngeal cancer, identifying 24 significant factors for more accurate predictions. Nasser and Abu-Naser[27] developed an ANN model for lung cancer identification after preprocessing and transforming data to enhance predictive analysis, with age emerging as a crucial factor.

Daoud and Mayo[28] presented the data preprocessing tools and architectures in recent ANN based cancer prediction models, showcasing ANNs' versatility as filters, predictors, and clustering methods. Takeuchi et al.[29] compared ANN and logistic regression analysis for prostate cancer diagnosis, emphasizing ANN' ability to prevent unnecessary biopsies and missed cancer cases. Muhammad et al.[30] created an ANN capable of calculating pancreatic cancer risk in the general population, utilizing readily available personal health data to identify high-risk individuals cost-effectively. Nayak et al.[1] integrated the Analysis of Variance (ANOVA) and Kruskal-Walis methods to evaluate relevant features, incorporating elephant herding optimization into ANN analysis across various cancer datasets such as breast, lung, and cervical cancer. Appaji et al.[31] employed diagonal correlation matrices to assess input attributes and described breast cancer diagnosis using deep learning approaches with Recurrent Neural Networks. Ma et al.[32] combined factorization machine and deep neural network structures to predict drug combination synergies, enhancing drug discovery. Prisciandaro et al.[33] presented that both fundamental research and clinical decision-making can greatly benefit from the use of ANN.

Madhu and Kumar[34] employed edge detection to preprocess graphical data, reducing data processing time and storage requirements for convolutional neural networks. Chuang et al.[35] developed a convolutional neural network model capable of categorizing normal and tumor samples from various cancer types. Lee et al.[36] identified predictive risk factors for lung cancer-related diseases using big data analytics and created a lung cancer prediction model based on the Deep Neural Network method. Tan et al.[37] utilized a fuzzy adaptive learning control network in conjunction with adaptive resonance theory to evaluate ovarian cancer and investigate proteome patterns using varying feature sets. Hamdan and Garibaldi[38] presented a hybrid methodology that combined the strengths of ANNs with fuzzy inference for survival modeling. Mahmoudi, Lahijan, and Kanan[39] employed Genetic Algorithms (GAs) and Particle Swarm Optimization (PSO) for gene selection in the ANFIS classifier, evaluating its robustness against noisy data. Hidayah et al.[40] used the ANFIS to classify colon cancer. Ziasabounchi and Askerzade[41] developed a hybrid learning algorithm to identify parameters in their ANFIS model, demonstrating its adaptability as a predictive mechanism for heart disease. Kalaiselvi and Nasira[42] introduced an approach for diabetes and cancer detection using the ANFIS and adaptive group-based k nearest neighbor. Wang et al.[43] utilized survival data to enhance the predictive performance of the ANFIS method, efficiently assessing functional relationships between covariates and time in complex prognostic scenarios. Rahouma et al.[44] employed K-means clustering for tumor segmentation, followed by feature extraction using a growing neural gas network. They used hybrid learning, combining descent and least square methods with ANFIS, to determine classification parameters.

Uyar et al.[45] used the GA based trained recurrent fuzzy neural network (RFNN) and ANFIS to predict breast cancer. The results of the study demonstrated that the RFNN with nine variables was the most accurate overall. Mishra and Bhoi[46] used the ensemble Kalman filter during the preprocessing phase. For classification, ANFIS was used. Furthermore, the newly evolved manta ray foraging optimization was hybridized with ANFIS during classification.

Numerous comparative studies indicate that the proposed ANN and ANFIS based methods consistently outperform alternative approaches in terms of prediction accuracy. The purpose of this study is to develop both the ANFIS and ANN methods for cancer prediction. There has not been a comparative study of ANN and ANFIS methods for predicting ovarian and lung cancer. Specifically, proposed methods employ HE4, CA-125, and CEA markers for ovarian cancer prediction and incorporate six variables for the development of methods for lung cancer prediction. The accuracy, sensitivity, and specificity of the proposed methods were computed, and the overall prediction ability of ANN and ANFIS was compared. The limitation of this study is that ovarian and lung cancer were taken into account. In addition, hybrid methods can be used to improve prediction performance in the future.

METHODS

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

In this study, two different cancer datasets were utilized to develop prediction methods. The main objective of this paper is to create a prediction method for lung and ovarian cancer by accurately computing, analyzing, and applying the most useful artificial intelligence tools, ANN

and ANFIS. The first dataset was obtained from Lu et al.[15] and contains various variables related to ovarian cancer. However, for the purpose of this paper, the focus was specifically on the biomarkers HE4, CA-125, and CEA, which are important indicators of ovarian cancer. Rows that contained missing values were removed from the dataset. The descriptive statistics of the ovarian cancer dataset are summarized in Table 1. These biomarkers were used as inputs for the prediction model developed in this study. The second dataset was obtained from[47] and specifically focused on lung cancer. Six different input variables were considered for this dataset, including smoking, anxiety, peer pressure, alcohol consumption, coughing, and chest pain. These variables were selected as potential predictors for lung cancer (Table 2). ANN and ANFIS were used to develop the prediction models.

Table 1. Descriptive statistics of ovarian cancer dataset

	Count	Minimum	Mean	Maximum
HE4	320	16.71	182.66	3537.6
CA-125	320	3.75	339.389	>5000
CEA	320	0.2	3.358	138.8

Human epididymis protein 4 (HE4), Carbohydrate antigen 125 (CA-125), Carcinoembryonic antigen (CEA)

Table 2. Descriptive statistics of lung cancer dataset

	Count	Min.	25%	50%	75%	Max.
Smoking*	309	1	1	2	2	2
Anxiety*	309	1	1	1	2	2
Peer pressure*	309	1	1	2	2	2
Alcohol consumption*	309	1	1	2	2	2
Coughing*	309	1	1	2	2	2
Chest pain*	309	1	1	2	2	2

* Yes=2, No=1

ANN

ANNs are computer algorithms. These algorithms are commonly used to sort a collection of patterns into one of several categories. The classification rules are learned by the network from examples rather than being written into the algorithm.[17] ANNs offer several benefits in various applications. The benefits mentioned are as follows: (i) Adaptive learning, (ii) Self-organization, (iii) Real-time operation, (iv) Fault-tolerance.[48]

The general application steps of ANN can be summarized as follows.[49] In the first step, a suitable ANN model is selected to begin the neural network design. In this paper, a feed forward back propagation neural network model was selected. In the second step, the number of hidden layers, hidden neurons, input parameters, and other parameters of ANN are determined. Once the network design process is completed, the proposed model is then initialized. The dataset is loaded, and the proposed ANN has learned from a training data set. The output of the proposed ANN is analyzed. The testing phase of the proposed ANN model is then initiated. Finally, the performance of the ANN is evaluated. The pseudocode of ANN is given in Figure 1.[50]

```
Input : ProblemSize, InputPatterns, iterations_max, learn_rate
Output : Network
Network ← ConstructNetworkLayer ();
Network_weight ← InitializeWeights(Network,ProblemSize);
for i = 1 to iterations_max do
        Pattern_i ← SelectInputPattern(InputPatterns);
        Output_i ← ForwardPropagate(Pattern,Network);
        BackwardPropagateError (Pattern,Output,Network);
        UpdateWeight (Pattern, Output, Network, learn_rate);
end
return network;
```

Figure 1. Pseudocode of ANN[50]

In this paper, ANN is used to predict ovarian cancer and lung cancer. The proposed ANN methods involve the use of a hidden layer consisting of 10 neurons. Hyperbolic tangent sigmoid transfer function is used as transfer function. Levenberg–Marquardt algorithm is employed to train the ANN methods. For ovarian cancer, two ANN methods are created using different input numbers. In first ANN method (ANN _2), two input including HE4 and CA-125 are utilized to create a prediction method for ovarian cancer (Figure 2). In the second ANN method (ANN _3), three input including HE4, CA-125, and CEA, are used for ovarian cancer (Figure 3). For lung cancer, six inputs including smoking, anxiety, peer pressure, alcohol, coughing, and chest pain, are used to create the ANN method (Figure 4).

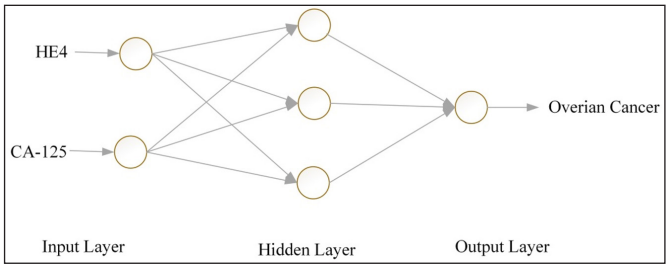

Figure 2. The proposed ANN_2 method for ovarian cancer

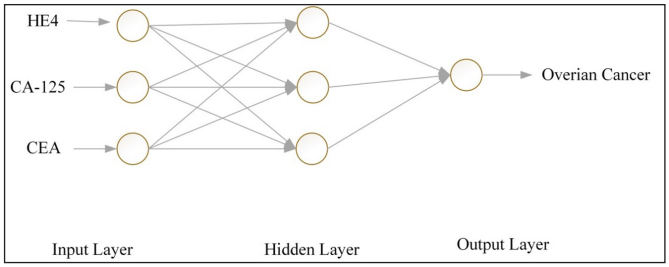

Figure 3. The proposed ANN_3 method for ovarian cancer

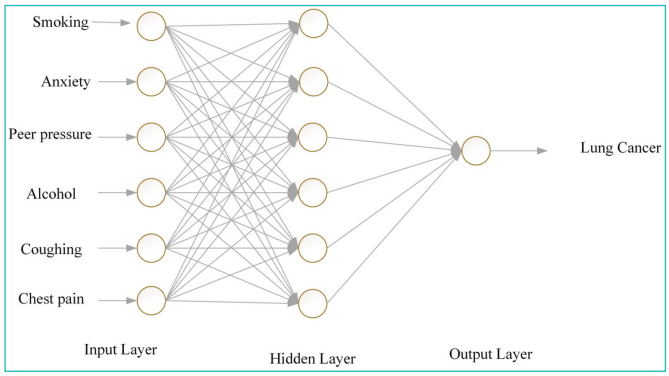

Figure 4. The proposed ANN method for lung cancer

ANFIS

The ANFIS is a powerful computational network that harnesses both the learning capabilities of ANNs and the decision-making proficiency of Fuzzy-Logic systems.[41] ANFIS is uniquely positioned to perform input-output mapping by amalgamating human knowledge with specified input-output data pairs through a hybrid learning approach. This integration combines rule-based systems with neural network learning capabilities to construct a fuzzy inference system (FIS) based on a set of input–output data. What sets ANFIS apart is its capacity to use explicit linguistic terminology for variables, simplifying the interpretation of modeling findings.[43] The ANFIS architecture is illustrated in Figure 5[51] and explained as follows.

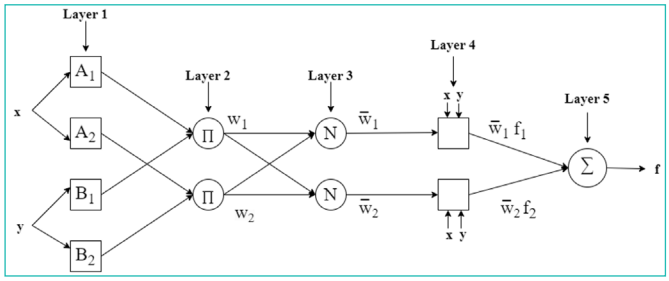

Figure 5. ANFIS architecture[51]

In Layer 1, each node i is an adaptable node whose node output is specified by

$$O_{1,i} = \mu_{A_i}(x) \quad \text{for} \quad i = 1,2 \quad \text{or} \qquad (1)$$

$$O_{1,i} = \mu_{B_{i-2}}(y) \quad \text{for} \quad i = 3,4 \qquad (2)$$

The node's input is represented by x (or y), while its associated fuzzy set is represented by A_i (or B_{i-2}). In Layer 2, every node is a fixed node labeled $\prod$. For instance,

$$O_{2,i} = w_i = \mu_{A_i}(x) \times \mu_{B_i}(y), \; i = 1,2 \qquad (3)$$

Every node's output indicates a rule's firing strength. In Layer 3, each node is a fixed node with the label N. Following equation is used in this layer:

$$O_{3,i} = \bar{w}_i = \frac{w_i}{w_1+w_2}, \; i = 1,2 \qquad (4)$$

In Layer 4, every node i is an adaptive node with a node function:

$$O_{4,i} = \bar{w}_i f_i = \bar{w}_i (p_i x + q_i y + r_i) \qquad (5)$$

The parameter set is $\{p_i, q_i, r_i\}$, and the output of Layer 3 is $\bar{w}_i$. In Layer 5, the fixed node with the labeled Σ is the single node. Following equation is used in this layer:

$$O_{5,1} = overall\ output = \sum_i \bar{w}_i f_i = \frac{\sum_i w_i f_i}{\sum_i w_i} \qquad (6)$$

Details about the architecture of ANFIS can be found in Jang and Sun[51] and Karaboga and Kaya.[52]

In this study, ANFIS plays a pivotal role in predicting both ovarian cancer and lung cancer. For ovarian cancer detection, we deploy two distinct ANFIS methods, each employing different sets of input variables. In the first ANFIS method (ANFIS_2), we utilize two inputs: HE4 and CA-125, to develop a prediction model. In the second ANFIS method (ANFIS_3), we expand the input two to three variables, incorporating HE4, CA-125, and CEA. The FIS structure for both ANFIS_2 and ANFIS_3 for ovarian cancer prediction is generated using the Trimf membership function. The fuzzy system is created using the grid partitioning method. For FIS training, we opt for the hybrid method and set the number of epochs at 1000. Additionally, we establish that each input should be associated with four membership functions.

Concerning lung cancer prediction, we rely on six input variables: smoking, anxiety, peer pressure, alcohol, coughing, and chest pain, to construct an ANFIS method. We configure the number of epochs for training at 10, and each input is associated with three membership functions. Similar to ovarian cancer prediction, we employ the hybrid method for FIS training and the grid partitioning method for constructing the fuzzy system. The Trimf membership function guides the development of the ANFIS method for lung cancer prediction.

RESULTS

Cancer is one of the leading causes of death worldwide. The impact of cancer is not limited to the patients alone but also extends to their families, friends, and communities. Cancer prediction plays a crucial role in the field of oncology. Accurate and early prediction of cancer can significantly impact the selection of appropriate treatment strategies for cancer patients. By identifying individuals who are at high risk or are likely to develop cancer, healthcare professionals can intervene at an early stage, potentially leading to improved outcomes and survival rates. A comparative analysis of methods in healthcare is given in Table 3. It is clearly seen that no exact method is available for use in healthcare. Each

method has a variety of advantages. In this paper, a comparative analysis of ANN and ANFIS is presented, considering two different cancer types.

Cancer prediction involves analyzing various factors, including patient demographics, medical history, genetic markers, biomarkers, and imaging data, among others. Machine learning techniques, such as ANNs, have been widely employed in cancer prediction models due to their ability to learn complex patterns and relationships from large datasets. In this study, ANN and ANFIS based methods are created to predict ovarian cancer and lung cancer.

In this study, a binary classification problem with two classes was created, and the results were classified as either positive or negative. Four results were possible.[53]

- True Positive (TP) refers to a situation where both the actual value and the prediction's results are positive.
- False Positive (FP) refers to a situation where a prediction provides positive results even though the actual value is negative.
- True Negative (TN) refers to a situation where both the actual value and the predictions' results are negative.
- False Negative (FN) refers to a situation where a prediction provides negative results while the actual value is positive.

The values of TP, FP, TN, and FN were given in Table 4 and Table 5. Accuracy, sensitivity, and specificity were calculated using the following equations, respectively[54]

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \quad (7)$$

$$Sensitivity = \frac{TP}{TP+FN} \quad (8)$$

$$Specificity = \frac{TN}{FP+TN} \quad (9)$$

Table 4. The performance metrics for prediction methods for ovarian cancer

Dataset	Methods	True benign	True cancer	Total	Accuracy rate
Ovarian cancer					
	ANFIS_2				0.939
	Predicted benign	84	4	88	
	Predicted cancer	3	23	26	
	Total	87	27	114	
	ANN_2				0.965
	Predicted benign	85	3	88	
	Predicted cancer	1	25	26	
	Total	86	28	114	
	ANFIS_3				0.939
	Predicted benign	84	4	88	
	Predicted cancer	3	23	26	
	Total	87	27	114	
	ANN_3				0.965
	Predicted benign	85	3	88	
	Predicted cancer	1	25	26	
	Total	86	28	114	

Artificial neural network (ANN), Adaptive-network-based fuzzy inference system (ANFIS)

Table 3. Comparative analysis of methods in healthcare

Author(s)	DT	ROMA	LR	ANN	TNM	ENN	BN	SMOTE	GA	CNN	DNN	XGBoost	ANFIS	RFNN	MaFO
Lu et al.[15]	✓	✓	✓												
Kappen and Neijt[16]				✓											
Floyd et al.[17]				✓											
Burke et al.[18]				✓	✓										
Kim and Cho[19]						✓									
Saritas[20]				✓											
Ecke et al.[21]				✓											
Enshaei et al.[22]	✓			✓			✓								
Hambali and Gbolagade[23]				✓				✓							
Hart et al.[24]				✓											
Charati et al.[25]				✓											
Nejatzadeh et al.[26]				✓					✓						
Nasser and Abu-Naser[27]				✓											
Madhu and Kumar[34]										✓					
Lee et al.[36]											✓	✓			
Ziasabounchi and Askerzade[41]													✓		
Kalaiselvi and Nasira[42]													✓		
Wang et al.[43]													✓		
Uyar et al.[45]									✓				✓	✓	
Mishra and Bhoi[46]													✓		✓
This study				✓									✓		

*Decision Trees (DT), Risk of Ovarian Malignancy Algorithm (ROMA), Logistic Regression (LR), ANN, Evolutionary Neural Network (ENN), Bayesian Network (BN), Synthetic Minority Oversampling Technique (SMOTE), Genetic Algorithm (GA), Convolutional Neural Networks (CNN), Deep Neural Network (DNN), Extreme Gradient Boosting (XGBoost), ANFIS, Recurrent Fuzzy Neural Network (RFNN), Manta Ray Foraging Optimization (MaFO)

In Table 4, the performance metrics for prediction methods for ovarian cancer are given. The results showed that the proposed methods predicted ovarian cancer with at least a 93.9% accuracy rate. In Table 5, the performance metrics of prediction methods for lung cancer are presented. In Table 5, the proposed methods predicted lung cancer with at least an 89% accuracy rate. The results of sensitivity and specificity are given in Table 6. The findings of the study indicate that the ANN method used in this study showed better results when applied to two specific cancer datasets. This suggests that the ANN approach has the potential to improve the accuracy and effectiveness of cancer prediction or classification compared to the ANFIS based method.

Table 5. The performance metrics for prediction methods for lung cancer

Dataset	Methods	True non-cancer	True cancer	Total	Accuracy rate
Lung cancer					
	ANFIS				0.89
	Predicted non-cancer	5	8	13	
	Predicted cancer	3	84	87	
	Total	8	92	100	
	ANN				0.92
	Predicted non-cancer	9	4	13	
	Predicted cancer	4	83	87	
	Total	13	87	100	

Artificial neural network (ANN), Adaptive-network-based fuzzy inference system (ANFIS)

Table 6. Comparison of proposed methods

Method	Sensitivity	Specificity
Ovarian cancer		
ANFIS_2	0.965	0.851
ANN_2	0.988	0.893
ANFIS_3	0.965	0.852
ANN_3	0.988	0.893
Lung cancer		
ANFIS	0.625	0.913
ANN	0.692	0.954

Artificial neural network (ANN), Adaptive-network-based fuzzy inference system (ANFIS)

DISCUSSION

Prediction accuracy varies according to the cancer types. For example, Faisal et al.[55] presented that a gradient-boosted tree was shown to achieve 90% accuracy, outperforming all other individual and ensemble classifiers for lung cancer. Lu et al.[15] determined that accuracy for ROMA, Decision Tree, and Logistic Regression were determined as 0.956, 0.921, and 0.974, respectively, for ovarian cancer prediction. Hassan et al.[56] achieved a maximum accuracy of 90.68% for breast cancer detection and prediction. In the light of previous studies, it can be said that it is possible to obtain a value above 90% accuracy in cancer prediction. Additionally, new prediction methods are needed to obtain better results in the prediction of all cancer types. In this study, the accuracy rate of cancer prediction by ANN was determined to be 96.5.

To properly evaluate the data, AI and machine learning techniques are needed. ANNs are used in most machine learning today. With the recent increase in processing power, ANNs have become incredibly common and can now be used almost anywhere.[57] ANFIS is a hybrid analytical method. In order to generate an output, ANFIS essentially learns the characteristics of the supplied data and adjusts the system parameters to meet the required error criterion of the system.[58] In this paper, ANN and ANFIS have been implemented for modeling and predicting lung and ovarian cancer. Millions of individuals suffer from the terrible effects of cancer every year, whether it be from cancer-related deaths or the challenges posed by the disease itself.[59] Therefore, even a little advancement in modeling and forecasting can make significant improvements.

CONCLUSION

The importance of accurate cancer prediction has attracted the interest of researchers, as it plays a crucial role in selecting appropriate treatment strategies and improving patient outcomes. While various methods for cancer prediction exist, no single method can effectively predict every type of cancer. In this study, two specific methods, namely the ANN and the ANFIS, were employed for cancer prediction. In literature, comparative research between the ANN and ANFIS methods for ovarian and lung cancer prediction has not yet been conducted.

The research findings indicate that both the ANN and ANFIS methods showed promising results in predicting cancer. These methods demonstrated their potential as effective tools for cancer prediction, although it is important to note that their performance may vary depending on the specific cancer type and dataset used. The accuracy rate of ANN based cancer prediction in this study was found to be 96.5. To improve results in the prediction of all cancer types, new prediction techniques can be created. To further enhance the prediction accuracy and effectiveness of these methods, future studies could explore the use of different parameters for constructing the ANN and ANFIS models. By optimizing the model parameters, researchers can potentially improve the prediction capabilities and overall performance of these methods.

In conclusion, the study's findings highlight the potential of ANN and ANFIS methods for cancer prediction.

Further research exploring different parameters, feature selection methods, and diverse cancer types will contribute to the development of more advanced and reliable prediction models in the future.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study does not require an ethics committee.

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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Novel insights into myocardial injury, diastolic pathology, and in-hospital mortality: the impact of H₂FPEF score in COVID-19 patients

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ABSTRACT

Aims: H₂FPEF score is a reliable tool for diagnosing heart failure with preserved ejection fraction (HFpEF) linked to diastolic dysfunction. Our objective was to explore the correlation between H₂FPEF score and in-hospital mortality, as well as parameters previously identified in association with COVID-19, among hospitalized COVID-19 patients.

Methods: This prospective, single-center observational study included 205 consecutive COVID-19 hospitalized patients. Data regarding patients' clinical status, comorbidities, and drug therapy were extracted from medical histories and records. Afterward, we calculated H₂FPEF score for each patient and subsequently grouped them based on the following score categories: low (0-1), medium (2-5), and high (6-9). Logistic regression and Kaplan-Meier survival curve analyses were conducted to assess in-hospital mortality and the presence of an intermediate-to-high H₂FPEF score.

Results: Death occurred in 46 (22.4%) patients. 79 participants (38.5%) fell into the low-risk category (0-1 points), 108 (52.7%) were classified as intermediate-risk (2-5 points), and the remaining 18 (8.8%) were in the high-risk category (6-9 points). Age, heart rate, body mass index, and co-morbidities exhibited a rising trend with increasing H₂FPEF scores ($p<0.05$ for all). Moreover, an escalation in the H₂FPEF category correlated with deteriorated echocardiographic parameters. Multivariable logistic regression analysis revealed that heart rate per minute (OR=1.048, p=0.022), H₂FPEF score (OR=1.396, p=0.018), and current smoker (OR=4.569, p=0.050) were independent determinants of in-hospital mortality. ROC curve indicated that the H₂FPEF score, with a threshold of ≥2, exhibited good discriminative capacity, demonstrating 80.4% sensitivity and 69.2% specificity (AUC=0.777, $p<0.001$). The pairwise comparison of ROC curves analysis demonstrated that troponin (AUC=0.819) exhibited better discriminative abilities than both D-dimer (AUC=0.737, p=0.029) and hemoglobin (AUC=0.691, p=0.007) in determining an intermediate-to-high H₂FPEF score.

Conclusion: COVID-19, recognized for its association with myocardial damage, could emerge as a significant risk factor for the onset of HFpEF. H₂FPEF score presents as a straightforward tool for rapid risk assessment upon hospitalization, potentially aiding in the evaluation of the risk for HFpEF development. Its utilization may facilitate early intervention, thereby contributing to a reduction in poor outcomes.

Keywords: COVID-19, heart failure with preserved ejection fraction, H₂FPEF score, in-hospital mortality, cardiac injury

INTRODUCTION

The coronavirus disease 2019 (COVID-19) remains a substantial challenge, contributing to widespread morbidity and mortality globally. It presents a complex clinical scenario with frequent cardiac symptoms and multi-organ involvement, and evidence suggests that heart damage is linked to increased major adverse cardiovascular events among individuals infected with COVID-19.[1]

Heart failure with preserved ejection fraction (HFpEF) has emerged as the predominant form of heart failure (HF) globally, closely linked to the aging of the general population and the escalating prevalence of obesity, diabetes, and hypertension.[2] HFpEF is characterized as a clinical syndrome hemodynamically associated with a heart incapable of pumping sufficient blood without elevated cardiac filling pressures. Currently, no universally accepted treatment modifies the clinical

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course of HFpEF.[3] Recent studies have demonstrated associations between COVID-19 and both systolic and diastolic dysfunction, as well as HF.[4,5]

The H2FPEF score is a simple scheme primarily developed for diagnosing HFpEF. A severe H2FPEF score includes obesity, atrial fibrillation, age >60, more than two antihypertensive treatments, an echocardiographic E/e' ratio >9, and echocardiographic pulmonary artery systolic pressure >35 mmHg. Moreover, correlations between this score, its constituent parameters, and various coronary phenomena have been demonstrated.[6] Notably, many parameters constituting this score have been associated with mortality in COVID-19. Thus, the scheme may elucidate the connection between COVID-19 and cardiac diastology and prove valuable in predicting poor prognosis. This study aims to assess the distribution and prognostic value of the HFpEF clinic and the H2FPEF score among patients with COVID-19.

METHODS

The study complied with the principles stated in the Helsinki Declaration, and the study protocol received approval from the local ethics committee and the Ministry of Health. Written informed consent was obtained from all participating patients. The Ethics Committee of Adana City Training and Research Hospital approved the study (Date: 24.03.2022, Decision No: 1860).

Study Population

This observational and prospective single-center study included 205 consecutive patients hospitalized with a diagnosis of COVID-19-associated pneumonia between February 2022 and June 2022.

