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Opinions and attitudes of pediatric nurses for missed nursing care in Türkiye

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Abstract

This study aimed to determine the attitudes of nurses working in the pediatric clinics located in the northern region of Turkey towards missed care needs. This was a descriptive study. It was carried out in the pediatric clinics of a university and a research and training hospital providing service Turkey between January 131, 2022. The sample of the study consists of 121 nurses. A Personal Information Form and Missed Nursing Care Survey-Pediatric Version were used to collect data in the study. The data were analyzed using SPSS 21.0. It was found that 40.5% of the nurses were providing care to more than 6 patients per nurse and the number of nurses were insufficient in the units where 86.8% of the nurses were working. The most common missed care needs were identified as "administration of drug 30 minutes before or after the specified time" at a ratio of 53.8%, "providing oral care" at a ratio of 34.7% and "providing emotional support to the child and/or family" at a ratio of 33.9%. It was found that mean total Missed Nursing Care Survey-Pediatric Version score of the participants was 2.35±0.68. Mean scores of the subscales were found to be 2.75±0.79 in Labor Resources, 2.12±0.74 for Material Resources and 2.05±0.77 in Communication/Teamwork, respectively. This study has shown that the most common reason of missed nursing care was workload and the least common one was communication. missed nursing care is an important problem that needs to be overcome in order to create patient safety culture and to ensure the quality of nursing care. Therefore, it is highly crucial to recognize missed nursing care clearly.

Keywords: missed nursing care, pediatric, nurse, patient safety

1. Introduction

Nursing care determines the quality of healthcare services. Nursing care, that is required in some situations, may be ignored in daily practice; and some situations that may put patient safety at risk may ocur (1). Inadequate nursing care reveals the concept of missed nursing care (MNC) needs (2). While neglecting or delaying some or all of the care that patients need has been described as "missed nursing care", this arising situation is evaluated as negligence error which affect patient and nurses in a negative way (3,4). The Agency for Healthcare Research and Quality (AHRQ) points out that negligence errors are quantitatively much more, recognized more difficultly and they are bigger and devastating problems in reality compared to the practical errors (5). Although missed care, that is accepted as negligence error, is directly correlational with health outputs such as quality of care and patient safety, it has been indicated that these type of errors remain confidential and overlooked unless investigated (6). However, missed care is an important nursing problem requiring emphasis and solution due to the reasons such as threatening quality of care and patient safety. Besides affecting the health of patients and nurses, this situation indirectly leads to an increase in the number of hospitalization days, and the use of supplemental treatments causes a significant increase in the cost allocated to care services (4). International literature include many studies regarding the most common missed nursing care and its causes. The results have shown that the most common missed care activities include mobilization of the patients, oral care, missed or delayed drug applications, provision of patient's mobility inside the bed, nutrition and excretion, planning discharge, providing emotional support, hygiene applications and integration of patients' acquaintances into the care (7-12). Emergence of missed care has been associated with individual characteristics of the nurse, institutional structure, working conditions and team communication (9,13-14). The number of nurses, adequacy of the sources and their effective use and the number of patients are the institutional factors affecting provision of care (7, 9,13,15,16). Missed care has negative effects on providers and recipients of the service as well as the institution. Patient falls, drug errors, nasocomial infections, pressure ulcers, accidents, mortality and repeated hospitalizations have been indicated as adverse patient outcomes (8,13,17,18). Besides affecting patient outcomes and quality indicators in a negative way, missed nursing care also arises some unwanted outcomes including nurse experiences such as spending a huge energy to hide the negligence error, intense stress and low self-esteem.

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Although nurses promote themselves with continuous trainings, it has been indicated that they experience conflicting feelings due to neglecting or delaying the needs of their patients and this leads to stress among the nurses and a decrese in their job satisfaction (5,19). It has been observed that there is a limited number of studies in the literature and a quite few studies performed in our country regarding the opinions and attitudes of pediatric nurses for MNC.

This study was carried out to determine the attitudes of nurses working in the pediatric clinics located in the northern region of Turkey towards missed care needs.

Answers were sought for the following questions in accordance with this purpose:

What is the level of nurses' attitudes towards missed care needs?

What are the most common missed care needs of the nurses?

2. Material and Methods

2.1. Type of the study

This was a descriptive study.

2.2. The sample and universe of the study

It was carried out in the pediatric clinics of a university and a research and training hospital providing service in a big city located in the northern region of Turkey between January 1-31, 2022. 228 nurses who were working in these clinics and who met the inclusion criteria (a working experience for at least one year, working as a clinic nurse and working in pediatric clinics outside the emergency, outpatient clinic and daily treatment areas) constituted the universe of the study. No sample was selected; it was tried to attain whole universe and finally, 121 nurses constituted the sample.

2.3. Data collection instruments

A Personal Information Form which was generated by the researchers in line with the literature and Missed Nursing Care Survey-Pediatric Version (MISSCARE) were used to collect data in the study.

Personal Information Form: It was composed of 12 questions regarding sociodemographic and professional characteristics of the nurses.

Missed Nursing Care Survey - Pediatric Version (MISSCARE): This scale was developed by Tubbs-Cooley et al. (2015) in order to measure the attitudes of nurses towards missed nursing care; and validity and reliability of its Turkish version were analyzed by Calikusu Incekar et al. (2020) (20,21). There are a total of 29 items of 5-likert type (5=always, 1=never and unapplicable) in the part A of the scale listing nursing activities. Data in this part are assessed with the sum of percentage values given to the scale statements. Part B consists of 17 items and three subscales including possible reasons of missed nursing care (labor resources, communication and material resources) and these items are graded as 4-likert type (4=important, 1=not important). Cronbach alpha coefficient of the scale is $\alpha = 0.91$. It was found to be 0.94 in this study.

2.4. Data collection

Data were collected by the researchers through face-to-face interviews in the nurse rooms of pediatric nurses working in the pediatric clinics of a university and public hospital within daily shift hours when they are available on January 2022. The completion of the data collection forms lasted for 10-15 minutes.

2.5. Assessment of data

Data were assessed by SPSS 21.0 statistical package program. Number, percentage, mean, standard deviation and median tests and Cronbach alpha coefficient test were used for descriptive statistics.

2.6. Ethical aspect of the study

The study was conducted after obtaining formal permissions for the study from Social Sciences and Humanities Research and Publication Ethics Committee (No:2021/900, Date: 11.26.2021). The nurses were informed about the purpose of the study, their questions were answered and their consents were taken. The authors of the original scale were asked for permission to use it through e-mail. The study was conducted in accordance with the principles of Helsinki Declaration.

3. Results

40.5% of the nurses were aged between 31- 40 years old; 96.7% were females; 81% were married; 80.2% were undergraduates and 52.1% were working in the public hospital. It was also determined that 47.9% of the participants had a professional experience between 11-20 years, 37.2% were working in the neonatal units, 86% were working during both day and night and 66.9% were working for 40 hours per week. Moreover, it was found that 40.5% of the nurses were providing care to more than 6 patients per nurse and the number of nurses were insufficient in the units where 86.8% of the nurses were working (Table 1).

Since participants provided no responses to "never and inapplicable" and "always" options of the scale, the rates of missed nursing care needs given to the options as "rarely, sometimes and often" were shown in Table 2. According to these, the most common missed care needs were identified as "administration of drug 30 minutes before or after the specified time" at a ratio of 53.8%, "providing oral care" at a ratio of 34.7% and "providing emotional support to the child and/or family" at a ratio of 33.9% (Table 2).

It was found that mean total MISSCARE score of the participants was 2.35±0.68, median value was 2.37, the lowest score was 1 and the highest score was 4. Mean scores of the subscales were found to be 2.75±0.79 in Labor Resources, 2.12±0.74 for Material Resources and

2.05±0.77 in Communication/Teamwork, respectively (Table 3).

Table 1: Descriptive characteristics of the nurses

X̄±Sd (Min-Max) **Duration of experience in the institution:** 12.88±7.86 (1-30) **Duration of experience in the pediatric clinic:** 12.29±7.79 (1-30)			
Characteristics		**Number (n)**	**Percentage (%)**
Age **X̄±Sd (Min-Max)** 37.52±7.73 (23-57)	20-30 years old	32	26.4
	31- 40 years old	49	40.5
	41 years old and more	40	33.1
Sex	Female	117	96.7
	Male	4	3.3
Marital status	Married	98	81.0
	Single	23	19.0
Educational status	Associate degree	13	10.7
	Undergraduate	97	80.2
	Master degree	11	9.1
Working institution	Public Hospital	63	52.1
	University Hospital	58	47.9
Duration of professional experience **X̄±Sd (Min-Max)** 16.02±8.28 (2-35)	1- 10 years	36	29.8
	11- 20 years	58	47.9
	21 years and more	27	22.3
Current working unit	Neonatal units	45	37.3
	Pediatric services	52	42.9
	Pediatric intensive care units	24	19.8
Working pattern	Only day	17	14.0
	Mixed (day+night)	104	86.0
Average weekly working hours	40 hours per week	81	66.9
	More than 40 hours per week	40	33.1
The number of patients per nurse in the current working unit	3-4 patients	44	36.4
	4-5 patients	28	23.1
	More than 6 patients	49	40.5
The status of the number of nurses working in the current unit	Sufficient	16	13.2
	Insufficient	105	86.8

Table 2: The reports of the nurses for the frequency of missed care during the last shift

	Rarely		**Sometimes**		**Often**	
	n	%	n	%	n	%
1. Attending daily bedside visits	26	21.5	8	6.6		
2. Raising and walking a child for three times a day if clinical conditions allow or in line with the nursing care plan	29	24.0	11	9.1		
3. Evaluating the efficacy of drugs administered	25	20.7	3	2.5		
4. Passive mobilization of the child every two hours or on request	26	21.5	14	11.6		
5. Providing oral care	29	24.0	13	10.7		
6. Including parents in the child's care	30	24.8	10	8.3		
7. Patient and family training	21	17.4	7	5.8		
8. The interview with the child and family regarding discharge plan and care at home	14	11.6	4	3.3		
9. Supporting neuro-evolutionary development of the baby based on age and clinical condition (for instance; neonatal care, cognitive and relational development of the child and adolescent)	24	19.8	13	10.7	2	1.7
10. Managing pain with pharmacological or non-pharmacological care approaches based on the protocol	21	17.4	5	4.1	1	0.8
11. Making drug requests within fifteen minutes	15	12.4	18	14.9		
12. Recording all required nursing data completely	10	8.3	8	6.6		
13. Forwarding all relevant information during shift change or handover	11	9.1	6	5.0	1	0.8

14. Meeting nutritional needs of the children according to their clinical conditions (eg. supporting oral feeding and/or nutrition according to newborn's request, encouraging proper feeding according to personal taste)	11	9.1	4	3.3		
15. Administering the drug 30 minutes before and after the specified time (eg. administration between 7.30 and 8.30 pm while specified time is 8.00 pm)	40	33.1	25	20.7		
16. Helping the child, who needs to go to toilet, within five minutes when he/she requests (eg. going to toilet with the baby or providing suitable instruments if bed-bound)	17	14.0	13	10.7	1	0.8
17. Responding to call light, request for intervention or alarm within five minutes (eg. monitor, infusion pump, ventilator)	9	7.4	5	4.1		
18. Providing emotional support to the child and/or family	29	24.0	12	9.9		
19. Taking biological samples on request	9	7.4	7	5.8	1	0.8
20. Body hygiene and skin care	12	9.9	5	4.1		
21. Checking central and peripheral catheter site according to the protocol	8	6.6	7	5.8		
22. Central and peripheral catheter care according to the protocol	6	5.0	4	3.3		
23. Taking necessary measures for infection control according to the protocol (Personal Protective Equipment, disinfectation of the equipment, isolation, correct disposal of waste)	6	5.5	2	1.7		
24. Follow-up of solid and liquid input-output	7	5.8	6	5.0		
25. Evaluating vital findings according to nursing care plan	8	6.6	3	2.5		
26. Making repeated assessments as focused during the shift in order to evaluate improvement or worsening in the condition of child	11	9.1	4	3.3		
27. Washing hands	8	6.6	1	0.8		
28. Evaluating practices made by the accompanying person	20	16.5	12	9.9	1	0.8
29. Checking safety and hygiene of bedside equipment once in every shift or according to the protocol (eg. bed, bedside table, devices)	5	4.1	9	7.4		

Table 3: Descriptive statistics of total MISSCARE and its subscales

Subscales and items	$\bar{X}$	S.d.	Median	Min	Max.
Communication/ Teamwork- 7 items	2.05	0.77	2.14	1	4
Labor Resources-6 items	2.75	0.79	2.80	1	4
Material Resources-4 items	2.12	0.74	2.25	1	4
MISSCARE Total	2.35	0.68	2.37	1	4

4. Discussion

In the study, nurses working in pediatric and neonatal fields indicated the most common MNC activities as failure to administer drug on time and providing emotional support to the parents. Similarly, failing to administer drug on specified time was reported as the first one among their care activities missed at last shift in a study conducted with pediatric nurses in Turkey (21). In a systematic review including fourteen studies on missed care in pediatric and neonatal units, the most common MNC activities were observed as oral care, preparation for discharge and parent training (22). In their study including pediatric nurses working in the USA, Lake et al. (2017) found that more than half of the nurses did not fulfill at least one nursing care in the previous shift and the most common missed care activities were developing a care plan and providing emotional support to the patient (23). Gathara et al. (2020) determined in their study with neonatal nurses that MNC activities were identified as examination of the newborn, cord care and changing the position and assessing the skin of the baby undergoing phototherapy (24). In the study by Tubbs-Cooley et al. (2019), the most common MNC activities were found as oral hygiene for babies undergoing mechanical ventilation, routine baby care such as bathing and parent training about nutrition and care in the neonatal intensive care units (25). Also in another study performed in the neonatal units, nurses stated MNC activities as preparation of the baby and family for discharge and pain management (26). In addition, Lake et al. (2020) reported that 36% of the nurses working in the neonatal intensive care units in the USA could not fulfill at least one nursing intervention in the previous shift and among these, the most common ones were relaxing/consulting the patient and parent training (27). Again in their study with the nurses working in the neonatal intensive care units, Tubbs-Cooley, Pickler, Younger et al. (2015) indicated that continuing daily care routine, oral care, bathing, including parents in the care and parent training were missed (20). Moreover, in a study conducted with nurse groups working in the adult units, MNC acitivities were identified as ensuring mobilization of the patient and providing emotional support to the patient and acquaintances (2). In another study with the nurses working in the adult units in the UK, most of the participants (86%) reported that they missed one or more care due to the inadequacy of time in their last shifts and the most common MNC activities were relaxing the patients, communicating with them and training them (14). The results of this study were found to be compliant with the relevant data in the literature; and it can be interpreted as nurses give priority to time-sensitive care interventions (drug management, oral

care, etc.). Again in the study, their failure to provide emotional support to the child and parents may show that family-centered care can not be given adequately due to the presence of high number of patients per nurse.

In the study, the most common reason of MNC was workload and the least common one was communication. Similarly in many national and international studies, the reasons of MNC were found to be associated with workload (7,19,27-29). In the study by Lake, Staiger, Cramer, et al. (2020), high workload in NICU, high dependency level of the patient and bad working environment were found to be possible reasons of missed care (27). In a review, nurse-patient ratios, insufficient number of nurses and individual subjective nurse workload were found to be effective as MNC reasons (22). Furthermore, a study performed in the neonatal field determined the most common reasons of MNC as frequent interruptions experienced during the shift, emergency patients and unexpected increases in the number of patients (25). Lake et al. (2017) have reported that MNC are observed among more than half of the pediatric nurses; their working environment affects this fact; the possibility of missed nursing care is 40% lower in better working environments and each additional patient increases the probability of missed nursing care by 70% (23). Tubbs-Cooley, Pickler, Younger et al. (2015) indicated the most common missed nursing care activities in the neonatal intensive care units as interruption of care, emergency patient, unexpected increase in the number of patients and density in the working unit (20). Srulovici and Drach-Zahavy (2017) found that the number of patients and personal characteristics of the nurse affected the frequency of missed care (30). Also in another research carried out in Turkey, the reasons of MNC needs of the nurses were indicated as the inadequacy in the number of staff working in labor resources (2).

The study was based on self-reports of the pediatric and neonatal nurses; and direct observation or health records were not used to identify MNC.

Pediatric nurses reported administration of drug on time, oral care and providing emotional support to the child and parent as their missed care. They stated the reason of these missed care activities as their workload.

MNC is an important problem that needs to be overcome in order to create patient safety culture and to ensure the quality of nursing care. Therefore, it is highly crucial to recognize MNC clearly; and it is important to identify its reasons by using MISSCARE scale, reporting its results quickly and honestly, sharing them with the nurses and using root analysis method. Providing the necessary improvements by decreasing the workload of pediatric nurses and the number of patients per nurse in our country will reduce MNC activities by affecting the quality of both working environment of the nurse and patient care in a positive way.

Ethical Statement

The study was conducted after obtaining formal permissions for the study from Social Sciences and Humanities Research and Publication Ethics Committee (No:2021/900, Date: 11.26.2021).

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: U.K., N.U.B., E.T.B., Design: N.U.B., E.T.B., Data Collection or Processing: E.T.B., U.K., Analysis or Interpretation: N.U.B., E.T.B., Literature
Search: U.K., N.U.B., E.T.B., Writing: U.K., N.U.B., E.T.B.

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Investigation of the relationship between clinical severity of acne and inflammatory parameters in patients with acne vulgaris

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Abstract

Objective In this study, we investigated whether the Neutrophil /Lymphosite (N/L) ratio, mean platelet volume (MPV) value, GADS (The global acne rating system) score and other inflammatory parameters are related in acne vulgaris patients. Methods One-hundred acne patients, aged between 18–40 years, who treated at the Karaman Training and Research Hospital Dermatology Polyclinic between January and March 2022 were included in the study. Age, sex, duration of disease, medications and additional systemic diseases were recorded. Acne patients included in the study were tested for N/L ratio, erythrocyte sedimentation rate (ESR, mm/hr), C-reactive protein (hsCRP, mg/L), platelet count (PLT/L) and (MPV/FL). GADS scores calculated. Results There was a significant difference in ESR ($p < 0.001$) and CRP levels ($p = 0.002$) in patients with high GADS scores compared to those with low GADS scores. In all patients, the mean GADS score was 24.77 ± 11.00, and GADS scores were positively correlated with ESR and CRP levels. In our binary logistic regression analysis of age, sex, time, N/L ratio, PLT/L, MPV and ESR for identifying risk factors for severe acne vulgaris, the model explained 25.7% of high GADS scores. A high CRP level was identified as a risk factor for having a high GADS score. Conclusion In our study, we evaluated acne patients because it affects many age groups, is common, and sometimes causes extensive inflammation.

Keywords: acne severity, inflammatory parameters, inflammatory diseases, acne vulgaris

1. Introduction

Acne vulgaris is a chronic inflammatory condition involving the pilosebaceous unit. It is frequently encountered in dermatology practice and affects 80% of the population at some point in their lives (1). While 60% of acne cases regress with short-term and local treatments, acne in the remaining 40% may persist into adulthood (2). Inflammatory indicators, such as the Neutrophil/Lymphosite (N/L) ratio, are associated with disease activity and prognosis in many dermatological diseases (3-5). Mean platelet volume (MPV) is a marker of routine complete blood count, and it is a frequently used value to determine platelet function and activation (6,7). Recent studies have stated that MPV can be used as an indicator of inflammation in different inflammatory conditions (8-10). The global acne rating system (GAGS) is used to determine the clinical severity of acne. In this study, we investigated whether the N/L ratio, MPV value, GAGS score and other inflammatory parameters are related in acne vulgaris patients.

2. Material and Methods

One-hundred acne patients, aged between 18–40 years, who were treated at the Karaman Training and Research Hospital Dermatology Polyclinic between January and March 2022 were included in the study. The exclusion criteria were pregnancy, lactation, kidney or liver dysfunction, the use of medication to treat such conditions, a history of systemic inflammatory disease, and/or systemic immunosuppressive treatment within the prior month.Informed consent was obtained from all patients prior to their inclusion in the study. Age, gender, duration of disease, medications and additional systemic diseases were recorded. Patient identification information was kept confidential. Ethical approval was obtained from the Non-Interventional Research Ethics Committee of Fırat University Rectorate (approval dated 05.01.2022, numbered 130133).

2.1. Lab tests

Acne patients included in the study were tested for N/L ratio, erythrocyte sedimentation rate (ESR, mm/hr), C-reactive protein (hsCRP, mg/L), platelet count (PLT/L) and mean platelet volume (MPV/FL) after 8 hrs of fasting. Serum hsCRP levels were determined using immunoturbidimetry.

2.2. Global Acne Assessment

In this scoring system, the upper parts of the face, chest, and back are divided into six parts. In addition, a coefficient for each region (chest and upper back = 3; forehead, right and left cheek = 2; nose and chin = 1) is assigned, which considers the region's width as well as the density and distribution of pilosebaceous units. Acne lesions are also graded between 0–4

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according to their type (no lesion = 0, ≥ 1 comedone = 1, ≥ 1 papule = 2, ≥ 1 pustule = 3, ≥ 1 nodule = 4). Each region is evaluated separately, and a score is determined for each region by multiplying the score according to the most severe lesion type in that region and the coefficient of that region; the global acne score is calculated as the sum of the scores of the six regions. Total scores range from 0 to 44, and acne severity is determined according to the GAGS score (0 points = no acne, 1–18 points = mild acne, 19–30 points = moderate acne, 31–38 points = severe acne, > 39 points = very severe acne) (11). In our study, patients with a GAGS score below 30 were classified as having mild to moderate acne, and those with a GAGS score above 30 were classified as having severe acne.

2.3. Statistical Analysis

Data analyses were performed with the SPSS Statistical Package, version 22.0 (IBM, Armonk, NY). For categorical data, the chi-square test was used, and data were summarised as frequency and percentage for each category. Numerical data were evaluated for normal distribution using the Kolmogorov-Smirnov test, and it was found that the data were not normally distributed. Therefore, the Mann-Whitney U test and Spearman's rank correlation coefficient were used. Binary logistic regression was used to determine the effective factors in determining the GAGS score. A p-value ≤ 0.05 was considered statistically significant.

3. Results

Of the 100 acne patients, 60 were women and 40 were men; mean age was 24.37 ± 7.74 years. In the mild to moderate GAGS group, 63.5% of the 33 patients were female, and the mean age of this group was 24.29 ± 8.05 years (Table 1).

Table 1. Age, gender, and time distributions of acne patients with low to high GAGS scores

		GAGS low	GAGS high	Total	p
Sex	Female	%63.5 (33)	%56.3 (27)	%60.0 (60)	0.462[a]
	Male	%36.5 (19)	%43.7 (21)	%40.0 (40)	
Age		24.29±8.05	24.46±7.47	24.37±7.74	0.777[b]
Time (year)		3.96±2.76	4.02±2.48	3.99±2.61	0.673[b]

[a]ChiSquare, [b]Mann Whitney U

Table 2. Comparison of laboratory data of patients with low and high GAGS values

	GAGS low	GAGS high	All patients	p
N/L	1.86±.67	2.036±0.79	1.94±0.73	0.315[b]
PLT/L	114.94±29.76	125.15±36.67	119.84±33.48	0.164[b]
MPV	9.22±.88	9.404±0.87	9.31±0.88	0.479[b]
ESR	7.27±12.07	10.71±7.86	8.92±10.36	**<0.001[b]**
CRP	2.40±3.05	7.371±7.51	4.788±6.15	**0.002[b]**

[a]ChiSquare, [b]Mann Whitney U

There was a significant difference in ESR ($p < 0.001$) and CRP levels ($p = 0.002$) in patients with high GAGS scores compared to those with low GAGS scores (Table 2). In all patients, the mean GAGS score was 24.77 ± 11.00, and GAGS scores were positively correlated with ESR and CRP levels (Table 3).

Table 3. Correlations between GAGS Score and Other Variables in Acne Vulgaris Patients

		Age	Time	GAGS	N/L	PLT/L	MPV	ESR	hsCRP
Age	r	1							
	p								
Time	r	.580**	1						
	p	<.001							
GAGS	r	-.018	.036	1					
	p	.855	.725						
N/L	r	-.097	-.036	.110	1				
	p	.337	.722	.275					
PLT/L	r	.013	.022	.151	**.555****	1			
	p	.897	.825	.135	**.000**				
MPV	r	-.108	.119	.191	.077	-.159	1		
	p	.283	.238	.057	.446	.113			
ESR	r	.028	.115	**.364****	**.316****	**.327****	.056	1	
	p	.779	.256	**.000**	**.001**	**.001**	.582		
CRP	r	.184	**.208***	**.276****	**.212***	.186	-.034	**.538****	1
	p	.067	**.038**	**.005**	**.035**	.063	.735	**<.001**	

r= correlation coefficient * $p \leq 0.05$ (2-tailed), ** $p \leq 0.01$ (2-tailed)

In our binary logistic regression analysis of age, sex, time, N/L ratio, PLT/L, MPV and ESR for identifying risk factors for severe acne vulgaris, the model explained 25.7% of high GAGS scores (Nagelkerke R squared = 0.257; Omnibus Tests of Model Coefficients, $p = 0.006$; Hosmer and Lemeshow test, $p = 0.446$). A high CRP level was identified as a risk factor for having a high GAGS score (Table 4).

Table 4. Risk factors for high GAGS scores.

	B	p	OR	95% CI for OR	
				Lower	Upper
Age	0.004	0.914	1.004	0.934	1.079
Sex (ref=Female)	0.280	0.564	1.323	0.510	3.431
Time (year)	-0.030	0.763	0.970	0.796	1.182
N/L	0.120	0.767	0.887	0.402	1.958
PLT/L	0.005	0.569	1.005	0.988	1.023
MPV	0.370	0.174	1.448	0.849	2.470
ESR	-0.012	0.659	0.988	0.938	1.042
hsCRP	0.188	**0.001**	1.207	1.078	1.352

4. Discussion

The main organ targeted in acne is the pilosebaceous follicle. Pathophysiological factors that play a role in acne formation include increased sebum production, abnormal follicular desquamation, Propionibacterium acnes colonisation and inflammation. Until recently, abnormalities in follicular desquamation responsible for the formation of microcomedones and hyperseborrhea were considered to be the most important of these factors. However, studies conducted in recent years have focused on the role of sebaceous lipids and inflammatory mediators, and it has been shown that immune changes and inflammatory responses occur in the early period before ductal hyperproliferation develops (2,12,13). Of the 100 acne patients included in our study, 60 were female and 40 were male. The mean age of the patients was 24.37 ± 7.74 years, and the mean ages of the mild to moderate and severe acne groups were comparable. These values are similar to those reported in the literature (14). hsCRP and ESR values are frequently used markers in the evaluation of acute phase response, due to their reliability and cost-effectiveness (15,16). In our study, the ESH and hsCRP values of patients with high GAGS scores were found to be significantly higher than those with low GAGS scores. Similar to our study, Namazi et al (17) compared a mild to moderate acne group with a severe acne group, and the mean hsCRP values were found to be significantly higher in the severe group (3.006 and 1.217 mg/l, respectively). It has been previously stated that hsCRP is a practical indicator used to evaluate the severity of cutaneous inflammation in acne (17). Some prospective epidemiological studies have indicated that, in addition to hsCRP being an inflammatory marker, high hsCRP levels are a strong indicator of cardiovascular risks (18,19). Since the skin is the largest organ in the body, inflammatory dermatological conditions have the potential to trigger systemic inflammation. MPV is studied as a part of routine complete blood count testing and is a common marker used to show platelet function and activation. In recent years, it has been reported that MPV can also be used as an inflammatory marker in different diseases (6,7). Different studies have reported that MPV has either a positive or negative correlation with inflammatory activity (8,9). In addition, there are studies investigating the effects of inflammatory diseases on haematopoiesis, and these effects are clinically observed most often in cases of anaemia and thrombocytosis. Anaemia and severe thrombocytosis have been found to be associated with some inflammatory diseases, and it has been shown that thrombocytosis may occur through proinflammatory cytokines and some growth factors (20-23).Neutrophils are activated by enzymes such as myeloperoxidase, elastase, and acid phosphatase. Circulating leukocyte rates vary based on inflammatory reaction states. Relative to neutrophilia, lymphopenia may occur. In the literature, it has been revealed that the N/L ratio is a prognostic marker in many important conditions such as cardiovascular diseases, diabetes mellitus, hypertension, and cancer (24). The PLT/L ratio is a parameter that can show inflammation more sensitively than the N/L ratio. Thrombocytosis is considered to be a negative prognostic factor in some cancer cases. An increased platelet count or an increase in the ratio of platelets to lymphocytes can be explained by the inflammatory process caused by neoplastic cells (25,26). Topal et al (26) stated that the number of lymphocytes may decrease with age. In the same study, it was emphasised that the use of the PLT/L ratio as a marker for inflammation varied between age groups. In our study, the MPV, N/L and PLT/L ratios of patients with mild to moderate acne and those with severe acne did not differ. We believe that this may be related to the low mean age of our patient group and the duration of disease. Acne is a condition that can continue long into adulthood, and it can become chronic and cause systemic inflammation (27,28). In our study, we evaluated acne patients because acne affects all age groups, is common, and sometimes causes intense inflammation.To evaluate the relationship between acne severity and inflammation, future studies with larger sample populations are needed.

The main organ targeted in acne is the pilosebaceous follicle. Pathophysiological factors that play a role in acne formation include increased sebum production, abnormal follicular desquamation, Propionibacterium acnes colonisation and inflammation. Until recently, abnormalities in follicular desquamation responsible for the formation of microcomedones and hyperseborrhea were considered to be the most important of these factors. However, studies conducted in recent years have focused on the role of sebaceous lipids and inflammatory mediators, and it has been shown that immune changes and inflammatory responses occur in the early period before ductal hyperproliferation develops

(2,12,13). Of the 100 acne patients included in our study, 60 were female and 40 were male. The mean age of the patients was 24.37 ± 7.74 years, and the mean ages of the mild to moderate and severe acne groups were comparable. These values are similar to those reported in the literature (14). hsCRP and ESR values are frequently used markers in the evaluation of acute phase response, due to their reliability and cost-effectiveness (15,16). In our study, the ESH and hsCRP values of patients with high GAGS scores were found to be significantly higher than those with low GAGS scores. Similar to our study, Namazi et al (17) compared a mild to moderate acne group with a severe acne group, and the mean hsCRP values were found to be significantly higher in the severe group (3.006 and 1.217 mg/l, respectively). It has been previously stated that hsCRP is a practical indicator used to evaluate the severity of cutaneous inflammation in acne (17). Some prospective epidemiological studies have indicated that, in addition to hsCRP being an inflammatory marker, high hsCRP levels are a strong indicator of cardiovascular risks (18,19). Since the skin is the largest organ in the body, inflammatory dermatological conditions have the potential to trigger systemic inflammation. MPV is studied as a part of routine complete blood count testing and is a common marker used to show platelet function and activation. In recent years, it has been reported that MPV can also be used as an inflammatory marker in different diseases (6,7). Different studies have reported that MPV has either a positive or negative correlation with inflammatory activity (8,9). In addition, there are studies investigating the effects of inflammatory diseases on haematopoiesis, and these effects are clinically observed most often in cases of anaemia and thrombocytosis. Anaemia and severe thrombocytosis have been found to be associated with some inflammatory diseases, and it has been shown that thrombocytosis may occur through proinflammatory cytokines and some growth factors (20-23).Neutrophils are activated by enzymes such as myeloperoxidase, elastase, and acid phosphatase. Circulating leukocyte rates vary based on inflammatory reaction states. Relative to neutrophilia, lymphopenia may occur. In the literature, it has been revealed that the N/L ratio is a prognostic marker in many important conditions such as cardiovascular diseases, diabetes mellitus, hypertension, and cancer (24). The PLT/L ratio is a parameter that can show inflammation more sensitively than the N/L ratio. Thrombocytosis is considered to be a negative prognostic factor in some cancer cases. An increased platelet count or an increase in the ratio of platelets to lymphocytes can be explained by the inflammatory process caused by neoplastic cells (25,26). Topal et al (26) stated that the number of lymphocytes may decrease with age. In the same study, it was emphasised that the use of the PLT/L ratio as a marker for inflammation varied between age groups. In our study, the MPV, N/L and PLT/L ratios of patients with mild to moderate acne and those with severe acne did not differ. We believe that this may be related to the low mean age of our patient group and the duration of disease. Acne is a condition that can continue long into adulthood, and it can become chronic and cause systemic inflammation (27,28). In our study, we evaluated acne patients because acne affects all age groups, is common, and sometimes causes intense inflammation.To evaluate the relationship between acne severity and inflammation, future studies with larger sample populations are needed.