The detection of SARS-CoV-2 RNA was conducted through a real-time reverse transcription polymerase chain reaction after collecting oropharyngeal and nasal samples following the protocols recommended by the Ministry of Health. Patient monitoring adhered to the COVID-19 treatment management guidelines specified by the Ministry of National Health of the Republic of Turkiye. Exclusions were made for patients with HF and reduced left ventricular ejection fraction, severe valvular disease, significant liver or kidney disease, hereditary coagulation disorders, active cancer or undergoing chemotherapy-radiotherapy, rheumatologic disease, and those below 18 years of age. A standard blood sample was uniformly collected from the antecubital vein for all patients. Comprehensive data regarding patients' clinical status, comorbidities, and drug therapy were directly extracted from their medical histories and records. Subsequently, H2FPEF score was calculated for each patient, and they were categorized into groups based on low (0-1), medium (2-5), and high (6-9) scores.

Transthoracic Two-dimensional Echocardiography

Echocardiographic parasternal and apical images, including 2D, M-mode, and Doppler echocardiography, were obtained in the in-patient clinics during hospitalization. The imaging occurred with patients in the left lateral decubitus position under stable conditions, utilizing the X5 transducer probe (Philips Epiq7; Philips Healthcare, Inc., Andover, MA, USA). The imaging was performed by three experienced cardiac sonographers who were blinded to the study data. Echocardiographic images were acquired employing four standard views (long-axis parasternal, short-axis parasternal, two-chamber apical, and four-chamber apical) following the techniques recommended by the American Society of Echocardiography. The assessment of left ventricular systolic function involved the calculation of the left ventricular ejection fraction from the apical two- and four-chamber views using Simpson's method. The end-diastolic and end-systolic endocardial borders were manually monitored.[7] Additionally, the left atrial diameter was measured at the end of systole from the parasternal long-axis window.

Tissue Doppler was utilized to measure the ratio of early transmitral flow velocity to early diastolic mitral annular velocity (E/e') and the ratio of early transmitral flow velocity (E) to late transmitral flow velocity (A). Tricuspid annulus plane systolic excursion (TAPSE) was performed using M-Mode, positioned opposite the lateral tricuspid valve annulus in a 4-chamber window. The assessment of right ventricular function involved the measurement of TAPSE. The estimated pulmonary artery systolic pressure was derived by summing the estimated right atrial pressure, determined from the size and collapse of the inferior vena cava during inspiration, with the peak velocity of the tricuspid regurgitation jet. The latter was obtained using continuous wave Doppler and applying the modified Bernoulli equation.

H2FPEF score

Throughout their hospitalization, two cardiologists, blinded to patient survival data, calculated each patient's H2FPEF score following the method described by Reddy et al.[8] This score integrates four clinical features and two echocardiographic parameters, including: (i) obesity (Body Mass Index >30 kg/m²- Heavy) (H); (ii) use of ≥2 antihypertensive drugs- Hypertensive (H); (iii) atrial Fibrillation (F); (iv) Pulmonary hypertension (Doppler echocardiographic estimated pulmonary artery systolic pressure >35 mmHg) (P); (v) age >60 years- Elder (E); and (vi) Filling pressure (Doppler echocardiographic E/e' >9) (F). Atrial fibrillation was assigned 3 points, obesity 2 points, and other variables 1 point each. The total score ranged from 0 to 9 points (**Table 1**). According to this scoring system, a score of 0-1 excludes the diagnosis of HFpEF. Scores between 2 and 5 indicate a moderate probability of HFpEF, while patients with a score of 6-9 are deemed to have a high probability of being diagnosed with HFpEF.

Table 1. The risk factors used in the H2FPEF score

H2FPEF score	H2	F	P	E	F
Clinical Variable	Heavy Hypertensive	Atrial Fibrillation	Pulmonary Hypertension	Elder	Filling Pressure
Values	-Body mass index>30 kg/m² -2 or more antihypertensive drugs	-Paroxysmal or persistent	-Doppler echocardiographic Estimated pulmonary systolic artery pressure>35mm Hg	-Age>60 years	-E/e'>9
Points Sum (0-9)	2 1	3	1	1	1

Statistical Analysis

Data analyses were conducted using IBM SPSS Statistics for Windows, Version 22.0 software (IBM Corp., Armonk, NY, USA). Continuous variables with a normal distribution were presented as mean ± standard deviation, while those with a non-normal distribution were expressed as median (interquartile range25-75). Categorical variables were reported as numbers (n) and percentages (%). Student's t-test or one-way ANOVA was employed for normally distributed parameters, and the Mann-Whitney U test or Kruskal-Wallis test was used for non-normally distributed parameters. The chi-square test or Fisher's exact test was applied to compare categorical variables. Univariable and multivariable logistic regression analyses were conducted to assess in-hospital mortality and the presence of an intermediate-to-high H2FPEF score (≥2). Variables with a significance value of p<0.05 in the univariable analysis were included in the multivariable analysis. Receiver operating characteristic (ROC) curve analysis was utilized to assess the predictive accuracy and performance of H2FPEF for in-hospital mortality and to identify intermediate-to-high H2FPEF score. The Youden index was utilized to determine the cut-off for the H2FPEF score in predicting in-hospital mortality. The DeLong method was employed for pairwise comparison of ROC curves. Survival analysis against the H2FPEF score was conducted using Kaplan-Meier analysis, and the log-rank test was employed for comparisons. Statistical significance was set at p<0.05.

RESULTS

Baseline Characteristics

The study included two hundred and five consecutive hospitalized patients with COVID-19 (52% male, mean age 51 years). According to the H2FPEF score, 79 participants (38.5%) fell into the low-risk category (0-1 points), 108 (52.7%) were classified as intermediate-risk (2-5 points), and the remaining 18 (8.8%) were in the high-risk category (6-9 points). The mean age (p<0.001), heart rate (p=0.014), and body-mass index (p<0.001) demonstrated an upward trend with increasing H2FPEF score categories. The prevalence of comorbidities, including coronary artery disease (p<0.001), diabetes mellitus (p<0.001), hypertension (p<0.001), hyperlipidemia (p<0.001), and chronic obstructive pulmonary disease (COPD) (p<0.001), also increased proportionally with the score category. Additionally, in the evaluation of echocardiographic parameters, left ventricular ejection fraction (p<0.001), resting E/A ratio (p<0.001), septal E/e' (p<0.001), and TAPSE (p<0.001) exhibited a gradual decrease, while interventricular septum thickness (p<0.001), end-systolic and end-diastolic diameters (p<0.001 for both), and left atrial anteroposterior diameter (p<0.001) showed a gradual increase.

In laboratory findings, hemoglobin and albumin levels were lower in the high H2FPEF score category, while white blood cell (WBC), C-reactive protein, D-dimer, and high-sensitive troponin T (hsTnT) values were higher (p<0.001 for all). Platelet count remained similar across the groups. Table 2 provides detailed information on the baseline, echocardiographic, and laboratory parameters of the study population based on the categorized H2FPEF score.

In-hospital Mortality and Determinants

Among the COVID-19 patients, 46 individuals (22.4%) experienced in-hospital mortality. In comparison to the survivors, the deceased cohort was characterized by advanced age (46 vs. 69 years, p<0.001) and a higher prevalence of smoking (p=0.002), coronary artery disease (p=0.001), diabetes mellitus (p=0.001), and COPD (p=0.047). Additionally, those who succumbed to the illness exhibited a statistically significant increase in the H2FPEF score (1.8 vs. 4.0 points, p<0.001). Furthermore, the individual parameters contributing to the H2FPEF score were also elevated in the deceased group (p<0.05 for all), except body mass index (p=0.452).

In terms of laboratory findings, the deceased group exhibited lower levels of hemoglobin (p<0.001), platelets (p=0.004), and albumin (p<0.001), while WBC count, C-reactive protein, D-dimer, and hsTnT levels were higher (p<0.001 for all). Table 3 provides an overview of the demographic and laboratory parameters, as well as H2FPEF score details, based on in-hospital mortality status.

Multivariable logistic regression analysis revealed that heart rate per minute (OR=1.048, 95% CI 1.007-1.091, p=0.022), H2FPEF score (OR=1.396, 95% CI 1.060-1.839, p=0.018), and current smoking status (OR=4.569, 95% CI 1.001-20.917, p=0.050) were independent determinants of in-hospital mortality among patients hospitalized for COVID-19 (Table 4).

Table 2. Baseline characteristics of the study population by the H2FPEF score

	All patients (n=205)	Low score (0-1 points) (n=79)	Intermediate score (2-5 points) (n=108)	High score (6-9 points) (n=18)	p-value*
Age, years	51.4±19.5	34.4±11.8	60.6±15.7	70.6±10.0	<0.001
Male gender, n (%)	107 (52.2)	44 (55.7)	57 (52.8)	6 (33.3)	0.227
Systolic BP, mm Hg	119.1±16.1	113.8±10.0	122.2±16.9	126.6±26.8	0.001
Diastolic BP, mm Hg	71.3±9.5	69.6±7.4	72.3±10.0	73.6±13.7	0.110
Heart rate, beats per minute	86.5±15.3	82.6±11.9	88.6±16.3	90.6±18.8	0.014
BMI, kg/m²	28.2±5.1	25.1±2.6	29.3±4.7	34.8±6.5	<0.001
Coronary artery disease, n (%)	30 (14.6)	2 (2.5)	20 (18.5)	8 (44.4)	<0.001
Diabetes mellitus, n (%)	41(20)	3 (3.8)	29 (26.9)	9 (50.0)	<0.001
Atrial fibrillation, n (%)	8 (3.9)	0 (0)	0 (0)	8 (44.4)	<0.001
Hypertension, n (%)	75 (36.6)	2 (2.5)	55 (50.9)	18 (100)	<0.001
Hyperlipidemia, n (%)	30 (14.6)	0 (0)	20 (18.5)	10 (55.6)	<0.001
COPD, n (%)	15 (7.3)	0 (0)	9 (8.3)	6 (33.3)	<0.001
Cerebrovascular accident, n (%)	2 (1)	0 (0)	1(0.9)	1(5.6)	0.096
Cancer, n (%)	4(2)	1 (1.3)	3 (2.8)	0 (0)	0.626
Current smoker, n (%)	32 (15.6)	14 (17.7)	12 (11.1)	6 (33.3)	0.045
LVEF, %	58.9±6.9	62.5±3.0	57.5±6.3	51.9±12.7	<0.001
IVS, mm	10.3±2.4	8.7±1.3	11.0±2.2	13.2±2.7	<0.001
LVDd, mm	47.0±4.2	44.7±3.2	48.1±4.0	50.3±4.9	<0.001
LVDs, mm	30.8±5.4	27.7±4.6	32.3±4.7	35.6±6.0	<0.001
LAD, mm	38.4±5.6	34.0±4.9	40.7±4.5	44.4±5.9	<0.001
E/A at rest	1.07±0.45	1.43±0.36	0.88±0.33	0.71±0.40	<0.001
E/e' septal	11.7±5.6	7.3±3.6	14.2±5.0	16.0±4.0	<0.001
sPAP, mm Hg	29.2±7.8	24.0±4.7	31.2±7.2	40.5± 5.7	<0.001
TAPSE, mm	19.8±5.1	23.1±4.1	18.4±4.5	14.1±3.6	<0.001
Hemoglobin, mmol/L	12.8±1.9	14.5±1.6	13.5±1.9	13.0±2.3	<0.001
WBC, ×10³/ul	6.8±3.3	6.2±2.0	6.8±3.4	9.1±5.6	0.004
Platelet count, ×10³/Ul	213.1±65.6	220.8±57.5	211.3±69.7	190.2±70.9	0.187
Albumin, g/L median, IQR	3.8 (3.5-4.1)	4.0 (3.8-4.3)	3.6 (3.2-4.0)	3.6 (2.9-3.9)	<0.001
C-reactive protein, nmol/L, median, (IQR)	1.00 (0.21-6.28)	0.22 (0.10-0.70)	2.01 (0.52-13.88)	5.48 (1.19-21.95)	<0.001
D-dimer, µg/ml, median, (IQR)	0.25 (0.06-0.97)	0.10 (0.0-0.26)	0.42 (0.11-4.23)	0.61 (0.17-8.19)	<0.001
hsTnT, pg/ml, median, (IQR)	2.8 (1.6-9.1)	1.7(1.3-2.5)	4.4 (2.3-15.8)	19.5 (6.9-99.0)	<0.001

Data are given as mean± standard deviation (SD), median (IQR25-75), or n (%). P value was calculated using one-way ANOVA test or Kruskal-Wallis test for continuous variables and a chi-squared test for categorical variables, as appropriate. *A p-value <0.05 was considered significant. Abbreviations: BMI, body mass index; BP, blood pressure; COPD, Chronic obstructive pulmonary disease; hsTnT, high-sensitive Troponin T; LAD, left atrium diameter; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; IVS, interventricular septum; IQR, interquartile range; sPAP, Systolic pulmonary artery pressure; TAPSE, Tricuspid annular plane systolic excursion; WBC, White blood count.

H2FPEF Score and Survival

The analysis of ROC curve indicated that the H2FPEF score, with a threshold of ≥2, exhibited good discriminative capacity, demonstrating 80.4% sensitivity and 69.2% specificity (AUC=0.777, 95% CI 0.710-0.844, p<0.001, Figure 1). Kaplan-Meier curve analysis, stratified by the H2FPEF score using this cut-off determined by the Youden index, revealed a higher in-hospital mortality rate among individuals with scores ≥2 (log-rank p<0.001, Figure 2).

The results of the multivariable logistic regression analysis aimed at identifying parameters associated with an intermediate-to-high H2FPEF score (≥2) revealed that diabetes (OR=5.775, 95% CI 1.534-21.735, p=0.010), hemoglobin (OR=0.782, 95% CI 0.637-0.961, p=0.019), D-dimer (OR=0.993, 95% CI 0.985-1.000, p=0.050), and hsTnT (OR=1.126, 95% CI 1.013-1.251, p=0.027) were associated with an increased H2FPEF score (Table 5). The pairwise comparison of ROC curves analysis demonstrated that hsTnT (AUC=0.819) exhibited better discriminative abilities than both D-dimer (AUC=0.737, p=0.029) and hemoglobin (AUC=0.691, p=0.007) in determining an intermediate-to-high H2FPEF score (Figure 3).

Table 3. Comparison of baseline characteristics among COVID-19 patients based on their mortality status

Variable	Survivor (n=159)	Non-survivors (n=46)	p
Age	46.3±18.0	69.0±13.4	<0.001
Male gender, n (%)	77 (48.4)	29 (63.0)	0.081
Systolic BP, mm Hg	117.6±13.1	127.3±25.08	0.058
Diastolic BP, mm Hg	70.7± 8.2	74.6± 14.2	0.163
Heart-rate, beats per minute	83.5± 10.9	96.9±22.3	<0.001
H_2FPEF score	1.8±2.0	4.0±2.1	<0.001
H_2FPEF score components			
BMI >30 kg/m²	39 (27.1)	15 (33.3)	0.452
E/e' >9	67 (45.9)	41 (91.1)	<0.001
Age >60, n (%)	39 (24.5)	38 (82.6)	<0.001
Hypertension	41 (28.8)	34 (73.9)	<0.001
SPAP>35 mm Hg, n (%)	36 (22.6)	28 (60.9)	<0.001
Atrial fibrillation, n (%)	3 (1.9)	5 (10.9)	0.015
H_2FPEF score point			<0.001
0- 1 (low)	77 (48.4)	2 (4.3)	
2-5 (intermediate)	73 (45.9)	35 (76.1)	
6-9 (high)	9 (5.7)	9 (19.6)	
Diabetes mellitus, n (%)	20 (12.6)	21 (45.7)	
Current smoker, n (%)	18 (11.3)	14 (30.4)	0.002
Coronary artery disease, n (%)	12 (7.5)	18 (38.1)	<0.001
Cerebrovascular accident, n (%)	0 (0)	2 (4.3)	0.049
COPD, n (%)	8 (5.0)	7 (15.2)	0.047
Cancer, n (%)	2 (1.3)	2 (4.3)	0.218
Hemoglobin, mmol/L	14.2±1.6	12.5±2	<0.001
WBC, $\times 10^3$/ul	6.1±2.4	9.0±4.8	<0.001
Platelet count, $\times 10^3$/ul	220.1± 60.3	188.9±77.2	0.004
Albumin, g/L, median, (IQR)	3.9 (3.6-4.2)	3.2 (2.6-3.6)	<0.001
C-reactive protein, nmol/L, median, (IQR)	0.51 (0.20-1.70)	22.15 (10.27-112.95)	<0.001
D-Dimer, µg /ml, median, (IQR)	0.13 (0.02-0.36)	441.50 (0.98-1842.50)	<0.001
hsTnT, pg/ml, median, (IQR)	2.3 (1.5-4.5)	24.0 (7.6-159.0)	<0.001

The data are expressed as number (%), mean ± standard deviation (SD), or median (IQR25-75). Statistical comparisons were performed using an independent samples t-test or the Mann-Whitney U-test for continuous variables, while categorical variables were analyzed using a chi-squared test or Fisher's exact test, as appropriate. A p-value <0.05 was considered statistically significant. Abbreviations: BMI, Body Mass Index; BP, blood pressure; COPD, Chronic obstructive pulmonary disease; hsTnT, high-sensitive Troponin T; WBC, White Blood Count.

Table 4. Univariable and multivariable logistic regression analysis of in-hospital mortality

Variable	Univariable analysis		Multivariable analysis+	
	OR (95% CI)	p value*	OR (95% CI)	p value*
Heart rate	1.064 (1.037-1.091)	<0.001	1.048 (1.007-1.091)	0.022
H_2FPEF score	1.573 (1.325-1.868)	<0.001	1.396 (1.060-1.839)	0.018
Diabetes mellitus	5.838 (2.769-12.308)	<0.001	2.591 (0.807-8.320)	0.110
Current Smoker	3.427 (1.545-7.604)	0.002	4.569 (1.001-20.917)	0.050
Coronary artery disease	7.875 (3.418-18.146)	<0.001	0.878 (0.220-3.509)	0.854
COPD	3.388 (1.158-9.914)	0.026	1.191 (0.219-6.474)	0.840
Hemoglobin	0.612 (0.498-0.754)	<0.001	0.796 (0.570-1.113)	0.183
WBC	1.274 (1.144-1.419)	<0.001	1.055 (0.863-1.289)	0.603
Platelet count	0.992 (0.986-0.997)	0.005	0.992 (0.983-1.002)	0.123
Albumin	1.087 (0.965-1.223)	0.169	-	-
C-reactive protein	1.048 (1.024-1.072)	<0.001	1.020 (0.999-1.043)	0.067
D-dimer	1.260 (1.125-1.412)	<0.001	1.080 (0.984-1.186)	0.107
hsTnT	1.087 (0.990-1.192)	0.079	-	-

*p-value <0.05 was considered significant. +Nagelkareke R square =0.656, -2log-likelihood=103, Omnibus tests of model coefficients p<0.001, Hosmer-Lemeshow test p=0.437 Abbreviations: CI, Confidence Interval; COPD, Chronic obstructive pulmonary disease; hsTnT, high-sensitive troponin T, OR, Odds Ratio; WBC, white blood cell.

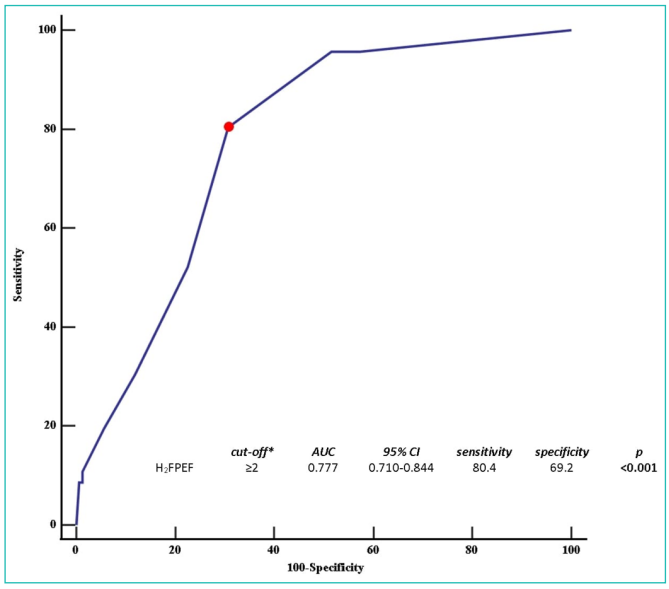

Figure 1. Receiver Operating Characteristic curve that illustrates the predictive ability of the H2FPEF score in identifying in-hospital mortality Abbreviations: AUC, Area Under the Curve; CI, Confidence Interval. *The threshold was determined using the Youden Index.

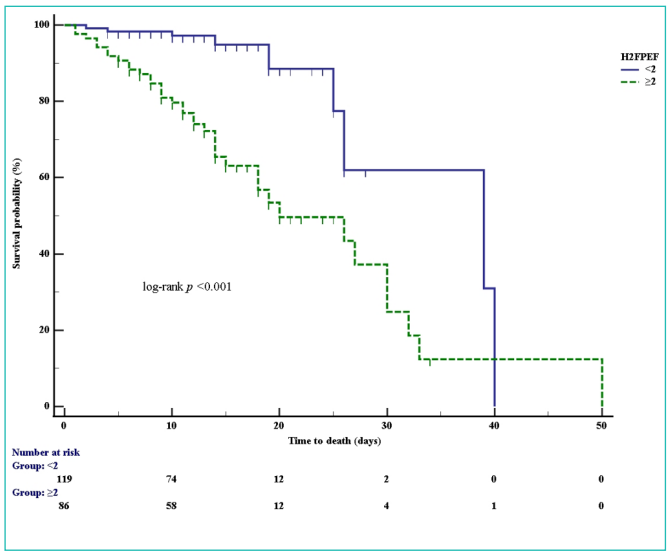

Figure 2. Kaplan-Meier survival curves of in-hospital mortality by H2FPEF score

DISCUSSION

Our objective was to explore the impact of the H2FPEF score on the distribution and clinical outcomes of COVID-19 patients. The H2FPEF score, designed as a straightforward tool for diagnosing HFpEF, was considered relevant given the occurrence of a clinical presentation resembling HFpEF in individuals affected by COVID-19. The key findings of our investigation are as follows: I) A substantial proportion of COVID-19 patients fell into the intermediate- and high-risk categories for HFpEF development. II) Non-survivors of COVID-19 exhibited significantly elevated H2FPEF scores compared to survivors (p<0.001). III) H2FPEF, heart rate, and smoking were established as independent

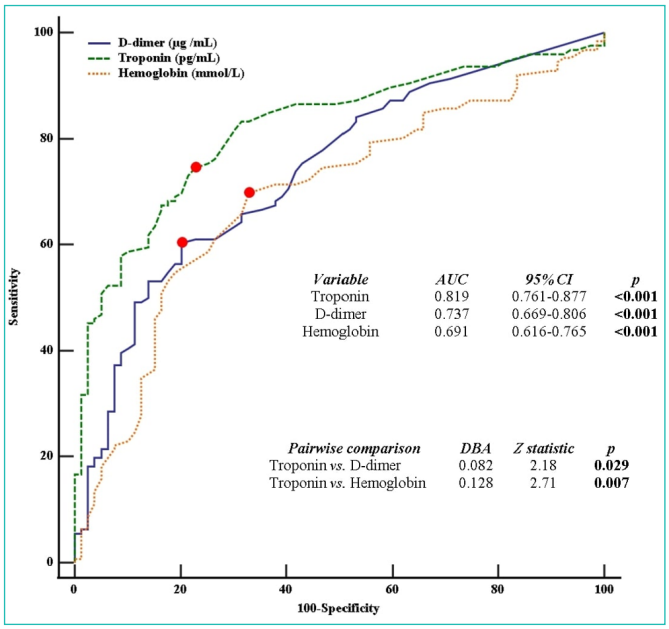

Figure 3. The results of the Receiver Operating Characteristic curve analysis that demonstrate the predictive accuracy of high-sensitivity cardiac troponin T, hemoglobin, and D-Dimer in the detection of intermediate-to-high H2FPEF scores (≥2) and the pairwise comparison of curves Abbreviations: AUC, Area Under the Curve; CI, Confidence Interval; DBA, Difference Between Areas. Note that the pairwise comparison analysis was conducted utilizing the DeLong method

determinants of in-hospital mortality. IV) Kaplan-Meier survival analysis revealed a significant increase in mortality among patients with an intermediate-to-high H2FPEF score (p<0.001 by log-rank test).

The activation of neurohormones and markers of myocyte necrosis, particularly in septic patients, has demonstrated a robust association with HFPEF.[9] Myocardial relaxation abnormalities in these patients could range from asymptomatic increases in cardiac filling pressures to clinical manifestations of classical HFpEF. COVID-19 has the potential to induce myocardial damage through inflammation and/or a cytokine storm, especially in cases with a severe course and pre-existing chronic diastolic dysfunction. This damage may further compromise myocardial relaxation, particularly in situations involving the administration of large volumes of intravenous fluids, ultimately leading to severe pulmonary involvement and concealed pulmonary edema beneath acute respiratory distress syndrome.[10] Consequently, it has been suggested that COVID-19 may contribute to HFpEF development through direct viral damage or autoimmune mechanisms.[5]

Research on the HFpEF clinic in COVID-19 patients is notably limited in the literature. The initial study, conducted with the most substantial patient cohort comprising 64 individuals, was the first to report a significant proportion of COVID-19 patients demonstrating a high risk of HFpEF. Correspondingly,

over half of the patients in this study were categorized into the clinically intermediate and high-risk groups for HFpEF, mirroring findings similar to ours.[11] Given this context, we believe that our study, including the largest number of patients on this subject, may provide a crucial contribution to the existing literature.

Elevated H2FPEF scores were linked to unfavorable clinical outcomes in our study. Moreover, the H2FPEF score exhibited robust performance in ROC analysis for determining in-hospital mortality among COVID-19 patients. Notably, the risk factors included in the H2FPEF score align with factors already recognized to be associated with COVID-19-related deaths. While our findings may not be entirely surprising in this context, the prevalence of moderate and high scores in our patient cohort, accounting for 61.5% of patients, underscores the substantial clinical risk for HFpEF. We posit that adjustments in the treatment strategy, considering HFpEF, could be beneficial in mitigating mortality in this patient group. Atrial fibrillation,[12] hypertension,[13] pulmonary hypertension,[14] and diastolic filling disorder,[15] all of which have established mortality associations in COVID-19 patients, render the H2FPEF score with its concise scoring system particularly pertinent in this patient group, alongside the documentation of advanced age and obesity.[16]

Notable increases were observed in both age and the prevalence of chronic diseases, corresponding to the escalation of the H2FPEF score in the present study. Furthermore, the H2FPEF score was significantly elevated in the non-survivor group. It is well-established that mortality rates in COVID-19 are higher among the elderly and individuals with significant comorbidities.[17,18] Remarkably, populations with poorer prognoses typically manifest at least one comorbidity, with hypertension, diabetes, chronic obstructive pulmonary disease, and heart disease ranking among the most prevalent.[19] We also found that the H2FPEF score appears to serve as a valuable predictor of morbidity and mortality, shedding light on the risk profile of the HFpEF clinic in COVID-19 patients. Furthermore, multivariable regression analysis revealed that, in addition to a high H2FPEF score, a high heart rate, and current smoker were independent determinants of in-hospital mortality. Similar to our findings, sinus tachycardia, the presence of diabetes mellitus, and a low platelet count have consistently emerged as independent risk factors for mortality in COVID-19, as demonstrated in numerous studies.[20-22]

A notable percentage (20% to 35%) of COVID-19 patients admitted to hospitals display elevated levels of cardiac biomarkers, such as hsTnT and natriuretic peptides.[23] Myocardial injury, defined by increased hsTnT values, has been linked to a more severe disease course and even death in COVID-19 patients.[24] Elevated D-dimer levels at admission are significantly associated with the severity of COVID-19 pneumonia and may serve as a predictor of mortality in hospitalized patients.[25,26] Serum CRP levels can effectively gauge disease severity and predict outcomes in patients with COVID-19.[27] In our study, patients with biochemical evidence of myocardial injury had higher H2FPEF scores, and it was observed that D-dimer, CRP, and particularly high-sensitive hsTnT levels, which impact COVID-19 severity and mortality, are robust predictors of high H2FPEF score. Therefore, increased hsTnT levels, especially in COVID-19 patients, may aid in identifying cardiac diastolic abnormalities caused by COVID-19.

Limitations

It is crucial to highlight that despite the prospective design of our study, it was conducted with a relatively small patient cohort. Nevertheless, conducting detailed echocardiograms in such a sizable sample of COVID-19-infected patients lends significance to the study. Another limitation of the study is the absence of certain laboratory parameters, such as N-terminal proB-type brain natriuretic peptide, and the lack of information on treatment protocols that could potentially influence the study outcomes. The majority of our patients presented with pneumonia necessitating hospitalization, and they exhibited a high risk of mortality during follow-up. Consequently, our findings may not fully represent the entire COVID-19 population, particularly those requiring outpatient treatment. Given that our study focused on the acute phase of COVID-19, additional investigations are warranted to elucidate the development of HFpEF in COVID-19 survivors during the chronic phase of recovery.

CONCLUSION

COVID-19 may emerge as a novel risk factor for HFpEF development, potentially triggered by systemic inflammation and autoimmune activation. It is advisable to conduct comprehensive assessments, including biomarkers and echocardiographic evaluations for HFpEF, in high-risk COVID-19 patients. In this context, the H2FPEF score may be valuable for rapid risk assessment upon hospital admission, providing insights to guide early treatment and facilitate close follow-up.

List of Abbreviations

A: Late transmitral flow velocity; COVID-19: Coronavirus disease 2019; CRP: C-reactive protein; E: Early transmitral flow velocity; E/e': Early transmitral flow velocity to early diastolic mitral annular velocity; HF: Heart failure; HFpEF: Heart failure with preserved ejection fraction; hsTnT: high-sensitive troponin; TAPSE: Tricuspid annulus plane systolic excursion.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Adana City Training and Research Hospital Clinical Researches Ethics Committee. (Date:24.03.2022, Decision No: 1860)

Informed Consent

Written informed consent was obtained from all participating patients.

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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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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Variant analysis of MiRNA regulatory genes in colorectal cancer

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ABSTRACT

Aims: The aim of this study was to investigate the clinical significance of mutations in *AGO2*, *DICER* and *DROSHA* genes, which are involved in miRNA biogenesis, as well as *TP53*, *KRAS*, *BRAF*, *PI3KCA* and *APC* genes, which are important in the pathophysiology of CRC, and their association with metastasis in patients diagnosed with sporadic colorectal cancer

Methods: DNA isolation was performed by taking 10-micron sections from paraffin-embedded tissue samples of 12 patients diagnosed with CRC and Kapa NGS DNA extraction kit was used for sequence analysis. The purity and concentration of the DNA obtained was measured by Qubit fluoremeter, and NadPrep DNA Universal Library Preparation Kit was used for high quality library preparation. Bioinformatics analyses were performed on the Genomize Seq platform.

Results: In our study, metastasis was detected in 42% of 12 colorectal cancer patients. Mutations in at least two miRNA biogenesis genes were detected in 80% of metastatic patients. In addition, variants detected in miRNA biogenesis regulatory genes and oncogenic genes were summarized according to pathogenicity status according to the American College of Medical Genetics and Genomics (ACMG) classification.

Conclusion: Genes involved in miRNA biogenesis and mutations of clinically relevant genes in CRC have important implications on disease prognosis and response to therapy. Mutations in these genes may be associated with the development of metastases and mechanisms of resistance to treatment and may be potential genetic markers for the development of personalized treatment strategies.

Keywords: Colorectal cancer, miRNA biogenesis, *AGO2*, *DICER*, *DROSHA*

INTRODUCTION

Cancer is a pathological condition caused by uncontrolled growth and division of cells and disruption of the mechanisms that regulate the normal behavior of the cell.[1] The first findings on the cellular changes involved in carcinogenesis were in the field of cancer genetics. It is now known that many factors leading to cancer initiation and progression are associated with genetic aberrations in oncogenes and tumor suppressor genes. Evidence from many years of research suggests that epigenetic changes play an important role in cancer development. In particular, epigenetic factors such as microRNAs (miRNAs) and histone proteins may play a role in the carcinogenesis process as a result of mutations and expression changes in the genes encoding them.[2,3]

miRNAs are a type of RNA that function as non-coding RNA molecules 22 nucleotides in length and regulate gene expression. These small RNA molecules play important roles in regulating various biological functions such as cell survival, cell proliferation, apoptosis, tumor growth and metastasis.[4] The cellular biogenesis of miRNAs is a complex process and starts with the transcription of miRNA genes through the enzyme RNA polymerase II.[5] This process starts with the formation of precursor molecules called pri-miRNA, which contain a hairpin structure.[6] The hairpin structure of the pre-miRNAs is then recognised and cut by *DROSHA* and the DGRCR8 complex, resulting in the formation of a 70-nucleotide pre-miRNA.[7] This pre-miRNA is transported from the nucleus to the cytoplasm by RanGTP/Exportin 5 complex.[8] Pre-miRNA is cleaved in the cytoplasm by the Rnaz III enzyme *DICER* and miRNA doubles of approximately 22 nucleotides are formed.[9] When miRNA duplexes are formed, they interact with the RNA-induced silencing complex (RISC) formed by Argonaute (Ago) proteins. As a result of this interaction, one strand of the 22-nucleotide RNA duplex remains in Ago as mature miRNA, while the other duplex strand is cleaved. RISC directs the single-stranded mature active miRNA to target mRNAs and enables it to take part in post-translational gene regulation.[10]

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Colorectal cancer (CRC) is the third leading cause of cancer death worldwide and is a serious cancer with more than 1.85 million cases and 850,000 deaths annually.[11] It is the second most common cancer in women and the third most common cancer in men in terms of gender.[12] The vast majority of colorectal carcinomas are adenocarcinomas that develop from epithelial cells, accounting for over 90%. Rare types of colorectal carcinomas include neuroendocrine, squamous cell, adenosquamous, spindle cell and undifferentiated carcinomas.[13] According to studies on CRC, *TP53*, *KRAS*, *BRAF*, *PIK3CA* and *APC* genes are among the most frequently studied genes. In addition, these genes are reported as the most frequently mutated genes in CRC cases according to the Catalogue of Somatic Mutation in Cancer (COSMIC) database.[14] Among these genes, *KRAS* has been identified as the most critical oncogenic factor and mutations in this gene have been associated with poor prognosis, undifferentiated tumor, distant metastasis, low survival and recurrence. Currently, patients are treated with combined therapies targeting *KRAS* mutations.[15]

Although research has provided some insights into CRC prognosis, there are still questions about recurrence, metastasis or survival.[16] This suggests that epigenetic factors may also play a role in this process.[17] In a study conducted on recurrent colon cancer tissue, miR-21, miR-106a, miR-155 and miR-200c expression levels were found to be increased compared to the control group, indicating that these miRNAs may be associated with the mechanism of recurrence in colon cancer.[18] In another study on colorectal cancer, low expression of miR-320 and miR-498 was associated with lower survival rates in the disease.[19] Studies in various solid tumors have shown that miR-21 is up-regulated especially in breast, lung, colorectal and pancreatic cancers and acts as an oncogene by targeting multiple tumor suppressor genes involved in cell proliferation, apoptosis and metastasis.[20] In contrast, miR-34a has been reported to play an important role in tumor suppression by targeting *TP53*.[21] According to the literature, miRNAs have been reported to play a role as oncogenes or tumor suppressors in various cancer types, but changes in key molecules involved in miRNA biogenesis have also been associated with the carcinogenesis process.[22] It has been reported that changes in the expression of *DICER* and *DROSHA* are associated with carcinogenesis and mutations in these genes may play a role in this process and cause changes in the expression of miRNAs.[23]

In this study, we performed sequence analysis of *AGO2*, *DICER* and *DROSHA* genes involved in miRNA biogenesis and *TP53*, *KRAS*, *BRAF*, *PI3KCA* and *APC* genes known to have clinical significance in CRC in 12 patients diagnosed with sporadic CRC and investigated the clinical significance of the variants detected.

METHODS

Ethics

The study was carried out with the permission of Selçuk University Faculty of Medicine Ethics Committee. (Date: 13.11.2023, Decision No: E-70632468-050.01.04-639070). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

DNA Isolation and Sequencing Analyses

The patients included in the study were all patients diagnosed with CRC who were referred to the Medical Genetics Department of Selçuk University Medical Oncology Department within the last 1 year. DNA isolation was performed from 10 micron sections of paraffin-embedded tissues obtained from the patients using the Kapa NGS DNA Extraction Kit manufactured by Roche Molecular Systems, Inc. (Germany). Translated with www.DeepL.com/Translator (free version) The purity and concentration of the DNA obtained were measured using a Qubit fluoremeter (ThermoFisher Scientific, USA). To generate a high-quality library from double stranded DNA (dsDNA), we used the NadPrep DNA Universal Library Preparation Kit (Nanodigmbio (Nanjing) Biotechnology Co., Ltd, China), which includes the Library Prep Module and Adapter Primer Module. NAD panels within 5' biotinylated probes, optimised for targeted capture applications in NGS, were used for libraries prepared using the NadPrep DNA Universal Library Preparation Kit (for MGI). In this study, 500 ng of DNA from each library was used for hybrid capture. After these procedures, a single-stranded circular DNA library was prepared using the MGIEasy Circularisation Kit (MGI Tech Co., Ltd, China).

Single-stranded circular DNAs were converted into nanoballs (DNBs) by rolling circle amplification using the DNB SEQ-G50RS high-throughput sequencing kit (MGI Tech Co., Ltd, China). The sequencing cartridge was then prepared, and the DNBs were placed in the DNB tube and inserted into the instrument. Samples were passed through the flow cell in the instrument and sequencing was performed on the DNBSEQ-G50RS instrument (MGI Tech Co., Ltd, China).

Statistical Analysis

Bioinformatic analysis of the data obtained from the study was performed on the Genomize Seq (v8.0.4) platform. The bioinformatic analysis of the data obtained from the study was carried out on the Genomize Seq platform.

RESULTS

In this study, we performed a comprehensive analysis on the miRNA biogenesis genes *AGO2*, *DICER*, *DROSHA* and *TP53*, *KRAS*, *BRAF*, *APC*, *PIK3CA* genes associated

with CRC clinic in the literature in 12 colorectal cancer patients. Our patient group consisted of 7 men and 5 women with a mean age of 66 years. One of the patients (case5) died during the process. The patient who died had metastasis and 5 mutations in *AGO2*, 4 mutations in *DICER*, 4 mutations in *DROSHA*, 1 mutation in *TP53*, 5 mutations in *BRAF* and 2 mutations in *PIK3CA* were detected. In this study, metastasis was detected in 42% of 12 colorectal cancer patients. Mutations in at least two miRNA biogenesis genes were detected in 80% of metastatic patients. No mutation was detected in miRNA biogenesis genes in 5 of 7 patients with no metastasis. In addition, the pathogenicity status of the variants detected in both miRNA biogenesis regulatory genes and oncogenic genes evaluated in our study according to the American College of Medical Genetics and Genomics (ACMG) classification are shown in Table 1-3.

Table 1. Distribution of cases according to the variants detected

CASES	*AGO2*	*DICER1*	*DROSHA*	METASTASIS	ACMG*
Case 1	c.700C>T c.2899C>T			✓	LP**/ VUS***
Case 2		c.2690T>C		✓	VUS
Case 3					
Case 4				✓	VUS
Case 5	c.1325T>C c.1228G>A c.1927G>A c.1861C>T c.2393C>T	c.4453A>T c.358A>T c.4666G>A c.2978C>T c.4426G>A c.2792T>G c.4561G>A c.3766C>T	c.859C>T c.2650G>A c.124C>T c.1555C>T	✓	VUS
Case 6					VUS
Case 7			c.3071A>C		VUS
Case 8					VUS
Case 9					
Case 10		c.5127T>G		✓	LP
Case 11					
Case 12	c.854C>T	c.4019A>C	c.1412C>T	✓	VUS/ VUS/VUS

*Pathogenicity according to SEQ Autopathogenicity Algorithm. VUS+ and VUS++ are new classifications developed by Genomize to let user see how close a variant to being pathogenic, **Likely pathogenic, ***Variant of Uncertain Significance

Table 2. Distribution of cases according to clinical and cultural characteristics

Cases	Gender	Age	Death	Metastasis	Histology
Case 1	Female	85			Colorectal
Case 2	Male	67		✓	Colorectal
Case 3	Female	76		✓	Colorectal
Case 4	Male	67		✓	Colorectal
Case 5	Male	73	✓		Colorectal
Case 6	Male	70		✓	Colorectal
Case 7	Male	49		✓	Colorectal
Case 8	Female	81			Colorectal
Case 9	Female	74			Colorectal
Case 10	Female	43		✓	Colorectal
Case 11	Male	55		✓	Colorectal
Case 12	Male	56		✓	Colorectal

DISCUSSION

CRC stands out as a major health problem, ranking third among cancer-related deaths worldwide.[24] Metastasis and drug resistance are among the main reasons for the high mortality rate and poor prognosis.[25] While 25% of patients show signs of metastasis at the time of initial diagnosis, metastatic spread is observed in 50% of CRC patients. Despite the use of multiple drugs in clinical practice, drug resistance is an important obstacle that cannot be overcome in CRC treatment. Drug resistance is a condition that cancer cells can develop through various epigenetic changes that occur before or during drug treatment. Moreover, epigenetic changes are associated with drug resistance by controlling key signaling pathways such as pro-survival signaling pathways and pro-apoptosis pathways. These mechanisms may help cancer cells to develop resistance to therapy, and epigenetic factors may play important roles in the metastatic progression of the disease.[26,27]

miRNAs can play different roles in various types of cancer. For example, miR-96 can be oncogenic in some cases, while in others it can act as a tumor suppressor. While miR-96 expression levels are increased in lung, prostate, bladder, colorectal and breast cancers,[28] was reported to be decreased in pancreatic cancer. Studies have shown that miR-96 may be effective on cell proliferation, metastasis and apoptosis by binding to the 3'UTR region of *KRAS* G12C mRNA in pancreatic cancer cells.[29] MiRNAs may also play tumor suppressor roles. For example, studies have shown that miR-30b acts as a *KRAS* tumor suppressor in CRC. In addition, decreased expression of miR-143 has been reported to contribute to CRC prognosis through suppression of *KRAS* expression. According to the results observed after treatment, inhibition of miR-143 increased cell proliferation in CRC cells. In contrast, increasing the levels of miR-143 decreased the proliferation capacity of the cells. These observed changes were attributed to the specific binding of miR-143 to the 3' end of *KRAS* mRNA and its inhibition of the activation of the ERK1/2 pathway.[30] Current research emphasizes the importance of miRNAs due to their critical role in CRC progression and points to miRNA biogenesis in this context.

Uncertainty regarding the regulation of *AGO2*, *DICER* and *DROSHA* during carcinogenesis, which are involved in miRNA biogenesis in various cancer types, has emerged as an important factor influencing cancer prognosis. However, the mechanisms of why these genes are up- or down-regulated in certain cancer types are not yet fully understood.[31] This is an important question for understanding the process of carcinogenesis. In limited studies, expression levels of *DICER*, *DROSHA* and *AGO2* genes have been associated with prognosis,

Table 3. Distribution of cases according to variants detected in oncogenic and tumor suppressor genes

Cases	*TP53*	*KRAS*	*BRAF*	*APC*	*PI3KCA*	ACMG*
Case 1			c.1208del c.2144A>G	c.6363_6365del		LP**/VUS
Case 2		c.436G>A	c.443A>C			VUS+/LP/LP
Case 3	c.320A>C c.400T>C		c.2144A>G		c.320A>C	LP/LP/LP/VUS
Case 4 Case 5	c.635_636del c.860A>T		c.2144A>G c.1570C>T c.1593G>T c.1505T>G c.1769T>G c.1592G>T		c.1636C>A c.1633G>A	P/LP/VUS VUS++/LP/LP/LP LP/LP/LP
Case 6	c.637C>T				c.3922A>T	LP/VUS++
Case 7	c.430C>T	c.35G>A			c.882T>G	VUS***/LP/LP
Case 8	c.743G>A		c.1799T>A			LP/LP
Case 9	c.844C>T	c.35G>C	c.1208del			LP/LP/LP/VUS+
			c.443A>C			
Case 10	c.578A>C	c.193A>C	c.1208del	c.573T>G	c.750T>G	P****/P/LP/LP/LP/LP
	c.902del	c.195T>G	c.1799T>A			LP/LP/LP/LP
			c.786A>C			LP/LP/VUS++/LP/LP
			c.770A>C			
			c.1388T>G			
			c.1798G>A			
			c.1589A>C			
			c.1445T>G			
			c.1798_1799delinsAA			
			c.750T>G			
Case 11	c.637C>T	c.35G>A	c.750T>G			LP/LP/LP
Case 12						

*Pathogenicity according to SEQ Autopathogenicity Algorithm. VUS+ and VUS++ are new classifications developed by Genomize to let user see how close a variant to being pathogenic, **Likely pathogenic, ***Variant of Uncertain Significance, **** Pathogenic

survival time and metastasis development. Upregulation of *DICER* expression level in CRC patients has been associated with reduced survival and poor prognosis.[31] The expression level of the *DICER* gene varies for tumors of different histological origin. While ovarian cancer patients have been shown to have reduced expression levels of the *DICER* gene, overexpression of the *DICER* gene in prostate cancer, leiomyosarcomas, CRC and neuroblastoma has been shown to make the tumor more aggressive.[32] This suggests tumor-specific regulation and function of the *DICER* enzyme. Studies in many solid tumors have revealed that mutations in *AGO2*, *DICER* and *DROSHA* genes, which play a role in miRNA biogenesis, may be associated with prognosis.[33-35] In a study, it was reported that rs11786030 and rs2292779 in *AGO2* gene, rs1057035 in *DICER* gene and rs874332 in *DROSHA* gene were associated with survival processes in breast cancer and rs2292779 variant in *AGO2* was associated with poor prognosis.[33] Ke et al.[36] showed that rs1187652 and rs11160231 variants in *DICER* are associated with cancer progression and recurrence in non-muscle invasive bladder cancer. In a study in Wilms tumor, it was reported that mutations in *DROSHA* and *DICER* lead to disruption of miRNA biogenesis, which results in decreased expression of tumor suppressor miRNAs.[37]

A study in metastatic CRC patients emphasized that the rs10719 variant in the *DROSHA* gene and some miRNAs may be a potential biomarker for treatment.[38] When *AGO2*, *DICER* and *DROSHA* genes are analyzed in terms of somatic mutations occurring in CRC, it is seen that they have different mutation profiles. According to the COSMIC database, 36 different mutations have been reported in *AGO2* gene, 55 in *DROSHA* gene and 100 in *DICER* gene in colorectal carcinoma.14 In our study, 6 different mutations in *AGO2* gene, 11 different mutations in *DICER* and 7 different mutations in *DROSHA* were detected in patients with CRC. Among these variants, only the c.1412C>T mutation in *DROSHA* was reported in the COSMIC database.

According to the Cancer Genome Atlas, *TP53*, *KRAS*, *BRAF*, PI3K and *APC* genes are the most important genes with critical importance in the pathophysiology of CRC and *KRAS* has been reported as the most critical oncogene according to COSMIC.[39] A total of 40 different mutations were detected in the clinically relevant genes of colorectal cancer patients examined in our study. *TP53* gene c.400T>C, c.635_636del, c.637C>T, c.430C>T, c.743G>A, c.844C>T, c.578A>C, c.902del, c.637C>T mutations; *KRAS* gene c.436G>A, c.35G>A, c.35G>C

mutations; *BRAF* gene c.1208 del, c.1799T>A, c.1798G>A, c.1798_1799delinsAA mutations; c.1636C>A, c.1633G>A in the *PIK3CA* gene and c.3922A>T mutations in the *APC* gene, previously reported in COSMIC in colorectal cancer cases. G12C mutation (**Figure 1**), a common mutation in the *KRAS* gene, was detected in patient Case.[7] The patient was initially treated with capecitabine and concurrent radiotherapy as neoadjuvant therapy. Despite the treatment, disease progression was observed. In the following period, the patient was switched to a chemotherapy regimen including 5-fluorouracil, oxaliplatin and bevacizumab. After 11 months of this treatment, the disease progressed again and the patient started a new treatment protocol including fluorouracil, irinotecan and aflibercept. However, there was no response to this treatment in the current situation. On the other hand, another patient in the cohort (Case2) was assigned to a treatment regimen including capecitabine and oxaliplatin. No information is yet available on the response of this patient and the efficacy of the treatment is awaited to be evaluated.

In our study, 58% of a group of 7 patients had mutations in at least one of the clinically relevant genes, while no pathogenic mutation was detected in miRNA biogenesis genes. Metastasis was observed in only 2 of these 7 patients. On the other hand, metastasis was observed in 3 of 5 patients with mutations in miRNA biogenesis genes. These results suggest that mutations in miRNA biogenesis genes may be associated with mutations in clinically known important genes and may be associated with metastatic behavior. In the present study, mutations in *AGO2*, *DICER* and *DROSHA* genes associated with miRNA biogenesis were analyzed in a cohort of patients with CRC. In addition, mutations in oncogenic genes *KRAS*, *BRAF*, *PIK3CA* tumor suppressor *TP53*, *APC* genes were also evaluated and their association with metastasis was investigated. The fact that the patient with *KRAS* G12C mutation (case7) did not respond to standard drug therapies suggests that mutations in these biogenesis genes may play an important role by contributing to the mechanism of treatment resistance by decreasing the expression levels of tumor suppressor miRNAs and increasing the activation of mutant *KRAS*. Our findings highlight the potential of these genes as important genetic markers in the development of personalized treatment strategies and management of treatment-resistant colorectal cancer cases.

Limitations

The findings of this study provide an important basis for understanding the association of variants detected in miRNA biosynthesis regulatory genes with CRC patients and their possible prognostic implications. However, limitations of the study include the use of a small cohort of patients and therefore statistical analyses could not be performed. In addition, we performed somatic

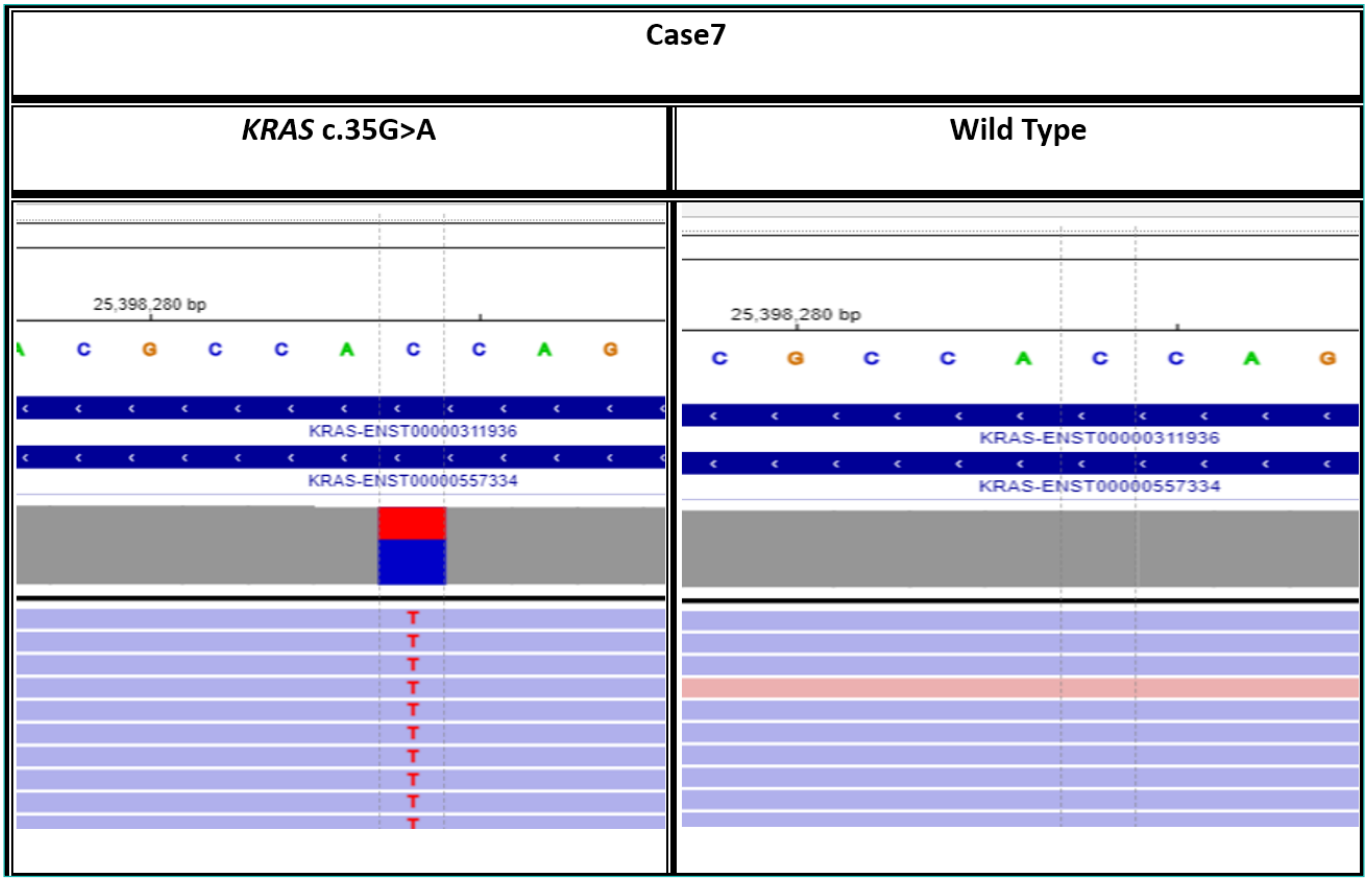

Figure 1. IGV image of *KRAS* G12C mutation

mutational analyses of key genes in miRNA biogenesis but not their expression levels. Therefore, these important findings need to be supported by larger patient groups and detailed molecular analysis.