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Ethical Statement

Ethical approval was obtained from the Non-Interventional Research Ethics Committee of Fırat University Rectorate (approval dated 05.01.2022, numbered 130133).

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: K.T.K., H.M.A., Design: K.T.K., Data Collection or Processing: K.T.K., H.M.A., Analysis or Interpretation: K.T.K., H.M.A., Literature Search: K.T.K., H.M.A., Writing: K.T.K., H.M.A.

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Oral cancer knowledge and awareness of dentists in Northern Cyprus: A survey study

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Abstract

Oral and oropharyngeal cancers have been recognized as a major global health problem and are the sixth most common cancers in the world, with increasing rates of incidence and mortality. Dentists have an important role and responsibility in the early diagnosis of oral cancer. This study aimed to assess knowledge, awareness, and attitudes regarding oral cancer among dentists in North Cyprus. This study was conducted via a questionnaire that was distributed to the dentists in different provinces of Northern Cyprus. The questionnaire comprised 19 main items about the demographic information, dentist's oral cancer knowledge, attitude, opinions, and practices. The study consisted of 120 dentists and an overall response rate of 81.6% (98) was achieved. Nearly, all respondents were knowledgeable about tobacco use (98%) and previous oral cancer history (98%). The percentage of correct answers to questions concerning knowledge about risk factors of oral cancer ranged from 4.1% (family history of cancer) to 98%. Two-thirds of the participants pointed out correctly that the tongue and floor of the mouth are the most affected sites for oral cancers and leukoplakia and erythroplakia are two lesions most commonly correlated with oral cancer. However, 47% of participants were able to identify squamous cell carcinoma as the most frequent form of oral cancer. In conclusion, dental faculty curriculums and continuing education programs should aim at managing oral cancer need to address not only increase the knowledge and awareness about oral cancers but also include the clinical cases and practices.

Keywords: awareness, knowledge, oral cancer, dentist, North Cyprus

1. Introduction

One of the worst diseases known as cancer has made a huge impact on public health, economies, and social aspects all over the world. Cancer is the abnormal growth and differentiation of cells and can arise from any organ or body structure. It is usually detected at a late stage or maybe incidentally diagnosed during routine laboratory tests or radiographic imaging performed for different reasons (1).

Oral and oropharyngeal cancers have been recognized as a major global health problem and are the sixth most common cancers in the world, with increasing rates of incidence and mortality (2). The geographic differences in the prevalence of lip and oral cavity cancers have been reported and it turns out to be a highly frequent incidence in Southern Asia as well as the Pacific Islands (3).

Many factors may play a role in the etiology of oral cancers however primary risk factors undoubtedly include smoking and alcohol consumption in patients over the age of 45 years (4). Other important risk factors are immunodeficiency, diet, and nutrition, socio-economic status, exposure to the sun, infection with human papillomavirus (HPV), and chronic trauma of the oral mucosa. Furthermore, potentially malignant disorders (PMDs) such as erythroplakia, non-homogeneous leukoplakia, erosive lichen planus, oral submucous fibrosis, and actinic keratosis, are known to harbor the development of oral cancers (5).

The most common initial site for oral cancer is the tongue in European, North American, and Asian countries, accounting for approximately 40-50% of all oral malignancies. Buccal mucosa in addition to gingiva is the second common location due to the betel nut and/or smokeless tobacco use among Asian populations and followed by the lip, floor of the mouth, hard and soft palate, and tonsils (6). Overall 90% of oral cancers are squamous epithelium in origin which results in oral squamous cell carcinomas (7).

Although the oral cavity is an easily identifiable area, early detection is not always possible due to the absence of pain, in the beginning, lack of the patient's awareness and dentist's knowledge, and more than half of the oral cancers are diagnosed in an advanced stage. The recognition and the early detection of oral cancer can lead to decreasing mortality and reducing morbidity related to the treatment (8). Patients with possible oral lesions or with potentially malignant disorders are

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often assessed first by general dental practitioners. However, the main reason for the lack of accurate and early diagnosis of oral cancers is an insufficient level of awareness. Dental practitioners must perform a thorough oral cancer examination as part of their clinical practice and should be aware of the early clinical signs and symptoms occurring with malignancy (9). This study is an attempt to determine the knowledge, attitude, and practices of dentists for the prevention and early diagnosis of oral cancers in the Turkish population of Cyprus.

2. Materials and Methods

2.1. Study design

This study was conducted via a questionnaire that was distributed to the general dental practitioners (%39.8) and specialist dentists (%60.2) in different provinces of Northern Cyprus. The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the Near East University (IRB approval number: YDU/89-1313).

2.2. Study Questionnaire

The questionnaire was designed and translated to the Turkish language following similar previous studies about oral cancer knowledge (9-13). All the dentists, who were registered Chamber of Turkish Cypriot Dentists, were invited to participate in the study. Altogether 120 dentists received the questionnaire. 98 out of 120 dentists accepted to participate in the study and two researchers (MM, MF) were personally surveyed in their offices or work station. The questionnaire comprised 19 main items and several lower ones. The first section included information about the participant's demographic information such as gender, age, number of years since graduation, nationality, working site, and level of education.

The next section contained 15 main questions and their sub-questions, about the dentist's oral cancer knowledge, attitude, opinions, and practices. Nine general knowledge questions were about the oral cancer risk factors, potentially malignant disorders, most common forms of oral cancer, most frequent age of diagnosis, most common sites of oral cancer, symptoms of both early, late stage of oral cancer, and the features of oral cancer metastases. The next three questions included the most commonly mentioned and reported symptoms by a patient with early stage of oral cancer, the characteristic features of the lymph node invasion, and related aspects of the medical history of oral cancer patients. Finally, in the last part, questions were associated with the attendance at oral cancer continuing education courses, and regarding their attitude and behavior about oral cancer patients.

All data entered into Microsoft Excel (Microsoft Corporation, Redmont, WA, USA), and then transferred into the SPSS program (version 22.0; IBM Corp., Armonk, NY, USA). A Chi-square test was used to compare descriptive statistics. Significance was assessed at $p < 0.05$ level.

3. Results

This cross-sectional study analyses the answers given by participating dentists in North Cyprus regarding early detection of oral cancer. The study consisted of 120 dentists and an overall response rate of 81.6% (98) was achieved. Among 98 dentists aged between 23 and 70 years, there were 49 (50%) female dentists and 49 (50%) male dentists. 60.2% percent of the participants were general dentists, while the remaining (39.8%) were specialists (Table 1).

Table 1. Demographic characteristics of participants

Variables	No. (%)
Gender	
Male	49 (50%)
Female	49 (50%)
Level of education	
Dental practitioners	59 (60.2%)
Specialist dentist	39 (39.8%)

To explore participant's knowledge, all dentists were asked about risk factors for oral cancers including tobacco use, alcohol use, previous history of oral cancer, family history of cancer, prolonged sunlight exposure, viral infections, bad oral hygiene, poorly fitting dentures, head and neck radiotherapy, chronic infection, being over 60 years of age, constant consumption of hot food and beverages, obesity, low consumption of fruit and vegetables and frequent consumption of spicy food. Nearly, all respondents were knowledgeable about tobacco use (98%) and previous oral cancer history (98%). The percentage of correct answers to questions concerning knowledge about risk factors of oral cancer ranged from 4.1% to 98% as shown in (Fig. 1). An item with the lowest percentage (4.1%) included knowing that a family history of cancer is not a risk factor for oral cancers. Only 37.8% and 59.2% of the participant correctly identified the low consumption of fruit and vegetables and older age as a risk factor respectively. However, 16.3% and 19.4% of the respondents, respectively, recognized bad oral hygiene and poorly fitting dentures as non-risk factors (Fig. 1).

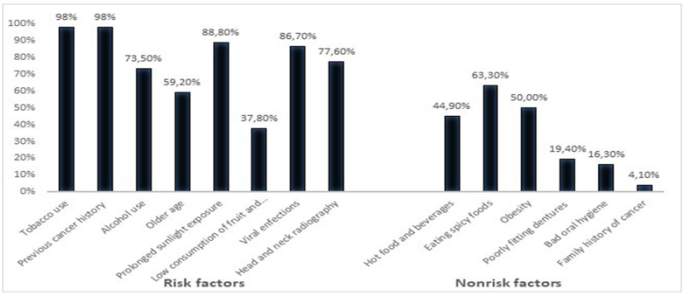

Fig 1. Knowledge about oral cancer risk factors of participants

67.4% of dentists pointed out correctly that the tongue and floor of the mouth are the most affected sites for oral cancers. However, approximately 47% of participants were able to identify squamous cell carcinoma as the most frequent form of oral cancer. When the participants were asked about two lesions most possible to be correlated with oral cancer, 69.4% of the respondent correctly recognized the leukoplakia and erythroplakia (Table 2).

Table 2. Distribution of the answers regarding clinical features of oral cancer

Variables		Percent
What is the most common type of oral cancer?	Squamous Cell Carcinoma	46.9%
	Lymphoma	7.1%
	Basal Cell Carcinoma	10.2%
	Adenocarcinoma	6.1%
	Kaposi Sarcoma	5.1%
	Do not know	24.5%
What is the most common site affected by the oral cancer excluding lip?	Tongue and floor of the mouth	67.4%
	Buccal mucosa	11.2%
	Soft palate	9.2%
	Gingiva	4.1%
	Tonsils	1%
	Do not know/Not sure	7.1%
What is the most age group affected by the oral cancer?	Under 18 years	0%
	Between 18 and 39 years	9.2%
	Between 40 and 59 years	39.8%
	Above 60 years	32.7%
	Do not know/Not sure	18.3%
What is the clinical properties of a prior oral cancer lesion?	Small, painful, white area	8.2%
	Small, painless, white area	44.9%
	Small, painful, red area	11.2%
	Small, painless, red area	27.6%
	Do not know/Not sure	8.2%
What is the most frequent clinical stage in which oral cancer is diagnosed?	Premalign	25.5%
	Initial/Local	35.7%
	Advanced/Metastasis	16.2%
	Do not know/Not sure	22.4%
Which symptoms most commonly expressed in early oral cancer?	Pain	7.1%
	Ulceration	49%
	Swelling	6.1%
	None/Asymptomatic	33.7%
	Do not know/Not sure	4.1%
Where is the most common site of distant metastasis of oral cancer?	Bone	24.1%
	Skin	22.9%
	Lung	42.2%
	Liver	10.8%
Which of the following two conditions are commonly related to the development of oral cancer?	Erythroplakia/Morbus Bowen	5.9%
	Leukoplakia/Erythroplakia	69.4%
	Blue Nevus/Leukoplakia	20%
	Morbus Bowen/Blue Nevus	4.7%
Lip cancers;	are related to sun exposure	59.2%
	are increasing each year	9.2%
	have a worse prognosis than most oral cancers	6.1%
	affect upper lip more frequently than the lower lip	5.1%
	have not been related to any form of tobacco use	2%
	Do not know/Not sure	18.4%
Which of the following findings in the neck lymph nodes indicate that oral cancer has metastasized?	Hard/Painful/Mobile	17.3%
	Hard/Painless/Fixed	50%
	Soft/Painful/Mobile	3.1%
	Soft/Painless/Fixed	8.2%
	Do not know/Not sure	21.4%

When the dentists were asked “Which of the following do you assess while taking a medical history of oral cancer patients?", more than %90 of them responded that they evaluate “present use of tobacco, history of cancer, previous use of tobacco, patient’s family history of cancer” (Table 3). Dental congresses were found to be the main source of oral cancer knowledge with 62.1 percent, followed by the textbooks (47.4%) (Table 4).

Table 3. Patient’s health history assessment by the dentist

	Yes	No
Patient’s current use of tobacco	94.9%	5.1%
Patient’s current use of alcohol	81.6%	18.4%
Patient’s history of cancer	95.8%	4.2%
Patient’s previous use of tobacco	91.8%	8.2%
Patient’s previous use of alcohol	72.4%	27.6%
Patient’s family history of cancer	92.8%	7.2%
Type&amount of alcohol used	71.4%	28.6%
Type&amount of tobacco	86.7%	13.3%
Diet type	69.4%	30.6%
Sun exposure	82.7%	17.3%

Table 4. Source/sources of oral cancer knowledge

	Yes	No
Educational courses	12.6%	87.4%
Scientific journals	42.1%	57.9%
Textbook	47.4%	52.6%
Dental congresses	62.1%	37.9%

Table 5 presents the breakdown of the participants’ answers to the questions about the attitudes towards oral cancer. While 37.7% of dentists strongly agreed/agreed that their knowledge about oral cancer is up to date, 93.9% reported they need further education. Only 10.2% of the respondents declared that their patients are sufficiently informed on risk factors for oral cancer and know the signs and symptoms of oral cancer. Answers regarding their role in diagnostic procedures showed that 59.2% of dentists either strongly agreed or agreed that they perform biopsy from the suspicious oral lesions.

Table 5. Attitude towards oral cancer of the participants

	Strongly agree	Agree	Uncertain	Disagree	Strongly disagree
I have a piece of updated knowledge about oral cancer	11.2%	26.5%	28.6%	23.5%	10.2%
Oral cancer examinations for those 40 years of age and older should be provided annually	55.1%	38.8%	5.1%	1%	0%
Oral cancer examinations for adults 18-39 years of age should be provided annually	34.7%	41.8%	14.3%	8.2%	1%
I am comfortable referring patients with suspicious oral lesions to specialists	87.8%	10.2%	2%	0%	0%
My patients are sufficiently informed on risk factors for oral cancer	1%	9.2%	25.5%	27.6%	36.7%
My patients sufficiently know the signs and symptoms of oral cancer	1%	9.2%	25.5%	27.6%	36.7%
Dentists are adequately trained to examine patients for oral cancer	20.4%	32.7%	28.6%	12.2%	6.1%
Doctors are adequately trained to examine patients for oral cancer	18.4%	33.7%	34.7%	10.1%	3.1%
Early detection improves 5-year survival rates from oral cancers	58.2%	29.6%	11.2%	1%	0%
Lesions associated with chewing tobacco resolve after tobacco cessation	7.1%	15.3%	48%	13.3%	16.3%
I routinely perform lymph node palpation in addition to oral cavity examination	17.3%	34.7%	16.3%	12.2%	19.4%
I perform biopsy from my patients with suspicious oral lesions	25.5%	33.7%	12.2%	6.2%	22.4%
I am adequately trained to perform an oral cancer examination	21.4%	26.5%	27.6%	12.2%	12.2%
I am adequately trained to perform patient’s lymph node palpation	26.5%	37.8%	19.4%	8.1%	8.2%
I advise my patients with suspicious oral lesions	71.4%	27.6%	1%	0%	0%
Dentists need further training to perform an oral cancer examination	69.4%	24.5%	3.1%	1%	2%
I am sure, I can diagnose oral cancer from the clinical appearances	12.2%	28.6%	41.8%	6.2%	11.2%
The dentist should inform their patients of their findings	78.6%	19.4%	2%	0%	0%

The 98 participants who participated in the questionnaire were grouped according to the number of correct answers which is shown in Fig. 2. According to the table, the level of the participants who answered between 0-9 is determined as low level and there is 1 dentist in this situation, the level of the participants who answered between 10-18 is determined as intermediate level and there are 74 dentists in this range, the level of the participants who answered between 19-27 is determined as high level and there are 23 dentists in this range.

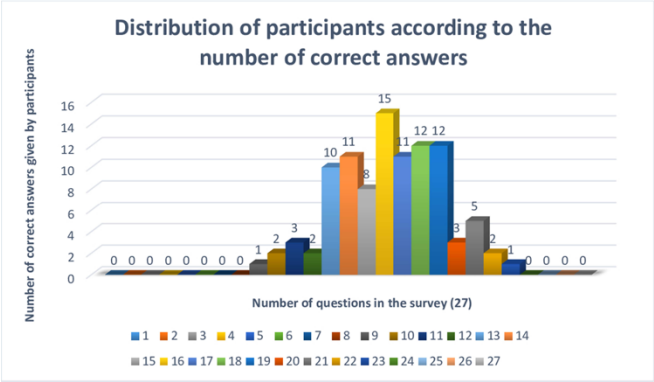

Fig 2. Distribution of participants according to the number of correct answers

The percentage calculation of the participants grouped as low, intermediate and high level is shown in Fig. 3. It was determined that 1.02 percent of the respondents were low level, 75.51 percent were intermediate level and 23.47 percent were high level.

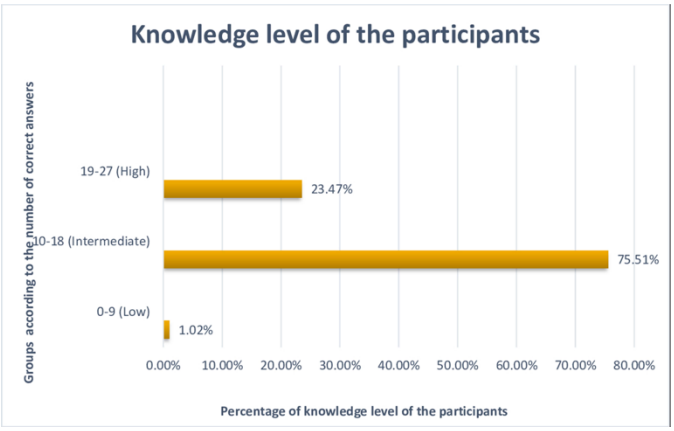

Fig 3. Knowledge level of the participants

4. Discussion

Dentists' awareness about symptoms, risk factors, and attitudes have a significant impact on the early detection of oral cancers to reduce high morbidity and mortality rates and improves the quality of life of the patient. Thus, measuring and understanding the dental practitioners' knowledge is of utmost importance. It would also help in developing postgraduate educational strategies. Many studies have documented the oral cancer awareness and knowledge level in a variety of participant groups from different countries (9-15). To the best of our knowledge, this is the first study that providing an overview of the current status of knowledge and skills regarding oral cancer among practicing dentists in North Cyprus.

In this study, a majority of the dentists rightly identified tobacco (98%) and alcohol (73.5%) use as the major risk factors for oral cancer. In a recent research, dentists identified tobacco (98.8%) and alcohol (95.3%) misuse as the major risk factors for oral cancer (13). Colella et al. similarly observed that 94.1% of dentists indicated tobacco usage, 79.2% of dentists indicated alcohol usage, and 89.5% of dentists indicated prior oral cancer lesion as risk factors (16). These findings are also consistent with the report on Kuwait (17), India (18) and Yemen (19).

In a study conducted by Negri et al reported that smoking, alcohol, and low β-carotene intake were responsible for 85-88% of oropharyngeal cancers in both genders in Northern Italy (20). Similar to this study Rodriguez et al showed that a combination of tobacco, alcohol, and low vegetable consumption were responsible for 85% of the oral and pharyngeal cancers in young adults (21). Frequent intake of fresh vegetables and fruits was inversely related to oral cancer risk. Also, Sánchez et al reported that the high intake of fruit and vegetables has a protective effect and reduced the risk of this neoplasm among current smokers and heavy alcohol drinkers (22). According to the results of our study, most dentists knew prolonged sunlight exposure, head-neck radiotherapy and previous oral cancer lesions as the main risk factors; on the other hand, low intake of fruit and vegetables was the least likely to associate with oral cancer in previous studies (11,12,15). Consistent with the literature, this research found that the low consumption of fruit and vegetables is the least known risk factor and most of the respondents were unaware of this link.

Second lower awareness level was determined in older age which is the strongest risk factor as well as tobacco and alcohol consumption. Nearly two-fifths of the present study participants could not identify older age as a risk factor. This finding is following the Keser and Pekiner, Kebabcıoğlu and Pekiner, and Decuseara et al. (10, 13, 23) Moreover, participants exhibited relatively better knowledge of oral cancer risk factors than non-risk factors. Family history of cancer (95.9%), bad oral hygiene (83.7%), and poorly fitting dentures (80.6%) were established as risk factors for oral cancer by a large number of dentists. Although tooth decay, bad oral hygiene, and ill-fitting dentures are not in themselves a relevant risk factor for oral cancer, there is no consensus about this issue in the literature. Despite adjustments for major known risk factors (tobacco and alcohol consumption), Moraes et al. found a statistically significant relationship between the extent and severity of chronic periodontitis and oral cancer (24). However, Meurman in a review study concluded that although specific bacterial, yeast, and viral infections associated with oral cancer and infectious agents associated both within the tumor cells and inflammation, the mechanism is not clear and the literature results are controversial (25). In

another review study, Colonia-Garcia et al reported that currently, the available evidence is limited and inadequate to derive definite conclusions about the role of the periodontal disease in oral cancer (26).

Two most common site of oral cancer was correctly identified as 'tongue and floor of the mouth' by 67.4% of the dentists. However, another diagnostic item, 'squamous cell carcinoma (SCC) is the most common type of oral cancer', which showed a low level of knowledge, was determined by only 46.9% of the respondents. In a study conducted with dentists in the Democratic Republic of Congo, it was known with a very low percentage that squamous cell carcinoma is the most common type of oral cancer, similar to ours (27). Similarly in Kuwait (17), Yemen (19), Iran (28), and Turkey (13) the majority of the dentists knew that floor of the mouth and tongue are at greatest risk of cancer development. The biological behavior of oral cavity cancer relies on the size, site, patient age, stage, and rate of growth as well as the tumor type among which site and tumor type are extremely important. The ventral surface of the tongue with the floor of the mouth (also known as coffin's corner) which is the common site of oral cancer development may easily be missed by cursory inspection (29). However, SCC in this region may be very aggressive and tend to invade and metastasize even if at an early stage (30, 31). Especially in young patients, tongue SCC has a worse prognosis and a high rate of metastasis (32,33).

Oral erythroplakia and leukoplakia are the most common oral potentially malignant disorders (OPMDs) in the oral cavity and are considered to have a high risk of malignant transformation. In a study of dentists attending the FDI World Dental Congress held in Istanbul, approximately two-thirds (64.1%) of dentists identified erythroplakia and leukoplakia as the lesions most likely to be precancerous, and approximately two-thirds (64.7%) of dentists considered that squamous cell carcinoma is the most common form of oral cancer (13). A study conducted by Shafer and Waldron (34) reported that 51% erythroplakia biopsy specimens were invasive carcinoma, 40 %were carcinoma in situ or severe epithelial dysplasia and 9% were mild or moderate epithelial dysplasia. In another study from Brazil, Pires et al retrospectively analyzed 684 OPMDs (leukoplakia, speckled leukoplakia, and actinic cheilitis) and found that more than half of these lesions showed a various degree of epithelial dysplasia (35). Although leukoplakia (white patches) is more common OPMD, erythroplakia (red patches) is more serious (36).

More than two-thirds of the participants of this study were familiar with leukoplakia and erythroplakias commonly related lesions for the development of oral cancer. However, only 26.7% of the dentists correctly identified the clinical properties of a prior oral cancer lesion as small, painless, and red areas while 44.9% of them select small, painless, and white areas. In India, most of the dentists (82%) considered white lesions as the most common seen oral cancer lesion and only 9.6% of dentists considered red erosions as the most common seen oral cancer lesion. In another study, Clovis et al. (23) observed that the apperance of early oral cancer lesions (small, painless, red lesions, in that order) was correctly identified by large numbers of respondents (77%) (13). Although most dentists know about what to look at theoretically, they do not know the clinical appearances of these lesions. Since early lesions of oral cancer are small, painless, and erythematous, most of the respondents failed to recognize the clinical appearances of oral cancer in North Cyprus.

The percentage of dentists who strongly agreed to inform patients with suspicious lesions were 79.5% and 64.3% of them strongly agreed to be adequately trained to be able to perform patient's lymph node palpation. In a similar study, Pekiner and Kebabçıoğlu stated that only 61.2% of the participating dentists informed their patients about suspected oral lesions (13). In other study, 12% of dentists referred patients with suspicious lesions to a specialist; 68% of them inform their patients on alcohol and tobacco usage as oral cancer risk factors and 37% of the dentists routinely palpated their patients' lymph nodes (18). In Yemen, 68.3 and 94.1% of dentists indicated that they are comfortable palpating lymph nodes in neck region and referring suspicious oral lesions to specialists, respectively (19).

Early detection of oral cancers increases the survival rate, and most of the participants (87.8%) are consistent with this finding. However, only 37.7% (strongly agree/agree) thought that their knowledge is current about oral cancer. Cervical lymph node palpation is an important part of the oral cancer examination. In this study, 52% of the physicians stated that they would routinely perform lymph node palpation in addition to oral cavity examination and 64.3% of them received sufficient training to palpate lymph nodes. In a study from Turkey, Keser and Pekiner (13) investigated oral cancer awareness among the undergraduate dental students, reported four-fifth of the students believed that they are not adequately trained to perform an oral cancer examination while 64.7% of them disagree or uncertain about adequate training to perform lymph node examination. A recent study conducted by Ozdemir-Ozenen et al. emphasized the importance of reorganization of the dental curriculum and courses for improving their skills, awareness, and knowledge of dental students about risk factors and early signs of oral cancers to prevent carcinogenesis and increase the survival rate (37).

The limitation of this survey study only reflects the theoretical knowledge of the participating physicians. In this study, the authors have some doubts about whether the theoretical knowledge of physicians increases the ability to recognize the lesion clinically.

In conclusion, dental faculty curriculums and continuing education programs should aim at managing oral cancer need to address not only increase the knowledge and awareness about oral cancers but also include the clinical cases and

practices such as performing the oral cancer examination, lymph node palpation, early characteristics of the potentially malignant disorders.

Ethical Statement

The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the Near East University (IRB approval number: YDU/89-1313).

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: K.O., S.A., Design: S.A., Data Collection or Processing: M.M.G., M.F., Analysis or Interpretation: M.M.G., M.F., Literature Search: S.A., M.M.G. Writing: S.A., M.M.G.

Ethical Statement

Approval was obtained from Near East University Scientific Research Ethics Committee, the study started. The ethics committee decision date is 25/03/2021 and the number of ethical committee decisions is 2021/89.

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Research Article

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Alginate-based hydrogel promotes neuronal survival and axon outgrowth of neuron-like cells

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Abstract

Alginate is a natural polymer preferred for biotechnological applications due to its properties, such as biocompatibility, biodegradability, and low toxicity. However, neurons do not possess surface molecules interacting with alginate; therefore, alginate-based materials have limitations for neurodegenerative applications. Thus, increasing neuronal survival and promoting axonal outgrowth in the alginate-based hydrogels are the primary purposes of this study. We also aim to study the performance of alginate extracted from bioresources to that of commercial alginate. Cell-embedded alginate-based hydrogels were formed with $CaCl_2$ and either mixed with collagen type I or supplemented with differentiation protocols such as the addition of NGF or FGF and serum withdrawal and retinoic acid (RA). Cells were observed by fluorescence imaging with acridine orange and propidium iodide, and upon dissolving the hydrogel with EDTA, cells were counted with trypan blue staining. In this study, commercial alginate and alginate extracted from seaweed were compared for their performance and were found to be comparable. We determined that adding collagen to the alginate hydrogel increased neuronal survival but not axon outgrowth. NSC-34 cell differentiation with NGF and FGF was successful in both commercial and extracted alginate, with both growth factors increasing neural survival and axonal outgrowth, despite the clustering of cells immediately after treatment. However, the SH-SY5Y differentiation protocol using serum withdrawal and RA treatment did not yield good results. Both extracted and commercial alginates showed comparable performance in terms of neuronal survival in our study, which was further increased upon collagen addition. We also showed that NGF and FGF differentiation protocol in alginate hydrogels resulted in successful axon outgrowth in NSC-34 cells.

Keywords: Alginate, Nerve growth factor (NGF), Fibroblast growth factor (FGF), hydrogel, NSC-34 motor-like neuron cell line, SH-SY5Y human neuroblastoma cell line

1. Introduction

The cells' natural environment is three-dimensional (3D); thus, mimicking the cellular environment is vital to understanding cellular behavior in nature. For example, 3D biomaterials are commonly used to simulate the extracellular matrix (ECM) (Frantz et al., 2010; Kular et al., 2014; Ravi et al., 2015). Each cell has a different ECM requirement; therefore, mimicking the environment is vital for each cell type. Thus, common ECM properties, such as a meshwork fibrous structure, are preferred. Hydrogels are among the most preferred biomaterials to mimic the cellular microenvironment (1,2) and can be obtained from synthetic (poly-(acrylic acid) (PAA), poly (ethylene glycol) (PEG)), or natural polymers (collagen, chitosan, alginate, hyaluronic acid) (1,3,4). Instead of synthetic polymers, natural polymers are preferred for biomedical applications due to their biocompatibility, low toxicity, and biodegradability properties (1,3,4).

Alginate is a natural polymer found in the cytoplasm of brown seaweeds to provide mechanical strength or flexibility to the algae (5,6). Alginate is preferably used in biotechnological applications due to its biodegradability, biocompatibility, and nontoxicity properties. Mainly, alginate is composed of two monomers: (1-4)-linked β-D-mannuronate (mannuronic acid, M) and L-guluronate (guluronic acid, G); however, the specific composition and ratio of these monomers in alginate vary depending on the algae type and harvesting time. The ratio of M residues to G residues determines the characteristics of the hydrogel due to crosslinking capacity (7,8). Alginate forms hydrogel if the environment has divalent cations, such as Ca^{2+}, $Sr^{2+,}$ or Ba^{2+}; primarily, Ca^{2+} is used to obtain alginate hydrogels in biotechnological applications (1,6,9). If the M/G ratio exceeds 1, the alginate hydrogel becomes more flexible, and vice versa. Dissolving the alginate hydrogels is possible by breaking the crosslinking; therefore, a calcium chelator provides the dissolution (5,10).

Besides several advantages of alginate, one disadvantage for neurobiology is that it is not recognized by neuronal cells

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due to a lack of receptors; thus, the hydrogel modifications are necessary for alginate to be used for neuronal model cells as a scaffold material (5,9). In this article, alginate extracted from *Cystoseira barbata* was mixed with collagen type I to increase the attachment of neuronal model cells to the hydrogel, and differentiation techniques were applied to create a 3D neuronal culture to increase cell survival and supportive cell axonal outgrowth. In addition, in the literature, two cell lines (NSC-34 and SH-SY5Y) were not studied with alginate material. Thus, this study investigated the comparison of extracted alginate to commercially available alginate in terms of alginate recognition improvement with neuron-like cells.