CONCLUSION

In this study, mutations in *AGO2*, *DICER* and *DROSHA* genes associated with miRNA biogenesis and alterations in *TP53*, *KRAS*, *BRAF*, *PI3KCA* and *APC* genes, genes clinically associated with CRC, were analyzed in sporadic CRC patients. Although pathogenic mutations in miRNA biogenesis genes were not detected in the majority of patients, the presence of mutations in clinically important genes was a finding that may be associated with metastatic behavior of the disease. Our study suggests that mutations in miRNA biogenesis genes are more common in CRC patients with metastatic disease and that these mutations may contribute to metastatic behavior by reducing the expression of tumor suppressor miRNAs. In particular, it has been emphasized that *KRAS* G12C mutation may play an important role in patients showing resistance to standard treatment protocols. Considering the limitations of our study, it is important to support the findings obtained with larger patient groups and functional studies. However, it supports the hypothesis that mutations in miRNA biogenesis genes can be used as potential genetic markers for the development of personalized treatment strategies and management of treatment-resistant CRC patients.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Selçuk University Faculty of Medicine Ethics Committee. (Date: 13.11.2023, Decision No: E-70632468-050.01.04-639070).

Informed Consent

This study was designed retrospectively and consent forms were also obtained from the 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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Does subscapularis tears combined with supraspinatus tears affect postoperative functional outcomes?

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ABSTRACT

Aims: Roughly 50% of rotator cuff tears includes a tear in the subscapularis tendon. We conducted a comparative analysis of the functional outcomes following arthroscopic repair in patients with a tear in both the supraspinatus and subscapularis tendons, as well as those with a rupture solely in the supraspinatus tendon. Our hypothesis posits that the functional outcomes after surgery for rotator cuff tears, specifically those with tears including subscapularis tendon, are inferior compared to tears that only involve the supraspinatus tendon.

Methods: We classified patients who had arthroscopic repair for a rotator cuff tear in our clinic from January 2017 to April 2022 into two groups. The study comprised patients who received arthroscopic surgery for a diagnosed rotator cuff injury. The study excluded individuals who were younger than 18, individuals with large tears that could not be repaired, individuals with paralabral cysts, individuals with glenohumeral arthritis, individuals who had undergone labrum tear repair or slap lesion repair, individuals with concomitant neurovascular damage, and individuals who had a follow-up period of less than 6 months, patients who benefit from physiotherapy and medical treatment for 6 months. Following the application of exclusion criteria, a total of 39 patients were selected to participate in the study. The presence of a rotator cuff injury was verified through arthroscopy after being detected using clinical tests such as Jobe, drop arm test, Neer sign, lift-off and belly-press tests, and radiographic evidence such as magnetic resonance imaging. Patients who received subscapularis repair together with a supraspinatus tear were categorised as Group 1 (n=14), while those who just had supraspinatus surgery without any subscapularis tears were categorized as Group 2 (n=25). We recorded the demographic information. At the last postoperative outpatient clinic follow-up, both groups were compared in terms of mortality, laboratory parameters, radiological findings, visual analogue scale (VAS), Constant-Murley score[1], American Shoulder and Elbow Surgeons score (ASES)[2], and University of California Los Angeles (UCLA) scores.[3] Postoperative satisfaction levels were categorised into four groups: very satisfied, satisfied, undecided, and dissatisfied.

Results: The groups did not show any significant differences in terms of age, gender distribution, and follow-up periods (p>0.05). There was no statistically significant disparity observed between the groups in relation to VAS, Constant-Murley score, ASES, and UCLA ratings assessed during the final postoperative outpatient clinic follow-up (p>0.05). There were no instances of re-rupture observed in any patient throughout the postoperative follow-up period. The postoperative satisfaction ratings show no significant difference between the two groups (p>0.05).

Conclusion: Our findings indicate that the outcomes of patients who received a surgical repair of rotator cuff tendons including subscapularis were at least as successful as those who underwent surgery for just supraspinatus tendon rupture. There is no basis to believe that arthroscopic repair will have a detrimental impact on the functional outcomes of individuals with rotator cuff tears which includes subscapularis tendon tears.

Keywords: Supraspinatus, subscapularis, shoulder arthroscopy, postoperative function, repair

INTRODUCTION

Surgical intervention is becoming increasingly common in the treatment of rotator cuff injuries, which are quite common.[4,5] Most rotator cuff injuries primarily affect the supraspinatus tendon, and as the size of the injury increases, it usually progresses posteriorly and involves the infraspinatus tendon.[6] The size of the rupture is the main determinant of postoperative functional outcomes. However, other factors such as age, fatty degeneration of muscle, diabetes and smoking also affect this situation.[7]

The subscapularis muscle is the strongest of the rotator cuff group and has been ignored for many years during the development of surgical treatment and has been called the "forgotten tendon".[7,8] The subscapularis muscle plays an important role in the anteroposterior dynamic stabilization of the shoulder with the infraspinatus and teres minor muscles,[8,9] and if it is damaged, joint biomechanics is significantly affected.[10] There are studies reporting that only 1% of rotator cuff tears consist of isolated subscapularis tears.[11,12] However, there have also been studies showing that more than half of rotator cuff tears are accompanied by subscapularis tears.[13,14] It is predicted that the expected functional results will be worse even after arthroscopic repair of this type of combined tears, and studies comparing post-surgical results seem limited in this regard.[7]

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In our study, we tried to examine the hypothesis that the functional results observed after arthroscopic repair of subscapularis tears accompanying supraspinatus tears would be more negative compared to cases with isolated supraspinatus tendon tears.

METHODS

The approval from the Clinical Researches Ethics Committee of Karabük University has been obtained (Date: 13.12.2022, Decision No: 2022/1204). The procedures adhered to the ethical guidelines and principles outlined in the Declaration of Helsinki.

We retrospectively reviewed the hospital database to review the records of patients who underwent arthroscopic rotator cuff repair in our clinic from January 2017 to April 2022. The study comprised patients who received arthroscopic surgery for a diagnosed rotator cuff injury. The study excluded individuals who were younger than 18, individuals with large tears that could not be repaired, individuals with paralabral cysts, individuals with glenohumeral arthritis, individuals who had undergone labrum tear repair or slap lesion repair, individuals with concomitant neurovascular damage, and individuals who had a follow-up period of less than 6 months, patients who benefit from physiotherapy and medical treatment for 6 months. Following the application of exclusion criteria, a total of 39 patients were selected to participate in the study. The presence of a rotator cuff injury was verified through arthroscopy after being detected using clinical tests such as Jobe, drop arm test, Neer sign, lift-off and belly-press tests, and radiographic evidence such as magnetic resonance imaging. Patients who received subscapularis repair together with a supraspinatus tear were categorised as Group 1 (n=14), while those who just had supraspinatus surgery without any subscapularis tears were categorized as Group 2 (n=25). We recorded the demographic information. At the last postoperative outpatient clinic follow-up, both groups were compared in terms of mortality, laboratory parameters, radiological findings, visual analogue scale (VAS), Constant-Murley score1, American Shoulder and Elbow Surgeons score (ASES)2, and University of California Los Angeles (UCLA) scores.[3] Postoperative satisfaction levels were categorised into four groups: very satisfied, satisfied, undecided, and dissatisfied.

Surgical Procedure

A single surgeon conducted all of the procedures. The surgeries were conducted using general anaesthesia while the patient was positioned on lateral decubitus position with 4 kilogrammes of longitudinal traction applied. The glenohumeral joint was examined by first opening the conventional posterior portal. The diagnosis of a rotator cuff injury was verified by inspecting the subscapularis and supraspinatus tendons by using a probe. Intraoperatively, all subscapularis tendons were evaluated and classified according to Lafosse et al.[15] in Group 1 The subscapularis tendon repaired to its insertion site by a suture anchor. The supraspinatus repairs in both groups were conducted using the trans-osseous equivalent technique, which was double-row suture bridge fixation according to Park et al.[16] Prior to the repair procedure, all patients received subacromial decompression, bursectomy, and acromioplasty. The supraspinatus attachment site was cleaned by removing debris using a shaver. Based on the tear's size, either one or two double-strand suture anchors were positioned just to the side of the articular cartilage. A knotless anchor was positioned 1 cm away from the supraspinatus attachment site in order to apply pressure to the rotator cuff. Every patient received antibiotic prophylaxis using a first-generation cephalosporin for a duration of 24 hours. Both groups received postoperative analgesia via intravenous and oral routes. Postoperative rehabilitation includes a 2-week period of strict immobilization with a sling with the shoulder in zero degrees of abduction, and postoperative periods 2-6. It involves gradual introduction of protected, passive range of motion over weeks, followed by restoration of active range of motion, followed by gradual strengthening starting at week 12 postoperatively.[17] Every patient was released on the first day following the surgery.

Statistical Analysis

The data was statistically analysed using the Statistical Package for the Social Sciences (SPSS) for Windows version 20.0 (Chicago, IL, USA). Categorical variables were represented using numbers and percentages, whereas numerical variables were provided as mean±standard deviation and minimum-maximum values. The eligibility of numerical data for normal distribution will be assessed using the "Kolmogorov-Smirnov" and "Shapiro-Wilk" tests. Parametric and non-parametric approaches are employed to analyse numerical variables that follow a normal distribution. In the analysis of numerical variables that do not exhibit a normal distribution, For data that follows a normal distribution, the "Independent Samples t test" was employed to determine the difference in means between two independent groups. On the other hand, the "one-way ANOVA" test was used to compare the means of more than two groups. To analyse numerical data that did not exhibit a normal distribution, the "Mann-Whitney U" test was used to determine the median difference between two independent groups. Additionally, the "Kruskal-Wallis H" test was employed to compare more than two groups. The examination of categorical variables among themselves was conducted utilising the "Chi-Square" test statistic when the "Chi-Square" criterion was satisfied and

the "Fisher's Exact Test" statistic when it was not satisfied. The correlation between two numerical datasets was assessed using either the "Pearson" or "Spearman" tests, depending on the fit to a normal distribution. Paired sample statistics, or Wilcoxon tests, were employed to make pairwise comparisons between groups that are dependent on each other. Tests were deemed statistically significant if the p-value, analysed at a confidence level of 95%, was below 0.05.

RESULTS

The patients' demographic and follow-up data are displayed in Table 1. There was no statistically significant disparity in age, gender distribution, or follow-up length between patients who underwent subscapularis repair and those who did not (p>0.05).

Table 1. Patient's demographic data comparison

	Total		Group 1 (n=14)		Group 2 (n=25)		p
	x̄±SD Mean (Min-Max)		x̄±SD Mean (Min-Max)		x̄±SD Mean (Min-Max)		
Age (year)	59.92±10.00 60 (37-87)		59.71±8.98 59.5 (43-79)		60.04±10.70 60 (37-87)		0.924[a]
Follow up (month)	11.67±3.22 10 (7-18)		10.64±3.27 9.5 (7-17)		12.24±3.11 11 (8-18)		0.062[b]
	n	%	n	%	n	%	
Gender							0.862[c]
Male	16	41	6	42.9	10	40	
Female	23	59	8	57.1	15	60	

a: Independent Samples t test, b: Mann Whitney U test, c: Chi-Square test

The average functional score findings of the groups are compared and displayed in Table 2. No statistically significant differences were seen between the groups in terms of VAS, Constant-Murley score, ASES, and UCLA scores evaluated at the last postoperative outpatient clinic follow-up (p>0.05). There were no instances of re-rupture observed in any patient throughout the postoperative follow-up period. The postoperative satisfaction ratings show no statistically significant difference between the two groups (p>0.05)(Table 3).

Table 2. Functional outcomes comparison

	Total	Group 1 (n=14)	Group 2 (n=25)	p
	x̄±SD Mean (Min-Max)	x̄±SD Mean (Min-Max)	x̄±SD Mean (Min-Max)	
VAS	1.79±2.23 1 (0-8)	1.93±1.85 1 (0-5)	1.72±2.45 1 (0-8)	0.443[b]
Constant Score	73.41±10.15 77 (39-86)	74.21±6.73 76 (58-81)	72.96±11.74 80 (39-86)	0.573[b]
UCLA Score	30.95±6.24 33 (8-35)	31.14±4.24 32.5 (20-35)	30.84±7.20 34 (8-35)	0.409[b]
ASES Score	26.74±6.65 29 (0-35)	27.57±2.82 28 (20-30)	26.28±8.07 29 (0-35)	0.633[b]

b: Mann Whitney U test

Table 3. Postoperative satisfaction levels compared

	Total		Group 1 (n=14)		Group 2 (n=25)		p
	n	%	n	%	n	%	
Level of satisfaction							0.598[c]
very satisfied	24	61.5	8	57.1	16	64	
satisfied	12	30.8	6	42.9	6	24	
undecided	1	2.6	0	0	1	4.0	
dissatisfied	2	5.1	0	0	2	8.0	

c: Fisher's Exact test

DISCUSSION

Our study has demonstrated that our hypothesis was not supported, as it revealed that the functional outcomes following surgery for rotator cuff tears with accompanying subscapularis tendon tears are inferior to those observed in cases of isolated supraspinatus tendon tears. The key discovery we made is that the existence of a subscapularis tendon tear did not result in a substantial alteration in the functional outcomes of rotator cuff repairs after surgery.

In a study conducted in South Korea in 2016, Park et al.[9] examined 92 patients to assess the condition of the subscapularis muscle in cases of extensive rotator cuff tears. The researchers categorised the patients into three groups based on the extent of subscapularis involvement: intact (n=42), less than half affected (n=22), and more than half or completely affected (n=28). They then compared the outcomes of arthroscopic repair among these groups. Previous biomechanical and clinical investigations have demonstrated the significance of maintaining a minimum of 50% integrity in the subscapularis muscle. As a result, these three groups were established with this criterion in mind.[9] According to reports, the functional outcomes after surgery for massive rotator cuff tears, where more than half of the subscapularis tendon is affected, are worse compared to the other two groups. Additionally, there is a higher likelihood of the repaired tendon tearing again, although this difference did not reach statistical significance.[9] The postoperative functional outcomes were comparable between the group of patients who underwent isolated supraspinatus surgery (n=25) and the group of patients who underwent combined subscapularis and supraspinatus repair (n=14) in our study. Simultaneously, there were no instances of rerupture observed in any of the cases included in our study.

Malavolta et al.[7] In their study including 326 patients in 2020, the patients were divided into two groups: the group with isolated posterosuperior rotator cuff repair (n=194) and the group with rotator cuff repair with the addition of subscapularis repair (n=132). At the end of

the 2-year follow-up period, ASES and UCLA scores were found to be similar in both groups, and no statistically significant difference was observed in postoperative functional results.[7] In another study conducted later, the functional results and rerupture rates of 107 patients with anterosuperior rotator cuff tears (Group A) and 119 patients with subscapularis repair (Group B) were compared at the end of a 3-year postoperative follow-up period.[18] Rupture rates were reported as 23.4% in the anterosuperior rotator cuff rupture group and 19.3% in the subscapularis repair group, but the difference did not reach statistical significance.[18] At the same time, the results were reported to be similar in both groups in terms of VAS, ASES and UCLA scores.[18] In our study, similar to these studies; There was no statistically significant difference between the groups in all functional scores ($p>0.05$). In the meta-analysis of Longo et al.[19] in 2021, they reported rerupture rates as 15% in the first 3 months after surgery, 16% between 6-12 months and after 24 months, and 21% between 3-6 months and 12-24 months. In this meta-analysis, advanced age, large size of the tear, and advanced fatty degeneration were reported as negative risk factors for rerupture.[19] In our study, we did not encounter any cases of rerupture in neither of the groups. We attribute the reason why we did not encounter rerupture in any patient to the short follow-up period, which is one of the notable shortcomings of our study, and to the fact that we did not classify according to tear size and preoperative fatty degeneration staging.

Ide et al.[20] conducted a prospective study in 2007 involving 20 patients, where they published the outcomes of arthroscopic repair for traumatic combined cuff tears, which also included subscapularis tendon tears (18 cases). They documented unsatisfactory outcomes in just 2 patients, with 1 moderate outcome and 1 unsatisfactory outcome. Within our research, a total of 14 patients were assigned to Group 1, out of which 8 expressed a high level of satisfaction and 6 indicated a moderate level of satisfaction. In contrast, within Group 2, consisting of 25 patients, 2 individuals reported a lack of satisfaction, while 1 patient remained uncertain about their level of satisfaction. Thus, whereas all patients who received subscapularis restoration expressed pleasure, the isolated supraspinatus repair group had a satisfaction percentage of 88%. While the observed differences in these values did not reach statistical significance, it is worth noting that patients with subscapularis tears exhibited more preoperative complaints and poorer functional conditions in comparison to patients with isolated supraspinatus tears. This disparity may have contributed to a greater level of postoperative satisfaction.

Limitations

The limitations of our investigation encompass the limited sample size, brief duration of follow-up, omission of tear size consideration, lack of analysis on fatty degeneration classification, and failure to measure preoperative functional scores.

CONCLUSION

Hence, it is evident that there is no basis to believe that arthroscopic treatment will have an adverse impact on the functional outcomes of patients with rotator cuff injuries accompanied by subscapularis tendon tears. Our findings indicate that the outcomes of individuals who underwent subscapularis tendon surgery were at least as favourable as those who had isolated supraspinatus tendon repair.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Karabük University Clinical Researches Ethics Committee (Date: 13.12.2022, Decision No: 2022/1204).

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

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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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Exploring normal urinary biomarker ratios in a pediatric population: insights into age and gender variations

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ABSTRACT

Aims: The assessment of urinary biomarker ratios, such as sodium/creatinine (Na/Crea), potasium/creatinine (K/Crea), calcium/creatinine (Ca/Crea), phosphorus/creatinine (P/Crea), uric acid/creatinine (Uric acid/Crea), magnesium/creatinine (Mg/Crea), and sodium/potassium (Na/K), holds significant importance in clinical and research contexts as they offer insights into physiological and pathological processes. This study aimed to establish the normal ranges of urinarymineral ratios across age and gender groups in a Turkish pediatric cohort.

Methods: This cross-sectional study was conducted involving 162 healthy children, with ages ranging from 2 to 15 years, at the Department of Pediatrics, Selçuk University. Demographic information, urinary biomarker ratios, and dietary features were recorded. Participants were divided into three age groups (2-5, 6-10, and 11-15 years), and statistical analyses were performed to determine relationships and variations.

Results: Gender distribution was uniform across age groups ($p>0.05$). Urinary creatinine levels stabilized after age 6. The mean urinary Na/Crea ratio was 0.33±0.22 mEq/mg, showing correlations with age and other ratios ($p<0.001$). Urinary K/Crea ratio was 0.13±0.15 mEq/mg, with significant differences between Group 1 and Group 3 ($p>0.05$). Urinary Na/K ratio was 3.5±2.4 mEq/mEq and correlated with uric acid and Ca/Crea ratio ($p<0.001$). Urinary calcium levels were consistent ($p<0.001$). Ca/Crea ratio correlated with other ratios ($p<0.001$). Urine P/Crea ratio differed significantly among groups ($p>0.05$). Uric acid levels differed between Group 2 and Group 3 ($p>0.05$), while uric acid//Crea ratio correlated with age and other ratios ($p<0.001$).

Conclusion: The findings provide insights into the normal ranges of urinary biomarker ratios in a Turkish pediatric cohort. The results align with previous studies and emphasize the impact of age, gender, and dietary factors on these ratios.

Keywords: Urinary mineral ratios, pediatric population, normal ranges, age and gender variations, mineral metabolism

INTRODUCTION

The assessment of urinary biomarker ratios, such as Na/Crea, K/Crea, Ca/Crea, P/Crea, uric acid//Crea, Mg//Crea, and Na/K, has crucial importance in clinical and research settings since they can provide valuable insights into various physiological and pathological processes. These ratios serve as important indicators of electrolyte balance, mineral metabolism, and renal function. Understanding the normal ranges of these ratios is essential for accurate diagnosis, monitoring, and management of numerous health conditions.

However, it is widely acknowledged that these urinary ratios are not fixed and can exhibit considerable variability. This variability can be attributed to a multitude of factors, including genetics, race, gender, age, dietary habits, and levels of physical activity.[1-4] Genetic predispositions and inherent physiological differences among individuals from diverse ethnic backgrounds and racial groups can contribute to variations in urinary biomarker ratios.[5-9] Furthermore, hormonal differences linked to age and gender play a pivotal role in influencing these ratios.[10,11] Additionally, lifestyle factors such as dietary preferences and levels of physical activity have been shown to exert significant impacts on urinary biomarker ratios, reflecting the intricate interplay between human physiology and environmental factors.[12-15]

Given the intricate interaction of factors influencing urinary biomarker ratios, it becomes imperative to establish comprehensive and accurate baseline ranges for these ratios across different demographic categories. These ranges would not only aid in distinguishing between normal and abnormal values but also provide a broader context for interpreting results in clinical and research settings. By shedding light on the extent of variations that can be attributed to genetic, racial, gender-related, age-

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related, dietary, and activity-related factors, healthcare professionals can make more informed decisions and tailor interventions that align with an individual's specific physiological makeup and circumstances.[16-18]

The aim of this study was to define the normal ranges of urinary Na/Crea, K/Crea, Ca/Crea, P/Crea, uric acid//Crea, Mg//Crea and Na/K ratios by age and sex in a Turkish pediatric cohort.

METHODS

Study Design

This cross-sectional study was conducted between June 2000 and December 2000 at the Department of Pediatrics, Faculty of Medicine, Selçuk University. The study included 162 healthy children with 5% to 95% percentiles in terms of weight and height, who applied to polyclinics due to various complaints and were found normal following a detailed clinical and laboratory assessment. Ethical standards were considered according to Declaration of Helsinki for the ethical principles for medical research. The study was produced from a thesis in 2001 and ethics committee approval was not requested for this study.

Measurements

Age, gender, Na/Crea ratio, K/Crea ratio, Ca/Crea ratio, P/Crea ratio, uric acid//Crea ratio Mg//Crea ratio, Na/K ratio were noted. The ratios were calculated by using random spot urine samples.

All children were examined in detail and their histories were taken. Patients who were found to have a systemic disease on examination and who had a history of any drug use, renal and metabolic disease in the family were excluded from the study.

Dietary features: Free-fed children who did not receive breast milk were included in the study. Children who had no vitamin d deficiency were considered for the assessment.

Participants were divided into three groups according to age groups; Group 1: 2-5 years, Group 2: 6-10, and Group 3: 11-15 years. Three groups were compared for urinary mineral excretion.

All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) for Windows 13.0 software was used for data analysis. Descriptive statistical methods (mean, standard deviation, frequency) were employed to evaluate the study data. The Shapiro-Wilk test was used to assess the normality of data distribution. The chi-squared test was used to assess the distribution of gender across the age groups. Differences in mean values among different groups were evaluated using analysis of variance followed by post hoc comparisons (ANOVA with posthoc Tukey and Kruskal-Wallis with Bonferroni). Correlations between urinary biomarker ratios were assessed using Pearson correlation coefficients. $p<0.05$ was accepted as significant.

RESULTS

Demographic Characteristics

The study comprised a total of 162 participants, including 86 girls (53.1%) and 76 boys (46.9%). The gender distribution yielded a female-to-male ratio of 1.13. The participants' ages ranged from 2 to 15 years, with a mean age of 7.5±3.3 years. The cohort was categorized into three age groups: Group 1 (2-5 years), Group 2 (6-10 years), and Group 3 (11-15 years). The gender distribution was uniform across these age groups (Table 1).

Table 1. Distribution of children according to groups and gender

Gender	Group 1		Group 2		Group 3		Total	
	number	%	number	%	number	%	number	%
Male	24	49	39	45.9	13	46.4	76	46.9
Female	25	51	46	54.1	15	53.6	86	53.1
Total	49	30.2	85	52.5	28	17.3	162	100

Urinary Creatinine Levels

The analysis of urinary creatinine levels revealed no significant gender-based differences ($p>0.05$). The mean urinary creatinine levels were 34.5±18.8 mg/dl for Group 1, 51.2±33.1 mg/dl for Group 2, and 52.8±33.9 mg/dl for Group 3. Notably, a statistically significant disparity in creatinine levels was observed between Group 1 and Group 2 ($p>0.05$). However, after the age of 6 years, the values appeared to stabilize and remain relatively similar across the age groups (Table 2).

Table 2. Mean, 5th and 95th percentile values of urinary creatinine level (mg/dl)

	Mean	SS (±)	5th percentile	95th percentile
Group 1	34.5	18.8	5.1	74.5
Group 2	51.2	33.1	10.4	118.4
Group 3	52.8	33.9	6.6	128.5

Urinary Biomarker Ratios

The mean urinary Na/Crea ratio was found to be 0.33±0.22 mEq/mg. Additionally, a significant correlation was established between the urinary Na/Crea ratio and age, Ca/Crea ratio, and K/Crea ratio ($r=0.44$, $p<0.001$).

The mean urine K/Crea ratio was 0.13±0.15 mEq/mg, displaying no significant gender-based difference. However, a statistically significant difference emerged

between Group 1 and Group 3 (p>0.05). Correlation analysis indicated associations between the urine K/Crea ratio and uric acid (r=0.27, p<0.001) and Na/Crea ratio (r=0.44, p<0.001), while no significant correlation was observed with the Ca/Crea ratio.

The urinary Na/K ratio demonstrated no substantial difference between the groups or genders, with a mean value of 3.5±2.4 mEq/mEq. Moreover, a negative correlation was noted with uric acid (r=-0.24, p>0.01), while positive correlations were observed with calcium and Ca/Crea ratio (r=0.38, p<0.001).

Urinary calcium levels showcased consistent mean values across gender and age groups, with an average of 5.1±6.2 mg/dl. Notably, positive correlations were identified between calcium and sodium, as well as magnesium (p<0.001).

The mean Ca/Crea ratio across the entire study cohort was 0.13±0.15 mg/mg. A positive correlation was established between the Ca/Crea ratio and P/Crea, Mg//Crea, and Na/Crea ratios. Conversely, a negative correlation was observed with uric acid//Crea ratio. The Ca/Crea ratio exhibited an increase with age, while the 95th percentile value demonstrated a non-significant decrease from 0.68 mg/mg to 0.42 mg/mg (p>0.05) (**Table 3**).

Table 3. Mean, 5th and 95th percentile values of urinary calcium/creatinine ratio (mg/mg)

	Mean	SS (±)	5th percentile	95th percentile
Group 1	0.16	0.18	0.018	0.68
Group 2	0.13	0.14	0.016	0.54
Group 3	0.10	0.10	0.021	0.42

The mean urine P/Crea ratio was determined to be 0.8±0.6 mg/mg. Comparative analysis indicated a significant difference between Group 1 and the other two groups (p>0.05).

Uric acid levels in urine revealed an average value of 31±18.5 mg/dl. A significant disparity was observed between Group 2 and Group 3 in urine uric acid levels (p>0.05). Gender had no substantial effect on uric acid levels (p>0.05). The mean uric acid//Crea ratio for the three groups was 1.01±1.06 mg/mg. Age-based differences were significant between Group 1 and Group 3 (p>0.05), while gender differences were not observed (p>0.05). The uric acid//Crea ratio displayed positive correlations with age, Na/Crea, K/Crea, and P/Crea ratios (p<0.001).

DISCUSSION

The findings of this study provide valuable insights into the normal ranges of urinary biomarker ratios across different age and gender groups in a pediatric population. These results have implications for clinical interpretation, health management, and future research in the field of pediatric nephrology and metabolism. In comparing our findings with existing literature, several noteworthy observations can be made.

Our study revealed that urinary biomarker ratios, such as Na/Crea, K/Crea, and Ca/Crea ratios, exhibited variations across different age groups in this Turkish pediatric cohort. Similar observations were reported by Simsek at et al.[2] in their study involving a pediatric population. Furthermore, the observed stability in urinary creatinine levels after the age of 6 years aligns with the findings of Simeckova et al.[19] who demonstrated a stabilization trend beyond this age.

Interestingly, the associations between urinary mineral excretion ratios and age, as well as correlations among different biomarker ratios, were similar to the findings of prior studies.[1] These correlations can be attributed to the intricate interplay of physiological processes governing mineral and electrolyte homeostasis.

The significant gender-based differences in urinary mineral excretion ratios were not observed in our study, consistent with results presented by Marwaha et al.[20] This contrasts with the earlier work of den Busche et al.[21] who reported significant gender-related variations in urine sodium, potassium, and creatinine. However, differences in study population, methodology, and demographics may contribute to such disparities.

Our findings also point to correlations between urinary element ratios and uric acid and calcium levels.[22] These correlations, as indicated in previous research, underscore the interconnectedness of various metabolic processes in the human body.

Limitations

It's important to acknowledge certain limitations. The relatively modest sample size and single-center nature of the study may limit the generalizability of the findings. Additionally, factors such as dietary habits and physical activity, which were not extensively examined, could also influence the observed variations in biomarker ratios.

CONCLUSION

This study contributes to the understanding of normal urinary biomarker ratios in pediatric populations, accounting for age and gender variations. The comparisons with previous studies provide context and support to our findings. These results enhance our ability to interpret urinary biomarker ratios, aiding in clinical decision-making and offering a foundation for future research in pediatric nephrology and metabolism.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was produced from the thesis of Dr. Abdulgani GÜLYÜZ. His thesis advisor is Prof. Dr. Ahmet ÖZEL. The thesis was conducted in 2001 and ethics committee approval was not requested for this study.

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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Original Article

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The role of preoperative serum CA-125 levels in predicting lymph node metastasis in patients undergoing treatment for endometrial cancer

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ABSTRACT

Aims: Endometrial cancer stands as the most prevalent gynecological malignancy in developed nations, often detected at an early stage, and generally carries a positive prognosis. The stage of the disease is important for survival, but many factors such as tumor grade, histopathology, myometrial invasion, age, and spread are also effective. Our objective was to assess the significance of preoperative Cancer antigen 125 (CA-125) concentrations in the prediction of lymph node metastasis in patients with endometrial cancer and to identify a suitable threshold value.

Methods: This retrospective analysis was carried out on 286 female patients diagnosed with endometrial cancer at a specialized gynecologic oncology facility from 2012 to 2022. We examined clinical-pathological and demographic attributes, including preoperative serum CA-125 concentrations, surgical interventions conducted for each patient, post-treatment physical assessments, imaging findings, and cytological outcomes. CA-125 was measured using electrochemiluminescence immunoassay.

Results: Statistically significant differences were observed in CA-125 levels among patients in terms of grade, invasion depth, lymph node involvement, cervical involvement, and stage (respectively, $p<0.001$, $p=0.042$, $p<0.001$, $p<0.001$, $p<0.001$). The FIGO advanced stage ratio was 30.6 times higher for serum CA-125 concentrations above the cutoff of 21 IU/ml (95% CI: 10.7-87.6) ($p<0.001$). Lymph node involvement was 29.7 times more likely for serum CA-125 values above the cutoff of 35 IU/ml (95% CI: 25.3-74.8) ($p<0.001$).

Conclusion: Early identification of high-risk endometrial cancer patients is vital for prognosis and guiding adjuvant therapy. CA-125, a tumor marker, has been found useful in assessing myometrial invasion depth, lymph node involvement, stage differentiation, and tumor grade.

Keywords: CA-125, endometrial cancer, metastasis, lymph node, predictive marker

INTRODUCTION

Endometrial cancer (EC) stands as the most common gynecological malignancy in developed nations.[1] The majority of cases are identified in the initial stages and typically carry a positive outlook. The 5-year survival rate for women with Stage I EC is around 80-90%, while for Stage III, it drops to only 50-60%, and for Stage IV, it decreases to 15-17%.[2] While standard treatments for EC typically involve surgical procedures such as bilateral salpingo-oophorectomy along with total hysterectomy, either with sentinel lymph node sampling or systematic lymphadenectomy; adjuvant therapy, including chemotherapy and radiotherapy, depending on the recurrence risk factor, is also considered.[3] Although the stage of the disease is the most critical variable affecting survival, various factors such as tumor grade, histopathology, depth of myometrial invasion (MI), the woman's age, extrauterine disease spread, surgical/pathological findings, and recurrence risk have been identified as significant contributors to survival.[4] Hence, it is imperative to identify patients with an elevated risk of recurrence for treatment and planning follow-up procedures.[5]

Cancer antigen 125 (CA-125) is a glycoprotein produced by the MUC16 gene situated on the short arm of chromosome 19 at 19p13.3.[6] CA-125 is a protein found in the bloodstream and is extensively employed for the

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early identification of ovarian cancer.[7] Evaluating CA-125 exhibits limited specificity for ovarian cancer diagnosis, yet it holds the capability to assess, track, and appraise responses to ovarian cancer treatment.[8] However, the use of CA-125 as a predictor for lymphovascular stromal invasion (LVSI) and lymph node metastasis in endometrial cancer has not been extensively studied.[9] This tumor marker has proven to be beneficial in women with endometrial cancer characterized by unfavorable prognostic factors, including high recurrence rates, grade 3 tumors, lymph node metastasis, and deep myometrial invasion.[10] The preoperative evaluation of CA-125 can serve as an additional resource in preoperative risk stratification for recognizing patients with adverse outcomes. Although some studies have proposed a cutoff of 35 IU/L for preoperative CA-125 levels to assess prognostic factors and survival in EC, different studies have employed different cutoff values.[11]

In this study, our primary aim is to measure the predictive capacity of preoperative CA-125 concentrations in anticipating lymph node metastasis in patients with endometrial cancer. Our secondary goal is to ascertain a suitable threshold value for our study population and assess the connection between preoperative serum CA-125 and postoperative histopathological results.

METHODS

The study was initiated with the approval of the İstanbul Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Researches Ethics Committee (Date:23.10.2023, Decision No:232). Additionally, written authorization was secured from the establishments where the research was carried out, and informed agreement was acquired from the patients. The study was conducted by the Principles of the Declaration of Helsinki.

This retrospective study involved the examination of medical records of 316 women diagnosed with endometrial cancer at a tertiary gynecologic oncology center between the years 2012 and 2022. A total of 286 women completed the study after excluding individuals with secondary malignancies (n=10), those with a history of blood transfusion or complications related to hematologic diseases within 3 months (n=10), patients who received radiotherapy or chemotherapy before surgery (n=3), those with a history of hormonal therapy within 12 months (n=2), complications related to any infectious diseases (n=0), and individuals who had surgery at an external center (n=5).

In all instances of primary endometrial cancer that required surgery, procedures included total abdominal hysterectomy, salpingo-oophorectomy, and staging surgery.[12] The selective lymphadenectomy based on the surgical algorithm is determined according to the histology of the tumor, grade, tumor size, degree of myometrial invasion, and the presence of extrauterine disease. Lymphadenectomy is not performed in patients with endometrioid tumors smaller than <2 cm and myometrial invasion less than 50%. The decision to administer postoperative radiotherapy, chemotherapy, or both is based on defined criteria and the final results of the pathological examination of surgical specimens and cytology. Following the completion of definitive treatment, all patients were subjected to a follow-up schedule, with assessments conducted every 3 months for 2 years, every 6 months for 2-5 years, and subsequently on an annual basis for routine check-ups.

For every patient, an extensive examination of clinical-pathological and demographic attributes was conducted, encompassing preoperative serum CA-125 concentrations, the surgical interventions carried out, post-treatment physical evaluations, imaging findings, and cytological outcomes. In addition, based on histopathological assessments, various factors such as tumor size, histological type, grade, depth of myometrial invasion (MI), peritoneal cytology, lymphovascular stromal invasion (LVSI), cervical involvement, adnexa involvement, parametrial involvement, and lymph node (LN) participation were documented. CA-125 was measured using electrochemiluminescence immunoassay (ECLIA).

Statistical Analysis

Statistical analysis was performed using the SPSS 15.0 for Windows program. Descriptive statistics were furnished, encompassing counts and percentages for categorical variables, as well as the mean, standard deviation, minimum, and maximum values for numerical variables. Comparisons of proportions between groups were conducted using the Chi-Square Test. Since the assumption of normal distribution was not met for the independent two-group comparisons of numerical variables, the Mann-Whitney U test was employed. Cut-off values were examined using ROC Curve Analysis. The alpha significance level was set at $p<0.05$.

RESULTS

The average age of women with endometrial cancer was 63.7±9.4 years, and 86% of them were postmenopausal. Based on surgical pathology results, 78.3% were diagnosed with stage I, 39.2% with grade I, and 60.8% with grade II-III tumors. Histopathology reports indicated that 87.4% of the patients had endometrioid endometrial cancer. Among surgical specimens, 98.3% had an invasion depth of >50%. Adnexal involvement was present in 1% of patients, and cervical involvement was observed

in 7%. Lymph node involvement was present in 17.48% of women with endometrial cancer. The median (IQR) number of lymph nodes collected during surgery was 16 (12-20) for pelvic and 4 (3-5) for paraaortic nodes. The mean CA-125 level for patients was 28.8±33.0.

Statistically significant differences in CA-125 levels were observed among patients in terms of grade, depth of invasion, lymph node involvement, cervical involvement, and stage (respectively, p<0.001, p=0.042, p<0.001, p<0.001, p<0.001). In particular, patients with Grade II and III exhibited elevated CA-125 levels in comparison to those with Grade I. Patients with invasion depth exceeding 50% displayed higher CA-125 levels than those with 50% or less invasion. Furthermore, patients with lymph node metastasis had higher CA-125 levels than those without, patients with cervical involvement showed higher CA-125 levels than those without, and patients in Stages II-III-IV had higher CA-125 levels in contrast to those in Stage I. The findings are summarized in Table 1.

The FIGO advanced stage ratio was 26.6 times higher for a serum CA-125 value above the cutoff of 20 IU/ml and 9 times higher for a cutoff of 35 IU/ml. This is summarized in Table 2.

According to the ROC Curve analysis in our study, a cutoff value of >21 was determined for the advanced stage with a sensitivity of 93.55% and specificity of 67.9%. The FIGO advanced stage ratio was 30.6 times higher for a serum CA-125 value above the cutoff of 21 IU/ml. The AUC is shown in Figure 1. The findings are summarized in Table 3.

Table 1. Histopathological results and CA-125 values

Variable	N(%)	Median (IQR) CA-125 Level (IU/ml)	Mean±SD (Min-Max) CA-125 Level (IU/ml)	p value
Histological tumor type				
Endometrioid	250 (87.4)	19 (11-33.25)	28.9±34.3 (4-299)	0.102
Serous	28 (9.8)	30 (13-43.5)	32.6±22.5 (7-96)	
Clear cell*	2 (0.7)	4 (3-5)	4.0±1.4 (3-5)	
Mucinous*	2 (0.7)	18,5 (9-28)	18.5±13.4 (9-28)	
Carsinosarcoma*	4 (1.7)	9,5 (8-23.75)	13.8±9.6 (8-28)	
Stage (FIGO)				
I	224 (78.3)	17 (10-28)	20.5±15.3 (3-116)	<0.001
II	37 (12.9)	33 (24.5-50)b	48.9±48.4 (9-265)	
III	18 (6.3)	39 (35.75-89)b	72.6±72.7 (25-299)	
IV	7 (2.4)	66 (57-111)b	74.3±28.8 (35-112)	
Grade				
I	112 (39.2)	13 (10-28)	22.4±23.1 (3-141)	<0.001
II	138 (48.2)	24 (14.75-37)a	32.1±33.3 (4-265)	
III	36 (12.6)	22,5 (14.75-37.5)a	36.1±51.0 (5-299)	
Depth of invasion				
<%50	5 (1.7)	10 (5-20)	12.0±9.5 (5-28)	0.042
>%50	281 (98.3)	19 (11-35)	29.1±33.2 (3-299)	
Lymph node involvement				
Absent	236 (82.52)	17 (11-26)	18.6±10.1 (3-46)	<0.001
Present	50 (17.48)	58 (44.75-85.5)	76.8±54.8 (33-299)	
Adnexal Involvement				
Absent*	283 (99)	19 (11-33)	28.3±32.7 (3-299)	-
Present	3 (1.0)	66 (35-112)	71.0±38.7 (35-112)	
Cervical involvement				
Absent	266 (93.0)	19 (11-32)	26.7±28.4 (3-265)	<0.001
Present	20 (7.0)	38 (33-49.25)	57.0±64.9 (5-299)	
CA-125 Mean±SD (Min-Max)		28.8±33.0 (3-299)		

Mann-Whitney U, * Inadequate sample, a : Different from Grade 1, b : Different from Stage 1, SD: Standard deviation

Table 2. Serum CA-125 values for FIGO stages based on cutoffs of 20 IU/ml and 35 IU/ml

Stage (FIGO)	CA-125<20 IU/ml n(%)	CA-125>20 IU/ml n(%)	Total n(%)	p-value
Early stage (I)	145 (64.7)	79 (35.3)	224 (78.3)	<0.001 OR:26,6
Late stage (II-IV)	4 (6.5)	58 (93.5)	62 (21.7)	(%95 CI:9.3-76)
Stage (FIGO)	**CA-125<35 IU/ml n(%)**	**CA-125>35 IU/ml n(%)**	**Total n(%)**	**p-value**
Early stage (I)	194 (86.6)	26 (13.4)	220 (76.9)	<0.001 OR:9
Late stage (II-IV)	30 (41.9)	36 (58.1)	66 (23.1)	(%95 CI:4.8-16.9)

Chi-Square Test

Table 3. Serum CA-125 value for FIGO stages based on a cutoff of 21 IU/ml

Stage (FIGO)	CA-125<21 IU/ml n(%)	CA-125>21 IU/ml n(%)	Total n(%)	p-value
Early stage (I)	152 (67.9)	72 (32.1)	224 (78.3)	<0.001 OR:30.6 (%95 CI:10.7-87.6)
Late stage (II-IV)	4 (6.5)	58 (93.5)	62 (21.7)	

Chi-Square Test

Table 4. Serum CA-125 values for lymph node involvement based on cutoffs of 20 IU/ml and 35 IU/ml

Lymph node involvement	CA-125<20 IU/ml n(%)	CA-125>20 IU/ml n(%)	Total n(%)	p-value
Absent Present	149 (63.1) 0 (0.0)	87 (36.9) 50 (100)	236 (82.5) 50 (17.5)	<0.001 -
Lymph node involvement	**CA-125<35 IU/ml n(%)**	**CA-125>35 IU/ml n(%)**	**Total n(%)**	**p-value**
Absent Present	218 (92.4) 2 (4.0)	18 (7.6) 48 (96.0)	236 (82.5) 50 (17.5)	<0.001 OR:29.7 (%95 CI:25.3-74.8)

Chi-Square Test, OR: Odds ratio

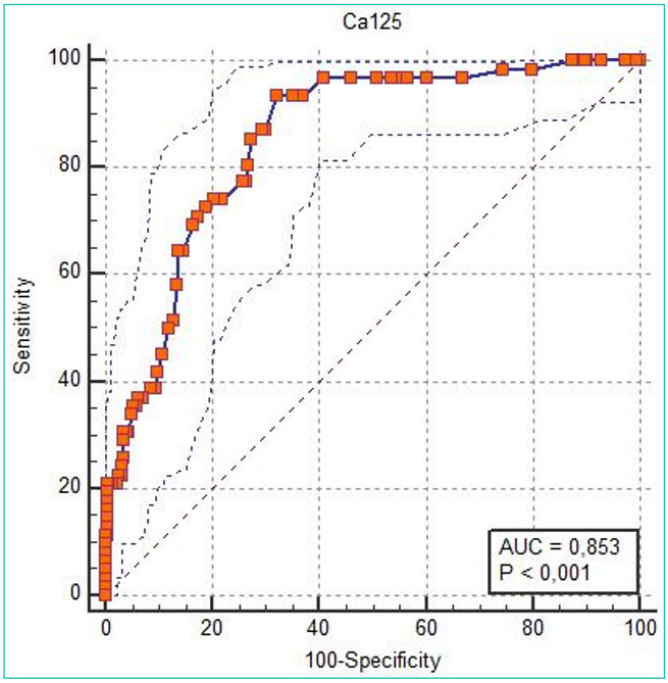

Figure 1. Receiver operating characteristic (ROC) examination of CA-125>21IU/ML

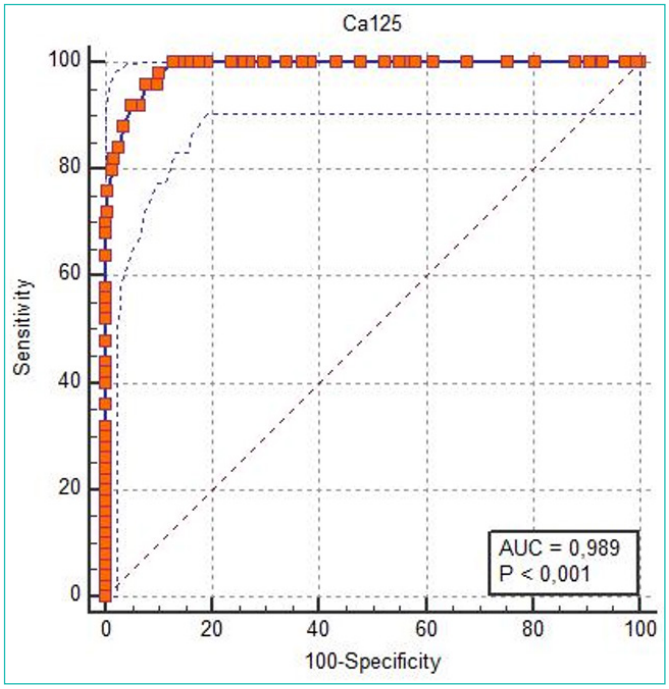

Figure 2. Receiver operating characteristic (ROC) examination of CA-125>35IU/ml

A cutoff value of >35 was determined for the advanced stage with 96% sensitivity and 92.4% specificity according to the ROC Curve analysis in our study. Serum CA-125 value was 29.7 times higher for lymph node involvement with a cutoff of 35 IU/ml. The findings are summarized in Table 4. The ROC curve for cutoff 35 is presented in Figure 2.

DISCUSSION

In the literature, the studies on the use of CA-125 as a tumor marker in endometrial cancer are limited, and it has been primarily employed in the diagnosis and postoperative follow-up of ovarian cancers.[13] Its elevation in benign pathologies, in addition to malignant events, makes it challenging to determine the optimal cutoff for this tumor marker. On the other hand, in benign ovarian cases such as endometriomas, its high elevation has led to a significant number of false-positive results.[14]

In our study, we found that the FIGO advanced stage ratio was 30.6 times higher in patients with a serum CA-125 value above the cutoff of 21 IU/ml. Additionally, concerning positive lymph nodes, we demonstrated that in endometrial cancer patients with a serum CA-125 value above 35 IU/ml, lymph node involvement was 29.7 times higher compared to those below the cutoff of 35. In the literature, many studies have associated poor prognostic factors and extrauterine spread with endometrial cancer.[15] On the other hand, in a study encompassing risk factors affecting overall survival and disease-free survival in early-stage endometrial cancer, it was found that CA-125 had no significant effect on overall survival and disease-free survival.[16]

The accurate staging of endometrial cancer relies on well-established and evidence-based surgical staging protocols. Clinical staging alone lacks precision and cannot evaluate critical factors such as LVSI, grade, or

lymph node metastasis. Thorough surgical staging, which includes lymph node assessment, is especially essential for delivering precise prognostic details in women with high-risk endometrial cancer. In a previous study, a preoperative CA-125 level above the cutoff of 21.2 was associated with lymphovascular stromal invasion in endometrial cancer patients.[17] In a recent study, similar to our results, CA-125 was considered a useful marker in predicting high-risk patients, including those with positive lymph nodes.[18]

For low-grade disease, the evaluation of myometrial invasion and tumor size is recommended to define high-risk cases. However, differences in sensitivity, specificity, and interobserver correlation coefficients between ultrasound (USG) and magnetic resonance imaging (MRI) in assessing preoperative myometrial invasion have suggested that CA-125 may be a suitable alternative for assessing myometrial invasion and tumor size.[19] In a similar study, it was found that in patients over 65 years old, with high tumor grade and high CA-125 levels, there was a correlation with decreased disease-free survival, and high tumor grade, non-endometrioid endometrial cancer, and high CA-125 levels were associated with increased disease-specific survival.[11,20]

A related study showed that in type 1 EC patients with negative prognostic factors, it may be more beneficial to choose a lower threshold value for CA 125 level (16 IU/L) instead of 35 IU/L.[21] In the literature, a respective number of studies have mostly included whole histological types of endometrial cancer and reported that elevated serum CA 125 levels might be useful in determining poor prognostic factors, such as extrauterine spread and LN metastasis in EC.[22]

In other study, preoperative serum CA-125 value of 16.75 U/ml in patients with EEC was 93% sensitivity and 57% specificity in predicting pelvic lymph node metastasis. Therefore, a preoperative serum CA-125 value of 16.75 U/ml may be useful in determining which patients would benefit from complete cytoreduction.[23] Bağcı et al.[24] treated 61 patients for endometrial cancer and were surgically diagnosed with stage I. They found a correlation between myometrial invasion and CA-125 values in postmenopausal patients. Atguden et al.[25] showed that CA-125 values could be used as a predictive test and alone as a prognostic factor in patients with early-stage EEC. Hsieh et al.[26] showed that 78% of endometrial cancer with lymph node metastases had high CA-125 levels. Thus, CA-125 levels can help to determine the extent of surgical staging, and if it is found to be high, it can be helpful as a marker in evaluating the response to subsequent chemotherapy.[27]

New findings in endometrial cancer have led to genomic analysis and immunohistochemistry, resulting in the current molecular classification of EC, which is divided into four groups: polymerase epsilon mutated (POLEmut), p53 abnormal (p53abn), mismatch repair deficient (MMRd), and no specific molecular profile (NSMP). Incorporating this molecular classification into the European Society of Gynaecological Oncology (ESGO) guidelines not only holds prognostic significance but also shows promise in guiding decisions regarding adjuvant therapy. Customizing treatments for endometrial cancer (EC) according to molecular and clinicopathological criteria is pivotal in striving for more refined and personalized outcomes.[28]

Limitations

The following limitations of the current study must be acknowledged. First, this was a retrospective study, and the intraoperative and postoperative management of patients with elevated serum CA-125 levels were not different from those with normal values. Second, we could not discriminate or identify false positive CA-125 elevations preoperatively. The patients might have had other medical comorbidities that contributed to elevated serum CA-125 levels independent of extrauterine disease. Third, elevations of tumor markers other than CA-125 were not evaluated in the study. In the coming years, advancements in technology will further aid our understanding of molecular oncology, contribute to the development of sensitive biomarkers, and provide valuable information, particularly in early-stage endometrial cancer prognosis, postoperative follow-up, and recurrence prediction.

CONCLUSION

CA-125 has been found to be useful in assessing deep myometrial invasion, lymph node involvement, differentiation between early and advanced stages, and grading for predicting high-risk patients. Preoperative assessment of CA-125 can be used as an additional tool in preoperative risk stratification to identify patients with poor outcomes.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of İstanbul Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Researches Ethics Committee (Date:23.10.2023, Decision No:232).

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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Can a unilateral approach replace the bilateral approach in percutaneous kyphoplasty?

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ABSTRACT

Aims: The main techniques used in balloon kyphoplasty include bilateral and unilateral approaches, but debate continues regarding their effectiveness. This study primarily aims to evaluate the clinical effects and safety profiles of unilateral and bilateral balloon kyphoplasty in osteoporotic and traumatic vertebral compression fractures and to compare patient satisfaction. The study's secondary aim is to identify the factors affecting prognosis, if any, in the general patient population. Our study compared the clinical and radiological results of patients with thoracolumbar vertebra fractures who underwent bilateral and unilateral balloon kyphoplasty operations in general and separately for patient subgroups.

Methods: Patients who underwent balloon kyphoplasty at the Neurosurgery Department of Atatürk Training and Research Hospital were investigated retrospectively and called for outpatient clinic control. Their current condition and satisfaction were evaluated. Clinically, patient satisfaction was assessed using a three-point Likert scale, Roland-Morris Disability Questionnaire, and VAS (Visual Analog Scale), and radiologically, preoperative, early postoperative, and late postoperative images in the sagittal plane, vertebral kyphosis angle, segmental kyphosis angle, Beck index, height loss, and axial canal compressions were examined and evaluated comparatively.

Results: A total of 77 patients who could be contacted and who were able to access the outpatient clinic were included in the study. Sixty-seven of the patients underwent unilateral surgery, and ten patients underwent bilateral surgery. In the final control, patients who underwent bilateral kyphoplasty had less height loss than patients who underwent unilateral kyphoplasty. There was no difference in other radiological outcomes. There was no significant difference in clinical outcomes between patients who underwent bilateral and unilateral kyphoplasty. Mean Visual Analogue Scale and Roland Morris scores at the final follow-up were significantly higher in female patients than in male patients.

Conclusion: There was no statistically significant difference in the satisfaction of patients who underwent bilateral kyphoplasty compared to unilateral kyphoplasty. This result may be due to the small number of patients in the study, and more extensive series are needed. The fact that the clinical results of female patients are worse than those of male patients may be a guide to giving realistic answers to the questions of patients' prognosis and pain expectations in the postoperative period.

Keywords: Kyphoplasty, unilateral approach, bilateral approach, vertebral compression fractures

INTRODUCTION

The balloon kyphoplasty (BKP) technique, developed for the treatment of progressive kyphotic deformity resulting from osteoporotic spinal fractures, consists of percutaneous inflation of a balloon placed in the fractured vertebral body to reduce the fracture and stabilization of the fracture by injection of a biomaterial into the body after balloon removal. Early results of this method, developed to restore sagittal balance by restoring vertebral body height in osteoporotic spinal fractures, have been reported to be quite favorable.[1-3]

Although the most common indication for BKP is painful acute and subacute osteoporotic compression fractures, traumatic compression fractures, aggressive spinal haemangiomas, multiple myeloma, and bone destructive metastases are also indications for kyphoplasty.[4-6]

Although these are widespread procedures, there is still controversy in the literature regarding the efficacy of bilateral and unilateral approaches to balloon kyphoplasty.