2. Materials and Methods

2.1. Alginate Solution Preparation

Alginate was extracted from *Cystoseira barbata* collected from Izmit Korfezi, Turkey, and lyophilized as described before (11). The lyophilized alginate extract and commercial alginate (Sigma, 9005-38-5) powders were dissolved in the distilled water at 500 rpm at 40°C for 30 min. The stock solutions were prepared using 0.9% NaCl (w/v) and 4.5% alginate (w/v) in distilled water. Also, 0.1 M $CaCl_2$ was prepared with distilled water to obtain alginate gel formation.

2.2. Sterilization Procedure

Alginate solutions and $CaCl_2$ were autoclaved at 121°C and 20 min to eliminate the contamination risk for cellular experiments. After autoclaving, the solutions were filtered under the Class II Laminar flow cabinet with a 0.45 µm syringe filter. After the sterilization procedure, the solutions were stored at 4°C for up to one week.

2.3. Hydrogel Preparation

Due to the alginate content, Ca^{2+} crosslinks with alginate and alginate solution turn the hydrogel form. For cellular and characterization experiments, the concentrations of alginate solutions were set to 1% with high glucose with 4.5 g/L D-Glucose, L-Glutamine, and sodium pyruvate Dulbecco's Modified Eagle Medium (DMEM) (Gibco) including 10% Fetal Bovine Serum (Gibco) and 1% penicillin-streptomycin (Gibco). The hydrogel was formed with the addition of $CaCl_2$, and the mixture was incubated for 10 minutes at room temperature. The alginate hydrogel formed from extracted alginate and the hydrogel formed from commercially available alginate (Sigma, 9005-38-5) are presented in Fig. 1a and 1b, respectively.

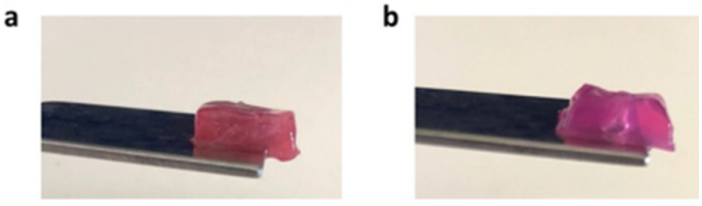

Fig. 1. Alginate hydrogels (1% w/w)prepared with DMEM and 1M $CaCl_2$. a. formed by using alginate extracted from *Cystoseira barbata*, b. formed by using commercial alginate

2.4. Cell Culture

NSC-34 (motor-like neuron cell line) and SH-SY5Y (human neuroblastoma cell line) were incubated for cell culture experiments at 5% CO_2 and 37°C. NSC-34 cells were grown in the DMEM high glucose without pyruvate with 10% FBS and 1% penicillin/streptomycin (Pen/Strep), and SH-SY5Y cells were seeded on DMEM high glucose with pyruvate, supplemented with 10% FBS and 1% Pen/Strep. When cells reached 80 % confluency, the existing medium was discarded, and the cells were collected with trypsin/EDTA (Gibco). Before centrifugation at 1500 rpm for 5 min, the cells were counted with Haemocytometer (Marienfield). The cell pellet was dissolved with the appropriate amount of DMEM to obtain $1x10^6$ cells/mL. The stock alginate solution (4.5% w/v) was diluted to 1% (w/v) with DMEM and cell solution (50,000 cell/well for NSC-34 and 75,000 cells/well for SH-SY5Y for 96-well plate). The prepared alginate/DMEM/cell solution was seeded onto the 96-well plate.

The indicated amount of the growth factors or the DMEM without FBS was applied during the dilution of the alginate solution and cell pellet dissolving as a differentiation medium for neuronal differentiation. The differentiation medium for NSC-34 contained 50 ng/mL nerve growth factor (NGF) or 10 ng/mL fibroblast growth factor (FGF). For SH-SY5Y, the differentiation medium contained retinoic acid (RA, Sigma R2625, final concentration at 10 µM) in DMEM with 1 % FBS. After cells reached 80% confluency, the cells were collected and seeded onto the flask and treated with DMEM with 1% FBS and 1% Pen/Strep for 24 h. Embedding the cells with alginate was achieved after 24h serum withdrawal, the cells were collected, and the cell pellet was dissolved with DMEM, including 1% FBS, 1% pen/strep, and 10 µM RA. The cell solution was blended with alginate, and this alginate/cell/differentiation medium solution was seeded onto the 96-well plates. The hydrogel form was obtained with the same procedure as described previously. Cell-embedded hydrogels were incubated at 37°C with 5% CO_2, and the differentiation medium was refreshed every two days for a 7-day treatment. The schematic representation of the cell embedding into the alginate hydrogel is shown in Fig. 2.

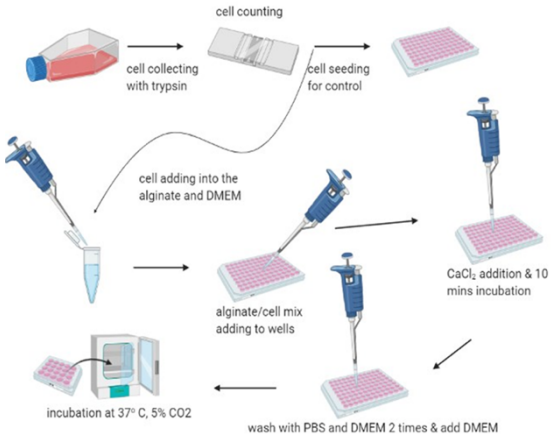

Fig. 2. The schematic representation of cell embedding into the alginate hydrogels

2.5. Fluorescence Staining

5 mg/mL Acridine Orange (AO) (Sigma, A6014) and 3 mg/mL propidium iodide (PI) (Sigma, P4170) were used to identify living cells and dead cells, respectively. The staining solution was prepared with 1x DPBS, and cells were treated with this solution for 15 min; after incubation, the existing solution was discarded, and the hydrogels were washed with 1x DPBS twice. Hydrogels were imaged under ZOE fluorescent cell imager with appropriate excitation/emission wavelength.

2.6. Dissolving Alginate and Cell Counting

Due to the Ca^{2+} crosslinking, the alginate hydrogel was dissolved with 0.5 M ethylenediaminetetraacetic acid (EDTA). After adding EDTA into the hydrogels, the solution was stained with trypan blue when the hydrogels dissolved completely. Trypan blue staining was applied to count the viable and dead cells via Haemocytometer after 5 min incubation at room temperature. While counting the cells, colored cells indicated dead cells, and the others represented living cells. The calculations were achieved with the percentage of the ratio of dead cell numbers over the number of live cells. A two-way ANOVA test was applied for both cell lines, with Sidak multiple comparison methods for comparing commercial and extracted alginate samples with a confidence interval of 95%.

2.7. Collagen/alginate Hydrogel Preparation

The initial concentration of collagen I, Rat Tail (Gibco), was 3mg/mL, and the working concentration of the collagen solution was diluted with 0.02 M acetic acid, 1x DPBS, with controlled pH of 1 mg/mL. Then, the collagen and alginate concentrations and ratios were adjusted using an appropriate amount of DMEM and cell solution.

The cell solution was added to the collagen/alginate mixture, and 100 µL of 50,000 cells (NSC-34) or 75,000 cells (SH-SY5Y) was seeded onto the 96-well plate for each well. To obtain collagen gelation, the seeded cell solutions were incubated at 37°C with 5% CO_2 for 1 hour to obtain collagen gelation. After incubation, 0.1 M $CaCl_2$ was added to the mixture, and 10 min incubation at room temperature was applied for gelation, and the hydrogels were washed with DPBS twice to reduce $CaCl_2$ existence. Finally, the plate was incubated under the same conditions with cell growth conditions after adding DMEM to the hydrogels.

3. Results

Fluorescence images were taken from cell-embedded alginate (extracted and commercial) hydrogels with acridine orange and propidium iodide stains. The staining results for NSC-34 motor neuron cells showed that cell viability decreased on Day 4 of embedding in hydrogels with both extracted and commercial alginate (Fig. 3a). In the case of SH-SY5Y cells, the number of the clustered cells was lower than that of NSC-34 cells, while cell survival was supported until Day 4 of embedding in both extracted and commercial alginate hydrogels, similar to the case in NSC-34 cells (Fig. 3b).

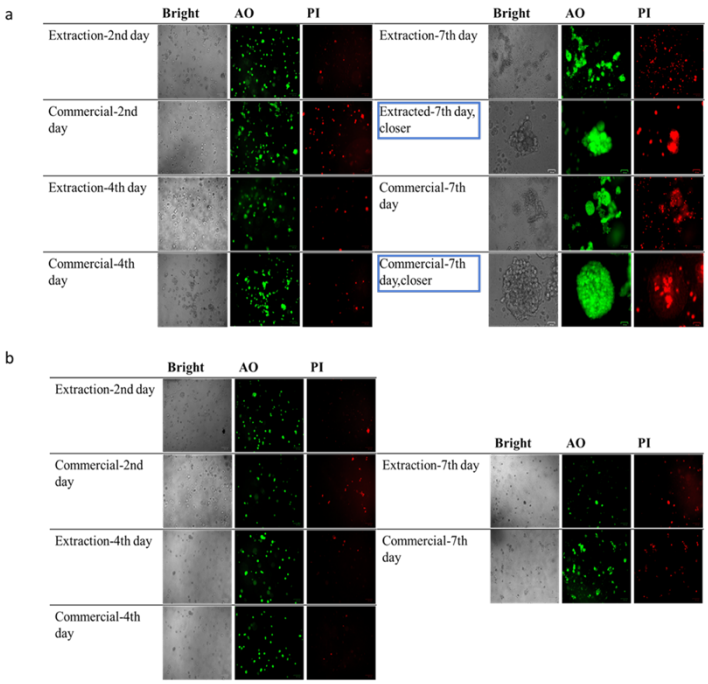

Fig. 3. Fluorescence imaging of a. NSC-34 and b. SY-SY5Y cells taken on the 2nd, 4th, and 7th days after embedding of either extracted (extraction) or commercial alginate hydrogels. Scale: 100 µm, for closer to 25 µm. Bright: brightfield; Green: Acridine orange (AO); Red: Propidium iodide (PI)

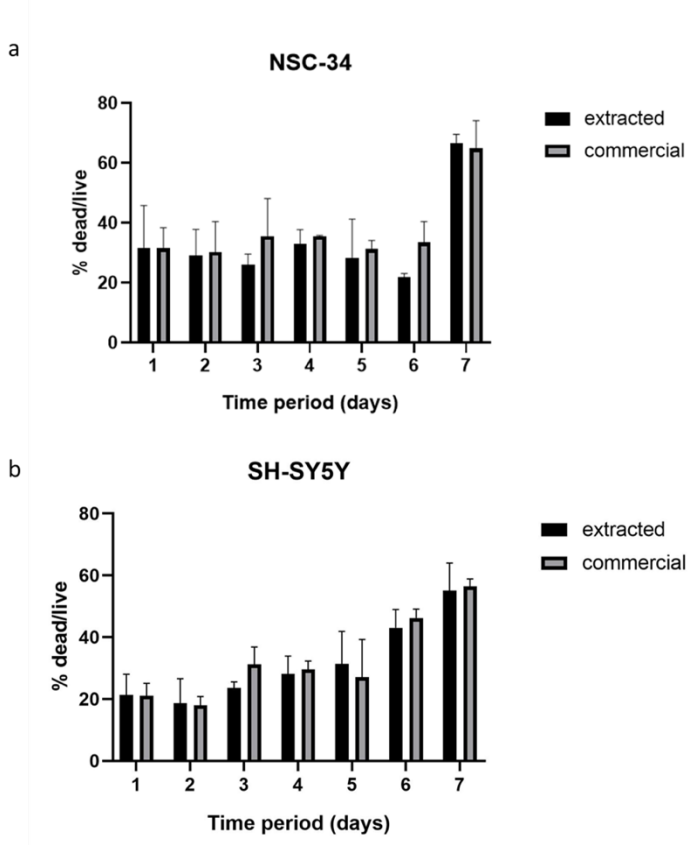

Fig. 4. The percentages of the ratio of dead cells to the living cells a) NSC-34 cells and b) SH-SY5Y for 7 days. Error bars indicate the standard deviation of 3 biological replicates

The live and dead cells were counted as described in Materials and Methods, and reported as a percentage of cell death for both NSC-34 and SH-SY5Y cells embedded in alginate hydrogels (Fig. 4). We observed that both hydrogels

exhibited similar performance in terms of supporting cell survival: While cell death appears to sharply increase after Day 6 for NSC-34 cells, SH-SY5Y cell death appeared to show a gradual increase (Fig. 4). Statistical analysis of the quantitative results indicated no significant differences between extracted alginate and commercial alginate results for both cell lines, ensuring the tissue culture applicability of the alginate extraction procedure.

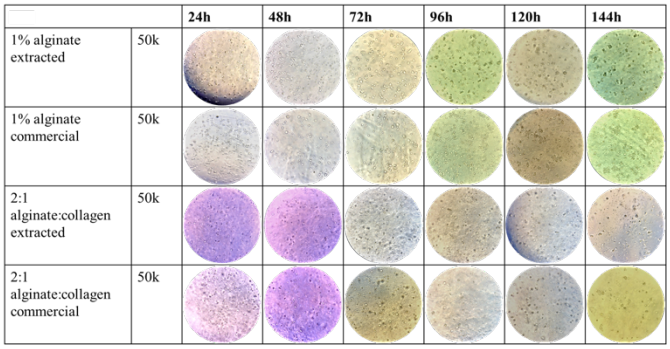

Fig. 5. The appearance of cell-embedded alginate hydrogels (%1 extracted alginate, %1 commercial alginate, extracted alginate:collagen (2:1), commercial alginate:collagen (2:1) under light microscopy during 6-days to identify the effect of collagen on cell survival and axonal outgrowth for NSC-34 cell lines. k: 1000 cells / well.

Cell-embedded alginate hydrogels were further observed under light microscopy during a 6-day period to identify the effect of collagen on cell survival and axonal outgrowth for both NSC-34 (Fig. 5) and SH-SY5Y (Fig. 6) cell lines. Alginate/collagen mixture at the ratio of 2:1 was used to embed the cells with the cell density of 50,000 cells per well in the 96-well plate. The cellular survival period was found to extend with the addition of collagen, decreasing cellular migration compared with only alginate hydrogels. Under these conditions, no significant axon outgrowth of the NSC-34 cells was observed either in alginate or alginate/collagen hydrogels (Fig. 5).

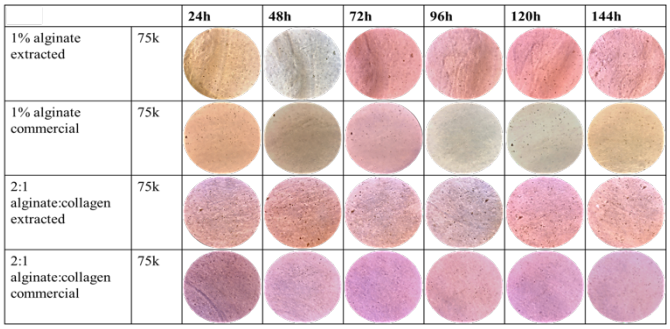

Fig. 6. Collagen mix with 2:1 ratio (alginate/collagen), SH-SY5Y cells embedded with alginate and alginate/collagen hydrogels. k: 1000 cells / well

Similarly, SH-SY5Y cells were embedded in the alginate (extracted and commercial) hydrogels with alginate/collagen hydrogels with a 75,000-cell density per well. Cell survival did not appear to be significantly enhanced in SH-SY5Y cells upon collagen addition. Therefore, no cellular proliferation or axonal outgrowth was observed during the 6-day period (Fig. 6).

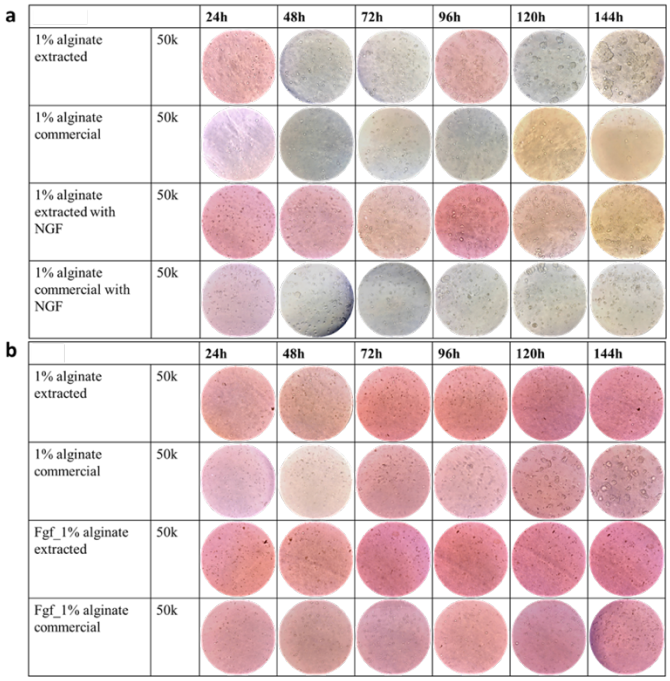

Fig. 7. NSC-34 cells embedded with & without included a. NGF b. FGF included medium. k: 1000 cells / well

Next, the effect of differentiation protocols on axon outgrowth in these hydrogels was studied. We initially treated NSC-34 cells with 10 ng/mL FGF or 50 ng/mL NGF to induce differentiation. Neuronal differentiation, as measured by axon outgrowth, was observed after Day 4 of embedding in FGF-treated cells (Fig. 7a), while NGF-treated cells in the hydrogel started to differentiate after the 3rd day (Fig. 7b). In addition, cells were observed to form cellular clusters earlier (about the 2nd or 3rd day) compared to non-NGF-treated hydrogels; moreover, cellular survival increased with NGF treatment.

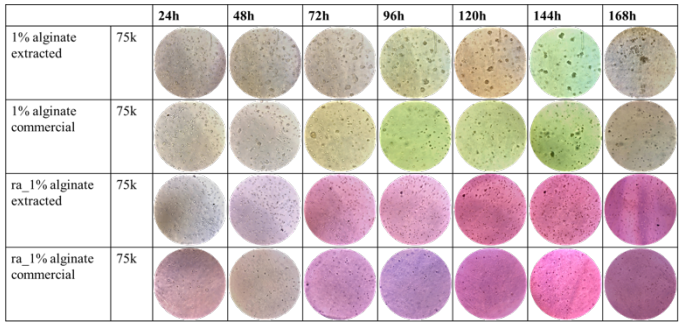

Fig. 8. SH-SY5Y cells were embedded in hydrogels, including RA, for 7 days. The medium exchanges were similar to usual embedding methods with medium, including RA. The scale bar of images is 450 µm. k: 1000 cells / well

The differentiation protocol used for SH-SY5Y cells included overnight serum withdrawal followed by RA treatment (12); however, neither in 3D nor in 2D conditions, the differentiation did not yield reliable axon outgrowth in SH-SY5Y cells (Fig. 8).

4. Discussion

The previously reported chemical and physical characterization (11), as well as the cellular results presented in this study, showed that the extracted alginate from *Cystoseria barbata* collected from Tuzla, Istanbul, Turkey, had

similar properties to commercially available alginate as a relatively cost-effective and locally produced alginate source.

The cell studies on the alginate hydrogels using two different neuronal model cells showed the applicability of alginate hydrogels and hydrogels of alginate/collagen mixtures, which were suitable for neuronal cell survival. It should be noted that fluorescence images of NSC-34 cells in this study also indicated that the cells migrate to each other to form a spheroid-like structure; this could be due to paracrine signaling to enhance survival (13). The cellular morphology of both NSC-34 and SH-SY5Y cells remained spherical after embedding in the hydrogels, which could be due to the lack of surface molecules that recognize and bind alginate (5), in turn lowering the cellular attachment to alginate hydrogel. Cellular proliferation or axonal outgrowth was not observed for either cell line in the alginate hydrogels alone.

Due to the low attachment of cells to alginate, we next mixed alginate with collagen in order to provide an extracellular matrix-like microenvironment to the cells. The results indicated that cellular survival was increased with the addition of collagen. More detailed work is necessary to determine optimum alginate mixtures suitable for different types of neuronal models.

We also studied the effect of differentiation protocols on axon outgrowth in hydrogels. NGF or FGF treatment of NSC-34 cells showed axon outgrowth in cells, indicating the hydrogels prepared in this study supported neuronal differentiation; however, the SH-S5Y5 differentiation protocol did not yield any axon outgrowth. Growth factor supplementation in neuronal differentiation protocol may be better suited in alginate-based hydrogels. The cellular clusters observed in the early periods of differentiation protocol can be due to the cells' need to maximize cell-to-cell communication and signaling as a means for cellular survival and differentiation (13,14).

This study aimed to create a 3D alginate-based environment that provides support for both neuronal survival and axonal outgrowth, using two different model cells, namely NSC-34 and SH-SY5Y cell lines. Our study showed that the collagen addition to the alginate hydrogel increased the cellular survival for both cell lines. Different ECM mimic molecules can be mixed with alginates in order to obtain optimum hydrogels for different neuronal cell types. We have also shown that neuronal differentiation of NSC-34 cells was achieved in alginate hydrogels supplemented with FGF and NGF, indicating alginate-based hydrogels can support neuronal differentiation, although optimum differentiation protocols need to be determined for different neuronal model cells and different alginate-hydrogel mixtures. In this study, we have also shown the biocompatibility of alginate extracted from algae in the Tuzla province of Türkiye as a local and cost-effective alternative bioresource.

Ethical Statement

Ethic commitee approval is not required for this study.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: B.D., I.K, M.I.H., Design: B.D., E.S., I.K, H.K., H.S., Data Collection or Processing: B.D., Analysis or Interpretation: B. D., Literature Search: B.D., Writing: B.D., I.K.

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Levels of osteopontin, suppression of tumorgenicity 2 and myeloperoxidase activity in acute coronary syndrome patients with fragmented QRS

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Abstract

In recent years, studies have focused on new markers to predict the risk of cardiovascular diseases (CVD). There are several studies analyzing biomarkers such as myeloperoxidase (MPO), suppression of tumorgenicity 2 (sST2), and osteopontin (OPN) in CVD, however; there are no studies that show their relationship in patients who have fragmented QRS (fQRS). It aimed to investigate the levels of OPN, sST2, and MPO activity in patients who were diagnosed with acute coronary syndrome (ACS) in the present study. Sixty ACS patients and 26 healthy individuals were included in the study. Patients diagnosed with ACS were divided into two groups; (+)fQRS (n=30) and (-)fQRS (n=30). Levels of OPN and sST2 were measured by ELISA, and MPO activity was measured by colorimetric methods. In ACS patients, serum activity of MPO (33.7 U/L), and levels of OPN (103.29 ng/mL) and sST2 (495.4 pg/mL) were found to be significantly higher than those in the control (23.14 U/L, 42.65 ng/mL, 344.11 pg/mL, respectively; $p<0.01$, $p<0.01$, $p<0.05$). However, there were no significant differences between the activity of MPO, levels of OPN, and sST2 in patients with (+)fQRS (respectively, 32.74 U/L, 101.89 ng/mL and 451.97 pg/mL) and (-)fQRS (respectively, 34.67 U/L, 104.69 ng/mL, 535.73 pg/mL). There were also positive correlations between MPO activity and platelet ($r=0.376$, $p<0.05$) levels in the (+)fQRS group, and sST2 and triglyceride levels in (-)fQRS group. When the diagnostic performances for ACS were examined, the sensitivity of CK-MB, troponin-I, MPO, OPN, and sST2 were 83%, 80%, 65%, 85%, 58%, and specificity was 96%, 100%, 72%, 96%, 80%, respectively. In conclusion, MPO, OPN, and sST2 may be included in the factors that may contribute to the diagnosis and development of ACS. In addition, the discovery of the high diagnostic sensitivity and specificity of OPN in acute coronary syndrome is a new finding obtained in this study.

Keywords: acute coronary syndrome, osteopontin, sST2, myeleporoxidase

1. Introduction

The term “acute coronary syndrome” (ACS) is a general term used for conditions that occur because of an interruption of blood flow to the heart. All clinical symptoms that develop due to acute myocardial ischemia are called ACS. The determination of the clinical type of ACS is based on electrocardiography (ECG) findings. According to ECG findings, there are three different types of ACS: unstable angina pectoris (UAP), non-ST-segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI) (1).

Fragmented QRS (fQRS) is a depolarization abnormality that can be easily detected from 12-lead ECG. fQRS detected in the routine ECG recordings of individuals with coronary artery disease or individuals with suspected coronary artery disease is an independent marker for mortality due to myocardial scar, arrhythmic events, and coronary artery diseases (2).

ST2 is an interleukin-33 (IL-33) receptor secreted by most living cells in response to cell damage, and it means "suppression of tumorgenicity 2". It has been revealed that it is secreted by cardiac cells in response to myocardial stress (3). ST2 has two main isoforms: transmembrane (ST2L) and soluble (sST2) forms. IL-33 acts directly as a transcriptional regulator and shows its effects by binding to the transmembrane receptor ST2L. Studies have demonstrated that the IL-33/ST2L interaction is cardioprotective and reduces myocardial fibrosis, cardiomyocyte hypertrophy, and apoptosis, as well as improving myocardial function (4,5). The levels of sST2, which is the other isoform of ST2, increase in inflammatory conditions, and studies have shown that high sST2 levels are consistently related to the mortality risk in acute/chronic cardiovascular conditions (6).

Osteopontin (OPN), an extracellular matrix protein, is released from osteoblasts and osteoclasts that are effective in

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bone formation in bone tissue. Several studies have been conducted to define the relationship between OPN and atherosclerotic cardiovascular diseases (7). OPN was first detected in the artery tissue by Giachelli et al. (8) and its relationship with cardiovascular diseases has not been elucidated yet. OPN, which is a multifunctional protein, has been shown to have important functions in cardiovascular disease, cancer, diabetes, renal lithiasis, infection, biomineralization, cell viability, and wound healing (9).

Myeloperoxidase (MPO) is a myeloid-based enzyme with strong antibacterial properties, and it is largely synthesized by neutrophils. MPO, a product of systemic inflammation, plays an important role in both the oxidative stress and inflammation process and takes part in the oxidation process of lipoproteins. Moreover, MPO contributes to the development of atherosclerotic plaques by disrupting endothelial function (10), LDL oxidation (11), generation of a thrombogenic environment (12), and thinning of the plaque fibrous cap (13).

The main approach to preventing the negative consequences of acute coronary syndromes is to detect high-risk individuals long before the development of disease-related complications. In this study, we aimed to investigate the activity of myeloperoxidase (MPO), osteopontin (OPN), and soluble ST2 (sST2) levels in acute coronary syndrome (ACS) patients with and without fQRS. We also aimed to investigate whether these biomarkers would be useful in the diagnosis of coronary diseases.

2. Materials and Methods

2.1. Study design and participants

This study concerned a total of 60 ACS patients who were admitted to the emergency department of Ordu University Training and Research Hospital with chest pain and then taken into the intensive care unit. After the approval of the Ordu University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 15/11/2018 and the number of ethical committee decisions is 2018/228. The patient population was divided into two groups: patients with fQRS (+fQRS) and patients with no fQRS (-fQRS). The control group consisted of 26 healthy individuals who are compatible with ACS patients due to age and gender.

A 12-lead ECG was obtained from all patients during their resting state. Exclusion criteria were as follows: patients with LV EF (left ventricular ejection fraction)<50%, presence of advanced valvular disease, patients with bundle branch block on ECG, patients with known cardiomyopathy and permanent pacemakers, patients with severe electrolyte imbalance, those with acute and chronic bacterial or viral inflammation, those with severe liver and kidney failure, patients with rheumatological and orthopedic diseases, patients receiving anti-inflammatory (other than aspirin) medications, hormones, cytokines or growth factors were excluded from the study. Fasting blood samples of patients were collected between the 12^{th} and 36^{th} hours considering the time at which the pain began. After waiting for 30 minutes, the samples were centrifuged in 1800 xg for 10 minutes and the serum was stored at -80℃ until analysis.

2.2. The Biochemical Analysis

Serum sST2 and OPN levels were measured by commercially available Enzyme-linked Immunosorbent Assay (ELISA) Kits (respectively, Thermo Fisher Scientific BMS2066/BMS2066TEN, USA; Elabscience Biotechnology Co., Ltd. E-EL- H1615, USA). Results were read at 450 nm wavelength on the ELISA reader (BioTek ELX800 reader, BioTek ELX50 washer, Winooski, Vermont, United States). Serum MPO activity was measured via methods developed by Bradley et al. (14). The principle of the method that O-dianicide, known as a peroxidase substrate, is oxidized by MPO to form a yellow-orange product in the presence of hydrogen peroxide (H_2O_2). This oxidation reaction was monitored at 460 nm with an increase in optical density depending on time.

Other parameters included in our study included fasting glucose, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), C-reactive protein (CRP) and creatine kinase (CK) were measured by the commercial kit and the auto-analyzer (Hitachi Cobas-c 501) produced by Roche Diagnostics Ltd., Japan. Troponin-I and mass CK-MB (creatine kinase-myocardial isoenzyme) were measured by the commercial kit and the autoanalyzer (Hitachi Cobas-e 601) produced by Roche Diagnostics. Hemoglobin, leukocyte, neutrophil, and platelet levels were measured by the commercial kit and the autoanalyzer (Sysmex XN-1000) produced by Sysmex Corporation Ltd., Japan.

The diagnosis of dyslipidemia was defined according to the National Cholesterol Education Program (NCEP, ATP III) final report (15).

2.3. Statistical Analysis

In this study, the primary variable was OPN for fQRS. According to previous studies, effect size (d) was assumed to be 0.96, and a Z value of 1.96 was used for the 0.05 types I error rate. It calculated that a 99% power value at d = 0.96 effect size and a 95% confidence level can be reached with at least 26 subjects (6).

The normal distribution control of the data was checked with the Kolmogorov-Smirnov test. The homogeneity of the group variances was checked by the Levene test. A comparison of the two groups was made using the Mann-Whitney U test, and the comparison of the three groups was made by one-way analysis of variance. After variance analysis, the Tukey HSD test was used as a multiple comparison test. The dependency between categorical variables was examined by the two-way chi-square test. Relationships between normally distributed variables were examined with the Pearson correlation coefficient and the relationships between non-normally

distributed variables were examined with the Spearman rank correlation coefficient. The results are presented as the mean± standard error (SE). Before performing multiple logistic regression analysis to determine the risk factors for acute coronary syndrome, LR was used to identify variables that significantly contributed to the model. Then, a multiple logistic regression analysis was performed with these variables. All calculations were made with the SPSS v25 (IBM Corp, Chicago, IL, USA) statistical package program. The statistical significance of all variables was established at $p<0.05$.

3. Results

The demographic and descriptive characteristics of the groups are shown in Tables 1 and 2. The comparison of sST2, OPN, MPO, and other biochemical parameters in groups are given in Table 3. There were no significant differences in terms of levels of sST2, OPN, and MPO activity between (+)fQRS and (-)fQRS groups ($p>0.05$). When the (+)fQRS and (-)fQRS groups were compared to the controls, OPN levels and MPO activity were found to be higher (p 0.01). In addition, the sST2 level increased significantly in the (-)fQRS group as compared to the controls ($p=0.03$).