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METHODS

The study was initiated with approval of the Katip Çelebi University Atatürk Training and Research Hospital Ethics Committee (Date: 27.03.2019, Decision No: 131). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study retrospectively analyzed patients who underwent balloon kyphoplasty in the Department of Neurosurgery of Atatürk Training and Research Hospital. Patients who could be reached and who came to the outpatient clinic for a control examination were included in the study. The inclusion criteria were the absence of neurological deficits and late outpatient clinic control availability.

Preoperative independent variables were age, gender, fracture site, fracture cause, level, fracture type, the time between trauma and hospitalization, the time between hospitalization and operation, and the time between operation and discharge.

Using PACS imaging software, vertebral kyphosis angle, segmental kyphosis angle, Beck index, and height loss in the sagittal plane, as well as canal compression in the axial plane, were evaluated in preoperative, early postoperative, and late postoperative images (**Figure 1, Figure 2**).

Vertebral kyphosis (α, degrees): angle between the upper and lower endplates of the fractured vertebral body

Segmental kyphosis (β, degrees): angle between the upper endplate of the vertebral body above and the lower endplate of the vertebral body below the fractured body

Beck index (%): anterior vertebral body height (a) / posterior vertebral body height (b)

Note: Segmental kyphosis was not measured if there was an additional fracture in the adjacent vertebra, above or below.

Height loss (%): average of the mid-heights of the two adjacent vertebrae (c and d) - the height at the center of the fractured vertebra (e) / average of the mid-heights of the two adjacent vertebrae (c and d)

Spinal canal compression (%): diameter of the most severe spinal canal compression (largest anteroposterior diameter of the retro pulse fragment in the spinal canal) / mean value of the spinal canal diameters at the level of two adjacent vertebrae above and below

Preoperative radiological findings were considered independent variables, and radiological findings in the early postoperative period and at the last follow-up were considered dependent variables.

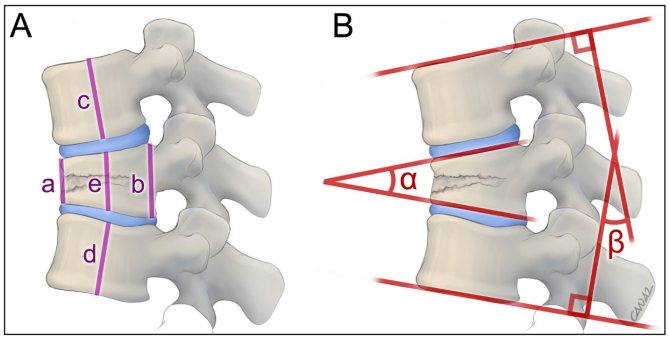

Figure 1. Schematic representation of radiological parameters (A-B). a: anterior vertebral body height. b: posterior vertebral body height. c: mid-height of the corpus of the upper adjacent vertebra. d: mid-height of the corpus of the lower adjacent vertebra. e: mid-height of the corpus of the fractured vertebra (A). α: vertebral kyphosis angle. β: Segmental kyphosis angle.

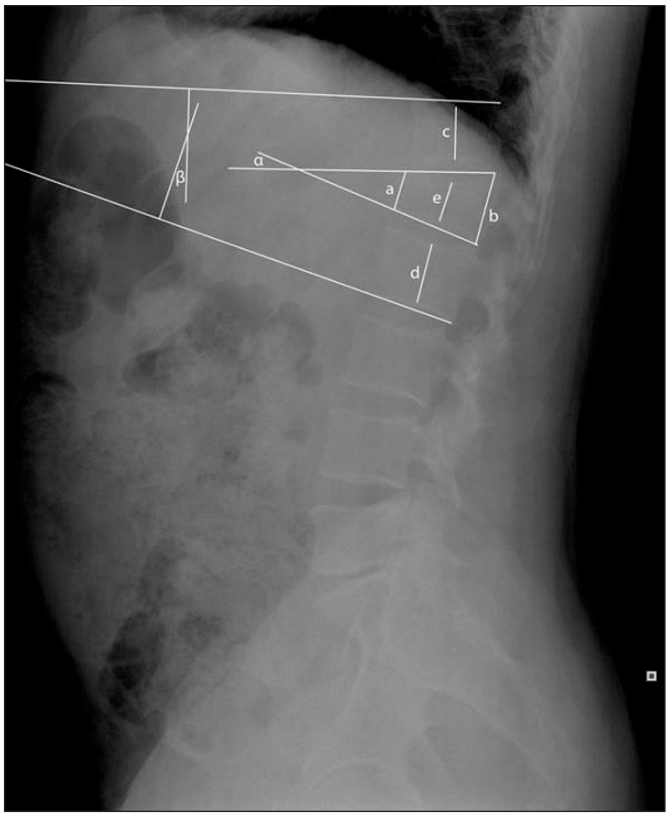

Figure 2. Representation of radiological parameters on the lateral radiograph. α: vertebral kyphosis angle (angle between the fractured vertebral body's upper and lower end plates). β: segmental kyphosis angle (angle between the upper end plate of the vertebral body above and the lower end plate of the vertebral body below the fractured body)

At the last follow-up visit, clinical evaluation was performed on three dependent variables: the Triple Likert Scale questionnaire for patient satisfaction, the Roland-Morris disability questionnaire, and the Visual Analogue Scale (VAS). While evaluating patient satisfaction, the patient was asked whether each of the three items was true for him/her. These items are: a) I am satisfied with the operation; I would have the operation again if I had the same situation. I would recommend that my relatives have surgery in the same situation. b) I was partially satisfied with the surgery; I am unsure if I would have surgery again in the same situation. I am not sure if I

would recommend that my relatives have surgery in the same situation. c) I am not satisfied with the surgery; I would not have surgery in the same situation again. I would not recommend that my relatives have surgery in the same situation.

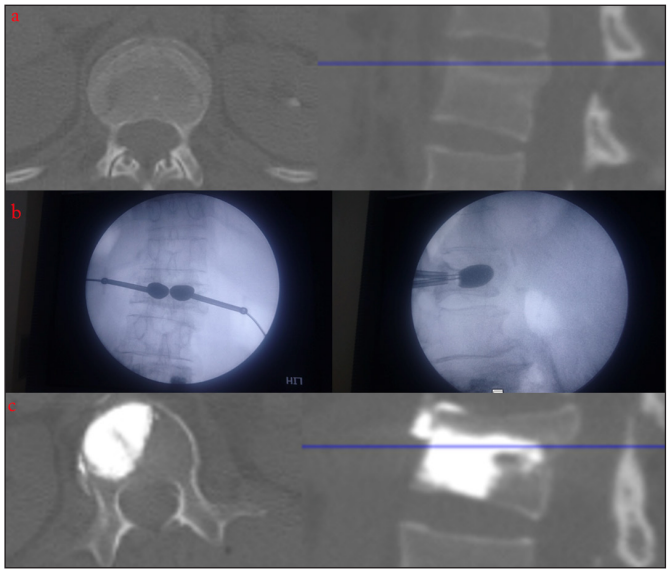

Figure 3. Preoperative, peroperative, and postoperative imaging of a patient undergoing kyphoplasty. a: preoperative tomography image, including axial and sagittal sections. b: peroperative anterior-posterior and lateral fluoroscopy images c: postoperative tomography image, including axial and sagittal sections

After the questioning of the patients, groups b and c were combined, and statistical tests were performed as the group "those who were not fully satisfied with the surgery."

The IBM SPSS Statistics 22 program was used. The chi-square test was used to compare a categorical independent variable (e.g., gender) with a categorical dependent variable (e.g., patient satisfaction). Pearson's bivariate correlation test was used to compare a numeric independent variable (e.g., age) with a numeric dependent variable (e.g., VAS). When one of the variables was categorical and the other was numerical, t-tests (independent sample or paired sample) were used.

RESULTS

Of the 77 patients who underwent kyphoplasty and were included in the study, 28 were male and 49 were female, and the mean age was 62.82 years (minimum: 16, maximum: 87, standard deviation: 15.04). A total of 100 percutaneous kyphoplasty procedures were performed in 85 sessions. All kyphoplasty procedures were performed under local anesthesia under fluoroscopic control.

One patient underwent kyphoplasty of 6 vertebrae (2+2+1+1) in 4 sessions. All kyphoplasty procedures were performed bilaterally in this patient. One patient had one kyphoplasty performed bilaterally in two sessions (2 kyphoplasty total). In addition, bilateral kyphoplasty was performed on one vertebra of 8 patients. Thus, 16 vertebrae of 10 patients underwent bilateral kyphoplasty in 14 sessions.

Additionally, unilateral kyphoplasty was performed on three vertebrae of two patients and two vertebrae of nine patients in the same session. Four patients underwent kyphoplasty in two separate sessions (2 kyphoplasty total). The remaining 52 patients underwent kyphoplasty of 1 vertebra in 1 session. Thus, unilateral kyphoplasty was performed on 84 vertebrae of 67 patients in 71 sessions. No patient underwent unilateral kyphoplasty on one vertebra and bilateral kyphoplasty on the other.

A total of 8 of these 77 patients who underwent clinical control could not undergo late follow-up CT because they refused (7 patients underwent unilateral kyphoplasty on one vertebra, and one patient underwent unilateral kyphoplasty on two vertebrae). Late radiological control was performed on 69 of the 77 patients who underwent clinical control. While the clinical results were evaluated based on the number of patients (77 patients), the radiological results were evaluated based on the number of vertebrae (91 vertebrae with late radiological control). The last radiological controls were performed after an average of 19.21 months (minimum: 2, maximum: 46, standard deviation: 10.67). Clinical controls were performed at an average of 18.26 months (minimum: 3, maximum: 46, standard deviation: 10.69).

When we analyzed the vertebrae treated, it was seen that kyphoplasty was performed on 1 Th5 vertebra, 3 Th7 vertebra, 1 Th8 vertebra, 3 Th9 vertebra, 4 Th10 vertebra, 7 T11 vertebra, 19 Th12 vertebra, 33 L1 vertebra, 12 L2 vertebra, 10 L3 vertebra, 4 L4 vertebra, and 3 L5 vertebra.

For both vertebral and segmental kyphosis angles, slight improvements without statistical significance were seen in the early postoperative period. Nevertheless, the kyphosis continued to rise following kyphoplasty, and more severe kyphosis angles were observed throughout the final follow-up in comparison to both the early postoperative and preoperative periods, with statistical significance. There was no difference between unilateral and bilateral kyphoplasty in vertebral and segmental kyphosis at any period. While there was a significant improvement in the early postop period, the mean Beck index became even lower than the preop period because of deterioration over time. There was no statistically significant difference at the last follow-up compared to the preop period. There was no difference in the Beck index between unilateral and bilateral kyphoplasty in any period. While there was a significant improvement in the mean height loss in the early postop period, it became even lower than in the preop period because of worsening over time. The values were statistically

significantly worse at the last follow-up than at the preop period. When the final control height losses were analyzed, it was noted that the height loss in unilateral kyphoplasty (0.426±0.149) was statistically significantly (P=0.042, independent sample T-test) higher than the height loss in bilateral kyphoplasty (0.309±1.91). There was no statistically significant difference in canal compression at the last follow-up compared to the preoperative period. There was no difference between unilateral and bilateral kyphoplasty in canal compression in any period (Table 1).

Prolonged hospitalization time was associated with worse preop height loss and worse early vertebral Cobb and Beck index values, while a long hospitalization-operation interval was associated with worse preop canal compression (Pearson Correlation; P, 0.05, 0.015, 0.005, 0.017, 0.008, respectively). However, there was no correlation between these intervals and the final control radiological values.

There was no difference between male and female patients regarding patient satisfaction, but female patients were worse than male patients at the last follow-up, according to both the Roland Morris scale and VAS. In addition, patients with spinal canal compression did worse than patients without compression, according to the Roland Morris scale at the last follow-up.

Although the mean age of the patients who were satisfied with the outcome (63.3±14.9) was higher than the mean age of the patients who were not satisfied (58.2±16.4), this was not statistically significant (Table 2). There was no significant correlation between age and Roland Morris and VAS values (Pearson Correlation Test, p: 0.051 and 0.122, respectively). There was no statistically significant difference between the clinical outcomes of the 67 patients who operated with the unilateral approach and the 10 patients who operated with the bilateral approach. Our study categorized fracture etiology under three headings: trauma, osteoporosis, and others, and osteoporosis and trauma groups were compared. There was no difference between the clinical results of patients who underwent single-level kyphoplasty and patients who underwent multiple-level kyphoplasty. Postoperative discharge of patients whose surgeries were delayed after admission was also delayed (Pearson Correlation, p: 0.038). However, there was no correlation between these delays and clinical outcomes. In our study, kyphoplasty was also performed in patients with A3-4 fractures, and although there was no difference in terms of patient satisfaction and VAS in the final postoperative controls of 45 patients without canal compression and 32 patients with canal compression (without neurological deficit), patients without canal compression were statistically significantly better than patients with canal compression in terms of Roland Morris score (Table 2).

Table 1. Analytical radiological results

	Preoperative (mean±SD)	Early postoperative (mean±SD)	p (preoperative/ early postoperative)	Last control (mean±SD)	p (Early postoperative/ last control; preoperative/ last control)
Vertebral Cobb	6.99±6.65	6.76±6.43	0.557	8.69±6.38	0.000; 0.003
Segmental Cobb	6.65±13.84	6.20±14.40	0.531	9.27±15.27	0.002; 0.006
Beck index	0.81±0.16	0.84±0.15	0.005	0.79±0.18	0.001; 0.260
Height loss	0.35±0.19	0.31±0.15	0.045	0.40±0.15	0.000; 0.036
Spinal canal compression	0.07±0.20	0.09±0.16	0.029	0.09±0.16	0.418; 0.077

Table 2. Clinical outcomes

	Satisfied	Unsatisfied	Roland Morris (Mean±SS)	VAS (Meana±SS)
Male (28)	25 (89.3%)	3 (10.7%)	6.85±6.88	24.11±22.56
Female (49)	42 (85.7%)	7 (14.3%)	11.68±6.75	41.28±30.42
P (Chi-square / IST/ IST)	0.654		0.004	0.013
Unilateral Approach (67)	58 (86.6%)	9 (13.4%)	10.03±7.13	36.59±28.60
Bilateral Approach (10)	9 (90%)	1 (10%)	9.20±7.58	24.90±30.08
P (Chi-square / IST/ IST)	0.763		0.735	0.236
Spinal Canal Compression (-) (45)	39 (86.7%)	6 (13.3%)	8.48±6.58	32.02±29.29
Spinal Canal Compression (+) (32)	28 (87.5%)	4 (12.5%)	11.81±7.52	38,94±28,29
P (Chi-square / IST/ IST)	0.915		0.046	0.311
Osteoporosis (23)	21 (91.3%)	2 (8.7%)	10.09±7.81	31,95±26,50
Trauma (41)	37 (90.2%)	4 (9.8%)	9.30±7.05	35,80±30,62
P (Chi-square / IST/ IST)	0.889		0.686	0.622
Single Level (60)	52 (86.7%)	8 (13.3%)	9.68±7.01	34,86±29,12
Multi-Level (17)	15 (88.2%)	2 (11.8%)	10.71±7.78	35,53±28,89
P (Chi-square / IST/ IST)	0.865		0.608	0.934

IST: Independent sample T-test, SD: Standard Deviation

DISCUSSION

Vertebral fractures are an essential health problem that may cause severe deterioration in quality of life and lead to morbidity and even mortality.[7-10] In recent years, percutaneous kyphoplasty has become the standard surgical procedure for vertebral fractures, but there is still controversy about the optimal surgical approach. It is unclear if the bilateral kyphoplasty is more effective than the unilateral kyphoplasty. For example, although the bilateral approach seems to provide better spreading of the injected cement within the bone, it is unclear to what extent this is reflected in the clinic.

In their study, Chen et al.[11] concluded that the bilateral approach was more effective in restoring the height of the vertebral body. On the contrary, there are also publications reporting that the results are not different.[12-18]

A study by Yilmaz et al.[19] looked at the differences in anterior wall vertebral height, midline height of the vertebral body, and posterior wall heights between patients who had surgery with a unilateral or bilateral approach. In the study, an increase in wall heights was observed in both approaches, and no statistically significant difference was found between the two groups. Our study revealed a statistically significant rise in the Beck index. This result may be related to increasing the height of the anterior wall and protecting it by placing the cement more anteriorly. In addition, when we compared the preoperative and early height loss, there was no statistically significant difference between the two groups.

In a study by Wang et al.[20] 203 patients with an approximate follow-up period of 12.7 months were analyzed, and it was observed that 38.9% of patients had recollapse. In the recollapse group, the mean midline vertebral height ratio and kyphotic angles changed statistically significantly during follow-up. It has been shown that pain scores decreased immediately after percutaneous kyphoplasty and generally remained low during follow-up. Our study found that late vertebral kyphosis and segmental kyphosis values worsened statistically significantly compared with preoperative values. This finding may be due to vertebral recollapse.

When the results of the patients with canal compression were analyzed, we found that early postoperative canal compression was statistically significantly increased compared to preoperative compression. Still, when we compared the early and late compression values, we found that the compression decreased statistically significantly over time. Finally, we found a statistically significant decrease when we compared the preoperative and late-postoperative compression values. We found no study comparing the early and late period data of patients with canal compression.

When the relationship between gender and the Roland Morris Disability Index was analyzed, it was found that the Roland Morris value was statistically significantly higher in women. We can say that this situation is related to the fact that osteoporosis is more common in women and comorbidities are more common. There was a correlation between age and Roland Morris. When the patients were divided into two groups, patients aged 65 years and younger and patients aged 66 years and older, the Roland Morris value was found to be 8.5 in the group of patients aged 65 years and younger and 11.8 in the group of patients aged 66 years and older. This result was statistically significant. Roland Morris's value was found to increase with increasing age. However, this result was thought to be related to the increase in additional morbidities with age.

CONCLUSION

There was no statistically significant difference in the satisfaction of patients who underwent bilateral kyphoplasty compared to unilateral kyphoplasty. However, when the pain parameters were analyzed, it was observed that the negative pain scores of female patients were higher than those of male patients in the long term. This result may be a guide for prognosis.

Although the progression to kyphosis in the early postoperative period seems to have been controlled in the early postoperative period, we observe that kyphoplasty is insufficient to prevent kyphosis progression in the final controls of the patients. Although there was no statistically significant difference in the Beck index at the last follow-up in our study, progressive worsening was noticeable. When choosing the surgical approach in either case, it is important to keep in mind that segmental and global kyphotic angulations may happen, angulations that are already there may get worse over time, and patients may have issues with their sagittal balance.

The fact that there is no significant worsening of canal compression in the long term suggests that kyphoplasty can be used as a safe method in patients without neurological findings.

The retrospective nature of our study and the small number of patients who underwent bilateral kyphoplasty are limitations in terms of evaluation. Larger series and multicenter studies with a higher level of evidence are needed to show whether there is a difference between the two methods and, if there is a difference, which method is superior in which patient group.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was initiated with approval of the Katip Çelebi University Atatürk Training and Research Hospital Ethics Committee (Date: 27.03.2019, Decision No:131).

Informed Consent

Written consent was obtained from the patients participating in this 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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Distribution of ABO and Rh blood groups in gynecological cancer cases

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ABSTRACT

Aims: To investigate the relationship between blood type and gynecological cancers (ovarian, endometrial, and cervical).

Methods: In the study, between 2017 and 2022, 457 patient files who underwent surgery for gynecological cancer at İstanbul Prof. Dr. Cemil Taşçıoğlu City Hospital were reviewed. Seventy-eight of these files were excluded from the study due to missing data. Out of the remaining files, 379 were considered suitable for the study. Twenty-five of these were further excluded due to being cases of other gynecological cancers. A total of 354 patients were included in the study (n=354). Participants' sociodemographic data such as age and gender, cancer type, and blood groups (ABO-Rh) were retrospectively collected from patient records and the hospital automation system. Cases with missing data or inaccessible sociodemographic records were excluded from the study. The study was planned retrospectively and observationally. Gynecological cancers were examined in the three most common groups: ovarian cancer, endometrial cancer, and cervical cancer. The patients' blood group, Rh status, and pathology reports were analyzed. Based on the pathological diagnosis, three groups were initially formed, and below them, Rh and blood group status were noted. The blood group and Rh status in cancer groups were first presented in numbers and then calculated as percentages.

Results: The AB+ ratio in cervical cancers was statistically significantly higher compared to the reference article and endometrial cancers (p=0.021, p=0.049).

Conclusion: There are studies indicating a significant relationship between blood groups and various diseases. The expression of blood group antigens on blood cells and other epithelial surfaces acting as receptors or signal transducers contributes to these findings. The possibility of ABO antigens serving as receptors in tumor structures caused by infections, such as cervical cancer, should not be overlooked. In this regard, the significantly higher prevalence of cervical cancer in individuals with AB Rh (+) blood type, carrying A, B, and Rh antigens, can be explained concerning the population.

Keywords: Gynecological cancers, blood group, cervical cancers

INTRODUCTION

Gynecological cancers are a collective term for cancers occurring in the cervix, ovaries, endometrium, uterus, fallopian tubes, vulva, or vagina. Endometrial cancer, ovarian cancer, and cervical cancer are the three most common gynecological cancers in women. A significant association has been found between the A Rh+ blood group and malignant melanoma, kidney, colorectal, breast, and ovarian cancers; whereas, pancreatic cancer is significantly associated with the O Rh+ blood group. It is speculated that a similar relationship may exist in gynecological cancers.

Cervix ca: The number of lymph nodes involved in patients undergoing surgical staging or lymphadenectomy also affects prognosis. In one report, five-year survival rates for patients with one, two, three to four, and five or more positive lymph nodes were 62, 36, 20, and 0 percent, respectively.[1] After radical hysterectomy and lymphadenectomy, five-year survival for patients with stage IB1 and IB2 disease is 91.6 percent and 83.3 percent, respectively, compared with 60.8 percent for those with pelvic nodal involvement.[2] Outcomes are worse in patients with para-aortic nodes (five-year survival, 37.5 percent).

Endometrium ca: The prognosis of endometrial carcinoma is determined primarily by the stage, grade and histology of the disease.[3] The prognosis for most patients with endometrial carcinoma is favorable, as the majority of patients have endometrioid histology and present with early-stage disease. Survival rates according to stages Localized 94.9% regional 69.8% distant 18.4% unknown 57.6%

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Ovarian ca: Approximately 80 percent of patients with early-stage disease do not experience recurrence within five years. However, most patients with advanced ovarian cancer experience recurrence. Mortality is high among those with recurrent disease. Five-year survival rates for epithelial ovarian and fallopian tube carcinomas by stage are shown in the table. Five-year survival is 89% in stage 1, 71% in stage 2, 41% in stage 3, and 20% in stage 4.[4]

Cancer is among the leading causes of death in developed countries. The role of ABO and Rhesus (Rh) blood groups in cancer biology has been studied by various researchers. ABO blood group antigens are expressed on the erythrocyte membrane and many other cell surfaces. When examining the pathophysiology between ABO blood groups and cancer, it is understood that irregularities in the enzymatic activities of Glycosyltransferase A and Glycosyltransferase B, responsible for the formation of membrane-derived signals in the immune response, lead to angiogenesis and tumor formation by modulating plasma levels of the Von Willebrand factor. The relationship between ABO antigens and intercellular adhesion molecules has been reported to be effective in tumor initiation and progression. Since the discovery of blood groups by Karl Landsteiner in 1901 and Aird's declaration in 1953 that the A blood group is associated with gastric cancer, blood groups have been the subject of research in many cancer types. ABO blood group genes are mapped to the 9q34.2 region, where genetic changes are common in many cancers. Therefore, blood group antigen expression can be influenced by genetic changes in the tumor. The glycoconjugate structures in RBCs have various functions, including receptors, carriers, channels, structural proteins, adhesion molecules, and enzymes for exogenous ligands, viruses, bacteria, and parasites.[5] However, the exact mechanisms explaining the relationships between blood group antigens and diseases in adhesion molecules are still unknown. Since infectious agents often use cell surface glycoconjugates as receptors for binding, glycosylation polymorphisms in ABO blood type can affect host-pathogen interactions and lead to sensitivity differences among individuals with different glycosylation profiles.[6] Previous studies suggest a possible relationship between the ABO blood group and some epithelial cancers, including pancreatic and gastric cancers. Various mechanisms, including inflammatory changes, intercellular adhesion, and membrane signal alterations, have been proposed to explain the observed relationship between ABO blood groups and cancer risk. However, it has been reported that the relationship between ABO blood group types and cancer risk is not definitive.

In this study, we aimed to evaluate the distribution of ABO-Rh blood groups according to gynecological cancer disease and its subtypes, which are accessible in the clinic.

In a study conducted in İstanbul, based on the results of blood type analysis of 123,900 individuals, the distribution is as follows: 47,496 (38.3%) individuals are A Rh (+), 6,793 (5.5%) individuals are A Rh (-), 36,427 (29.4%) individuals are O Rh (+), 5,451 (4.4%) individuals are O Rh (-), 16,294 (13.2%) individuals are B Rh (+), 2,560 (2.1%) individuals are B Rh (-), 7,971 (6.4%) individuals are AB Rh (+), and 908 (0.7%) individuals are AB Rh (-). Looking at the Rh blood group, 108,188 (87.31%) individuals are Rh (+), and 15,712 (12.69%) individuals are Rh (-). Conclusion: The demographics of İstanbul reflect a summary of Turkiye. The distribution of blood types in our region is similar to the overall rates in Turkiye and the İstanbul region.[7]

METHODS

The study was initiated with the approval of the İstanbul Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Researches Ethics Committee (Date:26.12.2022, Decision No:366). Additionally, written authorization was secured from the establishments where the research was carried out, and informed agreement was acquired from the patients. The study was conducted by the Principles of the Declaration of Helsinki.

In the study, between 2017 and 2022, 457 patient files who underwent surgery for gynecological cancer at İstanbul Prof. Dr. Cemil Taşçıoğlu City Hospital were reviewed. Seventy-eight of these files were excluded from the study due to missing data. Out of the remaining files, 379 were considered suitable for the study. Twenty-five of these were further excluded due to being cases of other gynecological cancers. A total of 354 patients were included in the study (n=354). Participants' sociodemographic data such as age and gender, cancer type, and blood groups (ABO-Rh) were retrospectively collected from patient records and the hospital automation system. Cases with missing data or inaccessible sociodemographic records were excluded from the study. The study was planned retrospectively and observationally. Gynecological cancers were examined in the three most common groups: ovarian cancer, endometrial cancer, and cervical cancer. The patients' blood group, Rh status, and pathology reports were analyzed. Based on the pathological diagnosis, three groups were initially formed, and below them, Rh and blood group status were noted. The blood group and Rh status in cancer groups were first presented in numbers and then calculated as percentages.

Statistical Analysis

The results were compared with the distribution of blood groups and Rh in the general population. SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics were provided for categorical variables in terms of number and percentage. Ratios in independent groups were compared using Chi-Square Analysis. A statistical alpha significance level of $p<0.05$ was determined.

RESULTS

Demographic findings are summarized in Table 1.