Table 1. Demographic characteristics of the groups included in the study

Parameters	Control (X±SE)	(+)fQRS (X±SE)	(-)fQRS (X± SE)	p
Age	59.62±1.55	63.90±2.14	64.33±2.76	0.289
Male	15(%57)	22(%73)	16(%53)	0.249
Female	11(%43)	8(%27)	14(%47)	

(+)fQRS: ACS patients with fQRS ; (-)fQRS: ACS patients without fQRS

Table 2. Descriptive characteristics of the groups diagnosed with ACS

Parameters	(+)fQRS (X±SE)	(-)fQRS (X±SE)	p
Diabetes Mellitus	12(%40)	8(%27)	0.224
MI	25(%83.3)	27(%90)	0.448
Hypertension	16(%55.2)	13(%44.8)	0.438
Dyslipidemia	19(%55.9)	15(%44.1)	0.297
SBP (mm Hg)	121.46±2.85	118.1±4.78	0.547
DBP (mm Hg)	76.56±1.84	75.36±3.08	0.741
Heart Rate (pulse/min)	77.56±2.1	71.43±3.45	0.135

(+)fQRS: ACS patients with fQRS ; (-)fQRS: ACS patients without fQRS; MI: a history of myocardial infarction; SBP: systolic blood pressure; DBP: diastolic blood pressure

The diagnostic performance levels of OPN, sST2, and activity of MPO were calculated in ROC analyses to differentiate the diagnosis of fQRS (as shown in Fig. 1). Lower and upper limit values for the area under the curve (AUC), sensitivity, specificity, and 95% confidence interval for MPO, OPN, and sST2 are also presented in Fig.1. There was no diagnostic value of MPO, OPN, and sST2 in the diagnosis of fQRS.

Table 3. Comparison of biochemical parameters among the groups

Parameters	Group	n	Mean	SE	p value
MPO (U/L)	Control	26	23.14	1.12	
	(+)fQRS	30	32.74 ^{a*}	3.14	0.007
	(-)fQRS	30	34.67 ^{a**}	2.84	
OPN (ng/mL)	Control	26	42.65	4.14	
	(+)fQRS	30	101.89 ^{a***}	6.18	0.000
	(-)fQRS	30	104.69 ^{a***}	4.66	
sST2 (pg/mL)	Control	26	344.11	49.06	
	(+)fQRS	30	451.97	49.81	0.03
	(-)fQRS	30	535.73 ^{a*}	45.60	
Glucose (mg/dL)	Control	26	98.00	1.2	0.002
	(+)fQRS	30	137.9^{a**}	8.92	
	(-)fQRS	30	126.33^{a**}	8.97	
BUN (mg/dL)	Control	26	13.47	1.03	0.049
	(+)fQRS	30	18.06^{a*}	1.28	
	(-)fQRS	30	18.24^{a*}	1.92	
TG (mg/dL)	Control	26	125.46	7.69	0.071
	(+)fQRS	27	152.56	11.5	
	(-)fQRS	29	175.86	21.4	
TC (mg/dL)	Control	26	185.54	6.15	0.6
	(+)fQRS	27	180.89	5.74	
	(-)fQRS	29	190.14	7.39	
HDL-C (mg/dL)	Control	26	51.77	2.26	0.001
	(+)fQRS	27	42.41^{a**}	2.31	
	(-)fQRS	29	41.45^{a**}	1.77	
LDL-C (mg/dL)	Control	26	110.72	5.62	0.113
	(+)fQRS	26	109.05	5.06	
	(-)fQRS	28	134.24	5.67	
CRP (mg/dL)	Control	26	0.33	0.04	0.009
	(+)fQRS	30	3.28^{a**}	1.0	
	(-)fQRS	30	1.56	0.4	
LDH (U/L)	Control	26	173.5	4.51	0.001
	(+)fQRS	30	399.03^{a**}	47.9	
	(-)fQRS	28	382.43^{a**}	54.59	
CK (U/L)	Control	26	80.18	6.49	0.002
	(+)fQRS	30	736.13^{a**}	183.2	
	(-)fQRS	30	416.43	76.12	
CK-MB (ng/mL)	Control	26	1.64	0.1	0.001
	(+)fQRS	29	56.68^{a**b*}	14.8	
	(-)fQRS	29	24.03	6.4	

*; $p<0.05$, **; $p<0.01$, *** ; $p<0.001$, a; when compared with control, b; when compared with (-)fQRS MPO; myleperoxidase, OPN; osteopontin, sST2; suppression of tumorgenicity 2, BUN; Blood urea nitrogen, TG; Triglycerides, TC; Total Cholesterol HDL-C, High density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; CRP; C-reactive protein, LDH; Lactate dehydrogenase, CK; creatinine kinase, CK-MB; creatinine kinase MB.

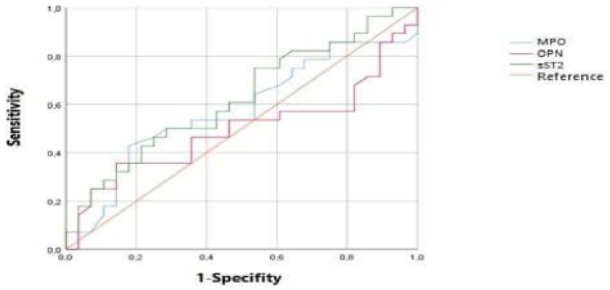

Fig 1. Diagnostic value of MPO, OPN ans ST2 for fORS

The sensitivity and specificity levels of Troponin-I (TnI), CK-MB, OPN, sST2, and, MPO activity were also calculated in ROC analyses (as shown in Figure 2). The results related to the diagnostic performance of Troponin-I, CK-MB, MPO, OPN, and sST2 are also given in Figure 2. In the ACS diagnostic performance analysis, AUC was 0.925 for OPN. When 73.64 ng/mL was taken as the cut-off value for the diagnosis, the sensitivity of OPN was 85% and the specificity was 96% (p=0.021; 95% CI, 0.534-0.792).

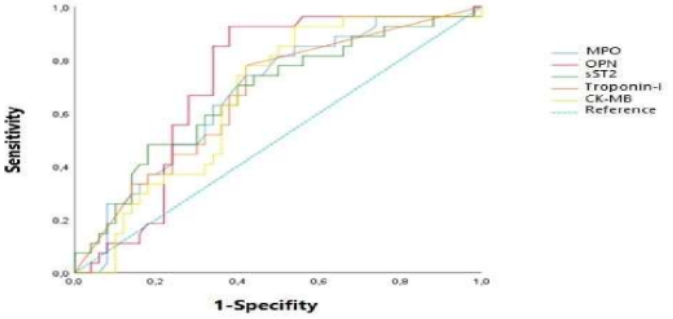

Fig 2. Diagnostic value of Troponin-I, CK-MB, MPO, OPN and sST2 for ACS

Table 4. Logistic regression analysis of the impact of various prognostic factors on acute coronary syndrome

Parameters	β	SE	Wald' s $\chi 2$	df	p	Odds Ratio (OR)
Neutrophil	1,169	0,552	4,485	1	0,034	3,217
Osteopontin	0,104	0,031	11,161	1	0,001	1,110
HDL-C	-0,222	0,087	6,539	1	0,011	0,801
Constant	-0,903	3,393	,071	1	0,790	0,405

Classification Table[a]			
	Predicted		
Observed	**Control**	**ACS**	**Percentage Correct**
Control	24	2	92,3
ACS	3	53	94,6
Overall Percentage (%)			93,9
Cox & Snell R Square = %62.4; Nagelkerke R Square= %87.6			

a: the cut value is 0.500, ACS; acute coronary syndrome.

Multiple logistic regression analysis was performed with variables (neutrophil, HDL-C, and OPN) that significantly contributed to the model, and the analysis results are summarized in Table 4. The odds ratio for a 1 unit increase in neutrophil level was 3.217 (95% CI: 1.09-9.48). Similarly, OR for OPN was found to be 1.11 (95% CI: 1.04-1.18). On the other hand, since the coefficient value of HDL-C is negative and OR is lower than one, a 1 unit decrease in HDL-C level increases the risk of getting ACS (1 / 0.8 = 1.25) 1.25 times.

4. Discussion

This study investigated the diagnostic importance of sST2, OPN, and MPO in patients with ACS. The principal findings of our study are as follows: (a); It was revealed that MPO, OPN, and sST2 may be included in the factors that may contribute to the diagnosis and development of ACS, but these parameters were not associated with the presence of fQRS. (b); OPN has higher diagnostic sensitivity than troponin I, CK-MB, MPO, and sST2 in patients with ACS. (c); Finally, we think this study is important in that it is the first study to exhibit the relationship between MPO, OPN, and sST2 and fQRS development.

fQRS is a non-invasive, easy-to-interpret, and cost-effective ECG parameter for clinicians. Extant studies have focused on the relationship of fQRS with myocardial ischemia and scars. Some studies have shown that the presence of fQRS on ECG is associated with myocardial ischemia (16). Further, some studies have shown a relationship between the presence of fQRS and the prognosis of cardiac events in patients with CAD. In a study conducted by Das et al. (17), the percentage of all-cause mortality and adverse cardiac events was found to be higher in patients with fQRS than in the group without fQRS.

sST2 levels increase in inflammatory diseases and heart diseases, therefore, the sST2 molecule is considered a valuable prognostic marker in both cases. In previous studies, a high sST2 level has been associated with the prognosis of acute coronary syndrome, pulmonary artery hypertension, and acute/chronic heart failure (18). As a result of our study, no significant difference was found between sST2 levels of (+)fQRS and (-)fQRS groups. Most patients with UA have coronary artery stenosis, and the main pathophysiological mechanisms that increase the degree of ischemia in the progressive process are plaque cracking, thrombus formation, lumen narrowing, and vasoconstriction. Demyanets et al. (19) have shown that patients diagnosed with ACS have higher levels of sST2 than individuals with stable coronary artery disease. The low sensitivity and specificity of sST2 can be considered the diagnostic value of sST2 insufficient for ACS. Marino et al. (20) compared sST2 and hs-cTnI concentrations and tested the short-term prognostic value of these two biomarkers in their study consisting of patients admitted to the emergency department with chest pain. As a result of their study, sST2 and hs-cTnI concentrations of ACS patients were found to be significantly higher than those in patients without ACS. Considering all ACS subclasses, sST2 showed a higher relative risk estimation than hs-cTnI. The authors concluded

that sST2 was found to have a greater prognostic value than hs-cTnI for cardiac mortality 30 days after discharge (20). Because of the results of our and previous studies (20,21), it is predicted that sST2 may be a more important parameter in estimating negative cardiac events of ACS and risk classification than having a diagnostic value for ACS.

Under basal conditions, OPN is found in very small amounts in the heart tissue (22). However, OPN expression in the heart tissue increases significantly under several pathological conditions (23). Mazzone et al. (24) reported that basal OPN levels were associated with rapid coronary plaque progression and OPN levels were also found in higher concentrations in ACS patients than in other groups. In the present study, while patients with ACS had higher OPN levels than the control group, there was no statistically significant difference in OPN levels between ACS patients with and without fQRS. Cardiac studies have been performed up to now and have focused on investigating the importance of OPN in the diagnosis of acute coronary syndrome. Yu et al. (25) reported that OPN levels were significantly higher in ACS patients as compared to controls, and also increased OPN levels were associated with an increase in the severity of ACS and the onset time of ACS symptoms. Our results have extended previous studies by demonstrating that increased OPN level is associated with an increased risk of ACS. Few studies have been found in the literature about the diagnostic value of OPN. While the diagnostic specificity of OPN was found at the same level as CK-MB, its sensitivity was found to be higher than CK-MB. In a study dealing with the diagnostic value of OPN in ACS, AUC was 0.897, the specificity value was 99.29% and the sensitivity value was 82.5% for OPN. Furthermore, in this study, the diagnostic accuracy of OPN in distinguishing ACS patients from controls was found to be 88.34% (26).

MPO is an inflammatory biomarker secreted by neutrophils, monocytes, and tissue-bound macrophages. It has been reported that MPO activity is increasing in AMI cases (27, 28). MPO activity reaches its peak level in a short time after AMI and, there is also a decrease in activity over time (29). In patients diagnosed with AMI it was determined that serum MPO activity increased 2 hours after the onset of chest pain (28). In the present study, although the MPO activity of ACS patients was higher than that of the control group, no significant difference was found between the ACS clinical subtypes and between the groups with and without fQRS. MPO levels were found to be significantly higher in ACS patients than in controls, and these results are compatible with the literature. When 48 U/mL is taken as the cut-off value in a study conducted with samples collected between the 4th and 6th hours after the onset of symptoms from patients by Gururajan et al. (30), it was observed that MPO had higher specificity (96%) and sensitivity (95%) values compared to TnI and CK-MB. Elevation of troponin levels takes 4-6 hours following myocardial injury. However, MPO, even in patients who are TnI negative, initially rises within 2 hours after symptom onset and peaks at the 4th hour (30). Therefore, in our study, it is an expected result that the diagnostic value of MPO is lower than the diagnostic value of TnI and CK-MB in the blood samples collected between the 12th and 36th hours. These findings suggest that increased MPO levels may be useful for the diagnosis of ACS and may be a marker for unstable angina before myocardial necrosis.

This study has certain limitations. First, our study population is small. Hence, with larger groups, more significant logistic regression analysis could be done. Second, we examined biomarkers associated with inflammation that might cause fQRS formation. However, we could not attain the results that would reveal this relationship. This suggests that these biomolecules may also be elevated due to causes independent of atrial fibrillation or scar tissue. Third, parameters related to inflammation were evaluated before starting any medical treatment. Since our study was performed in patients who are in the acute period of the ACS, it is recommended to examine patients with fQRS for long-term mortality and to investigate whether these parameters will change in the chronic period.

In conclusion, our study suggests that MPO, OPN, and sST2 may be included among the factors that may contribute to the diagnosis and development of ACS but they are not related to fQRS. High ACS diagnostic sensitivity and specificity of OPN is a new finding in this study. Also, this study is the first study that demonstrates the relationship between MPO, OPN, sST2, and fQRS development. Therefore, we believe that more comprehensive studies are needed to clarify this relationship and to develop new diagnoses and treatment methods for high-risk patients with elevated MPO, OPN, and sST2 levels.

Ethical Statement

Approval was obtained from Ordu University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 15/11/2018 and the number of ethical committee decisions is 2018/228.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: S.S.M., T.N., O.B., Design: S.S.M., T.N., Data Collection or Processing: S.S.M., T.N., O.B., Analysis or Interpretation: S.S.M., T.N., O.B., Literature Search: S.S.M., T.N., Writing: S.S.M., T.N.,

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Investigation of presence of O25B-ST131 clone and *in vıtro* efficacy of temocillin in *escherichia coli* isolates

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Abstract

Escherichia coli ST131 isolates associated with fluoroquinolone and cephalosporin resistance have increased in the last ten years. This increase has led to the emergence of multidrug-resistant *E. coli* isolates, resulting in treatment failures for urinary tract infections. The increasing antimicrobial resistance in gram-negative bacteria and the scarcity of new antimicrobials have brought old antimicrobials, such as temocillin, back into consideration. Temocillin has significant advantages and may serve as an alternative to carbapenems in treating serious Enterobacterales infections, such as systemic urinary tract infections. This study aimed to determine the presence of the O25b-ST131 clone in fluoroquinolone-resistant *E. coli* isolates and assess temocillin resistance. *E. coli* isolates obtained from urinary tract samples of patients hospitalized in the Faculty of Medicine Hospital of Ondokuz Mayıs University were included in the study. The presence of clone O25b-ST131 in these isolates was investigated using PCR. In addition, temocillin susceptibility in these isolates was determined using the Kirby-Bauer disk diffusion method. According to the PCR results, the prevalence of *E. coli* O25b-ST131 isolates was 40.8%. The findings of the antimicrobial resistance tests revealed that all isolates had low levels of carbapenem and fosfomycin resistance (2.04% and 3.06%, respectively). The temocillin resistance rate was determined to be 50%. It is known that clone O25b-ST131 is associated with fluoroquinolone and cephalosporin resistance; therefore, a high prevalence of the O25b-ST131 clone was expected in fluoroquinolone-resistant *E. coli* isolates included in the study. Temocillin is an antimicrobial agent widely used in many European countries, particularly for treating carbapenem-resistant *E. coli* infections. However, in our study, high rates of temocillin resistance (50%) were observed, despite this agent not being used in our country yet. These high resistance rates could be related to cross-antimicrobial resistance.

Keywords: *Escherchia coli*, fluoroquinolone resistance, O25b-ST131, temocillin

1. Introduction

Urinary tract infections (UTIs) are the most common type of community- and hospital-acquired infections, referring to infections in the kidney, ureter, urethra, bladder, and urinary tract. UTIs are estimated to affect approximately 150 million people annually, resulting in over 6 billion dollars in health expenditures. *Escherichia coli* is responsible for 70-90% of UTIs, and extraintestinal pathogenic *E. coli* (ExPEC) is a significant causative factor of UTIs in both developed and developing countries (1-4).

In 2008, a previously unrecognized clonal group of *E. coli*, known as 'sequence type 131 (ST131)', was identified in nine countries across three continents, including Canada, France, Portugal, Spain, Switzerland, India, South Korea, Kuwait, and Lebanon. ST131 was recognized as the dominant extraintestinal pathogenic *E. coli* (ExPEC) strain worldwide. This clone, referred to as a 'high-risk pandemic clone,' plays a significant role in the global spread of antimicrobial resistance, making it an important target for global surveillance studies. The dominant serotype of the ST131 clone is O25b:H4, which belongs to the phylogenetic group B2. This clone produces CTX-M-15 type extended-spectrum beta-lactamase (ESBL), exhibits high virulence, and is named 'O25b-ST131' (5-9).

E. coli ST131 isolates are responsible for causing community and hospital-acquired urinary tract infections and bacteremia worldwide. Moreover, the ST131 clone has been reported to be associated with other diseases, including intra-abdominal and soft tissue infections, meningitis, osteoarticular infection, myositis, and septic shock (9-13).

In the past decade, the emergence of multidrug-resistant ExPEC strains, mainly due to the increasing number of *E. coli* ST131 isolates, has posed challenges in treating UTIs, leading to antimicrobial therapy failure and increased morbidity and mortality (14-19). Carbapenems and fosfomycin are often considered appropriate treatment options for *E. coli* ST131 isolates. However, the widespread use of these antimicrobials has also led to the dissemination of carbapenem and fosfomycin resistance genes, resulting in increased resistance (1). As a result, there has been a need to explore alternative treatment options. The lack of novel antimicrobials for treating

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multidrug-resistant gram-negative bacterial infections has led to the resurgence of old antimicrobials, such as temocillin (20,21). Temocillin is a narrow-spectrum penicillin with limited activity against gram-positive, antipseudomonal, and anaerobic bacteria. It is recommended as an alternative antimicrobial agent for treating enteric bacterial infections. Temocillin has several advantages, including high resistance to hydrolysis by multiple ß-lactamases, including ESBL and AmpC, and minimal risk of causing *Clostridium difficile* infection. Due to these favorable properties, temocillin may be considered an alternative to carbapenems in treating severe *Enterobacterales* infections, such as systemic urinary tract infections (22-24).

This study aimed to assess the presence of the O25b-ST131 clone and temocillin resistance in fluoroquinolone-resistant *E. coli* isolates obtained from urinary tract samples.

2. Materials and Methods

2.1. Bacterial Isolates, Identification, and Susceptibility Testing

A total of 98 randomly selected fluoroquinolone-resistant *E. coli* isolates obtained from mid-stream urine samples were included in this study. These isolates were collected (one isolate per patient) at the Medical Microbiology Laboratory of Ondokuz Mayıs University between September 2020 and September 2021. Bacterial identification was performed using Vitek MS's automated system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing was conducted using the Vitek 2 Compact System (bioMérieux, Marcy l'Etoile, France). Prior to the detection of the O25b-ST131 clone, the boiling method was used for DNA extraction of the isolates, and the obtained DNAs were stored at -20°C for later use as template DNA for PCR.

2.2. Detection of O25b-ST131 Clone

The presence of the O25b-ST131 clone was detected by amplifying a 347 bp fragment of the pabB gene using primers *O25pabBspe*-F and *O25pabBspe*-R in all fluoroquinolone-resistant *E. coli* isolates. The primers used in this study were selected based on a literature search (25). PCR was performed with the following conditions: initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 5 seconds, annealing at 65°C for 10 seconds, and extension at 72°C for 5 minutes. The PCR products were then subjected to agarose gel electrophoresis on a 2% agarose gel. The DNA bands of the samples were compared with a 100 bp DNA marker and analyzed using an imaging instrument.

2.3. Temocillin Susceptibility Test

The temocillin susceptibility was determined using the Kirby-Bauer disk diffusion method, following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines on Mueller-Hinton agar. Disks containing 30 µg of temocillin were used. The zone diameters were interpreted according to the EUCAST breakpoints: isolates with zone diameters of ≥50 mm were considered susceptible, while those with zone diameters of <17 mm were considered resistant to temocillin (26).

2.4. Statistical Analysis

The data obtained from the study were analyzed using IBM SPSS Statistics software, version 22.0 (SPSS Inc., IL, USA). Descriptive statistics, including percentages, were used to present patients' demographic information, clinic distribution, and antimicrobial resistance rates. The relationship between the presence of the O25b-ST131 clone and antimicrobial resistance rates was analyzed using the Pearson Chi-square test. A p-value of <0.05 was considered statistically significant for the findings.

2.5. Limitations

One limitation of our study is that the minimum inhibitory concentration (MIC) value of temocillin was not determined by the microdilution method, which is considered the gold standard method for antimicrobial susceptibility testing.

3. Results

Demographic information of the patients included in the study is presented in Table 1. Of the total patients, 63.3% were female, and 36.7% were male. The distribution of urine samples based on the clinics are shown in Table 2, with 40.8% sent from internal medicine clinics and 25.5% from surgical clinics.

Table 1. Patients' demographic information

Variables		n (%)
Gender	Women	62 (63.3)
	Men	36 (36.7)
Age Groups	24-40 age	5 (5.1)
	41-65 age	36 (36.7)
	66+ age	57 (58.2)

Table 2. The distribution of samples according to clinics

Clinics	n (%)
Emergency	5 (5.1)
Surgery Clinics	8 (8.2)
Internal Medicine	31 (31.64)
Intensive Care	13 (13.26)
Infection Diseases	5 (5.1)
Chest diseases	3 (3.06)
Gynecology and Obstetrics Surgery	6 (6.1)
Cardiology	3 (3.06)
Neurology	4 (4.08)
Orthopaedics and traumatology	1 (1.02)
Oncology	9 (9.18)
Urology	10 (10.2)
Total	98 (100)

The resistance rates of the isolates to various antibiotics are as follows: 79.6% to third-generation cephalosporins, 55.1% to gentamicin, and 29.6% to trimethoprim-sulfamethoxazole. In contrast, the resistance rates to amikacin, fosfomycin, and carbapenems were lower, with 6.1%, 3.06%, and 2.04%, respectively. The temocillin disk diffusion test results showed that 50% of the 98 fluoroquinolone-resistant *E. coli* isolates were resistant to temocillin, while the other 50% were susceptible. The antimicrobial resistance rates of all isolates are presented in Table 3.

Table 3. The antimicrobial resistance rates of isolates

Antimicrobials	All of isolates (n:98)		O25b-ST131 isolates (n:40)		Non-O25b-ST131 isolates (n:58)		p-value*
	n	%	N	%	n	%	
Amikacin	6	6.12	3	7.5	3	5.17	0.158
Amoxicillin/Clavulanic Acid	58	59.18	26	65	32	55.17	0.331
Ampicillin	91	92.85	36	90	55	94.82	0.362
Ertapenem	2	2.04	1	2.5	1	1.72	0.789
Fosfomycin	3	3.06	-	-	3	5.17	0.237
Gentamicin	29	29.59	11	27.5	18	31.03	0.460
İmipenem	1	1.02	-	-	1	1.72	0.404
Meropenem	-	-	-	-	-	-	0.404
Nitrofurantoin	5	5.1	2	5	3	5.17	0.970
Piperacillin/Tazobactam	27	27.5	10	25	17	29.31	0.616
Cefixime	76	77.5	32	80	44	75.86	0.629
Ceftazidime	73	74.48	31	77.5	42	72.41	0.644
Ceftriaxone	75	76.53	32	80	43	74.13	0.501
Cefuroxime axetil	79	80.6	33	82.5	46	79.03	0.695
Cefuroxime	80	81.63	33	82.5	47	81.03	0.706
Trimethoprim/Sulfamethoxazole	54	55.1	23	57.5	31	53.44	0.692
Temocillin	49	50	22	55	27	46.5	0.411

*: The relation between the presence of O25b-ST131 clone and antimicrobial resistance rates was analyzed using Pearson Chi-square test. Statistically significant findings were assumed with $p < 0.05$

Upon amplification, 40.8% of the isolates were identified as *E. coli* O25b-ST131. The antimicrobial resistance rates of *E. coli* O25b-ST131 and non-O25b-ST131 isolates are shown in Table 3.

However, according to the statistical analysis, there was no statistically significant difference between O25b-ST131 and non-O25b-ST131 isolates in terms of antimicrobial resistance rates (Table 3).

4. Discussion

Urinary tract infections (UTIs) are infections associated with high morbidity and mortality. The most frequently isolated bacteria in UTIs are *E. coli*, responsible for approximately 70-90% of community-acquired UTIs (27). The emergence and rapid global spread of hypervirulent *E. coli* ST131 associated with antimicrobial resistance *seriously threaten* public health. ST131 is recognized as the predominant ExPEC strain worldwide. *E. coli* ST131 isolates are often resistant to broad-spectrum cephalosporins and FQs (7,28,29). Most studies investigating the ST131 clone have focused on broad-spectrum beta-lactamase (ESBL) producing or FQ-resistant isolates. The treatment options for these problematic isolates are limited. Therefore, it is crucial to monitor the prevalence of *E. coli* ST131 and the resistance of limited agents that can be used to treat bacteria belonging to this clonal group. This study aimed to determine the prevalence of the O25b-ST131 clone and investigate temocillin resistance in FQ-resistant uropathogenic *E. coli* isolates.

In studies conducted with uropathogenic *E. coli* isolates in China, Türkiye, and Iran, the prevalence of the O25b-ST131 clone was found to be 12.5%, 22%, and 24.7%, respectively (30-32). In a study conducted with uropathogenic FQ-resistant *E. coli* isolates in Japan, the prevalence of the O25b-ST131 clone was found to be >70% (33). The results of these studies support the existence of a high prevalence of O25b-ST131 in FQ-resistant isolates. On the other hand, studies reported that O25b-ST131 clone carriage is common in long-term hospitalized patients and that the prevalence of the clone was higher in inpatients than in outpatients. High *E. coli* ST131

carriage rates of 55%, 36%, and 24%, respectively, have been found in healthcare facilities in Ireland, the UK, and the USA. (34-36). In our study, the prevalence of the O25b-ST131 clone in FQ-resistant *E. coli* isolates was 40.8%. This high rate is expected because the isolates included in the study were FQ-resistant E. coli isolates obtained from inpatient samples.

Temocillin is a narrow-spectrum penicillin primarily active against the Enterobacterales order and resistant to many beta-lactamases, including most AmpC and ESBL. In the mid-2000s, ESBL-producing *Enterobacterales* isolates became widespread, and recently, carbapenem resistance has increased considerably. New treatment options were investigated for these reasons, and interest in this old antimicrobial has increased again (20, 37). The gold standard method for determining the temocillin susceptibility is the microdilution method. However, the Kirby-Bauer disk diffusion method can also be used to determine the temocillin susceptibility. In 1985 Fuchs et al. published the first interpretative criteria for temocillin susceptibility testing. According to Fuchs et al., with the 30 µg temocillin disk, 19 mm was the susceptible zone diameter breakpoint (38). Then, breakpoints for temocillin were published for the Clinical & Laboratory Standards Institute (CLSI), British Society for Antimicrobial Chemotherapy (BSAC), Comité de l'antibiogramme de la Société Française de Microbiologie (CA-SFM), and EUCAST (26, 39-41). The susceptibility diameter breakpoints for these guidelines are 19 mm, 12 mm, 20 mm, and 50 mm with the 30 µg temocillin disk, respectively.

In studies conducted with ESBL-producing *E. coli* isolates from Türkiye and France, temocillin resistance was reported as 20.7% and 28.7%, respectively (24,42). In a study conducted with uropathogenic *E. coli* isolates in Singapore, temocillin resistance was 7% (43). In another study conducted with uropathogenic *E. coli* isolates in Korea, temocillin resistance was reported as 9.2% in ciprofloxacin-resistant isolates and 1.5% in ciprofloxacin-susceptible isolates (44). In our study, temocillin resistance was found to be 50% in FQ-resistant *E. coli* isolates and 55% in FQ-resistant O25b-ST131 *E. coli* isolates. These results are relatively high, although temocillin is not used in Turkey. The high resistance rate may be due to cross-antimicrobial resistance. In addition, temocillin resistance was found to be relatively high compared to studies conducted in our country (24,45). This result may be because studies are based on different antimicrobial susceptibility tests, guidelines, and breakpoints. Our study was performed by the Kirby-Bauer disk diffusion method based on EUCAST breakpoints.

In conclusion, the prevalence of *E. coli* O25b-ST131 was determined to be 40.8% in our study. Since all isolates are FQ-resistant, the prevalence of O25b-ST131 is expected to be high in these isolates. According to the antimicrobial susceptibility results, carbapenem and fosfomycin resistance rates were still low in all isolates (2.04% and 3.06%, respectively). Although the resistance rates of these antimicrobials, which are essential for treatment, are pleasing, continuously monitoring the resistance rates is necessary. Moreover, significant resistance differences were not observed between O25b-ST131 and non-O25b-ST131 isolates. Temocillin is one of the critical agents used in many European countries, especially in treating carbapenem-resistant *E. coli* infections. Although this agent is not yet used clinically in Türkiye, a high rate of resistance to temocillin was found in our study. This high rate may be due to cross-antimicrobial resistance.

Ethical Statement

Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Ondokuz Mayis University before the start of the study (Number: B.30.2.ODM.0.20.08.667-679-707, Decision Number: 2022/449, Date: 12/10/2022).

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: K.H.Ü., Y.T.Ç., Design: K.H.Ü., Y.T.Ç., Data Collection or Processing: K.H.Ü., D.S., K.M., H.U., G.O., A.S., H.A.A.A., Analysis or Interpretation: K.H.Ü., D.S., K.M., H.U., G.O., A.S., H.A.A.A., Y.T.Ç., Literature Search: K.H.Ü., Y.T.Ç., Writing: K.H.Ü., Y.T.Ç.

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Somatosensory amplification, health anxiety and perceived social support levels in patients scheduled for hysteroscopy

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Abstract

This study examined the anxiety, health anxiety, somatosensory amplification, and social support levels perceived by hysteroscopy patients. This study included 100 women scheduled for diagnostic hysteroscopy and 70 women for control. Hospital Anxiety Depression Scale (HADS), Penn State Anxiety Scale (PSAS), Health Anxiety Scale (HAS), Somatosensory Amplification Scale (SAS), and Multidimensional Perceived Social Support Scale (MPSSS) were applied to all participants. We determined that the scores of the patients for SAS were higher ($p<0.001$). HAS all subscales were also calculated as higher than the healthy controls in the patients (p values: <0.001; 0.008; <0.001, respectively). It was also determined that MPSSS family support was perceived as adequate, and the subscales of special people and friends were perceived as inadequate. We found that women experienced health anxiety at a significant level before hysteroscopy. In addition, the somatization tendencies of these women increased. We recommended that these patients should be supported psychosocially before surgical procedures.

Keywords: hysteroscopy, anxiety, depression, somatization, health anxiety, social support

1. Introduction

Anxiety is a feeling similar to fear and is a state of worry that is unexplained, as if something negative/bad is going to happen without any actual reason. This clinical condition can be felt as very mild anxiety and intense enough to reach the panic level. During anxiety, autonomic nervous system activation and tension are seen to protect the body against danger. Accelerated breathing, palpitation in the heart, sweating because of body temperature, common body aches, weakness, and fatigue might occur (1). Health anxiety; is an individual's interpreting their body as if it is going to be seriously ill and being extremely concerned about their health (2). These concerns about one's health continue, although the individual is resistant and controls prove that the individual is healthy (3). Previous studies were conducted to report that health anxiety is prevalent in society and clinical settings (4, 5). Individuals with health anxiety feel extreme anxiety about their health, exaggerate bodily sensations or functional changes, and describe these as signs of disease (5). In addition, they also negatively interpret any bodily sensations even if there are no physical diseases (6). The "Somatosensory Amplification" concept emerged to explain the somatization in the distinction of spiritual/physical medicine (7). It is the condition in which a person responds physically to a stress factor, seeks medical attention, and feels their physical sensation as intense and harmful/threatening (8).

Failure, material losses, divorce, assault, accidents, as well as past diseases, hospitalization, and surgical procedures, are also perceived as stress factors in adulthood (9). The support an individual receives from friends and family to cope with stressful life events is defined as social support (10). The social support concept has become multidimensional according to how people perceive and understand events around them. Social support is expressed as an interpersonal relation protecting an individual from stressful settings and as information that makes them believe that they are cared for and are a member of a network of mutual responsibilities (11).

Many studies were conducted in the literature on anxiety and psychosocial problems perceived as stress factors before surgical procedures (12-14). However, no studies were detected in which health anxiety, somatosensory amplification, and perceived social support levels were examined together. In gynecology, hysteroscopy is a minimally invasive surgical intervention used frequently to diagnose and treat abnormal uterine hemorrhages, endometrial polyps, uterine anomalies, and sub-mucous myomas. Making the surgical procedure possible with an endoscope and hand tools that are placed in the endometrial cavity through trans cervical pathway without any incisions in the abdominal skin makes it possible for the discharge from the hospital on the same day with the

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intervention, fast return to social life, providing fast recovery. There is a limited number of studies examining the anxiety levels caused by hysteroscopy, which is seen as a minimally invasive surgical procedure from a surgical point of view with all its advantages (15, 16). The present study's first hypothesis is that women who are scheduled to undergo hysteroscopy have high anxiety levels and feel more intense health anxiety. Secondly, these patients will tend to amplify their bodily sensations because of the stress they experience. Finally, the social support they perceive is also low. Based on these hypotheses, our study aims to examine the anxiety, health anxiety, somatosensory amplification, and social support levels perceived by patients scheduled for hysteroscopy.

2. Subject and Methods

2.1. Sample

The study groups admitted to the hospital for diagnostic hysteroscopy because of menstrual irregularity were evaluated by the same psychiatric physician in the Obstetrics and Gynecology Service. The illiterate volunteers who could give written consent and fill out the forms were included in the present study. The individuals with poor general condition, chronic liver disease, chronic renal failure, chronic heart disease, mental retardation, and people who had any psychiatric disorders, who required treatment, who did not want to participate in the study, and those with alcohol-substance use disorders were excluded from the study. People who matched the Patient Group regarding sociodemographic data and those with no diagnosis of psychiatric diseases were also included as healthy.

2.2. Data collection tools

All participants signed the consent form. Then, Sociodemographic Data Form, Hospital Anxiety Depression Scale (HADS), Penn State Anxiety Scale (PSAS), Health Anxiety Scale (HAS), Somatosensory Amplification Scale (SAS), and Multidimensional Perceived Social Support Scale (MPSSS) were applied to the participants.

Sociodemographic Data Form: This form contained demographic data like age, marital status, educational status, residence, working status, and economic status. In addition to these data, it also included clinical evaluation questions like whether there was a psychiatric treatment before and during the study, whether the participant had a psychiatric disease, whether they had prior surgery, and whether they had an additional medical disease.

Hospital Anxiety Depression Scale (HADS): A 14-point self-notification scale applied to measure the symptoms of depression and anxiety the patient experiences. It was developed by Zigmond and Snaith and developed in Turkish form by Aydemir (17, 18).

Penn State Anxiety Scale (PSAS): This scale evaluates persistent, excessive, and uncontrollable anxiety levels. It is a Likert-type scale consisting of 16 items and is scored between 1-5 (19, 20).

Health Anxiety Scale-Short Form (HAS): This form is used to evaluate the level of anxiety a person experiences about their health. The first 14 items of the scale question the feelings and thoughts about health, and the last four items consist of questions on how the participant will feel and react if they have a severe illness. High scores on the scale indicate higher health anxiety levels. It was developed by Salkovskis et al. (4,6).

Somatosensory Amplification Scale (SAS): A Likert-type scale comprising ten questions evaluating the exaggeration of a person's common and usual bodily symptoms. A total exaggeration/somatization score is achieved by collecting the scores received (7, 8).

Multidimensional Perceived Social Support Scale (MPSSS): A 12-item scale that evaluates the adequacy of support from three different social support sources in a subjective manner. It is assessed in "family," "friend," and "a special person" sub-scales by assigning 4 points each. A high score indicates high perceived support (21, 22).

2.3. Statistical Analysis:

The Statistical Software SPSS for Windows 19 (Statistical Package for Social Sciences for Windows 19) was employed in the calculations. The qualitative variables of the study are demographic data like age, marital status, educational level, working status, and socioeconomic level, as well as having psychiatric treatment history in the participant or their family and any additional medical disease. The cross-table and Chi-Square Tests were used to evaluate whether there was a relation between the qualitative variables. The quantitative variables of the study are the scores from HADS, SAS, MPSSS, PSAS, and HAS. The Significance of the Difference Between Two Averages and the Pearson Correlation Coefficient were used to evaluate the association between quantitative variables. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Sample characteristics

One hundred sixty patients scheduled for hysteroscopy were interviewed. Since 20 of the patients who were evaluated refused to participate in the study, ten people continued their regular follow-ups and treatments in the psychiatry unit, eight people were illiterate, 15 people did not fill the forms, or the forms were incomplete, they were not included in the study. The remaining patients were excluded from the study because they met the exclusion criteria. One hundred patients scheduled for hysteroscopy were included as the study group, while 70 people who met the inclusion criteria were taken as the healthy control group (Fig. 1.)

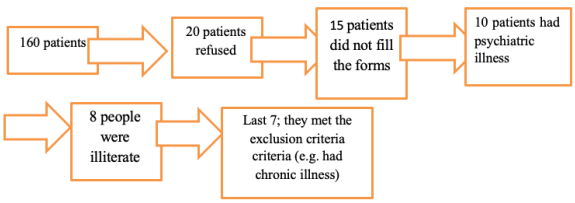

Fig. 1. Flowchart

All the participants were female. Three people in the patient group and five in the control group were single. No differences were detected between the participants' educational levels, working status, and socioeconomic status (p>0.05). There were no psychiatric disorders that required to receive treatment in any participants. None of the participants had any additional medical diseases. Thirty-seven people in the patient group had a previous operation, and for 63 people, it was the first operation. Thirty-nine people in the control group had previously undergone an operation, while 31 had not before (Table 1).

Table 1. Sociodemographic characteristics of the groups

	Control Group (n=70)(%)	The patient group scheduled for hysteroscopy (n=100)(%)	P
Mean Age (Mean±SD)	37.24±7.72	36.49±9.02	0.571
Marital status (married/single)	62.9/7.1	97/3	0.209
Educational status			
Primary school graduate	38.6	49	
High school graduate	34.3	25	0.324
University graduate	27.1	26	
Working status			
Part-time job	35.7	26	
Full-time job	7.1	2	0.070
Housewife	57.1	72	
Socioeconomic Status			
Low Level	14.3	12	
Moderate Level	81.4	75	0.157
High Level	4.3	13	
Residence			
City Center	72.9	50	
District	34.3	40	0.012
Village	5.7	10	
Past Surgery (Yes/No)	**55.7/44.3**	**63/37**	**0.340**

No psychiatric treatment in the family, no additional disease, none of the participants has psychiatric treatment. The Chi-Square Test was applied, the values in the table were calculated with %.

3.2. Scale scores

The participants' quantitative variables revealed no differences between the people scheduled for hysteroscopy and the control in any subscales of HADS (p>0.005). The Somatosensory Amplification Scale score in the patients scheduled for hysteroscopy was calculated as 31.37±8.14; and 25.07±8.87 in the control (p<0.001). The Health Anxiety Scale Body scores, negative results, and total scores were statistically and significantly higher in patients scheduled for hysteroscopy (p values: <0.001; 0.008; <0.001, respectively). The Multidimensional Perceived Social Support Scale scores were significant in specific subscales, and the Penn State Anxiety Scale did not differ at statistically significant levels (Table 2).

Table 2. Quantitative distributions of the groups

	Control Group (n=70) (Mean±SD)	Patient Group to Undergo hysteroscopy (n=100) (Mean±SD)	P
HADS			
Anxiety scale	6.63±2.39	6.56±3.88	0.896
Depression scale	5.14±3.23	5.55±3.61	0.451
Total Score	11.77±4.85	12.11±6.6	0.715
PSAS	41.47±11.34	44.07±11.07	0.138
SAS	25.07±8.87	31.37±8.14*	<0.001
MPSSS			
Special person	22.17±7.1	19.48±8.13*	0.027
Family Support	21.74±6.11	21.04±5.84	0.450
Friend Support	20.59±6.88	18.23±7.31*	0.036
Total score	64.64±18.77	58.18±18.45*	0.027
HAS			
Body Score	9.29±4.45	13.84±5.62*	<0.001
Negative Results	2.77±1.87	3.63±2.18*	0.008
Total score	**12.06±5.46**	**17.47±6.73***	**<0.001**

The abbreviations in the table: HADS: Hospital Anxiety Depression Scale, PSAS: Penn State Anxiety Scale, SAS: Somatosensory Amplification Scale, MPSSS: Multidimensional Perceived Social Support Scale, HAS: Health Anxiety Scale, (Mean±SD): Mean±Standard Deviation. The Significance of the Two Mean Values Test was applied. The values given in the table are presented as Mean±Standard Deviation.

3.3. The Pearson Correlation Analysis results

These results are presented as tables (Table 3 and Table 4). It was determined that there was a positive relation between SAS and PSAS (r=0.227; p=0.023). It was also determined that there was a positive relation between the total score, the Body Score, and the SAS and HAS (***r*** values=0.197; 0.190, respectively). No significant relations were detected between the Multidimensional Perceived Social Support Scale, SAS, HAS and PSAS (p>0.05).

Table 3. Pearson Correlation Analysis results of the patients scheduled for hysteroscopy - I

	Hospital Anxiety Depression Scale		
	Anxiety subscale	**Depression subscale**	**Total scor**
MPSSS			
Special person	-.010	-.100	-.060
Family Support	-.114	-.222*	-.189
Friend Support	-.085	-.102	-.106
Total score	-.049	-.138	-.104
PSAS	.419*	.236*	.376*
SAS	.095	.057	.087
HAS		11	
Body Score	.316*	.162	.275*
Negative Results	.363*	.312*	.384*
Total score	**.382***	**.236***	**.354***

The abbreviations in the table: PSAS: Penn State Anxiety Scale, SAS: Somatosensory Amplification Scale, MPSSS: Multidimensional Perceived Social Support Scale, HAS: Health Anxiety Scale. The values given in the table are the R values. The calculations were made with Pearson Correlation Analysis. *p<0.05

Table 4. Pearson Correlation Analysis results of the patients scheduled for hysteroscopy - II

	MPSSS-1	MPSSS-2	MPSSS-3	MPSSS-4	PSAS
SAS	.142	.140	.130	.142	.227*
Health Anxiety Scale					
Body Score	.055	-.093	-.053	-.014	.342*
Negative Result	.006	-.195	-.078	-.062	.360*
Total Score	**.048**	**-.141**	**-.069**	**-.032**	**.402***

The abbreviations in the table: MPSSS: Multidimensional Perceived Social Support Scale; 1: Special person; 2: Family Support; 3: Friend Support; 4: Total score; PSAS: Penn State Anxiety Scale, SAS: Somatosensory Amplification Scale. The values given in the table are the R values. The calculations were made with Pearson Correlation Analysis. *p<0.05

4. Discussion

We used HADS and PSAS in our research and detected no significant differences between the groups for HADS. Gambadauro et. al. showed that the anxiety levels of patients before hysteroscopy with HADS, patients received an average score of 6.8±4.2. Although this result was below the cut-off points, it was higher than the group scheduled for laparoscopic tubal ligation (15). Similar to this study, the anxiety scores of women scheduled for hysteroscopy were calculated as 6.56±3.88 on average in the present study. This score was below the cut-off points for the anxiety subscale. However, 16 (16%) of the participants who were scheduled for hysteroscopy exceeded the cut-off score for the anxiety subscale, and only one person exceeded the cut-off score in the control (1.42%). Although hysteroscopy is a minimally invasive procedure, it is a condition in which patients can be expected to worry before the procedure because it is a surgical procedure. In a study that examined the anxiety levels in patients who waited for hysteroscopy, it was reported that 65% experienced anxiety. However, an evaluation tool was not used in this study; instead, the participants were asked whether they had anxiety (23). The Spielberger State-Trait Anxiety Inventory (STAI) was used to evaluate anxiety levels before hysteroscopy in most of the studies conducted previously in the literature (24-26). STAI is an anxiety evaluation scale for measuring “state and trait anxiety” levels. The anxiety in specific situations is interpreted as “state anxiety,” and the tendency towards continuous anxiety and the future is interpreted as “trait anxiety” (27). In a study that examined the anxiety of people with this evaluation tool before hysteroscopy, the STAI score of the women before the procedure was reported as 45.7. This result was found to be higher than the patients who were admitted to the general gynecology clinic and lower than the patients who were evaluated for chronic pelvic pain (24). In a study conducted with STAI, the anxiety level was calculated as 41.50. This result was reported as a moderate anxiety level for the STAI scale in which 20 and 80 scores may be received (25).

Another study evaluated 18 women who underwent hysteroscopy for diagnosis in the postmenopausal period with STAI-short form. As a result, the anxiety scores were calculated as high compared to the control (26). The results in the literature showed changes per the measurement tools used to evaluate anxiety levels. Our study evaluated the anxiety levels of people with PSAS and HADS. The Penn Anxiety Scale STAI evaluates persistent, excessive, and uncontrollable anxiety. Scores can be between 16 and 80; the higher the score, the higher the anxiety levels are (19, 20). No significant differences were detected between the participants for PSAS in our results. However, although no participants received 80 maximum points in the control, two people (2%) received maximum points in the patient. In addition, 30 people (30%) scored 50 or more in the patient, and this rate was calculated as 14 (20%) in the control. Scores of 50 or more are considered moderate anxiety levels for an anxiety evaluation tool with ratings between 16 and 80.

No significant differences were detected in our results in terms of the subscale of depression of the participants. Studies on depression levels before surgical or diagnostic interventions are limited in the literature (28-30). A previous study determined that the depression and anxiety scores of people before interventional procedures for diagnostic purposes were high (28). Another study showed that the diagnosis of preoperative depressive disorder adversely affects the recovery times after the surgery (29, 30). Studies on anxiety and depressive disorders report higher incidence in the female gender. Factors like the psychosocial role, pregnancy, and childbirth, and biological differences related to premenstrual periods of women within the society were held responsible for this (31, 32). Although there were no differences in our results in terms of depression scores between the patient and the control, 20 people (20%) exceeded the cut-off score for the depression subscale in the group that was scheduled for hysteroscopy. This rate was calculated as eight people in the control (11.40%). Since the menstrual cycle is irregular, it was interpreted as an expected condition that there were increases in the depression scores of people who were scheduled for hysteroscopy for diagnostic purposes.

Although the participants did not have any differences in terms of both anxiety evaluation tools and the depression subscale, their Health Anxiety and Somatosensory Amplification levels were calculated to be higher than the control that was scheduled to undergo hysteroscopy. Health anxiety occurs when a person interprets existing physical symptoms as a severe disease even if they do not have any physical illness diagnosed, and when they over-worry the negative results of the condition they believe to exist (6, 21). Somatosensory amplification is the tendency to perceive normal bodily sensations in an extreme, harmful, disturbing, and depressing way (7, 8). Previous studies revealed that somatization is associated with psychiatric disorders like depressive disorder, panic disorder, and hypochondriasis, as well as other medical diseases like fibromyalgia and chronic pain syndrome (8). There are studies in the literature arguing that there is a difference between genders and that women are

more prone to somatization (33, 34), as well as studies reporting the opposite, namely, men are more prone to somatization (35). Some studies reported that there is no gender difference for somatization (4, 7, 8). No other studies in the literature examined health anxiety and somatization before hysteroscopy. In a study examining somatization and health anxiety levels before invasive intervention/angiography for diagnostic purposes, the somatization and health anxiety levels of the group scheduled for angiography were higher than the control (28). A study conducted on pregnant women found that the somatosensory amplification levels of pregnant women were higher than the non-pregnant group (36). Although some studies reported a relation between anxiety and depression scores and somatization (28, 36), the results of some studies were similar to ours (7, 8); namely, no relations were detected between anxiety, depression, and somatization scores. Although the surgery is minimally invasive, the thought of surgery in the patient's consciousness and the possibility of being diagnosed with a postoperative malignancy might lead individuals to consider bodily sensations more seriously.

Finally, the perceived social support levels were also examined in the study. Significant differences were detected in the group scheduled to undergo hysteroscopy compared to the control in specific subscales. No previously conducted studies were found in the literature examining the perceived social support levels of patients scheduled for hysteroscopy. However, studies were conducted to examine the perceived social support levels before surgical procedures or diagnostic interventions. These studies emphasized that the recovery times of patients with good support were shortened in addition to the importance of preoperative social support, especially family support (37-39). In addition, these studies showed that the family support perceived by patients was adequate before the operation, similar to our results (37-39). Our results revealed insufficient perceived social support - special human and friend subscales. This result may stem from patients who underwent hysteroscopy, a minimally invasive surgery with fast discharge from the hospital, not notifying people other than their families.

Our results should be considered with some limitations in mind. The first one of these limitations is the number of patients. Other limitations are the inclusion of only women in the study, the random selection of the universe, the lack of SCID-5-CV (Structured Clinical Interview for DSM-5), and the failure to conduct long-term interviews after the hysteroscopy. These limitations prevent our findings from being generalized. Further studies are needed with a much larger number of participants in larger sampling groups.

Our results evinced that although the anxiety and depression levels of women did not increase before hysteroscopy, they experienced significant health anxiety. In addition, we also determined that the somatization tendencies of these women increased, and the social support levels they perceived decreased, especially for the "friends" subscale. In light of these results, we recommend psychosocially supporting such patients to help them more in diagnostic procedures before surgery, tolerating possible postoperative malignancy diagnosis, and facilitating compliance with treatment.

Ethical Statement

Approval was obtained from Gaziosmanpaşa University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 19/02/2019 and the number of ethical committee decisions is 2019/02.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: F.O., A.Z.O., Design: F.O., A.Z.O., Data Collection or Processing: S.U., K.E.U., O.U., Analysis or Interpretation: F.O., N.K., Ş., K., Literature Search: F.O., Writing: F.O.

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The comparison of PAP smear test results of women according to anti-mullerian hormone levels

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Abstract

This study investigates OR investigated the relationship between PAP smear test results and anti-mullerian hormone (AMH) levels. The PAP smear test is used to screen for cervical cancer. This test identifies malignant or pre-malignant cells of the cervix and permits timely diagnosis and treatment. This retrospective cross-sectional study included 104 women of reproductive age who applied for a PAP smear test. This study was conducted between October 2019 and December 2020. Patients were clustered as subjects: 1) with an AMH <1ng/ml, 2) with a 1<AMH<3, and 3) with an AMH>3ng/ml. The mean age of the study group was 28.45 ± 3.31 years. There was a statistically significant association between AMH levels and PAP smear test results ($p<0.05$). AMH>3ng/ml group had a significant relationship with the AGUS PAP smear results. There was a statistically significant association between AMH between 1 and 3 and normal PAP smear test results. In conclusion, the results indicated that the serum AMH level and PAP smear test results had a significant relationship.

Keywords: Cervical cancer, anti-mullerian hormone, pap smear test, precancerous lesions

1. Introduction

The number of new cases of cervical cancer as a global public health problem is ranked 9th among all cancers, of which the incidence is estimated to be 604,127 and the mortality is estimated to be 341,831 in 2020 all over the world (1). There has been a decline in cervical cancer incidence in several high-resource countries with organized cervical screening programs (2). Approximately half a million new cervical cancer cases are detected yearly because of well-integrated human papillomavirus (HPV) vaccine programs (3, 4). PAP smear test is used for fast diagnosis of the disease and reduces its effects to a great extent. PAP smear test is a screening test to diagnose cervical cancer in apparently healthy women and is considered a health-promoting behavior (5). This test is performed every three years on women who have been or are sexually active. The implementation of the screening program in several countries for a term of five years has shown that the PAP smear test can reduce the death rate of cervical cancer by 60% (6).

Müllerian inhibiting substance (MIS), or anti-mullerian hormone (AMH) is a member of the superfamily of transforming growth factor beta (TGF-β) discovered by Alfred Jost (7, 8). AMH is produced exclusively in the ovarian small antral and preantral follicles' granulosa cells (9-11). One of the hormones which has attracted attention recently as a marker for predicting ovarian response before using assisted reproductive methods is AMH (12, 13). This hormone is produced by the ovarian granulose cells, and after puberty, its rate gradually decreases at a slow rate and disappears during menopause (14). Among the functions of this hormone are inhibition of primary follicle application, Follicle stimulating hormone (FSH)-dependent growth inhibition, and selection of perinatal follicles and small antral follicles (12).

Since AMH serum levels are associated with the number of primary antral follicles, this hormone is used to evaluate fertility potential and ovarian response in IVF, which stands for in vitro fertilization (11). We believe that there is a relationship between AMH levels and PAP smear test results. Physicians should recommend patients perform the PAP smear test by observing AMH values.

The present study assessed the relationship between AMH levels and PAP smear test results. The present study is significant in identifying the factors affecting the incidence of cervical cancer to provide preventive treatments. This study assesses the relationship between AMH levels and PAP smear test results.

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2. Materials and Methods

This retrospective cross-sectional study was approved by the local ethics committee of the university. (Date: 13/01/2021 Decision no: 2021/0027). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was performed on one hundred-four women between October 2019 and December 2020.

After the Institutional Review Board gave the approval, all surgical pathology files and PAP smear files were searched for "routine" PAP smears. We classified abnormal routine PAP smears into the following categories based on the latest Bethesda system: Atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells of undetermined significance (AGUS), low-grade squamous intraepithelial lesion (LGSIL), high grade squamous intraepithelial lesion (HGSIL), and cervicitis. We correlated each abnormal PAP smear with a tissue biopsy from the cervix, if available, for each abnormal PAP smear. We collected the grade and presence of dysplasia and the utility of immune stains on tissue biopsy, particularly HPV-16 (Clone Cam Vir-1, ready to use, BioGenex) and P16 (Clone E6H4, ready to use, Roche) in ruling out or confirming HPV infection, if available. AMH values were recorded from hospital data. Details about the serum AMH levels were collected from medical records. AMH enzyme immunoassay (Instrumentation Laboratory and Beckman-Coulter, Vienna, Austria) was used to specify serum AMH (ng/ ml). The scale for measuring hormones was nanograms per milliliter (ng/ml). Patients were clustered as subjects: 1) with an AMH<1, 2) with a 1<AMH<3, and 3) with an AMH>3 ng/ml.

2.1. Statistical analysis

The Kolmogorov-Smirnov test was performed to check the normality. The mean and standard deviations (SD) were measured to check each continuous variable, including age, body mass index (BMI), and AMH. The Mann-Whitney U test was performed to study the difference between the two groups. Pearson's chi-squared test was used to decide whether there was a statistically significant difference between the Pap smear test results and AMH levels. The Paired Samples Z-Test was used to determine the significance of AMH levels in different Pap test results. SPSS v22 was used for statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

To calculate the sample size with the G-Power 3.1 program, two groups' total mean was measured based on the Mann-Whitney test with a power of 95%, an effect size of 50%, and 0.05 type 1 error for at least 92 patients (15).

3. Results

This study included one hundred and four age-matched (28.45±3.31) and BMI-matched (25.19±2.25) women. Table 1 shows descriptive statistics of study parameters. Table 2 shows the frequency of PAP smear test results of AMH values in each PAP smear test result. As stated in Table 2, the highest frequency of PAP smear results was cervicitis (37.5%), normal (34.6%), ASCUS (14.4%), AGUS (8.7%), LGSIL (3.8%), and HGSIL (1%). As stated in Table 3, a chi-square test found a statistically significant association between AMH levels and Pap smear test results ($p<0.05$). The Pairwise Z-Tests found that the percentage of women who had the normal test result was significantly higher for those who had AMH between 1 and 3 (55.3% from n = 21) than for those who had AMH greater than three and lower than one. The percentage of women with the AGUS test result was significantly higher for those with AMH greater than three (20.6% from n=7). Women with AGUS PAP smear test results had serum AMH levels (2.8±1.14). AMH levels were not significantly different in ASCUS, LGSIL, HGSIL, and cervicitis PAP smear test results. Fig. 1 shows AMH levels in different PAP smear test results. More than 50% of normal PAP smear test results appeared between one and three in AMH.

Table 1. Descriptive statistics of study parameters in women (n=104)

Study parameters	median (range) / mean ± SD
Age	28 (22-36) / 28.45±3.31
BMI	25 (20-30) / 25.19±2.25
AMH	1.85 (0.2-4.6) / 2.02±1.25

SD, standard deviation

Table 2. The frequency of PAP smear test results

Study parameters		N (%)	AMH (mean ± SD)
PAP smear test results	Normal	36 (34.6)	2.01±1.07
	AGUS	9 (8.7)	2.8±1.14
	ASCUS	15 (14.4)	2.16±1.3
	LGSIL	4 (3.8)	0.9±0.62
	HGSIL	1 (1)	0.8±0.0
	Servisit	39 (37.5)	1.94±1.38

Table 3. The relationship between PAP smear test results and AMH levels

Study parameters		AMH<1 n (%)	1<AMH<3 n (%)	AMH>3 n (%)	*p*
PAP smear test results	Normal	7 (21.9)	**21 (55.3)**†	8 (23.5)	0.010*
	AGUS	1 (3.1)	1 (2.6)	**7 (20.6)**†	
	ASCUS	5 (15.6)	4 (10.5)	6 (17.6)	
	LGSIL	2 (6.3)	2 (5.3)	0 (0.0)	
	HGSIL	1 (3.1)	0 (0.0)	0 (0.0)	
	Servisit	16 (50.0)	10 (26.3)	13 (38.2)	

*A Chi-square test. † Pairwise Z-Tests

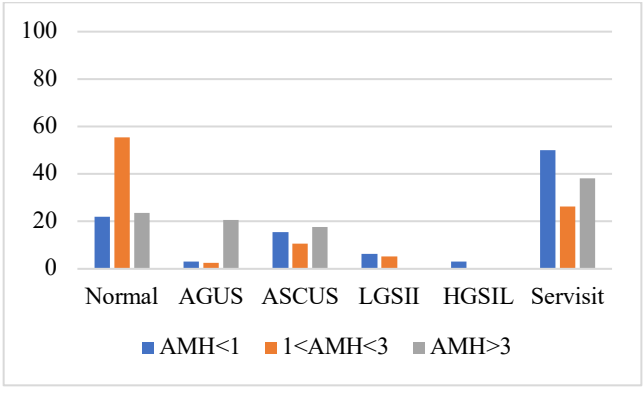

Fig. 1. AMH levels in different PAP smear test results

4. Discussion

Our study investigated the relationship between PAP smear results and AMH levels in sexually active women. Of the 104 sexually active women, 36(34.61%) had normal cytology results, and 68(65.39%) women had abnormal cytology. The frequency of abnormal cytology was 8.7% (n=9) for AGUS, 14.4% (n=15) for ASCUS, 3.8% (n=4) for LGSIL, 1% (n=1) for HGSIL, and 37.5% (n=39) for cervicitis. There was a significant association between AMH and PAP smear results. AMH levels as a marker for predicting ovarian response were between one and three in women who had normal cytology. The AGUS result was significantly higher for women who had AMH greater than three.

It is essential to know the risk factors of cervical cancer to reduce the mortality and morbidity related to the cervical cancer. The researchers have evaluated the factors affecting the PAP smear test results. Cervical cancer can be easily diagnosed and wholly treated in the early stages, but if its diagnosis is delayed, it will be challenging to treat and sometimes unsuccessful. Early diagnosis of this cancer is possible through a PAP smear test (16). The warning items that women should observe to perform this test are essential. Based on the findings, women can be suggested to take the test by monitoring their levels of AMH. However, to prove this finding, more research with a higher number of samples of different ages is needed. One can predict the qualitative and quantitative aspects of controlled ovarian stimulation and menopausal age, with the polycystic ovary syndrome (PCOS) diagnosis of serum AMH levels as a biomarker to evaluate ovarian reserve in women. Factors such as a history of ovarian surgery, PCOS, obesity, chemotherapy, and vitamin D deficiency also impact serum AMH levels (16). Several studies have been conducted on the extent and impact of these factors (16). This study aims to evaluate the possible relationship between PAP smear results and serum AMH levels. The following sections highlight a few related works investigating the relationship between serum AMH levels and the clinical characteristics of women.

Cervical cancer is one of women's leading causes of death (17). A PAP smear test is used for the early detection and diagnosis of this cancer. Researchers studied the factors influencing women's awareness of the PAP smear test for years (18). Age, multiparity, economic conditions, education, and job status were reported as factors affecting the PAP smear test (19-21). Factors affecting PAP smear test results are considered in limited studies. In our study, measures of the level of AMH in the blood and its effect on PAP smear test results were presented. AMH between one and three had a statistically significant relationship with normal PAP smear results. Therefore, knowing the AMH levels can make recommending a PAP test more serious.

Sachan et al. (22) studied the demographic profile of patients that affects pap smear results. Their study demonstrated that LGSIL and HGSIL were primarily seen in women 41–50 years of age, and multiparity (>3) is a considerable risk factor for cervical carcinoma. Sharif (23) reported low smoking rates, conservative sexual behavior, and religious norms affect the PAP smear results.