Table 1. Age Distribution by endometrium, ovarian, cervical cancer and blood types

Blood Type	Age					p
	N	Avg.	SD	Min	Max	
Total						0.490*
A	156	59.0	11.4	32	93	
AB	30	60.1	10.0	34	76	
B	60	60.3	11.2	32	82	
O	108	57.7	11.4	31	84	
Endometrium						0.579*
A	72	58.3	11.6	32	93	
AB	10	59.8	6.8	52	72	
B	33	61.3	10.4	43	82	
O	47	60.5	11.9	32	84	
Ovarian						0.122*
A	65	59.5	12.2	33	84	
AB	12	63.8	9.4	40	76	
B	22	59.3	13.1	32	77	
O	49	55.6	11.3	31	77	
Cervical						0.462**
A	19	59.7	7.6	41	71	
AB	8	54.8	12.6	34	72	
B	5	57.8	8.1	52	72	
O	12	55.3	7.8	41	65	

*One Way ANOVA **Kruskal Wallis Test

In our study (n=354), the blood type distribution is as follows: A Rh (+) 137 (38.70%), A Rh (-) 19 (5.36%), O Rh (+) 94 (26.55%), O Rh (-) 14 (3.95%), B Rh (+) 55 (15.53%), B Rh (-) 5 (1.41%), AB Rh (+) 26 (7.34%), AB Rh (–) 4 (1.12%). In terms of Rh factor, 309 individuals (87.28%) are Rh (+), and 42 individuals (11.86%) are Rh (-).

In the endometrial cancer distribution of our study (n=162), the blood type breakdown is as follows: A Rh (+) 64 (39.50%), A Rh (-) 8 (4.93%), O Rh (+) 39 (24.07%), O Rh (-) 8 (4.93%), B Rh (+) 29 (17.90%), B Rh (-) 4 (2.46%), AB Rh (+) 9 (5.55%), and AB Rh (-) 1 (0.61%).

In the ovary cancer distribution of our study (n=148), the blood type distribution is as follows: A Rh (+) 56 (37.83%), A Rh (-) 9 (6.08%), O Rh (+) 43 (29.05%), O Rh (-) 6 (4.05%), B Rh(+) 21 (14.18%), B Rh(-) 1 (0.67%), AB Rh(+) 10 (6.75%), and AB Rh(-) 2 (1.35%).

In the cervix cancer distribution of our study (n=44), the blood type distribution is as follows: A Rh (+) 17 (38.63%), A Rh (-) 2 (4.54%), O Rh (+) 12 (27.27%), O Rh (-) 0 (0%), B Rh (+) 5 (11.36%), B Rh (-) 0 (0%), AB Rh (+) 7 (15.90%), and AB Rh (-) 1 (2.27%).

The AB+ blood type ratio in cervical cancers was found to be statistically significantly higher compared to the reference article and statistically significantly higher than endometrial cancers (p=0.021, p=0.049, respectively). Findings are summarized in Table 2 .

DISCUSSION

In our study, the blood type distribution in patients was similar to the blood type distribution in the Marmara region. Findings in endometrial and ovarian cancers were consistent with blood type distribution, and no statistically significant difference was observed. However, in cervical cancer, the AB Rh (+) ratio was found to be significantly higher compared to the blood type distribution in the Marmara region and endometrial cancer (p=0.021, p=0.049).

Table 2. Cancer areas and reference evaluations according to blood types

	Endometrial	Ovarian	Cervical	Reference Article	Ref. vs. Endometrial	Ref. vs. Ovarian	Ref. vs. Cervical
	n (%)	n (%)	n (%)	n (%)	p	p	p
O-	8 (4.93%)	6 (4.05%)	0	5451 (4.4%)	0.738	0.838	0.265
O+	39 (24.07%)	43 (29.05%)	12 (27.27%)	36427 (29.4%)	0.137	0.926	0.757
A-	8 (4.93%)	9 (6.08%)	2 (4.54%)	6793 (5.5%)	0.761	0.749	1.000
A+	64 (39.50%)	56 (37.83%)	17 (38.63%)	47496 (38.3%)	0.759	0.901	0.967
B-	4 (2.46%)	1 (0.67%)	0	2560 (2.1%)	0.719	0.380	1.000
B+	29 (17.90%)	21 (14.18%)	5 (11.36%)	16294 (13.2%)	0.074	0.709	0.726
AB-	1 (0.61%)	2 (1.35%)	1 (2.27%)	908 (0.7%)	1.000	0.296	0.277
AB+	9 (5.55%)	10 (6.75%)	7 (15.90%)	5451 (4.4%)	0.649	0.873	0.021
N	162	148	44	123900			

Cervical vs. Endometrial p=0,049 Cervical vs. Ovarian p=0,073

As observed for over 35 years, aberrant glycosylation occurs essentially in all types of experimental and human cancers, and many glycosyl epitopes constitute tumor-associated antigens. Whether abnormal glycosylation is a consequence or a cause of cancer is a long-standing debate. Many recent studies indicate that abnormal glycosylation, in some but not all cases, is a consequence of initial oncogenic transformation and is also a key event in the initiation of invasion and metastasis.[8]

ABO may also be associated with cancer risk because the A antigen can be detected in tumor cells from non-A individuals; Glycosylation, in turn, can lead to conformational changes in proteins such as the epidermal growth factor receptor or alter the immune recognition of natural killer cells. helps tumor formation.[9]

Studies on survival and cancer have revealed that patients with blood type A have a longer survival time than other blood types.[10] A retrospective analysis of 968 women affected by gynecological tumors was performed to evaluate the existence of a difference in survival between patients with different blood groups. Data on 237 cases of endometrial cancer, 92 cases of ovarian cancer, and 639 cases of invasive cervical cancer are presented, detailing their ABO blood antigenic phenotypes, stage of neoplasia, and treatment received. In terms of endometrial cancer, significantly better 5-year and 10-year survival is associated with blood type O compared to blood type A. This finding is more evident when considering 5-year survival among patients affected by ovarian cancer. Regarding cervical cancer, the analysis showed that a survival rate of slightly better than 5 years was associated with the 0 blood phenotype; On the contrary, better survival is associated with the A blood phenotype, considering survival of 10 years or more. This study confirms the evidence of the association between blood type A and gynecological tumors. Endometrial and ovarian cancer is more common in women with blood type A than in other blood groups, and blood type A is also associated with poor prognosis in the same tumors. The possible reason for these findings is currently discussed in detail, considering the possible biological importance given to the ABO group system in the complex activities of the immune system.[11]

According to a study conducted in India, blood group B and marriage age between 11 and 20 years were significantly associated with cervical carcinoma.[12] Region of residence, parity and religion reveal a varying risk for cervical carcinoma. Another study concluded that blood group B could be considered a risk factor for cervical carcinoma.[13] Another study also shows that early-stage cervical cancer patients with non-O blood type have poorer 5-year survival than those with O blood type, and this has been proven during the first 5 years.[14]

Tyagi et al.[15] found that AB blood group has a significant higher risk compared to the stable blood group O in relation of carcinoma cervix. In our study, AB blood group was found to be significantly higher in patients with cervical cancer.

They reported the presence of an A-like antigen (MRG-1) in cervical tissues and suggested that persons with blood group A and AB, thereby lacking antiA antibodies are more susceptible to tumours.[16]

A systematic review and meta-analysis found that the risk of developing ovarian cancer is significantly increased in individuals with blood types A and AB. However, no significant effect of ABO blood groups on the overall survival of ovarian cancer patients was found.[17]

In a subgroup of patients with high-grade serous adenocarcinoma, blood groups B and AB were associated with a better 5-year cancer-specific survival rate compared with blood groups A and 0 [(60.3±8.6% vs. 43.8±3%) ,6). p=0.04)].[18]

Another study showed that the presence of B antigen (B/AB) was an unfavorable prognostic factor in ovarian carcinoma, especially in FIGO stages I, IV and menopausal patients.[19]

While blood group A was a positive factor for endometrial cancer patients in two studies.[20,21] In another study, no relationship was found.[22]

Blood type screening, when compared to the current gold standard, is unlikely to assist in the early-stage diagnosis of endometrioid endometrial carcinomas. Furthermore, a specific blood type does not increase the risk of recurrence or undifferentiated type endometrial carcinoma.[23]

Loss of expression of normal A, B, and O (ABO) blood group antigens in tumor tissue has been associated with the clinical behavior of certain epithelial cancers. Early recurrence is observed in 78% of patients with loss of blood group antigens. The loss of blood group antigens is the most significant variable associated with early recurrence.[24]

It has been suggested that blood group-associated antigens play a role in the adhesion of trophoblasts, inflammatory cells, and metastatic tumor cells to endothelial cells in the vascular system.[25]

In general, non-O blood groups are more susceptible to diseases compared to blood group O. Increasing awareness among people about this could be beneficial because individuals with high-risk blood groups can be screened, and they can be educated to modify their lifestyles, health behaviors, and habits.[26]

Additional studies are needed to clarify whether blood types are associated with increased cancer risk and to determine how antigen expression affects tumorigenesis.

Study Limitations

The limitations of our study arise from the small patient cohort and single center. Prospective studies with large cohorts will contribute more to science.

CONCLUSION

There are studies indicating a significant relationship between blood groups and various diseases. The expression of blood group antigens on blood cells and other epithelial surfaces acting as receptors or signal transducers contributes to these findings. The possibility of ABO antigens serving as receptors in tumor structures caused by infections, such as cervical cancer, should not be overlooked. In this regard, the significantly higher prevalence of cervical cancer in individuals with AB Rh (+) blood type, carrying A, B, and Rh antigens, can be explained concerning the population.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of İstanbul Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Researches Ethics Committee (Date:26.12.2022, Decision No:366).

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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Review

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Enhancing dermatology: the current landscape and future prospects of augmented and virtual reality technologies

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ABSTRACT

This article aims to provide a comprehensive assessment of the current status and future potential of augmented and virtual reality (AR/VR) technologies in the field of dermatology. We conducted an extensive review of the existing literature, encompassing studies and case reports related to the utilization of AR/VR in dermatology. This analysis encompassed diverse applications, including medical education, diagnostics, and dermatologic surgery, to offer a holistic view of their current implementations. Despite the significant interest generated within the dermatological community, the integration of AR/VR technologies in dermatology has not advanced at the same pace as in surgery. Our review underscores the current applications of AR/VR, which encompass improving medical education through interactive simulations, enhancing diagnostic precision, and facilitating complex dermatologic surgical procedures. Additionally, we address the challenges and constraints associated with their practical implementation in clinical settings. Augmented and virtual reality technologies possess immense potential to transform the landscape of dermatology. While their adoption has been gradual, these technologies have showcased their ability to enhance medical education, diagnostics, and surgical interventions. The future holds promising prospects for further developments in AR/VR applications, positioning them as valuable assets for dermatologists and aspiring dermatologists alike. However, it is imperative to address issues related to accessibility, cost-effectiveness, and patient acceptance to foster their widespread integration into clinical practice.

Keywords: Augmented reality, virtual reality, dermatology, digital dermatology, medical education, diagnostics

INTRODUCTION

Augmented reality (AR) and virtual reality (VR) are integral components of virtual environments (VE) that provide users with immersive experiences.[1] While VR fully immerses users in artificial settings, AR overlays virtual elements onto the real world, seamlessly merging the virtual and the physical. Over the past few decades, the utilization of AR/VR applications has surged significantly. These technologies have found substantial application in various medical fields, notably in plastic surgery.[2] Despite several compelling reasons, the adoption of AR/VR in the field of dermatology has remained relatively limited. First and foremost, the skin, as an easily accessible organ, offers an ideal canvas for AR applications. Secondly, dermatology heavily relies on visualization, making the potential impact of AR/VR profound. Lastly, the dermatological sector represents a multibillion-dollar industry in the United States alone, offering substantial economic opportunities.[3-5] The primary applications of AR/VR in dermatology can be broadly categorized into three main areas: education,[6-8] dermatologic surgery,[9,10] and diagnostics.[11] In a pioneering work published in the early 2000s, Gladstone and colleagues envisioned the twenty-first-century applications of VR in dermatology.[10] They envisioned a future where dermatologists could train using patient-specific skin and skeletal data just before performing complex Mohs surgery. Students would not only visualize lesions in 3D but also physically interact with them using haptic feedback systems. Such tools held the potential to revolutionize telemedicine. Moreover, VR technology would enable practitioners to explore lesions from various angles, thereby enhancing diagnostic accuracy.[5,8]

In this article, we review the current status of AR and VR in dermatology and provide insights into their potential future developments.

METHODS

All procedures were carried out in accordance with the ethical rules and the principles (of the Declaration of Helsinki).

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A rigorous literature review was undertaken, drawing from the Web of Science Core Collection and the MEDLINE® databases. Our research encompassed descriptors such as "virtual environments," "virtual reality," "augmented reality," "dermatology," and "dermatological science." To narrow our focus on the application of AR and VR in dermatology, the term "skin" was deliberately excluded. The parameters of our search were titles, abstracts, and author-specific keywords, without any date, document type, or other limitations. However, we concentrated only on English publications. This method resulted in a compilation of 64 papers, of which 35 were recent (within the past 5 years), and 51 were categorically aligned with "Dermatology." Each article was individually assessed, with selection based on relevance inferred from their titles and summaries.

RESULTS

Education

Improving dermatological education presents a significant challenge due to the difficulty of retaining visual memory without continuous exposure. Primary care physicians often exhibit suboptimal diagnostic accuracy for common skin conditions.[10,12] The integration of 3D silicon-made phantoms has shown substantial promise in enhancing diagnostic skills and knowledge retention compared to traditional 2D images.[13] Therefore, similar benefits are expected with the incorporation of AR.8 However, recent studies have produced unexpected results, with minor variations in knowledge acquisition and slightly improved knowledge retention among groups utilizing AR-based tools compared to textbooks.[7] This outcome could potentially be enhanced by integrating AR with haptic feedback, a common feature in surgical training tools.

A promising approach to training the next generation of medical professionals involves creating realistic 3D models that showcase skin diseases and abnormalities. These models would feature high-resolution clinical textures applied to newly generated 3D models with anatomically accurate proportions. They could be accessible on smartphones, tablets, or VR headsets, allowing for the adjustment of environmental conditions (e.g., lighting) to enhance realism. Additionally, synthetic clinical images could enhance these models by addressing the challenges of data scarcity and the lack of training on rare dermatological conditions and particular Fitzpatrick skin types. To illustrate, the incidence of melanoma is comparatively low among Black populations, which is significant because it contributes to their lower survival rates when contrasted with non-Hispanic White populations in the US. This gap underscores the necessity for diverse training data to improve diagnostic accuracy and treatment outcomes across all skin types.[14] Such disparities have garnered significant media attention.[15] The DermGAN project aims to enhance diversity in clinical skin images by generating images that exhibit the characteristics of specific skin conditions, locations, and underlying skin colors, which can then be transformed into 3D visualizations.[16]

In addition to healthcare professionals, some studies have explored AR/VR as educational tools for patients or the general public to raise awareness about skin disease prevention. For instance, Elke Hacker's group in Australia developed a virtual reality (VR) game that incorporates preventive skin cancer messages for youth and young adults.[17] This initiative was prompted by the persistence of high rates of sunburn among young populations despite successful public health campaigns in the 1980s. [15]

Another innovative application of VR in dermatology is the creation of the "Virtual Derm" VR app, which immerses students in a virtual dermatology practice.[18] This app is compatible with standard Android smartphones, ensuring accessibility for most students. It offers a unique learning experience, with students having the opportunity to solve 100 patient vignettes. [16]

Dermatologic Surgery

Dermatologic surgery, a specialized field within dermatology, focuses on surgical procedures aimed at repairing or enhancing the function and appearance of the skin.[19] While plastic surgery has significantly harnessed the potential of AR/VR technology,[20] its application in dermatologic surgery remains relatively limited. Apart from surgical training applications[21] (discussed in the preceding section on education), AR and VR have been employed in Mohs surgery to alleviate patient anxiety[22] and as surgical assistance tools.[23]

In the context of patient care, VR has been employed to mitigate pre- and intraoperative anxiety. Generic VR sequences, capable of reducing anxiety in various scenarios, have been used effectively in surgical contexts.[22] VR also holds promise as a patient education tool, offering patients a clearer understanding of surgical procedures, including complex interventions such as flap-based surgical defect reconstructions. However, detailed investigations into the impact of providing extensive surgical procedure information on patient anxiety are warranted.[22]

For medical professionals, VR serves as a valuable training tool across various surgical specialties, including dermatologic surgery. The realistic visual representation offered by VR, coupled with tactile sensations (haptic feedback), facilitates the training of dermatologic surgical

procedures, mirroring the training methods employed in other surgical domains, notably plastic surgery. AR has demonstrated successful application in improving communication between surgeons and pathologists in Mohs surgery cases, especially when the surgeon did not directly interpret pathology slides.[23] Additionally, AR holds substantial potential in other facets of Mohs surgery and dermatologic surgery. After acquiring theoretical knowledge and training on artificial models, the most effective learning in surgical procedures occurs through hands-on experience with real patients.[21] However, due to limitations in staffing and scheduling, junior surgeons may occasionally find themselves performing procedures beyond their comfort level without the immediate supervision of senior faculty members. AR can bridge this gap by enabling senior faculty to remotely monitor operations and provide precise guidance to junior surgeons using superimposed AR images on the surgical site. In larger institutions, this could optimize the allocation of senior staff resources, allowing experienced surgeons to oversee multiple surgeries simultaneously.

Diagnostics

Artificial intelligence (AI) has played a significant role in dermatology for several decades, particularly in the realm of skin cancer detection. Convolutional neural networks (CNNs) have demonstrated dermatologist-level classification proficiency for cutaneous lesions, encompassing both dermoscopic and non-dermoscopic images.[24] AI has proven particularly valuable when integrated with VR reconstruction of the body's surface. An exemplary instance is the Canfield® Vectra WB360 device, which amalgamates 2D photographs from 46 stereo-vision pods, resulting in a comprehensive VR reconstruction of the entire body that can be manipulated at will. The system further employs machine learning-based segmentation and classification to identify nevi and other cutaneous lesions, evaluating their probability of malignancy. Lesion information, along with its anatomical position, is cataloged, enabling the tracking of changes over time. This technology actively aids in the early detection of developing melanomas. A study (Melanoma Detection in Switzerland With VECTRA-MELVEC, ClinicalTrials.gov Identifier: NCT04605822) is currently underway to compare the diagnostic accuracy of dermatologists with and without the support of artificial intelligence. Patients receive their VR reconstructions and results, which they can review from the comfort of their homes. This innovative approach, thus far unique in dermatology, has garnered enthusiastic feedback from professionals in the field.

AI-driven smartphone applications, or "apps," have proven to be practical tools for enhancing diagnostics,25-28 despite some limitations regarding melanoma screening accuracy.[27] In a recent study,[25] a mobile augmented reality system was employed to provide real-time diagnostic support for melanoma lesions using deep learning. Parameters such as lesion diameter, color, and asymmetry are displayed in real-time within the camera view (**Figure 1**). Preliminary evaluations of the app have yielded encouraging results.[25]

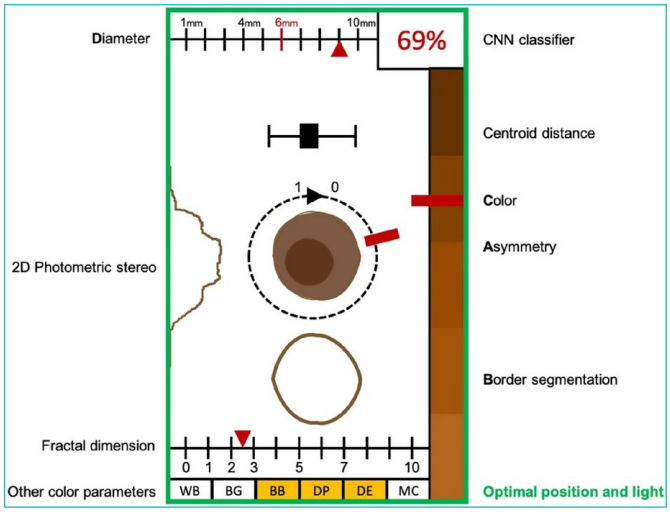

Figure 1. Proposed layout sketch of the augmented reality app for melanoma diagnostics[25]

These models are meticulously designed to incorporate high-definition clinical textures onto 3D models that accurately reflect anatomical proportions. Such models can be seamlessly accessed via a range of devices, including smartphones, tablets, and VR headsets. The dynamic nature of these models allows users to fine-tune environmental conditions, such as lighting, which further enhances the realism of the models. In addition, by incorporating synthetic clinical imagery, these models address crucial challenges like the dearth of data and insufficient training related to infrequent dermatological conditions and distinct Fitzpatrick skin categorizations. It's noteworthy that melanoma, for example, has a lower incidence among black demographics, which unfortunately leads to decreased survival rates when contrasted with non-Hispanic whites in the US.[14] The significant media spotlight has underscored such disparities.[15] A notable endeavor, the DermGAN project, is poised to bolster diversity in clinical skin imagery. It's designed to create images that vividly display specific skin conditions, localizations, and the intrinsic colors of the skin, which can subsequently be converted into 3D visualizations.[16]

The potential of AR/VR in dermatology is greatly amplified with the inclusion of haptic feedback. Often simply referred to as "haptics," this technology facilitates tangible interaction with the virtual environment, transmitting tactile nuances and force to the user.[29] Data related to skin attributes like temperature, texture, and rigidity can provide invaluable additional insights

to bolster diagnostic evaluations.[30] In line with this, research by Kim and associates yielded a technique that can transform a singular image into a 3D tactile interface, supporting instantaneous rendering.[31,32]

DISCUSSION

VR has been proven valuable in patient care, particularly in alleviating preoperative and intraoperative anxieties. Standardized VR sequences, known to decrease anxiety across diverse settings, have found efficacy in surgical environments.[22] VR's potential extends to patient education, affording patients a lucid comprehension of surgeries, even intricate procedures like flap-based surgical defect reconstructions. For medical practitioners, VR stands as an indispensable training tool in numerous surgical specialties, inclusive of dermatologic surgery. Its capacity to combine lifelike visual depictions with haptic feedback enables robust training paradigms, reminiscent of the training techniques used in other surgical fields such as plastic surgery. The merit of AR is evident in enhancing dialogue between Mohs surgeons and pathologists, especially in cases where the surgeon might not directly interpret pathology findings.[23]

AR and VR, with their transformative capabilities, have ushered in a wave of advancements across various aspects of dermatology, spanning from education and patient engagement to surgical procedures. Their significant potential remains somewhat under-tapped, but the uptick in research over recent years offers a promising horizon. As these technologies mature, evolve, and become more accessible, they are poised to spark further innovations in the field.

Limitations

Addressing the constraints of AR and VR in dermatology requires a nuanced perspective, concentrating predominantly on applications while sidelining potential health repercussions from user interaction with these technologies. Challenges in leveraging AR/VR for dermatological learning mirror those in broader medical education. Recent extensive studies, possibly propelled by the challenges posed by the COVID-19 pandemic, have delved deep into these limitations.[33-35] For instance, Parsons and MacCallum conducted a deep dive into the potentials and constraints of augmented reality in medical academia, shedding light on the multifaceted utility of AR/VR in this sector while accentuating the necessity to prudently choose affordances during the development phase to amplify benefits.[33]

In dermatologic surgery and diagnostics, AR and VR technologies do face challenges that echo those in general surgical practices.[20] Current technological bottlenecks, like image fidelity, battery longevity, and ergonomics, can be impediments, but continuous technological progress is anticipated to alleviate these issues. Just like any other electronic medical data, the sanctity of patient confidentiality and legal compliance remains non-negotiable.

CONCLUSION

Virtual reality (VR) has demonstrated its substantial value in enhancing patient care, particularly in alleviating preoperative and intraoperative anxieties. The use of standardized VR sequences has consistently proven effective in reducing anxiety levels in diverse healthcare settings, including surgical environments. VR's potential extends beyond anxiety reduction, encompassing patient education, where it provides patients with a clear understanding of complex surgical procedures, such as flap-based surgical defect reconstructions. Additionally, VR serves as an indispensable training tool for medical practitioners in various surgical specialties, revolutionizing training paradigms with its lifelike visual depictions and haptic feedback, akin to those employed in fields like plastic surgery. Augmented reality (AR) has also demonstrated its merit in dermatology, particularly in enhancing communication between Mohs surgeons and pathologists. This is especially valuable in cases where the interpretation of pathology findings may require collaborative input. The transformative capabilities of AR and VR have ushered in a wave of advancements in dermatology, impacting education, patient engagement, and even surgical procedures. While their full potential is yet to be fully realized, the increasing volume of research in recent years points towards a promising future. As these technologies continue to mature, evolve, and become more accessible, they are poised to ignite further innovations in the field of dermatology, ultimately leading to improved patient care and outcomes.

ETHICAL DECLARATIONS

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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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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Review

HEALTH SCIENCES
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Exploring the relationship between orthostatic hypertension and diabetes mellitus: a literature review

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ABSTRACT

Diabetes mellitus is a swiftly escalating health problem both nationally and internationally. Diabetic neuropathy, a prominent microvascular complication of diabetes, frequently occurs. Autonomic cardiac neuropathy, which arises from diabetic neuropathy, is a substantial cause of mortality and morbidity. It is also linked with orthostatic hypertension, a condition characterized by an abnormal increase in blood pressure upon standing. There is, however, no consensus on the clinical and pathological characteristics of orthostatic hypertension. Blood pressure and pulse display a circadian rhythm, varying throughout the day. This literature review seeks to explore the intricate relationship between diabetes mellitus and orthostatic hypertension. Special attention is given to the potential impact of circadian rhythm on these conditions, as this aspect could provide essential insights into the disease mechanism and patient management. The review will cover the existing literature, aiming to identify knowledge gaps and illuminate potential areas for future research. Such exploration could lead to the development of enhanced preventive measures and therapeutic strategies, improving patient outcomes in these prevalent and interconnected conditions.