Gosh et al. (24) reported that the PAP smear results were associated with a more youthful age group, a younger age at sexual debut, and a lower socioeconomic status. The reason for the positive relationship between infections and young age is that younger women have more sexual activity, making them susceptible to sexually transmitted infections. Many studies reported socioeconomic status influencing PAP smear results because of poor hygiene and few health check-ups (19-21). Studies found that socioeconomic status and age were critical factors in cervical cancer screening that could change PAP smear test results.

We accept the limits of our examination. All samples have been recovered from a single organization, and the number of tests conducted for abnormal PAP smears was low. Another limitation is that the AMH test is more expensive than the PAP smear test and is more difficult to obtain. Physicians could use these results as good advisors and suggest patients conduct PAP smear tests by seeing AMH values in routine controls.

In conclusion, the results showed that the serum AMH level and the PAP smear test results had a statistically significant relationship. More research is required to evaluate the impact of serum AMH levels on the PAP smear test results. The research results can be used for a better understanding of cervical cancer risk factors in sexually active women. The current study will provide as a strong foundation for further investigation into the potential effects of blood AMH levels on the outcomes of PAP smear tests in the future.

Ethical Statement

The study was carried out with the permission of Medeniyet University non-interventional clinical researchs ethics committee (Date:13/01/2021 Decision no: 2021/0027).

Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contributions

Concept: İ.Ö., A.A.M., E.D., Design: İ.Ö., A.A.M., E.D., Data Collection or Processing: İ.Ö., A.A.M., E.D., Analysis or Interpretation: İ.Ö., A.A.M., E.D., Literature Search: İ.Ö., A.A.M., E.D., Writing: İ.Ö., A.A.M., E.D.

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Comparison of lower extremity lymphedema patients and healthy individuals with plantar pressure sense, balance, and fall risk: A pilot study

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Abstract

Plantar pressure is sensed by the receptors on the sole. It is still not clear how the changes after the skin involvement and the effect of this on balance and fall risk in lower extremity lymphedema. To compare patients with lower extremity lymphedema and healthy individuals in terms of plantar pressure sense, balance, and fall risk. A total of 31 participants were included in the study. The plantar pressure sense was evaluated with the Semmes-Weinstein Monofilament Test, the balance was made with the Berg Balance Scale, and the fall risk was assessed with the Tinetti Fall Efficacy Scale. Differences were found when the lower extremity lymphedema group and healthy individuals' plantar pressure sense were compared ($p<0.05$). A significant difference was detected between the groups when the balance and fall values were compared ($p<0.05$). No significant results were detected when the affected and unaffected extremities were compared in terms of plantar pressure sense in patients with lower extremity lymphedema ($p>0.05$). This study showed that patients with lower extremity lymphedema had decreased plantar pressure sense and balance and increased fall risk. Early detection of these parameters in patients with lymphedema and their management is essential for rehabilitation.

Keywords: Lymphedema, plantar pressure sense, balance, fall risk

1. Introduction

Lymphedema causes frequent recurrent infections, psychological stress, and cosmetic deformity, as well as functional limitations such as a feeling of heaviness, decreased joint range of motion, and fatigue because of edema in the affected extremity (1, 2).

The accumulation of protein-rich fluid in the interstitial space results in chronic inflammation and perilymphatic fibrosis over time in lymphedema. The skin thickens and its turgor increases and becomes rough (3). Excessive skin thickness also causes loss of sensation (4). Sensory disturbances and skin sensitivity were observed with the increased volume in the arm with lymphedema in patients with upper extremity lymphedema (5, 6).

Plantar pressure sense stimulates the sensory receptors under the sole directly (7). Mechanoreceptors on the sole carry this vital information to the Central Nervous System, which uses this information during walking and standing. For this reason, this information is critical in maintaining balance. A balance disorder may develop when there is a malfunction in the transmission of this information (8, 9). It was shown that the sensitivity of the sole is also related to the distribution of pressure under the sole (7), and decreased underfoot sensation is associated with falling by changing the plantar pressure distribution during walking and causes an increased body sway (10).

It was also shown in the literature that postural stability and balance are affected negatively in lymphedema, which occurs as a post-surgical complication in cancer patients (11-14). Basar et al. (10) emphasized in their study that postural stability decreased in pre-elderly female patients who had unilateral lymphedema in the upper extremity and that asymmetric fluid distribution may affect postural sway negatively and cause falls in these individuals. Celenay and Kaya (12), on the other hand, reported that balance and posture improved after four weeks in cases when they applied complex unloading physiotherapy after mastectomy. Bowman et al. (14) evaluated the psychosocial well-being and quality of life in individuals with lower extremity lymphedema in their review and stated that the symptoms of lower extremity lymphedema and increased severity of edema were associated with worse quality of life and negative psychosocial well-being. In their study, Doruk and Kaya (15) compared healthy patients with lower extremity lymphedema patients and found that there was a loss of balance in patients with lower extremity lymphedema but showed that it did not increase the risk of falling. However,

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to the best of our knowledge, there is no study investigating the sense of plantar pressure sense, balance, and fall risk in patients with lower extremity lymphedema. This study hypothesizes that the determination of plantar pressure sense in patients with lower extremity lymphedema may be necessary in terms of balance and fall variables. This study aimed to compare patients with lower extremity lymphedema and healthy individuals in terms of plantar pressure sense, balance, and fall risk.

2. Materials and Methods

Before the study commenced, approval and informed consent form were obtained from all participants, with the decision dated B.08.6.YOK.2.US.0.05.0.06/2017/351. The study was conducted in line with the Declaration of Helsinki Principles.

2.1. Individuals

Sixteen patients (15 female, one male) diagnosed with unilateral lower extremity lymphedema and 15 healthy volunteers (14 female, 1 male) with similar characteristics, aged between 21 and 68 years, were included in the present study. Those with a severe cardiac diagnosis, uncontrollable hypertension, an orthopedic and neurological disease that would impede walking, and cognitive problems were not included (13). Individuals were randomly selected using the closed envelope method among individuals diagnosed with lower extremity lymphedema who applied to the clinic at Uskudar University NP Medical Center between March 2018 and March 2019. The individuals were asked to hit the ball placed in the middle of the feet in the standing position. The extremity on the kicking side was accepted as the dominant side to determine the lower extremity dominance of the individuals included in the study (16).

2.2. Evaluation Methods

Edema Evaluation

The circumference measurement method was used in the evaluation of lymphedema. All patients were rested supine with the affected elevated extremities for 30 minutes before the measurement. Then, when the patients were in the semi-sitting position and the foot-ankle was in the neutral position, circumference measurements were made at 5 cm intervals from the medial malleolus level of the ankle to the proximal. A measuring tape measuring 150 cm long and 7 mm wide was used. The extremity volume was determined by calculating the obtained values with the Frustum formula ((V=) [h x ($R1^2$+R1.R2+$R2^2$)] / (12 x π) (V: Volume of each conical segment, h: Range used in circumference measurement, R1: Base circumference measurement of the conical segment, R2: upper circumference measurement of the conical segment, VT: Leg volume, n: Conical segment number) (17).

According to the severity of the patients' edema in both extremities, less than 250 ml was classified as mild lymphedema, between 250-500 ml as moderate lymphedema, and over 500 ml as severe lymphedema (18, 19).

Plantar Pressure Sense

The Semmes-Weinstein Monofilament Test is a diagnostic test used to detect sensory problems by objectively measuring the touch threshold. In the present study, it was evaluated using the Semmes Weinstein Monofilament Test Kit (SWM) (North Coast Medical, San Jose, CA, USA) from 3 regions of the sole (1st metatarsal head, 5th metatarsal head, and midpoint of the heel). Firstly, the lightest 2.83-number monofilament was used for the test. Monofilaments were tested by touching the test sites for 1.0-1.5 seconds using monofilament numbers between 2.83-6.65 in 3 repetitions in sequence. The number of this monofilament was recorded when individuals correctly sensed two stimuli out of 3 trials. The tester switched to another monofilament when there was no sensation (6, 20).

Balance Evaluation

The balance evaluation was made with the Turkish version of the Berg Balance Scale (BBS) (21). The BBS includes 14 movement parameters for the assessment of balance. The level of proficiency for each activity is scored between "0: The patient cannot do this" and 4: "The patient does this independently and safely" (0-4). In this survey, which has a total of 56 points, high scores indicate good balance, low scores indicate poor balance, 0-20 points indicate balance disorder, 21-40 points indicate an acceptable balance and 41-56 points indicate a good balance (22, 23).

Fall Evaluation

It was evaluated with the Turkish version of the Tinetti Fall Efficiency Scale (TFES) (24). It is asked the person how safe they feel while taking a bath, lying down on a shelf, preparing meals, walking around the house, getting into and out of bed, answering the door or the phone, sitting in or standing up from a chair, dressing or undressing, doing light housework, doing simple shopping. The person is also asked to mark from 1 to 10 (10 completely unsafe, 1 extremely safe), and when all scores are added, a total score between 0 (low fall-related activity) and 100 (high fall-related activity) is obtained (25).

Tinetti VAS

The VAS is a self-reported scale consisting of a horizontal line (10 cm long) with anchor points of "no pain" and "worst possible pain." The patient was asked to put a mark on the line that best describes their pain severity (24, 25).

2.3. Statistical Analysis

The values to be used in power analysis were determined according to the suitability of the study because there is no study in this field in the literature. In the power analysis, alpha significance level [i.e. Type I error (α=0.05)] was taken as the power value desired to obtain [Type II error (β=0.80) (α and β values were similar in articles close to the subject of the study)]. These operations were performed by using the G*Power 3.1.9.2 Software. The effect size was taken as 0.90 as the value that accepts a high degree of difference according to Cohen's ***d*** standards because no detailed article on this subject was conducted before. The groups in the study were

lymphedema patients and healthy controls. The sample size obtained from these variables was 32 individuals (16 lower extremity lymphedema, 16 healthy controls).

The data were analyzed using the IBM SPSS Statistics 22 Software. The conformity of the variables to the normal distribution was examined using the analytical method Kolmogorov-Smirnov, and the mean and standard deviation and minimum-maximum values of these variables were given in the representation of descriptive statistics. Also, number (n) and percentage values were given for categorical variables such as gender, dominant extremity, and lymphedema severity. The demographic data of lymphedema patients and healthy individuals were compared using the Independent-T Test. Since the plantar pressure sense, balance, and fall data of lymphedema patients and healthy individuals did not comply with the normal distribution, they were evaluated using the Mann-Whitney U Test. The Plantar pressure sense of the affected and unaffected sides of the lower extremity lymphedema patients was compared with the Chi-Square Test (26).

3. Results

The mean age of the lower extremity lymphedema patients was 38.63±11.53 years, their Body Mass Index (BMI) was 29.82±6.84 kg/m², and the diagnosis time of lymphedema was 13.50±10.90 years in the present study. According to the severity of lymphedema, 18.75% of the patients had mild lymphedema, 18.75% moderate lymphedema, and 62.5% severe lymphedema. Furthermore, 75% of the dominant sides of lymphedema patients were right, 25% were left, 62.5% of the affected extremities were right and 37.5% were left extremities. The mean age of the healthy individuals in the control group was 34.93±9.32 years, the mean BMI was 24.90±3.74 kg/m², and 73% of the dominant sides were on the right and 27% on the left. No significant differences were detected between the lower extremity lymphedema group and the control group in terms of gender, age, BMI, and dominant extremities (p>0.05) (Table 1).

When the dominant and non-dominant plantar pressure senses of the lower extremity lymphedema group and healthy individuals in the control group were compared (1st metatarsal head, 5th metatarsal head, and heel midpoint), differences were detected between the two groups in terms of other senses except for the heel midpoint on the dominant and non-dominant sides (p<0.05). Also, a significant difference was found between the groups when the balance and fall values were compared (p<0.05) (Table 2).

However, when the plantar pressure senses of the affected and unaffected extremities (1st metatarsal head, 5th metatarsal head, and midpoint of the heel) of lower extremity lymphedema patients were compared, no significant differences were detected (p>0.05), (Table 3).

Table 1. Demographic and clinical characteristics of patients with lower extremity lymphedema and healthy individuals

Evaluation Features		Patients with Lower Extremity Lymphedema (mean±SD)	Healthy Individuals (mean±SD)	p
Age (years)		38.63±11.53	34.93±9.32	0.826
Gender n (%)	**Female** **Male**	15 (93.75) 1 (6.25)	14 (93.33) 1 (6.66)	0.928
Height (cm)		165.25±7.33	166.92±6.13	0.976
Body Weight (kg)		81.07±17.88	68.80±9.97	0.076
BMI (kg/m²)		29.82±6.84	24.90±3.74	0.176
Dominant Limb n (%)	**Right** **Left**	12 (75.00) 4 (25.00)	11 (73.33) 4 (26.66)	0.839
Lymphedema Diagnosis Time (years)		13.50±10.90		
Lymphedema Severity n (%)	**Light** **Middle** **Severe**	3 (18.75) 3 (18.75) 10 (62.50)		

p>0.05; Independent T test; Mann-Whitney U Test

Table 2. Comparison of dominant and non-dominant sides plantar pressure sensation, balance and fall risk in patients with lower extremity lymphedema and healthy individuals

Evaluation Features	Patients with Lower Extremity Lymphedema Median (min-max)	Healthy Individuals Median (min-max)	p
Dominant Side Plantar Pressure Sense			
1. Metatarsal Head	4.31 (2.83-4.31)	2.83 (2.44-4.08)	**0.001****
5. Head of Metatarsal	4.31 (2.83-4.31)	3.61 (2.36-3.84)	**0.001****
Heel Midpoint	4.31 (3.61-4.56)	4.31 (2.83-4.56)	0.358
Non-Dominant Side Plantar Pressure Sense			
1. Metatarsal Head	4.31 (2.83-5.07)	3.22 (2.44-3.61)	**0.001****
5. Head of Metatarsal	4.31 (2.83-5.07)	3.22 (2.36-3.84)	**0.002****
Heel Midpoint	4.31 (2.83-5.07)	4.08 (2.83-4.56)	0.417
Berg Balance Scale Total Score	3.50 (30-35)	56.00 (54-56)	**0.000****
Tinetti Fall Activity Scale Total Score	90.00 (64-100)	10.00 (10-14)	**0.000****
Tinetti VAS	1.50 (0-6.50)	0.00 (0-0)	**0.000****

*: p<0.05, **: p<0,01; Mann- Whitney U test; VAS: Visual Analogue Scale

Table 3. Comparison of plantar pressure sense of affected and unaffected extremities in patients with lower extremity lymphedema

Evaluation Features	Affected Extremity Median (min-max)	Unaffected Extremity Median (min-max)	p
Plantar Pressure Sense			
1. Metatarsal Head	4.31 (2.83-5.07)	4.31 (2.83-4.31)	0.515
5. Head of Metatarsal	4.31 (2.83-5.07)	4.31 (2.83-4.31)	0.515
Heel Midpoint	4.31 (3.61-5.07)	4.31 (2.83-4.56)	0.239

P<0.05* Chi-Square Test

4. Discussion

A comparison of the plantar sense, balance, and risk of falling of patients with lymphedema in the lower extremities and healthy individuals revealed that patients with lower extremity lymphedema had lower plantar pressure sense and balance levels, and the risk of falling increased. No difference was found when the affected side and intact side plantar pressure senses of the individuals with lower extremity lymphedema were compared. These results can be a guide for healthcare staff working with lymphedema patients. This study is the first to examine the effect of plantar pressure sense on balance and falling in patients with lower extremity lymphedema.

Lymphedema is a severe condition threatening lifestyles and reducing the quality of life by causing cosmetic deformities, functional losses, and psychological disorders in individuals (27, 28). Sensory disturbances in the extremities, pain, loss of strength, flexibility, movement limitation, susceptibility to infection, and skin sensitivity develop because of the chronic nature of lymphedema (29). It was shown that edema-related sensory disorders and skin sensitivity develop in patients with upper extremity lymphedema (5, 6). Baran et al. (30) reported in their study that there was a decrease in sensory perception in patients with upper extremity lymphedema. Dai et al. (31) showed that the use of compression stockings against skin problems decreased the sense of temperature in patients with lower extremity lymphedema. To the best of our knowledge, no study was conducted in the literature in which plantar sensory pressure evaluation was performed in patients with lower extremity lymphedema. As a result of the present study, lower extremity lymphedema patients showed a decrease in Plantar pressure sense compared to healthy individuals. This can be explained by reasons such as volume increase, swelling, and cellulite caused by lower extremity lymphedema.

Previous studies showed that postural stability is affected, and spinal posture and balance are impaired in patients with upper extremity lymphedema (11, 12). The decreased balance of lower extremity lymphedema patients included in the present study was consistent with the results of patients with upper extremity lymphedema in the literature (5, 15). Doruk and Kaya (15) reported in their study that postural sway increased, and there was a loss of balance in patients with lower extremity lymphedema. This study showed that balance losses occur in patients with lower extremity lymphedema compared to healthy individuals. We think that the cause of balance loss is because of decreased mobility and musculoskeletal disorders.

The risk of falling, which was investigated in different patient groups, negatively affects physical, functional, and psychological status, leading to a limitation of activities of daily living, decreased independence, and limitation of social activities (14, 32). Altas and Demirdal (32) reported that the risk of falling was increased in patients with upper extremity lymphedema. In their study on patients with lower extremity lymphedema, Doruk and Kaya (15) found no differences in the risk of falling, although there was increased postural sway and balance loss of the patients compared to the healthy group. The present revealed that the risk of falling was high in patients with lower-extremity lymphedema, which showed that falls should be evaluated holistically in patients with lower-extremity lymphedema, regardless of the plantar pressure sense factor. Patients with lower extremity lymphedema should also be evaluated, considering that the increased risk of falling may have physical and psychological reflections (14, 33).

It is already known that the presence of cellulitis causing lymphedema, ulcer, and lymphorrhea negatively affects patients' skin quality and flexibility (13). Fluid retention in lymphedema and consequent tissue swelling stemming from the lymphatic system causes skin thickening. Skin fibrosis is a clinically severe pathological secondary lymphedema process. In their study, Sun et al. (33) showed that early examination of skin fibrosis could reveal significant skin changes in patients with lower extremity lymphedema. Based on this, the present study considered that the plantar pressure sense would be affected in patients with lower extremity lymphedema. However, no differences were detected between the affected and unaffected extremities in patients with lymphedema. Although there was a difference when compared to healthy individuals, the absence of a difference in the unaffected extremity suggests a decrease in the plantar pressure sense in the intact extremities of individuals with lower extremity lymphedema.

The number of individuals participating was small because the present study was a pilot scheme, so further studies on this subject on a larger population are needed. Also, the exposure levels of different lymphedema intensities can be compared by increasing the sample size. Since postural swing disorder may affect balance and fall risk, these changes should be considered.

In conclusion, the present study evinced that the plantar pressure sense decreased, the balance was negatively affected, and the risk of falling was increased in patients with lower extremity lymphedema. Patients with lower extremity lymphedema should be evaluated in more detail, and plantar pressure sense, balance, and falling parameters should also be

considered during the treatment and rehabilitation process. Sensory training should be added to the existing physiotherapy and rehabilitation program, considering that the decreased sense of plantar pressure sense will prevent the activities of the individual in daily life in the future.

Ethical Statement

This study was approved ethically, and informed consent forms were obtained from all participants, with the decision dated B.08.6.YOK.2.US.0.05.0.06/2017/351. The study was conducted in line with the Declaration of Helsinki Principles

Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

Concept: D.K.Y., Design: D.K.Y., B.P., Data Collection or Processing: B.P., D.K.Y., Analysis or Interpretation: T.Y.S., D.K.Y., Literature Search: B.P., T.Y.S., Writing: D.K.Y., T.Y.S.

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Research Article

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Evaluation of the relationship between functional low back pain level and urinary incontinence severity

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Abstract

The study was designed to investigate the relationship between functional low back pain levels and urinary incontinence severity. Participants aged 18 and older with both functional low back pain and urinary incontinence were given a survey with scales determining the level of functional low back pain and the severity of urinary incontinence on social media platforms. The total number of study participants was 174, of whom 52.3% were male and 47.7% were female. The mean score for urinary incontinence was 6.03, and the mean score for functional low back pain was 32.58. The functional low back pain score and other variables had no statistically significant effect on the urinary incontinence severity score ($p>0.05$). The functional low back pain score and urinary incontinence score had no statistically significant relationship ($p=0.480$). Functional low back pain and urinary incontinence are both disorders that can seriously affect the quality of life. In the present study, no correlation was found between the severity of low back pain and urinary incontinence, but more comprehensive and extensive studies are required.

Keywords: Low back pain, patient outcome assessment, urinary incontinence, coexistent disease

1. Introduction

Urinary incontinence, previously defined as "loss of bladder control that can be voluntary," is now defined as "involuntary leakage of urine," with a prevalence ranging from 30-45%. There are classifications for urinary incontinence, and there are also scales/methods for determining its severity (1, 2). Many factors influence the severity of urinary incontinence, ranging from causes that increase intra-abdominal pressure to various mechanical pains, and studies show that functional low back pain may also influence the severity of incontinence (3). There are also different scales/methods for determining the severity of functional low back pain (4). Urinary incontinence and functional low back pain are significant because they have a great impact on the quality of life (1, 3). The more the causes of functional low back pain and the severity of urinary incontinence are linked, the more personalized the patient's follow-up and treatment can be. The aim of this study was to examine the link between functional low back pain and the severity of urinary incontinence. The relationship between these two symptoms should be investigated, and treatment protocols should be arranged according to the existence of the relationship.

2. Materials and methods

The population of the study consisted of people aged 18 and older with both urinary incontinence and low back pain who agreed to participate in the survey on social media platforms. Approval was obtained from Hacettepe University Non-Interventional Clinical Research Ethics Committee on April 5, 2022, under the 2022/06 meeting number, the GO 22/288 project number, and the 2022/06-16 decision number before the study. The study was conducted between April 6, 2022, and October 1, 2022.

Those who agreed to participate in our study were given our three-section questionnaire. The first five questions on our 21-question questionnaire were about sociodemographic information, the second two were about the Incontinence Severity Index (ISI), and the third fourteen were about the Functional Low Back Pain Scale. Participants with missing data and those under the age of 18 were excluded from the study.

2.1. Incontinence Severity Index (ISI)

The score of the scale, consisting of 2 questions developed to be used in epidemiologic and clinical studies to identify women with urinary incontinence, is obtained by multiplying the score in the first question by the score in the second question. 1.2 points: mild; 3.6 points: moderate; 8.9 points: severe; and 12 points are classified as very severe. The Turkish validity and reliability study of the scale was conducted by Uyar Hazar and Şirin (2). Both genders were included in our study.

2.2. Functional Low Back Pain Scale

It is a scale designed to measure how much low back

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discomfort interferes with a patient's ability to perform. Each item has a score between 0 and 5. The scale score can vary between 0-60 and a full score of "60" indicates that any performance activity is not difficult. The Turkish validity and reliability study of the scale was conducted by Koç and Bayar (4).

2.3. Statistical Method

Data were analyzed with IBM SPSS V23. Compliance with normal distribution was examined by coefficients of skewness and kurtosis. The Pearson Correlation Coefficient was used to examine the relationship between scale scores and variables. Linear Regression Analysis was used to analyze the independent variables affecting the Urinary Incontinence Severity Score. The results of the analysis were presented as frequency and percentage for categorical variables and mean ± standard deviation and median (minimum-maximum) for quantitative variables. The significance level was accepted as $p<0.05$.

3. Results

The total number of study participants was 174, of whom 52.3% were male and 47.7% were female. The marital status of 47.1% of the participants was single, while 52.9% were married. The proportion of those with mild urinary incontinence was 16.7%, moderate 41.4%, severe 37.9%, and very severe 4% (Table 1).

Table 1. Frequency distributions of demographic data

	Frequency (n)	Percentage (%)
Sex		
Male	91	52.3
Female	83	47.7
Marital Status		
Single	82	47.1
Married	92	52.9
Urinary Incontinence Severity Level		
Mild	29	16.7
Moderate	72	41.4
Severe	66	37.9
Very Severe	7	4

The mean age of the participants in the study was 40.46 years. It was determined that the participants had incontinence for an average of 3.52 years. When functional low back pain was analyzed, it was seen that the participants had low back pain for an average of 3.03 years. The mean urinary incontinence score was 6.03, while the mean functional low back pain score was 32.58 (Table 2).

Table 2. Descriptive statistics of variables

	Mean±SD	Median (min-max)
Age	40.46±14.23	41.5 (18-65)
Duration of Urinary Incontinence (years)	3.52±1.77	4 (1-6)
Duration of Functional Low Back Pain (years)	3.03±1.41	3 (1-5)
Urinary Incontinence Score	6.03±2.92	6 (1-12)
Functional Low Back Pain Score	32.58±18.25	33 (1-60)

The age of the participants and the duration of urinary incontinence were found to have a statistically significant but very weak relationship ($r=0.158$; $p=0.037$). The duration of functional low back pain and age had no statistically significant relationship ($p=0.347$). The duration of functional low back pain had no statistically significant relationship with the duration of urinary incontinence ($p=0.532$). There was no statistically significant relationship between the participants' Urinary Incontinence score and their age ($p=0.473$). The functional low back pain score and urinary incontinence score had no statistically significant relationship ($p=0.480$). There was no statistically significant relationship between the participants' Urinary Incontinence score and functional low back pain duration ($p=0.330$). The score of functional low back pain and the participants' age had no statistically significant relationship ($p=0.795$). The functional low back pain score and urinary incontinence score had no statistically significant relationship ($p=0.768$). There was no statistically significant relationship between the participants' functional low back pain score and functional low back pain duration ($p=0.240$). The functional low back pain score and urinary incontinence score had no statistically significant relationship ($p=0.480$) (Table 3). It was anticipated that there may be differences in terms of gender, but there was no statistically significant relationship between the functional low back pain score and the urinary incontinence score when gender was evaluated together.

Table 3. Examination of the relationship between scale scores and variables

		Age	Urinary Incontinence Duration	Duration of Functional Low Back Pain	Urinary Incontinence Score
Duration of Urinary Incontinence (years)	r	0.158	-	-	-
	p	**0.037**	-	-	-
Functional Low Back Pain (years)	r	-0.072	-0.048	-	-
	p	0.347	0.532	-	-
Urinary Incontinence Score	r	0.055	0.023	0.074	-
	p	0.473	0.760	0.330	-
Functional Low Back Pain Score	r	-0.02	-0.023	0.09	0.054
	p	0.795	0.768	0.240	0.480

Pearson Correlation Coefficient

The independent variables affecting the urinary incontinence severity score were analyzed by linear regression analysis, and the regression model was found to be statistically significant ($F=140.764$; $p<0.001$). In the regression model, the enter method was used, and the independent variables explained 87.9% of the dependent variable. It was found that those with moderate urinary incontinence severity scores were 3.362 units higher than those with mild urinary incontinence

severity scores (p<0.001). Those with severe urinary incontinence had a urinary incontinence severity score that was 6.914 units higher than that of those with mild urinary incontinence (p<0.001). The severity of urinary incontinence in those with very severe urinary incontinence was 10.416 units higher than in those with mild urinary incontinence (p<0.001). The functional low back pain score and other variables had no statistically significant effect on the severity of urinary incontinence (p>0.05) (Table 4). It was an unexpected result to see that functional low back pain had no effect on the variables we questioned in our study.

Table 4. Examination of independent variables affecting urinary incontinence severity score by Linear Regression Analysis

	β_0 (%95 CI)	S. Error	β_1	t	p	r^1	r^2	VIF
Fixed	1.622 (0.824-2.421)	0.404		4.012	**<0.001**			
Age	-0.001 (-0.012-0.01)	0.006	-0.007	-0.256	0.798	0.055	-0.02	1.062
Duration of Urinary Incontinence (years)	0.01 (-0.078-0.098)	0.044	0.006	0.224	0.823	0.023	0.017	1.038
Duration of Functional Low Back Pain (years)	-0.044 (-0.154-0.067)	0.056	-0.021	-0.78	0.437	0.074	-0.061	1.041
Functional Low Back Pain Score	0.002 (-0.006-0.011)	0.004	0.015	0.557	0.579	0.054	0.043	1.056
Sex (Male)	Reference							
Female	0.053 (-0.261-0.366)	0.159	0.009	0.333	0.740	-0.007	0.026	1.062
Marital Status (Single)	Reference							
Married	0.058 (-0.25-0.365)	0.156	0.01	0.37	0.712	-0.005	0.029	1.021
Urinary Incontinence Level (Mild)	Reference							
Moderate	3.362 (2.916-3.808)	0.226	0.569	14.879	**<0.001**	-0.311	0.758	2.092
Severe	6.914 (6.463-7.365)	0.229	1.153	30.252	**<0.001**	0.666	0.921	2.078
Very severe	10.416 (9.564-11.268)	0.431	0.703	24.148	**<0.001**	0.42	0.883	1.214

F=140.764, p<0.001, R^2=88.5%, Adjusted R^2=87.9%, β^0: Unstandardized beta coefficient, β^1: Standardized beta coefficient, r^1: Zero-order correlation, r^2: Partial correlation

4. Discussion

Both low back pain and urinary incontinence have a great effect on the quality of life, and it is thought that the coexistence of both can decrease the quality of life. However, it has been observed that the severity of these symptoms did not affect each other in our study.

One of the risk factors for urinary incontinence is sexual intercourse status and women have a higher rate of risk factors (5, 6). Although it is considered that single women have less sexual intercourse, 47.8% of women with urinary incontinence were found to be married in our study, similar to the study by Sinan et al. in 2018, and the rate of single women was found to be higher.

The rate of patients with moderate urinary incontinence was found to be the highest in our study, and most studies have reported that the severity of urinary incontinence varies according to the type of incontinence (7, 8).

A variety of factors, including lifestyle, occupation, age, and genetics, affect the duration of low back pain (9-13). In our study, the mean duration of functional low back pain was 3 years, and the mean score was around 30 points, which is consistent with many other studies (4, 10, 12).

Pelvic floor muscles weaken as age advances, and thus the prevalence of urinary incontinence increases with age (13). Although the mean age of urinary incontinence was found to be 40 years in our study, the age of onset of incontinence is multifactorial and may date back to childhood (14-16).

Low back pain should be taken into consideration in patients admitted with serious symptoms like urine incontinence, even if there was no statistically significant link between the low back pain score and the urinary incontinence score in our study (17). Furthermore, studies are showing that women with overactive bladder syndrome have a higher incidence of low back pain and pain severity compared to asymptomatic women (18).

Treatment effectiveness is crucial when urinary incontinence and functional low back pain are present since both conditions have a considerable negative influence on quality of life. Despite the fact that our research revealed no correlation between the two diseases, it is still important to improve patient quality of life and raise the efficacy of treatment procedures to lessen the severity of both diseases. Larger and multicenter studies are needed, despite the fact that our study found no correlation between the coexistence of two disorders and their effects.

Ethical statement

Ethical approval was obtained from Hacettepe University Non-Interventional Clinical Research Ethics Committee on April 5, 2022, under the 2022/06 meeting number, the GO 22/288 project number, and the 2022/06-16 decision number before the study.

Conflict of interest

The authors have no conflicts of interest to disclosure.

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Authors' contributions

Concept: İ.F., H.A., Design: İ.F., D.A.B., Data Collection or Processing: İ.F., Analysis or Interpretation: H.A., D.A.B., Literature Search: İ.F., D.A.B., H.A., Writing: İ.F., H.A.

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Research Article

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Convalescent plasma transfusion in severe covid-19 patients admitted to intensive care unit: A single center study

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Abstract

Initial difficult times of novel coronavirus disease 2019 (COVID-19) had clinicians actively seeking appropriate treatment. Convalescent plasma (CP) has been studied for treatment of past coronavirus pandemics and was successful with promising efficacy and safety. This study aims to measure the efficacy of convalescent plasma transfusion in severe COVID-19 patients, determined by alleviation of symptoms, improvement in radiologic findings, and laboratory parameters. Cross-sectional study conducted involving 23 severe COVID-19 patients admitted to Udayana University Hospital intensive care unit in 2020. Patients received a minimum 200 cc CP transfusion, dexamethasone, and remdesivir. Data were retrieved from patient's medical records. Patients mean age was 54.04 years. Mean time from onset of illness to transfusion and length of stay were 11.09 and 16.70 days respectively. No adverse effects were observed during treatment. Twelve patients (52.2%) showed alleviation of symptoms and recovered, with 15 days median time from transfusion to recovery. Post-transfusion chest x-ray examination showed varying degrees of absorption of lung lesions in 10 patients (43.5%) and was associated with the patient's outcome (p=0.001). Significant changes in c-reactive protein (p=0.000) and procalcitonin level (p=0.024) was found as compared to pretransfusion. Despite these findings, almost half the patients (47.8%) did not receive benefits from CP transfusion and dead. CP transfusion has shown remarkable improvement in radiologic, inflammatory, and prognostic parameters but was unable to improve patient clinical outcomes and mortality rate.

Keywords: Convalescent plasma therapy, COVID-19, critical care, outcome

1. Introduction

Indonesia has reported a total of 1,012,350 cases and 24,468 deaths (2.8% confirmed case fatality rate) up to January 26, 2021, since the first two laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections were reported on early March 2020, of which 25% (254,580) of cases and 17% (4077) of deaths were in the capital city of Jakarta. The number of novel coronavirus disease 2019 (COVID-19) cases and fatalities in Jakarta rapidly increased during the first two months of the outbreak (March–April 2020), and they have continued to rise steadily through January 2021 (1). The COVID-19 pandemic has had a massive negative impact on the Indonesian economy, particularly in Bali Province, where tourism is the primary source of income (2), and tourism, on the other hand, poses a risk of COVID-19 spreading. The increasing death rates and such impact on the economy caused by the pandemic have led to high demands to develop new potential therapies and made clinicians actively seek appropriate treatments.

During this early COVID-19 pandemic, clinicians still lacked evidence, especially randomized controlled trial (RCT) studies, to support which drugs would be helpful (3). Indonesia's second edition of the COVID-19 management guideline has included convalescent plasma (CP) transfusion for consideration in severe COVID-19 patients (4). For more than a century, CP transfusion as a traditional adaptive immunotherapy has been used to prevent and treat a variety of infectious diseases, including severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), and the 2009 H1N1 pandemic, have all been successfully treated with CP therapy over the past 20 years with satisfying efficacy and safety (5–8). Compared to placebo or no treatment, CP transfusion significantly reduced the pooled odds of mortality (odds ratio, 0.25; 95% confidence interval, 0.14-0.45) in a meta-analysis of 32 studies on SARS

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coronavirus infection and severe influenza (9). SARS, MERS, and COVID-19 have similar virological and clinical traits. Therefore, CP transfusion was a promising treatment for managing severe COVID-19 cases (10). However, in the middle of 2020, it was unclear whether using convalescent blood products during COVID-19 would be clinically advantageous or harmful. In the absence of a well-designed large multicenter RCT, a systematic review published in April 2020 has shown that CP transfusion appears safe, clinically effective, and reduces COVID-19-related mortality (11). Although numerous studies have been reported since then, the World Health Organization (WHO), on its seventh update of WHO's living guidelines on COVID-19 therapeutics, recommends against the use of CP to treat COVID-19. CP transfusions were found to cause significant costs and neither increase survival nor decrease the need for mechanical ventilation. While there is no question that convalescent plasma has no benefit for non-severe COVID-29 patients, this is not yet clear in the case of severe and critically ill patients (12).

Considering the potential benefit of CP transfusion in managing severe COVID-19 patients back in early 2020, one of the referral hospitals for managing severe COVID-19 in Bali Province, Udayana University Hospital, has done CP transfusion to severe COVID-19 patients admitted to the intensive care unit (ICU). In this study, we aim to report the efficacy and safety of CP transfusion for severe COVID-19 patients admitted to the ICU of Udayana University Hospital.

2. Materials and Methods

2.1. Study Population, Setting, and Data Collection

We did this study on 23 severe COVID-19 patients admitted to the ICU of Udayana University Hospital, Bali Province, Indonesia, in 2020. Research participants were laboratory-confirmed COVID-19 patients using nasopharyngeal swabs of SARS-CoV-2 real-time polymerase chain reaction (RT-PCR). Severe COVID-19 patients in the ICU have been empirically treated with dexamethasone (6 mg/day for ten days) and remdesivir [200 mg intravenous drip (day 1) continued by 100 mg intravenous drip (day 2-10)], which was recommended by the COVID-19 management guideline in Indonesia (4). The inclusion criteria were severe COVID-19 patients admitted to the ICU. Patients were defined as having severe COVID-19 by referring to Indonesia's COVID-19 management guidelines (4), which were patients that show clinical signs of pneumonia (fever, cough, shortness of breath, rapid breathing) plus one of: respiratory rate >30 breaths/minute, severe respiratory distress, or <93% oxygen saturation in room air. Exclusion criteria were (a) patients allergic to plasma products; (b) pregnancy; (c) breastfeeding mother; (d) patients with known thrombosis; (e) severe heart failure with risk of volume overload; (f) septic shock; and (g) kidney failure with ongoing dialysis. Demographic data, medical history, length of stay, laboratory, and radiology examination results were taken from medical records retrospectively. The Ethical Review Board of the Faculty of Medicine, Udayana University, has approved this study with an ethical clearance letter number 1010/UN14.2.2.VII.14/LT/2020. Written informed consent was obtained from each participant.

2.2. Convalescent plasma donors and transfusion

Convalescent plasma was obtained from patients who had recovered from COVID-19 through plasmapheresis. Relief from symptoms and negative results for SARS-CoV-2 nucleic acid in at least one RT-PCR test were considered signs of recovery. The donor recruitment was done by contacting previously recovered severe COVID-19 patients through voluntary participation. Donor criteria were 18-59 years old male or female who has never pregnant. The donors must test seropositive for anti-SARS-CoV-2 and seronegative for hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and syphilis. The CP was also found free of any remaining SARS-CoV-2 by RT-PCR. As soon as we had access to available ABO-compatible CP, transfusion to eligible patients was initiated. Patients received a minimum of 200 mL CP transfusion, with each transfusion given over 30 minutes.

2.3. Safety and outcome measurements

Adverse events associated with convalescent plasma transfusion were assessed before and at 15, 30, and 60 minutes after CP transfusion. The safety measurements in CP transfusion included vital signs and transfusion reaction symptoms (fever, dyspnea, chest pain, cyanosis, wheal/urticaria, bleeding, and changes in urine color). The efficacy of CP transfusion for severe COVID-19 patients was determined by alleviation of symptoms and improvement in chest x-ray findings and laboratory parameters during 24 hours pre-CP transfusion and 48 hours post-CP transfusion. Laboratory parameters included in the analysis were hemoglobin, white blood cell count (WBC), differential neutrophil percentage, differential lymphocyte percentage, neutrophil-to-lymphocyte ratio (NLR), platelet count, prothrombin time (PT), c-reactive protein (CRP), D-Dimer, procalcitonin, alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), blood urea nitrogen (BUN), and serum creatinine (SC). Normal references from the hematology analyzer were also recorded. The NLR value was counted from the differential neutrophil and lymphocyte count, and the normal reference range was set to 0.78-3.53 according to the study by Forget et al. (13). Chest x-ray findings pre and post-CP transfusion were categorized as "decreased lesions," "persistent lesions," and "increased lesions."

2.4. Statistical Analysis

Variables with categorical scales were reported in the frequency and percentage distribution, while numerical variables were reported as mean (± standard deviation) if the data were normally distributed or median (and interquartile range) if the data were not normally distributed. The numerical data normality of distribution test was performed using the Shapiro-Wilk test. The data distribution is not normal if the p-value <0.05. Mean differences between pre-CP transfusion and post-CP transfusion laboratory parameters were analyzed with Paired sample T-test in normally distributed data; otherwise, analysis was carried out using the Wilcoxon test. Chi-square analysis was used to evaluate the association between variables with categorical scales. Statistical analysis was performed using the IBM Statistical Package for the Social Sciences® software version 17.

3. Results

3.1. Patient's Characteristics

The patient's mean age was 54.04 (±13.07) years, ranging from 26 to 76 years old, and more than half were male (65.2%). Most patients had the O blood group (47.8%), while the AB blood group was the least common (8.7%). Patient's initial symptoms before ICU admission were mostly dyspnea (100%), fever (91.3%), and cough (95.7%), while the less common symptoms were rhinorrhea (13%), nausea and vomiting (17.4%), headache (8.7%), odynophagia (8.7%), lethargy (17.4%), and diarrhea (4.3%). The mean time from initial symptoms to ICU admission was 6.74 (±4) days. Patients were reported to have several comorbidities during treatment, including a history of type 2 diabetes mellitus, hypertension, obesity, bronchial asthma, hypokalemia, hyponatremia, pregnancy, and pulmonary edema.

Patients received CP transfusion with a median time of 4 (IQR=2) days from ICU admission (ranging from 0-13 days) or a mean of 11.09 (±4.28) days from initial symptoms. Most of the mean or median of the laboratory parameters value before CP transfusion was abnormal. Pre-transfusion median white blood cell count was 10.78 $\times 10^3/\mu L$ (interquartile range (IQR)=2.92), neutrophil differential percentage 90.80% (IQR=5.80), lymphocyte differential percentage 5.1% (IQR=3.80), D-Dimer 1889 ng/mL FEU (IQR=2921), procalcitonin 0.29 ng/mL (IQR=0.4), SGOT 49 U/L (IQR=48), SGPT 71 U/L (IQR=74), and SC 0.64 mg/dL (IQR=0.20). The mean NLR was 17.57 (±7.54) and CRP 129.40 mg/L (± 87.95). Baseline characteristics were further compared between the recovered and dead patients, as shown in Table 1 and 2.

Table 1. Patient baseline characteristics

Patient characteristics at ICU admission	Recovered (n=12)	Dead (n=11)
Age[a] - years	50.92±12.78	57.45±13.11
Sex – N (%)		
Male	9 (60)	6 (40)
Female	3 (37.5)	5 (62.5)
Comorbidities – N (%)		
Type 2 Diabetes Mellitus	2 (40)	3 (60)
Hypertension	1 (25)	3 (75)
Obesity	1 (33.3)	2 (66.7)
Pulmonary Tuberculosis	0 (0)	1 (100)
Pneumonia	2 (66.7)	1 (33.3)
Bronchial Asthma	1 (33.3)	2 (66.7)
Hypokalemia	0 (0)	1 (100)
Hyponatremia	0 (0)	1 (100)
Pregnancy	0 (0)	1 (100)
Lung Edema	0 (0)	1 (100)
Initial symptoms – N (%)		
Fever	10 (47.6)	11 (52.4)
Cough	11 (50)	11 (50)
Rhinorrhea	2 (66.7)	1 (33.3)
Nausea and vomiting	2 (50)	2 (50)
Headache	0 (0)	2 (100)
Odinophagia	2 (100)	0 (0)
Lethargy	1 (25)	3 (75)
Diarrhea	1 (100)	0 (0)
Interval from initial symptoms to ICU admission – days	1-14	1-14
Interval from ICU admission to CP transfusion – days	0-13	1-5
Interval from CP transfusion to outcome – days	8-22	3-22

[a]Mean±SD

3.2. Outcomes of Therapy

The mean overall length of ICU stay was 16.70 (±6.09) days. The mortality rate in this study reached 47.8%. Almost half of the patients did not receive the benefit of CP transfusion. Alleviation of symptoms was found in the recovered groups (52.2%), with median times 15 days (IQR=9) from transfusion to recovery. Post-transfusion statistically significant changes were observed in WBC (p=0.002), CRP (p=0.000), and procalcitonin value (p=0.024), as compared to pre-transfusion. Further detailed data on laboratory parameter changes are presented in Table 3. Improvement in radiological findings was found only in 10 patients (43.5%) and was associated with patient outcomes (p=0.003). No adverse events related to CP transfusion were found in this study. Comorbidities were not associated with the patient's mortality rate (p=0.278)

Table 2. Pre and post-CP transfusion changes between outcome groups

Parameters	Pre CP Transfusion		Post CP Transfusion		Normal References
	Recovered	Dead	Recovered	Dead	
Laboratory parameters					
Hemoglobin[a] – g/dL	13.15 (±1.29)	12.65 (±1.74)	12.33 (±1.63)	12.50 (±2.46)	13.2-17.3
WBC[b] – x10^3/µL	10.45 (4.49)	10.79 (2.85)	12.48 (6.34)	17.30 (7.08)	3.80-10.6
Differential neutrophil[b] – %	91.15 (4.35)	90.20 (6)	86.45 (7.38)	92.40 (3.60)	50-70
Differential lymphocyte[b] – %	5.05 (3.07)	6.10 (5.40)	8.70 (7.23)	3.20 (4.40)	25-40
NLR[a]	18.42 (±6.74)	16.64 (±8.55)	12.58 (±7.44)	27.45 (±12.25)	0.78-3.53
Platelet count[a] – x10^3/µL	312 (±123.02)	270.55 (±133.06)	335.58 (±121.45)	310.64 (±147.95)	150-450
PT[b] – seconds	9.75 (1.10)	9.20 (1.70)	9.50 (1.15)	10.10 (4.40)	7.9-10.3
CRP[b] – mg/L	69.30 (133.40)	146.10 (87.40)	4.30 (3.50)	54.80 (51.10)	≤10
D-Dimer[b] – ng/mL FEU	2104.50 (3148)	1111 (2991)	1112.50 (1738)	3195 (8250)	<500
Procalcitonin[b] – ng/mL	0.35 (1.20)	0.24 (0.44)	0.04 (0.14)	0.21 (1.14)	<0.05
SGPT[b] – U/L	85.50 (147)	59 (59)	92 (146)	65 (282)	<40
SGOT[b] – U/L	53.50 (76)	44 (26)	44.50 (43)	32 (66)	<41
BUN[b] – mg/dL	22 (12)	14 (10)	17.50 (15)	20 (13)	6-20
SC[b] – mg/dL	0.65 (0.38)	0.58 (0.28)	0.59 (0.45)	0.66 (0.56)	0.67-1.17
Radiologic changes – n (%)					
Decreased lesions			9 (90)	1 (10)	
Persistent lesions			2 (50)	2 (50)	
Increased lesions			1 (11.1)	8 (88.9)	

WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; PT, prothrombin time; CRP, c-reactive protein; SGPT, alanine aminotransferase; SGOT, aspartate aminotransferase; BUN, blood urea nitrogen; SC, serum creatinine

[a]Mean (±SD); [b]Median (IQR)

Table 3. Pre and post-CP transfusion changes in laboratory parameters

Parameters	Median (Min-Max)	Mean ± SD	Mean Differences (95% CI)	p
Hemoglobin – g/dL				
Pre-transfusion	12.60 (9.90-16.40)	12.91 ± 1.51	0.50 (-0.10 – 1.10)	0.099[a]
Post-transfusion	12.40 (7.40-16.70)	12.41 ± 2.02		
WBC – x10^3/µL				
Pre-transfusion	10.78 (4.36-23.69)	11.25 ± 3.66		**0.002**[b]
Post-transfusion	15.47 (5.77-47.09)	16.60 ± 8.48		
Differential neutrophil – %				
Pre-transfusion	90.80 (81.60-93.60)	89.59 ± 3.54		0.976[b]
Post-transfusion	90.50 (67.90-94.40)	88.18 ± 6.97		
Differential lymphocyte – %				
Pre-transfusion	5.10 (2.90-16.40)	6.30 ± 3.27	-0.39 (-2.76–1.97)	0.732[a]
Post-transfusion	5.50 (2.10-20.20)	6.70 ± 4.41		
NLR				
Pre-transfusion	18 (5-32)	17.57 ± 7.54		0.843[b]
Post-transfusion	17 (3-44)	19.70 ± 12.39		
Platelet count – x10^3/µL				
Pre-transfusion	260 (88-523)	292.17 ± 126.74	-31.47 (-76.40–13.44)	0.160[a]
Post-transfusion	322 (122-549)	323.65 ± 132.24		
PT – seconds				
Pre-transfusion	9.70 (7.80-13.90)	9.89 ± 1.36		0.314[b]
Post-transfusion	9.60 (8.20-17.50)	10.45 ± 2.46		
CRP – mg/L				
Pre-transfusion	116.40 (5.10-360)	129.40 ± 87.95		**0.000**[b]
Post-transfusion	15.20 (0.60-162.70)	33.82 ± 41.12		
D-Dimer – ng/mL FEU				
Pre-transfusion	1889 (283-50322)	5728.96 ± 11089.62		0.412[b]
Post-transfusion	1542 (323-50381)	5307.22 ± 10939.68		

Table 3. Pre and post-CP transfusion changes in laboratory parameters (Continue)

Parameters	Median (Min-Max)	Mean ± SD	Mean Differences (95% CI)	p
Procalcitonin – ng/mL				
Pre-transfusion	0.29 (0.07-4.81)	0.64 ± 1.02		**0.024**[b]
Post-transfusion	0.07 (0.01-19.73)	1.90 ± 5.62		
SGPT – U/L				
Pre-transfusion	71 (25-538)	105.52 ± 117.70		0.855[b]
Post-transfusion	70.20 (22-520)	131.14 ± 127.23		
SGOT – U/L				
Pre-transfusion	49 (24-138)	60.57 ± 32.71		0.191[b]
Post-transfusion	36 (8-830)	107.98 ± 203.42		
BUN – mg/dL				
Pre-transfusion	17 (6-48)	18.91 ± 9.38	-2.69 (-8.32–2.93)	0.331[a]
Post-transfusion	19 (6-54)	21.61 ± 12.38		
SC – mg/dL				
Pre-transfusion	0.64 (0.28-1.11)	0.65 ± 0.20		0.681[b]
Post-transfusion	0.60 (0.27-25)	1.87 ± 5.12		

WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; PT, prothrombin time; CRP, c-reactive protein; SGPT, alanine aminotransferase; SGOT, aspartate aminotransferase; BUN, blood urea nitrogen; SC, serum creatinine

[a]Paired sample t-test; [b]Wilcoxon test

4. Discussion

This study has found that convalescent plasma transfusion, along with dexamethasone and remdesivir, causes a significant decrease in inflammatory and prognostic markers, CRP and procalcitonin. Aside from these satisfactory changes, the mortality rate was still high. This study result demonstrated that CP transfusion could not improve overall outcomes in severe COVID-19 patients. Initial RCTs have also shown no statistically significant reduction in mortality compared to standard treatment alone or placebo. Despite the negative results, the characteristics of CP used in these RCTs were heterogenous, particularly its antibody content and the stratification of the recipient's serologic status (14–16). In a recent Cochrane living systematic review with a varying dose and total volume of plasma administered in 11 RCTs, there is a high certainty of evidence that CP transfusion to moderate-severe COVID-19 patients does not reduce all-cause mortality at up to 28 days, little to no impact on clinical improvement, and does not reduce the need for invasive mechanical ventilation (17). Therefore, the result of this study is in line with available evidence against the transfusion of CP for severe COVID-19 patients.

Transfusion of CP in this study appeared to be safe. There have been raised concerns in the early pandemic about the potential harm of CP transfusion to COVID-19, including transfusion-associated circulatory overload (TACO), coagulation, and antibody-dependent enhancement of COVID-19 (18). However, no recognized risk of plasma transfusion has been reported in the early RCTs (14–16). Although it has been reported to be safe, it is still difficult to draw a concrete conclusion due to the heterogeneous characteristics of CP. Cochrane's systematic review stated that there is uncertainty about whether CP reduces or increases the risk of serious adverse events (17). There was insufficient high-quality evidence reported to conclude the safety of CP.

Given the absence of benefits of CP transfusion to severe COVID-19, it is reasonable to recommend against its use in clinical settings. While it has undeniably high costs, applying CP transfusion outside the research context will shift healthcare resources away from other priorities in managing severe COVID-19 cases and offering false hope to patients (18).

Although this study revealed that CP transfusion has little to no benefit in severe COVID-19 patients, this study has several limitations to consider. First, this study has no control group; therefore, we could not see if the mortality rate would be the same or higher than the treatment group. Second, the number of samples in this study was relatively small, and study results were obtained from a single center. Third, the participants in this study had several comorbidities with varying fatality present during the observation; although chi-square analysis revealed no significant association, they may have affected the outcome of this study.

Ethical statement

The Ethical Review Board of the Faculty of Medicine, Udayana University, has approved this study with an ethical clearance letter number 1010/UN14.2.2.VII.14/LT/2020. Written informed consent was obtained from each participant

Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions
Concept: I.B.Y.B., C.A.W.P., Design: I.B.Y.B., C.A.W.P. Data Collection or Processing: I.B.Y.B., Analysis or Interpretation: I.B.Y.B., C.A.W.P., N.M.D.D.S., A.A.A.Y.G., I.M.S.U., I.K.A.S., I.G.N.M.A., K.T.P.M., Literature Search: I.B.Y.B., Writing: I.B.Y.B.

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Disease severity and serum endocan levels in fibromyalgia patients

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Abstract

The purpose of this study was to look into the link between serum endocan levels and disease severity in fibromyalgia syndrome (FMS).We evaluated 45 patients with FMS according to the 2010 ACR FMS criteria and 28 controls. Disease severity was evaluated with the symptom severity scale (SSS), the widespread pain index (WPI), and the visual analog scale (VAS). Serum endocan, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and CRP values of the patient and control groups were measured. Endocan levels were significantly higher in the FMS group compared to controls. Serum endocan levels were 0.99 ± 0.28 ng/mL (range: 0.1-2.2) in FMS patients and 0.63 ± 0.17 ng/mL (range: 0.4-1.1) in controls ($p<0.05$). In our study, endocan levels that were higher in patients with FMS compared to controls supported the use of endocan as an important potential marker for FMS.

Keywords: fibromyalgia syndrome, endocan, symptom severity scale, inflammation, pain

1. Introduction

Fibromyalgia syndrome (FMS) is a chronic rheumatic disease characterized by widespread pain and different clinical comorbidities. Fibromyalgia syndrome can be seen as a comorbidity with other rheumatic diseases. FMS may be associated with clinical conditions such as pain, sleep disturbance, depression, genitourinary disorders, and irritable bowel syndrome (1, 2). What are the causes of FMS? The answer to the question is still under investigation. Genetic, environmental, and neurohormonal factors and inflammation are thought to play a role in the etiopathogenesis of FMS (3, 4). Inflammatory mediators cause the inflammatory response, which is one of the defense processes required for human survival (5). Although FMS is thought to be a non-inflammatory rheumatic disease, the potential role of inflammation in the pathogenesis of FMS is being investigated (5-7). Recent studies have found a relationship between oxidative stress, inflammation, and endothelial dysfunction in FMS (8, 9).

Endocan is a biomarker of endothelial activation and is secreted from endothelial cells as a soluble proteoglycan (10-12). Studies investigating endocan as a blood and tissue-based biomarker for cancer and inflammation have found results supporting this view. (13-15). Most patients admitted to the hospital have a complete blood count (CBC) and laboratory tests as part of their routine evaluation. The blood test results are important in inflammatory processes (16). Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are non-invasive, promising, and cost-effective prognostic and diagnostic biomarkers for cardiovascular disease (18), cancer (19, 20), rheumatological diseases (21), and some neurological diseases (22, 23). This study evaluated inflammatory biomarkers in FMS patients; NLR, PLR, and serum endocan levels. The presence of inflammation in FMS, the availability of endocan as a biomarker, and the relationship between endocan levels and disease symptom severity were investigated.

2. Material Methods

Individuals aged ≥18 years old and over who applied to the Physical Medicine and Rehabilitation Clinic between October 2020 and October 2021. The study was conducted by including 45 patients who met the 2010 ACR (24) FMS criteria, and 28 controls without a diagnosis of FMS. Smokers and patients with diagnosed arterial hypertension, inflammatory disease, diabetes mellitus, metabolic syndrome, thyroid dysfunction, anemia, local or systemic infection, kidney or liver dysfunction, malignancy, pregnancy, and coronary artery disease were excluded from the study. The study complied with the Declaration of Helsinki, and informed consent was obtained from all participants. Endocan and laboratory parameters were measured in all subjects. The symptom severity scale (SSS), the widespread pain index (WPI), and the visual analog scale (VAS) were evaluated.

Symptom Severity Scale (SSS): Fatigue, restless sleep, cognitive symptoms, a six-month headache, abdominal pain,

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depression, and other somatic symptoms were questioned (25). Scoring is between 0 and 12. The continuation of the symptoms for at least 3 months is necessary for the diagnosis.

Widespread Pain Index (WPI): Right and left; there is pain in at least 4 of the following: the jaw, shoulder, upper arm, forearm, hip (trochanter, gluteal region), upper-lower leg, neck, back, waist, chest, and abdomen. For each region, areas of continuous pain in the last seven days are marked. The score is between 0 and 19. Widespread pain index (WPI) ≥7, symptom severity scale (SSS) 5, or WPI = 3-6, and SSS ≥ 9 are required for diagnosis (26).

Visual Analog Scale (VAS) It is a table used for digitizing values that cannot be measured numerically. Marks where a patient's condition is appropriate on a 100 mm line. It is a common, reliable test accepted in the literature. It can be applied easily (27).

After 12 hours of fasting, samples taken from the 5 ml antecubital vein from patients for a complete blood count were analyzed by dropping them into vacuum tubes containing ethylene diamine tetraacetic acid (EDTA)-anticoagulation tubes (BD Vacutainer K2E; Becton Dickinson, UK). CBC parameters were evaluated with the Beckman Coulter DxH 800 hematology analyzer (Beckman-Coulter, Brea, CA). CRP levels were assessed with IMAGE 800 (Beckman, USA). Blood samples from the control and patients. The blood materials of the participants were placed in tubes with aprotinin (BD Vacutainer SST II Advance, BD, Plymouth, UK) to determine endocan levels and centrifuged at 4000 rpm for 10 minutes. The resulting plasma (containing Endocan) was placed in small-volume Eppendorf tubes for analysis and stored at -80 °C until runtime. Plasma endocan levels were assessed using the Endothelial Cell-Specific Molecule 1 (ESM1) ELISA Kit (CLOUD-CLONE CORP. (CCC, USA, Wuhan), Item No.: SEC463Hu) in accordance with the working procedure outlined in the kit's catalog. Absorbance was evaluated using the Chromate 4300 Microplate Reader (Awareness Technology, Palm City, USA). The minimum detection limit of Endocan was 0.065 ng/mL. The intra-assay coefficient of variation for Endocan was <10%, and the inter-assay coefficient of variation was <12%.

2.1. Statistical analysis:

All statistical analyses were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL). The results were given as median (range), and mean ± SD. Data that showed normally distributed differences between the fibromyalgia and control groups were evaluated using the independent samples t-test. A $p < 0.05$ was considered statistically significant.

3. Results

The patient and control subjects were similar in age and body mass index (BMI). Participants with FMS had significantly higher serum endocan levels. Endocan mean serum levels in FMS patients were 0.99± 0.28 ng/mL (range: 0.1-2.2) and 0.63± 0.17 ng/mL (range: 0.4- 1.1) in control subjects (p :0.05). The mean age of FMS patients was 44.71± 8.8 years, while the control group's mean age was 43.22 9.3 years (p: 0.49). The neutrophil/lymphocyte ratio in patients was 1.93±0.7 in FMS patients and 2.1±0.63 in controls (p = 0.261). The relationship between inflammatory markers and VAS, WPI, and SSS, which show the severity of the disease, is shown in Table 1. The ROC analysis shown in Fig. 1. [AUC (%95 GA): 0.89 (0.81-0.97), sensitivity: % 68.9, specificity: %96.

Table 1. Clinical and laboratory values in the patient and control groups

	FMS	Control	p
Age	44.08±9	43.8±9.2	0.9
CRP (mg/dl)	5.2±4.5	5.6±6.7	0.7
Monocyte/Lymphocyte	0.2±0.08	0.2±0.14	0.7
Platelet/Lymphocyte	133.1±47.2	133.7± 41.7	0.9
Endocan (mg/dl)	0.9±0.2	0.6±0.1	0.00
VAS	6.4±2.9	0.03±0 .1	0.00
Widespread pain index	11.8±5.9	0	0.00
Symptom severity scale	7.3±3.6	0	0.00
BMI	27.6±4.8	28.7±3.7	0.3

FMS: Fibromyalgia Syndrome, CRP: C-Reactive Protein VAS: Visual Analog Scale, BMI: Body Mass Index, mg: milligram, dl: deciliter, p: p value

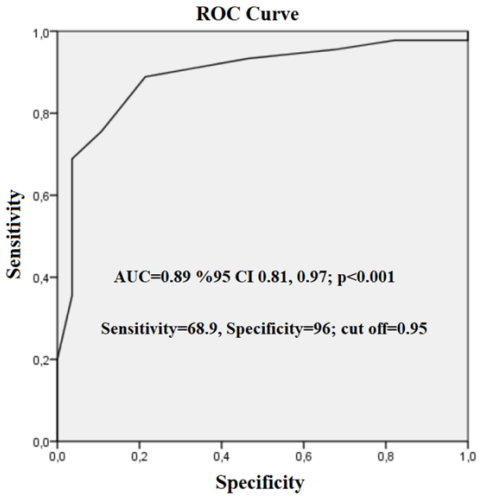

Fig. 1. ROC analysis for endocan level in the FMS group

4. Discussion

In our study, we determined significantly higher endocan levels in FMS patients compared with healthy individuals. Endocan is an essential immunomodulatory protein secreted by human vascular endothelial cells that has been proposed as a biomarker to demonstrate endothelial dysfunction. (12,13). According to our reviews, there is only one study in the literature that demonstrates elevated serum endocan levels in patients with FMS (28). Unlike the current study, the effects of endocan levels on FMS severity were examined in our study. We evaluated the subclinical inflammation suggested to exist in FMS with the endocan level, CRP, platelet-lymphocyte ratio

(PLR), and neutrophil-lymphocyte ratio (NLR) (16). CRP, PLR, and NLR values were similar in patients and controls. CRP is typically only significantly elevated during acute inflammation or infection. High-sensitivity CRP (hs-CRP) measurement is useful in low-grade and chronic conditions as it allows accurate measurement at low levels (29). Focusing on chronic inflammation in FMS may explain the similarity of CRP values. Studies in which hs-CRP measurements can be made will help in this regard. On the other hand, endocan levels were significantly different between groups. Our findings support previous studies showing that these markers alone cannot help us determine the pathophysiology of the disease in FMS, but may be supportive in the diagnosis (30–32). The low number of our patients may explain this situation. We found no evidence that PLR and NLR changed disease severity (SSS and WPI). Similarly, Jayakrishnan et al. reported that PLR and NLR were not associated with disease severity. Our study is consistent with these findings (31). High serum endocan levels have been associated with subclinical inflammation and endothelial cell dysfunction in cancer, sepsis, diabetes, psoriasis (33), Behçet's syndrome (34), and cardiovascular diseases (35), as in FMS.