Keywords: Diabetes mellitus, orthostatic hypertension, autonomic cardiac neuropathy, diabetic neuropathy

INTRODUCTION

Today, diabetes is emerging as a health problem of increasing importance worldwide, due to its prevalence and the issues it creates. Alongside lifestyle changes and shifts in dietary habits, the prevalence of diabetes is rapidly rising in all countries.[1]

Autonomic neuropathy, a chronic microvascular complication of diabetes mellitus, can clinically manifest as unawareness of hypoglycemia, resting tachycardia, orthostatic hypotension, reduced heart rate variability, silent myocardial infarction, sudden cardiac death, gastroparesis, constipation, diarrhea, fecal incontinence, erectile dysfunction, neurogenic bladder, and sudomotor dysfunction.[2] Autonomic cardiac neuropathy is associated with significant morbidity and mortality in diabetes mellitus.[3] Unlike postural hypotension, a common feature of advanced autonomic neuropathy in diabetes mellitus, the prevalence and clinical importance of early-onset orthostatic hypertension in diabetic patients have not been fully understood.[4,5] Orthostatic hypertension, defined as a consistent increase in blood pressure occurring 1 to 5 minutes after standing up, is a cardiovascular disorder that has been less studied and is increasingly recognized. As one transitions from a supine to standing position, a rapid increase compensates for the transient drop in blood pressure, facilitated by autoregulatory mechanisms modulated by the autonomic nervous system. Despite representing the opposite of OHT, OHT similarly arises from autonomic nervous system (ANS) dysfunction.[6] Pathophysiology of orthostatic hypertension is considered responsible for increased peripheral vascular resistance, high levels of norepinephrine, impaired arterial and cardiopulmonary baroreceptor sensitivity, increased activation of the renin-angiotensin-aldosterone system (RAAS), and increased vasopressin secretion.[7]

Cardiovascular parameters such as blood pressure, pulse, and coronary tone are influenced by circadian rhythms throughout the day.[8] According to ambulatory blood pressure monitoring data of healthy individuals, blood pressure reaches its highest values in the morning, shows a slow decrease throughout the day, and is at its lowest at night.[9] This circadian rhythm in blood pressure has led to a new classification. In this classification made with ambulatory blood pressure monitoring, a decrease of 10% or more in blood pressure values measured at night compared to daytime values is named dipper hypertension, and a decrease of less than 10% is named

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non-dipper hypertension.[10] Recently, it has been shown that 24-hour ambulatory blood pressure measurements (ABPM) are more valuable than clinic measurements in assessing cardiovascular risk.[11]

The objective of this review is to comprehensively analyze the existing literature on the relationship between diabetes mellitus and orthostatic hypertension, placing particular emphasis on autonomic neuropathy and the effects of circadian rhythms on these conditions. Despite growing acknowledgment of orthostatic hypertension as a prevalent complication in diabetic patients, there remains a considerable gap in comprehensive literature reviews exploring its prevalence and clinical implications. This lack of holistic understanding hampers the progression towards formulating effective treatment and preventative measures for diabetic patients susceptible to orthostatic hypertension. By synthesizing the findings on the nuances of this relationship, including the impacts of circadian rhythms on blood pressure and pulse, we intend to illuminate a facet of research that has often been overshadowed. Highlighting these connections can guide improved patient management protocols, aiming to reduce the morbidity and mortality associated with these intertwined conditions. Furthermore, with the global rise in diabetes cases, grasping its complications such as orthostatic hypertension is vital for worldwide health. Consequently, this review holds considerable relevance in the realm of diabetes research and its practical application, offering insights that might shape policy and healthcare directives.

DIABETIC NEUROPATHY

It is defined as symptoms and signs of the peripheral nervous system that cannot be attributed to anything other than diabetes in a diabetic patient. It is the most common microvascular complication in diabetes. Neuropathy is seen in at least half of the patients who have had diabetes for more than ten years.[12]

Prevalence

The prevalence in diabetic patients is on average 30%, and symptomatic peripheral neuropathy develops in about half of the patients during follow-up. Autonomic neuropathy accompanies peripheral neuropathy in 30 to 50% of patients.[13]

The most common form of neuropathy is distal sensory and autonomic polyneuropathy. The most common of the mononeuropathies is carpal tunnel syndrome. Other causes of neuropathy should be ruled out for diagnosis.[14]

Pathogenesis

Metabolic and vascular factors are considered together responsible for the pathogenesis of diabetic neuropathy. However, the most significant metabolic factor is undoubtedly chronic hyperglycemia. There is plenty of evidence that hyperglycemia increases this risk.[15,16]

Multiple mechanisms are held responsible for the development of microvascular complications. One of the pathological reactions triggered by hyperglycemia is the activation of the polyol pathway. With the activation of the polyol pathway, sorbitol increases in the tissues, while myo-inositol levels decrease. As a result of these two parameters, there is a slowdown in nerve conduction speed.[17]

When there is prolonged high blood sugar, or chronic hyperglycemia, compounds called Advanced Glycation End Products (AGEs) are formed. A commonly referenced example of an AGE is HbA1c. What happens is that glucose attaches to proteins in a way that can't be reversed. This attachment can disrupt the normal structure and function of these proteins. Additionally, when AGEs interact with their designated receptor, known as RAGE, it triggers an increase in the production of certain inflammatory substances like cytokines and molecules that help cells stick together, which are released from monocytes and the lining of blood vessels, the endothelium. When RAGE is activated, it also impacts the creation of enzymes called matrix metalloproteinases, which can, in turn, harm nerve fibers.[18]

Other pathological mechanisms triggered by hyperglycemia are decreases in growth factors, activation of protein kinase C, and increased oxidative stress. [19]

One of the most important factors implicated in neuropathy is neural ischemia. Studies have found that the presence of macrovascular disease is a risk factor for the development of neuropathy. The increases in nerve conduction speed after revascularization interventions on large vessels are direct evidence for the role of ischemic factors in neuropathy pathogenesis.[20,21]

Oxidative stress, both experimental data and mitochondrial (SOD2) and extracellular (SOD3) superoxide dismutase gene polymorphism, increase the risk of neuropathy. Some data on the positive effects of antioxidant agents support the role of this mechanism.[22-25]

Symptoms vary according to the affected sensory fiber class. While small fiber involvement leads to pain, burning and tingling, large fiber involvement results in numbness and loss of sensation.[26]

Risk Factors

In type 1 and type 2 diabetes, advanced age, the duration and severity of hyperglycemia are major risk factors for the development of diabetic neuropathy. Additionally, obesity, metabolic syndrome, glycemic variability, dyslipidemia, and smoking are considered risk factors.[27]

Screening

In type 1 DM, it is recommended to perform neuropathy screening every year, starting 5 years after the diagnosis, and in type 2 DM, starting from the time of diagnosis. Neuropathy screening should be done with physical examination, 10-gram (10-g) monofilament, and tests like tuning fork.[28]

Diagnosis

Early diagnosis of diabetic polyneuropathy is important in preventing many complications. Patients should be questioned about the type of symptoms, onset time, progression duration, symmetry, alcohol use, family history. The presence of autonomic symptoms such as constipation, urinary retention, changes in sweating pattern, blurred vision, and bloating in the abdomen should be evaluated. Neuropathy screening, in addition to the physical examination, can be done with simple clinical tests like the monofilament test, which evaluates small nerve fiber function by applying 10 g pressure once a year, and the tuning fork test, which evaluates large nerve fiber function by testing the vibration sense. Electrophysiological tests or referral to a neurologist is rarely required except in cases with atypical symptoms or unclear diagnosis. In all patients with diabetic neuropathy, causes of neuropathy other than diabetes, toxins, neurotoxic drugs, vitamin B12 deficiency, hypothyroidism, kidney disease, malignancies, infections, chronic inflammatory demyelinating neuropathies, hereditary neuropathies, and vasculitis should be ruled out.[26]

Classification

Diabetic neuropathies:[29]

A. A. Diffuse
   1. Distal symmetric polyneuropathy
      - Primary small fiber neuropathy
      - Primary large fiber neuropathy
      - Mixed small and large fiber neuropathy (most common)
   2. Autonomic
      - Cardiovascular
      - Decreased heart rate variability
      - Resting tachycardia
      - Orthostatic hypotension
      - Sudden death
      - Gastrointestinal
      - Diabetic gastroparesis
      - Diabetic enteropathy
      - Chronic hypomotility
      - Urogenital
      - Diabetic cystopathy
      - Erectile dysfunction
      - Female sexual dysfunction
      - Sudomotor dysfunction
      - Distal hypohidrosis/anhidrosis
      - Unawareness of hypoglycemia
      - Abnormal pupillary function

B. Mononeuropathy
   - Isolated cranial or peripheral nerve
   - Mononeuritis multiplex

C. Radiculopathy or polyradiculopathy
   - Radiculoplexus neuropathy
   - Thoracic radiculopathy

DIABETIC AUTONOMIC NEUROPATHY

The signs and symptoms of autonomic neuropathy should be carefully identified during the history taking and physical examination. The main symptoms of diabetic autonomic neuropathy include unawareness of hypoglycemia, resting tachycardia, orthostatic hypotension, gastroparesis, constipation, diarrhea, fecal incontinence, erectile dysfunction, neurogenic bladder, and sudomotor dysfunction with increased/decreased sweating.[28]

The clinical symptoms of autonomic neuropathy usually appear long after the onset of diabetes. While symptoms suggestive of autonomic dysfunction can be common, they are often due to reasons other than actual autonomic neuropathy. Nevertheless, subclinical autonomic dysfunction can emerge within one year after diagnosis in type 2 diabetic patients and within two years in type 1 diabetic patients.[30]

Cardiac Autonomic Neuropathy

Cardiac autonomic neuropathy is associated with mortality, independent of other cardiovascular risk factors.[31,32] It is suggested that a significant portion of sudden cardiac death cases in diabetic patients is due to silent cardiac autonomic neuropathy.[33]

In its early stages, cardiac autonomic neuropathy may be entirely asymptomatic. Advanced disease can be associated with resting tachycardia (>100 beats/min) and orthostatic hypotension.[28]

In most patients, parasympathetic dysfunction is detected before sympathetic dysfunction. Ewing and colleagues developed an assessment scale that evaluates both sympathetic and parasympathetic autonomic dysfunction together.[34]

In epidemiological studies, diabetes has been found to be an independent risk factor for cardiac hypertrophy, systolic dysfunction, and heart failure.[35,36]

ORTHOSTATIC HYPERTENSION

Contrary to postural hypotension, a common feature of advanced autonomic neuropathy in diabetes mellitus, the prevalence and clinical significance of orthostatic hypertension in diabetic patients have not yet been

fully understood. The difficulty in determining the true prevalence of OHT is due to various factors, such as different definitions used and differences among the populations studied to date.[37]

Definition of Orthostatic Hypertension

Most researchers have suggested that OHT can be defined as an increase of at least 20 mmHg in systolic blood pressure (SBP) after standing.[38] However, recent large-scale studies and in line with the definition of orthostatic hypotension, an orthostatic increase of at least 10 mmHg in diastolic blood pressure (DBP) has also been included in the OHT definition.[39,40] It emerges as a result of abnormal regulation of blood pressure during postural changes and was first defined in 1980.[41]

Pathogenesis of Orthostatic Hypertension

The pathophysiology of orthostatic hypertension is not yet fully understood. Orthostatic hypertension represents the hemodynamic opposite of hypotension but similarly originates from ANS dysfunction. Opposite functioning common mechanisms play a role in both disorders. The reason why common triggering factors and mechanisms support the development of orthostatic hypertension in some patients and orthostatic hypotension in others remains unclear. OHT is closely associated with cardiovascular, cerebrovascular diseases and central nervous system damage, and is considered a new risk factor for cardiovascular and cerebrovascular diseases in adults. It is thought that conditions that increase ANS degeneration are factors supporting the development of OH.[7] These factors are;

- Activation of the Sympathetic Nervous System
- Aging
- Essential hypertension
- Diabetes mellitus
- Neurological disorders

When moving from a supine to a standing position, approximately 500-1000 ml of blood pools below the diaphragm, and hydrostatic pressure shifts fluids from the intravascular area to the interstitial area. In healthy individuals, compensatory autonomic reflex mechanisms withdraw cardiac parasympathetic impulses and increase cardiac and vascular sympathetic activity, leading to an increase in heart rate, a decrease of <20 mmHg in systolic blood pressure, and a small increase in diastolic blood pressure. Studies suggest that orthostatic hypertension results from an excessive increase in vascular resistance when standing. Observations that plasma norepinephrine increases more with standing in patients with orthostatic hypertension suggest that the response is mediated through excessive sympathetic activation while standing.[42] In patients with orthostatic hypertension, due to higher sympathetic activity, vasoconstriction is also greater, and as a result, blood pressure increases. Autonomic nervous system dysfunction leads to insensitivity of arterial and cardiopulmonary baroreceptors and/or inability to adjust the baroreflex to normal blood pressure values, while excessive vasoconstriction mediated by alpha-adrenergic vascular hyperactivity is believed to be another important mechanism. Another proposed mechanism is nephroptosis. In these patients, the renal artery is strained and bent when standing up. As a result, renal blood flow decreases and RAAS is activated.[43]

Signs and Symptoms of Orthostatic Hypertension

Unlike orthostatic hypotension, orthostatic hypertension (OHT) is usually asymptomatic and does not produce any symptoms. Rarely, syncope, feelings of emptiness, dizziness, headaches, palpitations, nausea, and sweating may occur.[44]

Orthostatic Hypertension Treatment

Due to the lack of evidence associated with negative outcomes, there are no specific recommendations for the management and treatment of OHT. There is no direct evidence that the treatment of OHT improves prognosis. If it is considered that OHT is linked to masked and future hypertension, patients with OHT should be closely monitored for the rapid detection of essential hypertension.[7]

Ambulatory Blood Pressure Measurement

Ambulatory blood pressure measurement (ABPM) is considered the gold standard for the diagnosis of hypertension. In healthy individuals, nighttime blood pressure values are usually lower than daytime values, and a reduction of the night-time blood pressure by 10-20% or more compared to the daytime average is considered as a 'dipper' pattern. Non-dipper refers to less than a 10% decrease, while reverse dipper is defined as no decrease or even an increase in nighttime pressure values. It has been reported that non-dipper and reverse dipper patterns are associated with increased cardiovascular mortality.[45]

CONCLUSION

Diabetic neuropathy, the most common microvascular complication in diabetic patients, presents a significant challenge in terms of its diagnosis, management, and long-term impact on patient quality of life. It is a multifaceted disorder, with a prevalence rate of approximately 30% among diabetic patients and often accompanied by symptomatic peripheral and autonomic neuropathy.

The complex pathogenesis of diabetic neuropathy involves both metabolic and vascular factors, with chronic hyperglycemia being the most significant metabolic factor. Various mechanisms contribute to the development of microvascular complications, such as the

activation of the polyol pathway, advanced glycosylation end products (AGEs), oxidative stress, and alterations in growth factors. Furthermore, neural ischemia and the presence of macrovascular disease contribute to the risk of neuropathy development.

Diagnosis of diabetic neuropathy is particularly challenging due to its varied symptoms, which depend on the type of affected sensory fiber. Early diagnosis can prevent many complications, so understanding the type and onset of symptoms, progression, alcohol usage, family history, and the presence of autonomic symptoms can be crucial for diagnosis.

Screening is recommended yearly starting 5 years after diagnosis for Type 1 diabetes and at the time of diagnosis for Type 2 diabetes. Various risk factors can contribute to the development of diabetic neuropathy, including older age, duration and severity of hyperglycemia, obesity, metabolic syndrome, glycemic variability, dyslipidemia, and smoking.

Finally, diabetic neuropathy can be classified into diffused neuropathy (distal symmetric polyneuropathy and autonomic neuropathy), mononeuropathy (isolated cranial or peripheral nerve or mononeuritis multiplex), and radiculopathy or polyradiculopathy (radiculoplexus neuropathy and thoracic radiculopathy).

Understanding the intricate pathophysiology of diabetic neuropathy is vital for the development of effective treatments and interventions. There remains a need for more comprehensive research to further illuminate the mechanisms involved and potentially discover novel therapeutic strategies. Improving patient outcomes will require ongoing efforts in early diagnosis, personalized treatment plans, and disease management education for patients. Ultimately, the objective should be not just to treat diabetic neuropathy, but also to prevent its onset through effective management of diabetes and its associated risk factors.

HIGHLIGHT KEY POINTS

Diabetic neuropathy is a common microvascular complication in diabetic patients, with a prevalence rate of around 30%. Its complex pathogenesis involves both metabolic and vascular factors, with chronic hyperglycemia being the most significant metabolic factor.Different mechanisms including the activation of the polyol pathway, advanced glycosylation end products (AGEs), oxidative stress, and alterations in growth factors contribute to neuropathy development.Diagnosis is challenging due to the varied symptoms, which depend on the type of affected sensory fiber.Screening is recommended yearly starting 5 years after diagnosis for Type 1 diabetes and at the time of diagnosis for Type 2 diabetes.Numerous risk factors contribute to the development of diabetic neuropathy, including older age, duration and severity of hyperglycemia, obesity, metabolic syndrome, glycemic variability, dyslipidemia, and smoking.

Diabetic neuropathy can be classified into diffused neuropathy, mononeuropathy, and radiculopathy or polyradiculopathy.Further comprehensive research is needed to understand the intricate pathophysiology of diabetic neuropathy and develop effective treatments. The ultimate goal should be not just to treat diabetic neuropathy, but also to prevent its onset through effective management of diabetes and its associated risk factors.

ETHICAL DECLARATIONS

Referee Evaluation Process

Externally peer reviewed.

Conflict of Interest Statement

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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Case Report

HEALTH SCIENCES
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Combination of substance addiction and Fournier's gangrenia: a case report

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ABSTRACT

Fournier's gangrene is a bacterial and rapidly progressive necrotizing fasciitis of the perianal and perineal region. It still has high morbidity and mortality. We aimed to present Fournier's gangrene, which developed in a 49-year-old male patient with substance abuse. The patient was brought to the emergency department with complaints of abdominal pain and pain in the perineum and perianal region on the 3rd day after foreign body trauma to the perianal region. Crepitation was detected under the skin in the suprapubic region of the patient. Acute abdomen was present in the abdominal examination. In the abdominal tomography, minimal fluid was detected between the bowel loops in the pelvis. Diffuse edema, heterogeneity and emphysematous changes were observed in the skin and subcutaneous tissues in the perineum, and in the rectus muscle of the anterior abdominal wall. The patient underwent an emergency laparotomy. Abscess material extending from the back of the rectus muscle to the pelvis was seen in the abdomen. Abscess and necrotic areas in the abdomen and perineum were cleaned. A protective Hartman end colostomy was opened to the patient. In the postoperative period, VAC was applied to the perianal region for 3 sessions with 72 hour intervals. Then, the wound in the perineum was closed primarily. After 3 months, his colostomy was taken into the abdomen. Early and aggressive surgical debridement, broad-spectrum antibiotics, and fluid resuscitation are critical in Fournier's gangrene. Because of this patient's late admission to the hospital and his long-term immunosuppression associated with substance abuse, his clinical condition deteriorated rapidly. It should be kept in mind that skin infections in the perianal region may progress to Fournier's gangrene in patients with conditions that may cause immunosuppression, such as substance abuse.

Keywords: Fournier's gangrene, substance abuse, necrotizing fasciitis

INTRODUCTION

Fournier's gangrene is a rapidly progressive necrotizing fasciitis of bacterial origin of the genital, perineal, and perianal regions.[1] It is typically due to the development of vascular necrosis following polymicrobial infection of the genitourinary and anorectal region, followed by further progression of bacterial infection due to localized ischemia.[2]

Mortality of Fournier's gangrene has decreased from a very high rate of 50% to approximately 10% over the years.[1] It can be said that this decrease in mortality is due to more aggressive surgical debridement, effective intravenous (IV) antibiotic administration, and improvements in intensive care techniques.[3]

The incidence of the disease is 1.6-3/100,000, it varies according to various studies, and it is seen approximately 10 times more frequently in men than in women.[4,5] Many patients have an immunosuppressive condition such as diabetes, alcoholism, substance abuse, obesity, peripheral vascular disease, local trauma, or urethral stricture that may predispose the area to polymicrobial necrotizing fasciitis.[6]

In this case report, we aimed to draw attention to the fact that Fournier's gangrene may spread to the perianal region, anterior abdominal wall and also to the abdomen, and may progress aggressively, rather than a single localization in an immunosuppressive patient with substance addiction.

CASE

A 49-year-old male patient presented to the emergency department with complaints of abdominal pain and pain and redness in the perianal region on the 3rd day after trauma with a foreign body in the perianal region. It was learned from the history of the patient that he was diagnosed

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with antisocial personality disorder and had been a substance addict for 10 years. The patient's complaints were abdominal pain that continued to increase on the 3rd day after the beating, pain in the anus area, redness and warmth. In the examination of the patient, arterial blood pressure was 140/70 mmHg, pulse was 122/min, oxygen saturation was 94%, respiratory rate was 18/min, and body temperature was 38.2°C. Abdominal examination revealed rebound and defense. Infection and necrotizing fasciitis in the perineum and perianal region had extended over the pubic bone to the posterior wall of the rectus and advanced into the abdomen. In the abdominal tomography, minimal fluid was detected between the bowel loops in the pelvis. Diffuse edema, heterogeneity and emphysematous changes were observed in the skin and subcutaneous tissues in the perineum, and in the rectus muscle of the anterior abdominal wall. In the laboratory tests of the patient, no abnormal values were found except WBC: 27,600/µL, CRP: 450.76 mg/dl, procalcitonin: 6.32 ng/ml. The patient was admitted to the general surgery service. He was operated on with the diagnosis of acute abdomen and perianal abscess. Wound culture was taken for treatment arrangement. Abscess and necrotic areas in the abdomen were debrided. Necrotic areas in the rectus muscle on the anterior abdominal wall were cleared. Necrosis and abscess areas in the perineum were debrided. After debridement, it was washed with plenty of saline and oxygenated water. Oxygenated water and saline water were used by mixing them in a one-to-one ratio. An end colostomy was performed. A protective Hartman end colostomy was opened because of the protection of the wound area from fecal contamination. In the post-operative period, 3 sessions of Vacuum Assisted Closure (VAC) were applied for 72 hours. Wound debridements were repeated when necessary between VAC applications. In this process, the treatment was arranged according to *E. coli* and Str. Proteus that cultured in wound. According to the results, meropenem and tazobactam were started as antibiotics and this treatment continued for 7 days. On the seventh postoperative day, when the clinical condition of our patient improved, the abdominal drains were removed. As a result of debridement of the perianal region and VAC applications, living tissues began to appear at the wound sites. After 16 days, the wound was closed primarily. Although hyperbaric oxygen therapy is used today, it could not be used because it was not available in our center. No adverse events were observed in the patient controls, and the colostomy was closed after 3 months.

DISCUSSION

Fournier's gangrene is a rapidly progressive necrotizing fasciitis that develops in the perianal and genitourinary regions. It general affects men more.[2] Necrotizing fasciitis is divided into three subgroups according to the type of causative microorganisms: Type 1 is polymicrobial and anaerobic and aerobic bacteria are responsible for this picture, Type 2 is usually caused by streptococci or staphylococci, and in Type 3, vibrio strains are responsible for the infection.[7]

The key to successful outcomes in complicated cases of Fournier's gangrene is clinical suspicion, aggressive and early surgical debridement, broad-spectrum antibiotics, fluid resuscitation, and early multidisciplinary organization.[2]

Our case was a middle-aged, long-term substance addicted and immunosuppressive patient. Due to his late admission to the hospital, we saw that the infection in **Figure 1, 2** progressed very rapidly and spread from the perineum to the anterior abdominal wall and into the abdomen.

As a result, Fournier Gangrene is a very important and a surgical emergency with high mortality rates

it should be known. primary care service emergency physicians as well as family physicians and dermatology doctors, this disease the necessity of having knowledge about it is obvious.

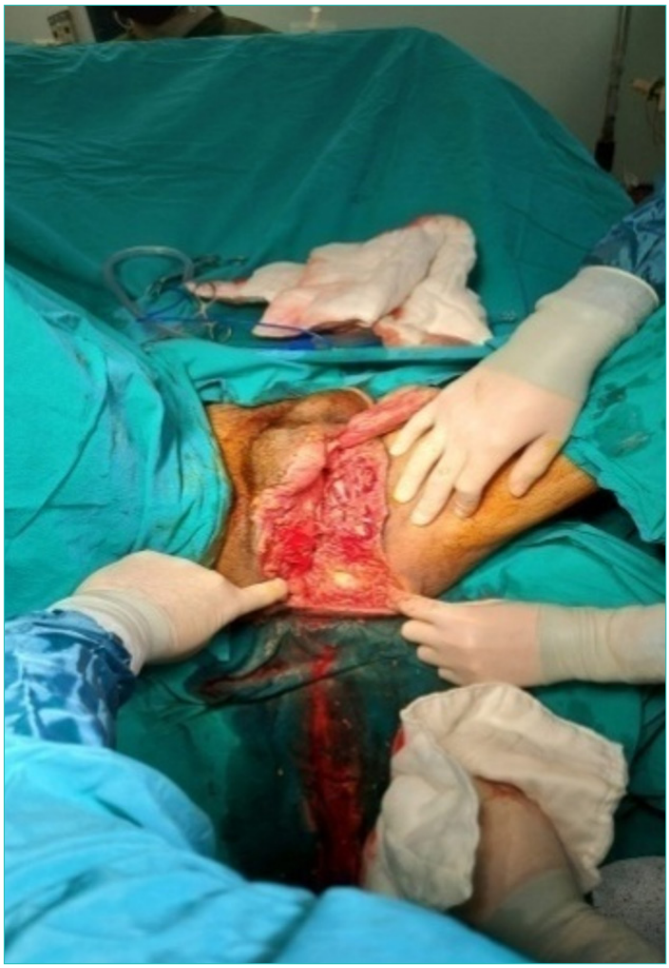

Figure 1. Intraop visual of patient's wound with Fournier's gangrenia

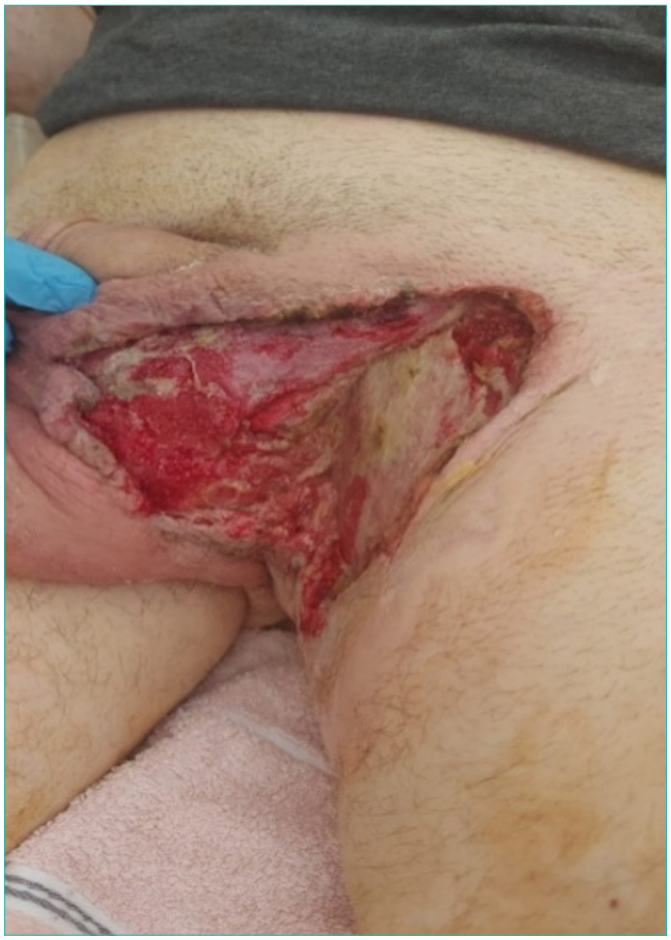

Figure 2: Postop visual of patient's wound with Fournier's gangrenia

CONCLUSION

It should be kept in mind that skin infections in the perianal regions of patients with comorbidities that may cause immunosuppression, such as substance abuse, may rapidly progress to Fournier's gangrene.

ETHICAL DECLARATIONS

Informed Consent
All patients signed and free and informed consent form.

Referee Evaluation Process
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The authors have no conflicts of interest to declare.

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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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