Studies have shown that increased IL-6 and IL-8 levels correlate with the severity of symptoms in FMS cases (2). A recent review highlighted high levels of cytokines in FMS. All these results clearly show that inflammation is effective in the pathogenesis of FMS. The relationship between the chemokine-cytokine network and FMS is important for determining effective treatment modalities (36-38). Endocan had a sensitivity and specificity for FMS diagnosis in our study's ROC analysis. [AUC (%95 GA): 0.89 (0.81-0.97), sensitivity: % 68,9, specificity: %96. Mertoğlu et al. evaluated the possibility of endocan as a diagnostic marker in FMS, the sensitivity was 88.5% and the specificity was 89.7% (39).

We determined a significant relationship between endocan levels and SSS and WPI, reflecting cognitive impairment, pain, fatigue, and mood disorders in FMS. This demonstrated the link between disease severity and endocan levels. In our literature search, we did not find any studies evaluating the effects of endocan levels on disease severity in FMS. Taylor et al. In their study evaluating inflammation and pain in FMS, they stated that high levels of inflammation increase perceived pain (40). The relationship we found between endocan levels and the parameters we evaluated for the severity of FMS; supports the hypothesis that subclinical inflammation affects the severity of the disease in the pathophysiology of FMS. Our study also supported the utility of endocan as an important potential marker for FMS. More comprehensive, multicenter studies are needed to explain the inflammation network in FMS and its possible role in the diagnosis and/or treatment of FMS. Recent research should look into the levels of biomarkers, cytokines, and autoantibodies that are more specific to inflammatory processes in patients with pre-diagnosed FMS.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: N.P.T., R.A.B., P.Ö., Design: N.P.T., P.Ö., Data Collection or Processing: N.P.T., Ö.A., F.F.Ş Analysis or Interpretation: N.P.T., R.AB., Literature Search: N.P.T., R.AB., Ö.A.,F.F.Ş Writing: N.P.T., R.A.B

Ethical Statement

Approval was obtained from Fırat University Noninterventional Research Ethics Committee, the study started. The ethics committee decision date is 19/04/2019 and the number of ethical committee decisions is 31.

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Research Article

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Which one to choose for anti-cytokeratin immunohistochemistry in small gastric biopsies: Bleached periodic acid schiff- Alcian blue (PAB) or May Grunwald Giemsa (MGG) sections?

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Abstract

Endoscopic biopsies are commonly used for diagnosis in gastrointestinal tractus diseases. The dimensions of the biopsy samples taken during endoscopic examination may sometimes not allow additional examinations or the tissues may become smaller during routine technical procedures. In gastric endoscopic biopsies, hematoxylin & eosin (H&E) staining, periodic acid schiff-Alcian blue (PAB) histochemistry for intestinal metaplasia, and giemsa histochemistry for Helicobacter pylori are used routinely in many in laboratories. In cases where tumor is suspected, immunohistochemical staining is performed in addition to these stainings. The most commonly performed staining is anti-cytokeratin. For various reasons, additional staining applied to materials with reduced tissue size may not yield results. In this case, the patient may need to undergo an endoscopic examination again for definitive diagnosis. To avoid or at least minimize this situation, this study is planned to evaluate the diagnostic usability of restaining with anti-cytokeratin on bleached PAB and MGG sections that were performed for routine examination.

Keywords: Anti-cytokeratin, bleaching, restaining, periodic acid schiff- Alcian blue, May Grunwald Giemsa

1. Introduction

Hematoxylin & eosin (H&E) has been used as the main staining in pathology laboratories since Wissowzky published the first study in the 1870s in which hematoxylin and eosin were used together (1). However, as scientific developments have increased, various histotechnical methods have emerged and support H&E in diagnosis. In earlier times histochemistry, later immunohistochemistry, and nowadays molecular methods serve as beneficial tools for a definitive diagnosis and enable subtyping to guide treatment.

There are many detection methods for the nature and/or content of the tissue in histochemistry applications. These methods, also called histochemical stains, are still frequently used in pathology laboratories (2). Immunohistochemical (IHC) applications also function as important building blocks of many pathology laboratories. Immunohistochemistry, whose basic logic is based on the formation of antigen-antibody complexes, is applied to sections on positively charged slides due to the chemicals used. There are many pre-staining, inter-staining and post-staining factors to obtain appropriate stained sections (3).

The fixation solution, the quality and dimensions of the tissues can be counted among the factors affecting the pre-staining. Occasionally, there are cases where there is no tissue left for IHC staining after H&E sections are taken from small tissues. For this reason, in some routinely applied histochemical examinations, slides are prepared before removing the block and trimming after the H&E section and kept unstained until they are needed. Gastric endoscopic biopsies are one of the best examples of this situation. In many pathology laboratories, 1 H&E section for routine histopathological evaluation in gastric endoscopic biopsies, 1 Giemsa stained section for helicobacter pylori evaluation and 1 Alcian blue (AB) or periodic acid shiff-Alcian blue (PAB) stained section for intestinal metaplasia evaluation are prepared. The small tissue in these biopsy materials, which require IHC from time to time, especially in neoplastic conditions, create technical problems. The tissue in the new sections taken may not be sufficient for evaluation. In such cases, previously stained slides are bleached and IHC staining is performed in these bleached slides.

Since the main histopathological evaluation stain is H&E, bleaching the histochemical stained slides seems to be a more reasonable choice to make comparisons. However, the compatibility of post-bleached slides with directly stained slides should be evaluated in terms of IHC standardization and

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quality. For this reason, in this study, it was aimed to compare the IHC staining of the gastric endoscopic biopsies proven to be neoplastic, in slides stained after the histochemically stained slides bleached, and the direct IHC staining of the new slides.

2. Materials and Methods

48 cases diagnosed with adenocarcinoma in endoscopic gastric biopsies performed in a tertiary healthcare institution between 2014 and 2017 were included in the study. Slides that were in the pathology archive of the cases and stained histochemically with PAB (Facepath) and May Grunwald Giemsa (MGG) (Biognost) according to the steps in table 1 were bleached using the technique in table 1. Anticytokeratin staining (Anti-cytokeratin Cocktail, Biogenex, USA) was performed manually on these bleached slides and new slides obtained from paraffin blocks of the cases, immunohistochemically as given in Table 2. Anticytokeratin stained slides obtained by direct staining of the new section and anticytokeratin stained slides obtained after histochemical staining bleaching were evaluated for staining intensities semi quantitatively (0: no staining, 1: mild staining, 2: moderate staining, 3: strong staining), staining frequencies (0: no staining, 1: 1-33% staining, 2: 34-66% staining, 3: 67-100% staining) and H-scores. The H-score was evaluated both numerically and categorized as 0: no staining, 1-3: mild staining, 4-6: moderate staining, 7-9: strong staining. Statistical analyzes were performed with SPSS package program 15.0 (Released 2006. SPSS for Windows, Version 15.0. Chicago, SPSS Inc.). In the analyzes performed at the 95% confidence interval, the conformity of the data to the normal distribution was evaluated with the Shapiro-Wilk test, and the Spearman test was used in the correlation analysis, and $p<0.05$ values were considered statistically significant.

Table 1. May Grunwald Giemsa (MGG) and Periodic acid schiff-Alcian blue (PAB) staining protocols and histochemical staining bleaching protocol

Order of process	MGG staining	PAB staining	Bleaching
1	4 µm thickness slides were kept in 70 ˚C oven for 15 minutes.	4 µm thickness slides were kept in 70 ˚C oven for 15 minutes.	The slides were kept for 3-4 hours in a 60-degree oven in xylol inside the chalet to open the coverslips.
2	Kept in xylol for 5 minutes.	Kept in Alcian Blue-Ph 2.5 solution for 30 minutes.	The opened coverslips were taken
3	Kept in 100% alcohol for 2 minutes.	Washing was done in tap water for 2 minutes.	It was kept in xylol for 1-2 hours until the entellan on the tissue was completely dissolved.
4	Kept in 95% alcohol for 1 minute.	Kept in Periodic Acid solution for 10 minutes.	Absolute alcohol 10 times dipping
5	Kept in 90% alcohol for 1 minute.	Washing was done in distilled water.	95% alcohol 10 times dipping
6	Washing was done in distilled water for 1 minute.	Kept in Schiff's solution for 30 minutes.	Tap water 10 times dipping
7	Kept in May Grunwald stain for 5 minutes.	Washing was done in tap water for 5 minutes.	1% acid alcohol (with 70% alcohol) was left for 5 minutes-1 hour
8	Washing was done in tap water until the color ran clear.	Passed through distilled water.	The acid was removed under running water for 10 minutes.
9	Kept in 10% Giemsa stain for 20 minutes.	Kept in Harris Hematoxylin solution for 3 minutes.	Passed through alcohol series.
10	Washing was done in tap water until the color ran clear.	Washing was done in tap water for 2 minutes.	Clearing was done with xylene.
11	After the slide was passed through alcohol series and cleared with xylene, it is closed with mount.	After the slide was passed through alcohol series and cleared with xylene, it is closed with mount.	The slide is closed with mount.

Table 2. Anti-cytokeratin immunohistochemical staining protocol

Order of process	Anti-cytokeratin immunohistochemical staining protocol
1	Sections of the tissues were taken on 4 micron thick poly-lysine slides. Sections were kept in an oven at 80 degrees for 1 hour.
2	Deparaffinization was done.
3	Antigen retriever was performed with 1/10 Citrate buffer solution using a pressure whistle.
4	Tissues were taken into distilled water and kept for 2 minutes.
5	The tissue was scratched with Pappen. 3% hydrogen peroxide was dripped and left for 10 minutes.
6	They were incubated in phosphate buffered solution (PBS) for 2 minutes.
7	Kept in Wblock for 5 minutes. Washing was not done.
8	Antibody was dripped and waited according to the time written in the datasheet.
9	Washing was done with PBS for 4 minutes and biotin was incubated for 10 minutes.
10	Washing was done with PBS for 4 minutes and streptavidin was incubated for 10 minutes.
11	Washing was done with PBS for 4 minutes and chromogen was incubated for 10 minutes.
12	Slide was washed in distilled water for 5 minutes and kept in Mayer hematoxylin for 5 minutes.
13	Slide was washed in tap water for 5 minutes, passed through alcohol series, cleared with xylene, and then closed with a mount.

3. Results

The IHC staining profiles of the cases after PAP and MGG bleaching and the direct IHC staining profiles are given in Fig. 1. In Fig. 2 and 3; H&E sections of tumor tissues, direct IHC staining, IHC staining after PAB and MGG bleaching of two cases are demonstrated. In direct immunohistochemical staining, mild frequency staining was not observed. The mean frequency of direct immunohistochemical staining was calculated as 2.58±0.50, mean intensity was 2.58±0.65, and mean H-score was 6.92±2.62. IHC staining after PAP bleaching revealed mean frequency 1.50±0.65, mean intensity 2.08±0.87, mean H-score 3.58±2.72. The mean IHC staining frequency after MGG bleaching was 1.23+0.43, mean intensity was 1.75±0.84, and mean H-score was 2.23±1.55.

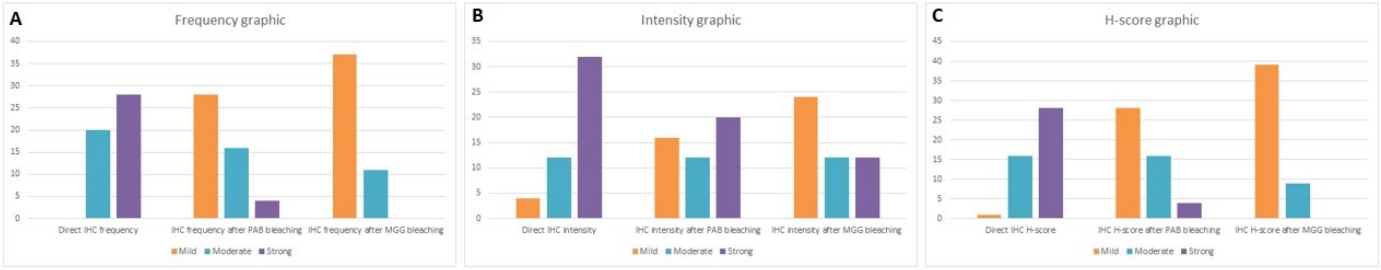

Fig. 1. A: The frequency of staining scores of direct IHC, IHC after PAB bleaching, IHC after MGG bleaching; B: The intensity of staining scores of direct IHC, IHC after PAB bleaching, IHC after MGG bleaching; C: Categorized H-scores of staining scores of direct IHC, IHC after PAB bleaching, IHC after MGG bleaching

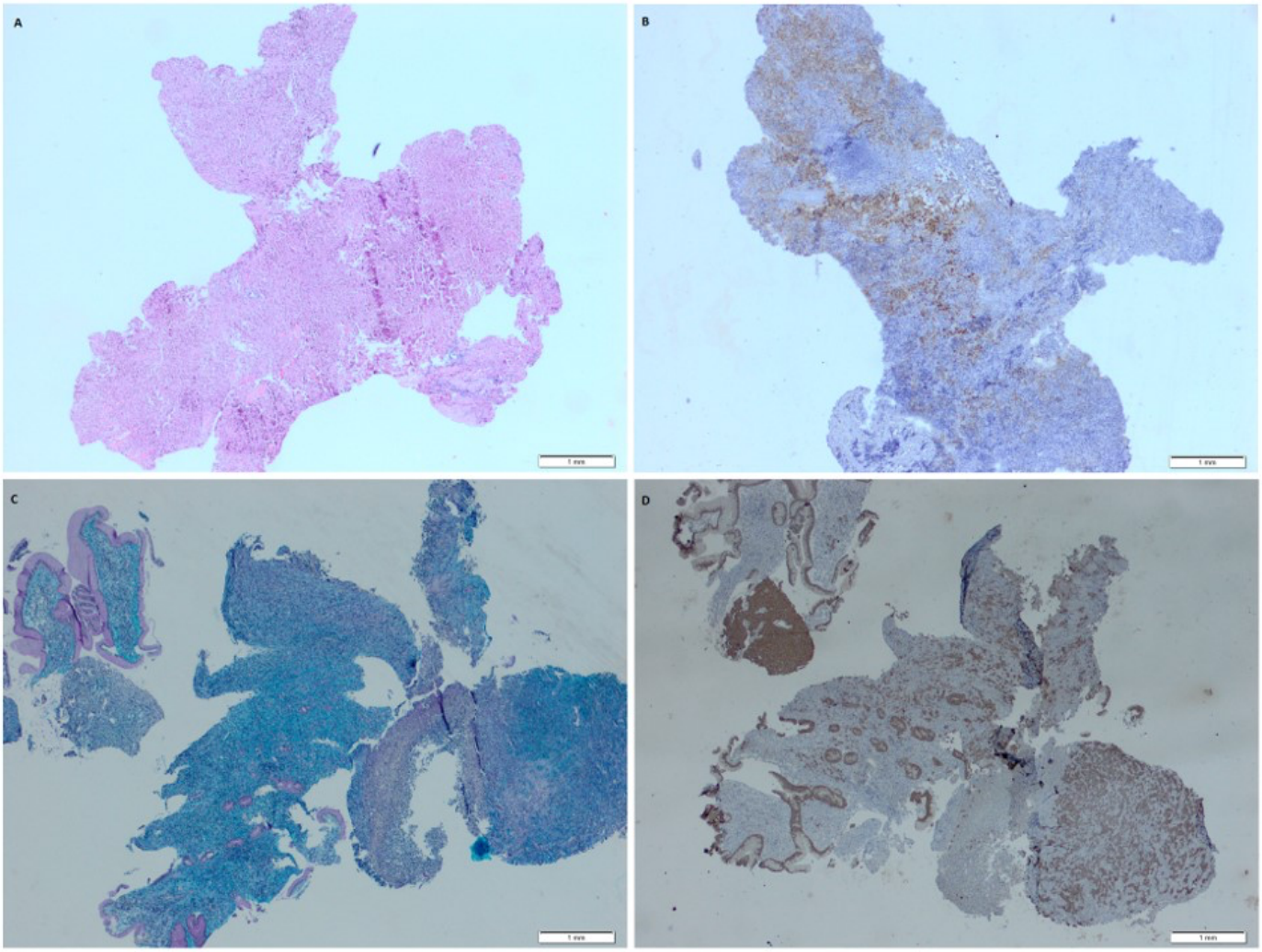

Fig. 2. A: H&E stained slide of endoscopic biopsy material diagnosed as adenocarcinoma, 40x; B: Direct anti-cytokeratin stained slide endoscopic biopsy material of figure 2A, 40x; C: PAB stained slide of endoscopic biopsy material of figure 2A, 40x; D: Anti-cytokeratin stained slide of figure 2C after PAB bleaching, 40x

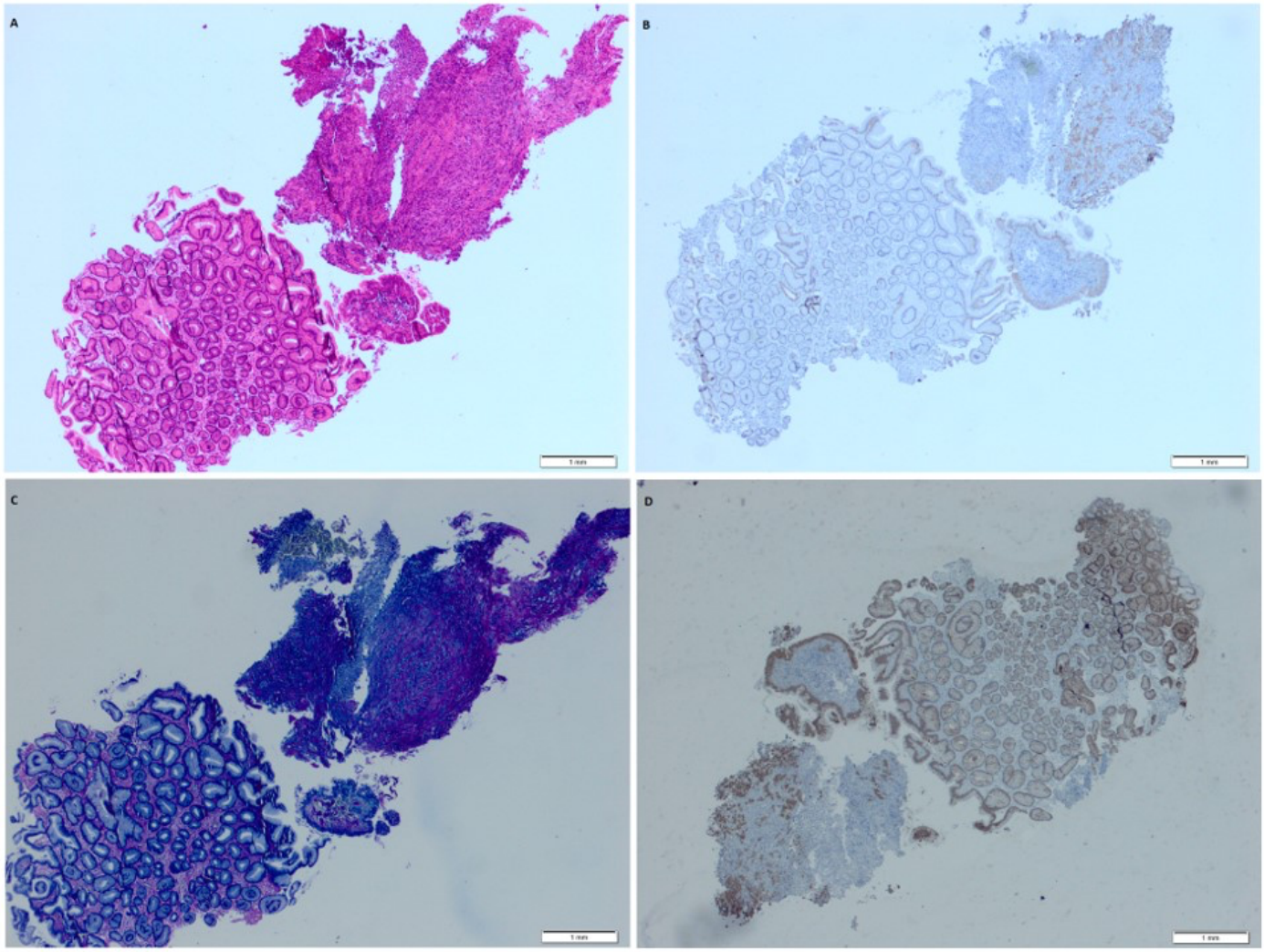

Fig. 3. A: H&E stained slide of endoscopic biopsy material diagnosed as adenocarcinoma, 40x; B: Direct anti-cytokeratin stained slide endoscopic biopsy material of figure 3A, 40x; C: MGG stained slide of endoscopic biopsy material of figure 3A, 40x; D: Anti-cytokeratin stained slide of figure 3C after MGG bleaching, 40x

Spearman's test was used in correlation tests, since it was determined that the data were not normally distributed in Shapiro-Wilk test -even though data were not far from the normality-. While direct IHC staining and the staining frequency after PAB bleaching were correlated in the analyzes (p= 0.006, correlation coefficient 0.391), the same correlation was not found with the staining frequency after MGG bleaching (p= 0.777, correlation coefficient -0.420). Additionally, while the intensity of direct IHC staining and the intensity of staining after PAB bleaching were correlated (p= 0.000, correlation coefficient 0.515), the same correlation was not found with the staining intensity after MGG bleaching (p= 0.255, correlation coefficient 0.168). Furthermore, the numerical evaluation and categorical evaluation of the H-score are similar, and the numerical (p= 0.004, correlation coefficient 0.411) and categorized (p= 0.004, correlation coefficient 0.407) analysis of direct IHC H-scores and IHC after PAB bleaching show a statistical correlation. No correlation was found between the numerical H-score of direct IHC and IHC after MGG bleaching (p= 0.404, correlation coefficient 0.123) as well as categorized H-score (p= 0.953, correlation coefficient 0.009)

4. Discussion

Endoscopy and histopathological examinations, which are the most important methods in diagnosing stomach diseases, are frequently performed today. The dimensions of the biopsy samples taken during endoscopic examination may sometimes not allow additional examinations or the tissues may become smaller during routine technical procedures. Therefore, the presented study evaluated the diagnostic usability of restaining with anti-cytokeratin on bleached PAB and MGG sections that were performed for routine examination.

Although the continued progress of molecular techniques has led to a breakthrough in the pathological examination of neoplasms, the immunohistochemical staining technique, which has evolved with many different steps since Marrack's production of reagents for Typhus and cholera microorganisms (4), is now used quite frequently in many pathology laboratories in addition to routine sections. In the past, immunohistochemistry was referred to as the "Brown revolution" in pathology laboratories, which has many uses

such as the differentiation of benign and malignant lesions, subgrouping of neoplasms or determination of the nature of undifferentiated tumors, finding the primary focus in metastatic lesions, providing therapeutic and prognostic data (5-8).

In immunohistochemical examinations, many pre-, inter- and post-staining factors starting from taking the biopsy affect the staining result (3, 9). Small biopsy materials are vulnerable to many negative factors in terms of staining results, especially shedding and tissue loss. In this case, bleaching the existing sections and staining them again immunohistochemically comes to the fore. In one of the limited studies in the literature on the subject, H&E sections of 105 materials in prostate tru-cut materials were bleached and re-stained. It has been reported that a definitive diagnosis was obtained in 58% of the bleached and restained sections (10). Another study in which H&E is bleached and restained was performed on rat liver and kidney sections. In this study, it was reported that an image similar to the H&E imaage could be obtained in restaining with iNOS (11). In a relatively newer study, it is stated that archived H&E sections can be re-used for multiplex protein biomarker analysis (12). Moreover, novel methods are defined for decolorization of the H&E and masson-trichrome which can be re-used for additional tests including immunohistochemistry (13). However, a study on the semi-quantitative evaluation of immunohistochemical examination performed after histochemical bleaching method in small biopsy materials such as gastric tumors does not draw attention in the literature.

In the present study, the staining frequency, intensity and H-scores of direct IHC staining and IHC staining after PAB and MGG bleaching were evaluated. As a result, it was determined that IHC staining after PAB bleaching showed a positive correlation with direct IHC staining in terms of frequency, intensity and H score, but it was noted that the same correlation was not observed with IHC staining after MGG bleaching. The bleaching of MGG was harder than PAB and PAB showed relatively better bleaching than MGG. Therefore, the lack of correlation detected in MGG bleached sections can be associated with this situation.

Since H&E is the most frequently used stain routinely, it is obvious that H&E sections will be preferred over PAB or MGG stain as an archive or consultation material due to revealing cytoplasmic and nuclear details more clearly and in terms of eye familiarity for pathologists. In the light of the data, we have obtained as a result of current study, it is suggested that IHC staining in PAB bleached sections can be used when IHC examination is required in small endoscopic biopsy materials in order to protect H&E sections as an archive or consultation material. The use of IHC staining after MGG bleaching that does not correlate with direct IHC staining, therefore it is not recommended.

Ethical statement

This study was approved ethically, with the decision number 80576354-050-99/148 and dated 27.09.2017 by Kafkas University, Faculty of Medicine, Ethics Committee.

Conflict of interest

The authors report there are no competing interests to declare.

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None to declare.

Authors' contributions

Concept: Y.A., Design: Y.A., H.B., Data Collection or Processing: Y.A., H.B., Analysis or Interpretation: Y.A., H.B., Literature Search: Y.A., H.B., Writing: Y.A.

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Relationship between gestational diabetes, vitamin D levels, and maternal age

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Abstract

Our study aimed to determine the vitamin D level of pregnant women was associated with gestational diabetes mellitus (GDM) and whether maternal age affects this. This cohort study retrospectively examined the electronic records of 200 pregnant women. The patients were divided into two groups: the GDM group (n: 35) as the case group and the healthy group (n: 165) as the control group. Pregnant women aged 19-44 years were included in the study. Vitamin D levels are deficient at <12 ng/mL, insufficient at 12-20 ng/mL, and sufficient at>20 ng/mL. Results found a statistically significant association between vitamin D levels and GDM in women ($p < 0.05$). The vitamin D level in the GDM group (M=16.19; SD=5.59) was lower than the vitamin D level in controls (M=25.48; SD=10.01). The percentage of women whose vitamin D was within the acceptable range in GDM was 11% compared to 63% of healthy pregnant women. There was no statistically significant association between age and GDM ($p > 0.05$). Results showed that the vitamin D levels in the GDM group were significantly lower than in the control group.

Keywords: Gestational diabetes mellitus, vitamin D, pregnancy, advanced maternal age

1. Introduction

Gestational diabetes mellitus (GDM) is defined as impaired *glucose* tolerance (IGT) and insulin resistance, first diagnosed during pregnancy. GDM affects 6-25% of pregnant women (1, 2). GDM usually has no symptoms but is associated with a higher risk of neonatal and pregnancy complications (3). Based on the performed studies, the reduction of vitamin D levels leads to an increase in the risk of GDM by 45%(4).

Epidemiological factors such as the rising maternal age and increasing background rates of obesity among women of reproductive age lead to a continually increasing prevalence of GDM worldwide. Recently, the World Health Organization has allocated GDM to global health research priorities in terms of its sudden increasing prevalence among women of reproductive age and its relationship with increased risk of neonatal complications, birth difficulties, and abnormal intrauterine growth, including neonatal hypoglycemia, macrosomia, fetal demise, neonatal respiratory distress syndrome, hyperbilirubinemia, the cardiovascular disease after pregnancy, and the onset of type 2 diabetes (1).

Vitamin D is a vitamin soluble in fat that contributes to the mineralization of bone, phosphorus, and calcium intake, immune system function, and parathyroid status (5). The role of vitamins in women's health is one of the main topics in various research (6, 7). Vitamin D deficiency may be due to different factors, including gender, age, BMI, time of sun exposure, level of sun exposure, season, diet, skin type, and sleep pattern (8). Possible mechanisms to adjust glucose homeostasis by vitamin D may include stimulating physiological insulin secretion in interaction with insulin-like growth factor, increasing duodenal intake and calcium renal reabsorption, and facilitating insulin intracellular signal transmission (9).

The high-income countries have an increased rate of women giving birth over the age of 35 over time, raising concern about the effect of advanced maternal age (AMA) on pregnancy outcomes (10). There is an association between AMA and extensive adverse pregnancy outcomes, including stillbirth, chromosomal abnormalities, miscarriage, fetal growth restriction (FGR), preterm labor, GDM, preeclampsia, and increased cesarean section rates (CS). In developing countries, we see an increase in the age of marriage and childbearing among women every year. This issue requires more studies on the increased risks for the baby and the mother.

Information on the role of vitamin D in glucose metabolism in pregnancy and its relationship with the development of GDM is insufficient and contradictory. The study aimed to determine the vitamin D level of pregnant women was associated with gestational diabetes mellitus (GDM) and

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whether maternal age affects this.

2. Materials and Methods

The Ethics Committee of Medipol University Non-Interventional Clinical Research Ethics Committee approved this retrospective study (Date:13.9.2022 Decision no:789). All procedures were carried out by the ethical rules and the principles of the Declaration of Helsinki. Two hundred women participated in this study between July 2018 and May 2022.

Maternal ages were divided into four groups; 18-21, 22-35, 36-40, and >40 years old. Pregnant women whose 25 (OH) vitamin D levels were checked in the first trimester and had an oral glucose tolerance test (OGTT) at 24-28 were included in the study. The study did not include pregestational diabetic women who did not have the recommended OGTT or whose result was not in the file. Pregnant women who had OGTT but were not screened for vitamin D levels in the first trimester and for whom no vitamin D was screened were excluded from the study.

The pregnant women included in the study were screened for GDM with the two-stage diagnostic approach recommended by the American Diabetes Association (11). In the two-stage diagnostic approach, if the plasma glucose level is ≥140 mg/dL 1 hour after drinking 50 g of glucose liquid randomly during the day, it is suspicious for diabetes. A 3-hour OGTT with 100 g glucose should be performed to confirm the diagnosis in pregnant women with a positive 50 g screening test. In our study, the evaluation of 100gr OGTT was evaluated according to the Carpenter-Coustan criteria in the "American College of Obstetricians and Gynecologists" (ACOG) and "American Diabetes Association" (ADA) guidelines. Fasting, first, second, and 3rd-hour cut-off values in the Carpenter-Coustan assessment are ≥95 mg/dL - ≥180 mg/dL - ≥155 mg/dL - ≥140 mg/dL, respectively. Those with two positive values out of four were diagnosed with GDM, and those with one positive value on the OGTT were called a single pathological value in the OGTT. A diagnosis of 'impaired glucose tolerance' was made. Two-step tests 24-28 were applied during pregnancy weeks. It was determined that pregnant women with a high risk for GDM were screened with 100 g OGTT in earlier weeks.

Vitamin D levels are deficient <12 ng/mL, insufficient 12-20 ng/mL, and sufficient >20 ng/mL(12-14).

2.1. Statistical Analysis

SPSS 26.0 package program was used for statistical analysis. Evaluation of the data, percentage distributions, mean(M), standard deviation (SD), Kolmogorov Smirnov test as a normality test, Mann-Whitney U test, and Kruskal-Wallis H test were used.

We utilized the GPower 3.1 program to calculate the example size. The two groups' total mean was calculated based on the Mann-Whitney test with a power of 80%, an effect size of 50%, and a beta/alpha ratio of 1 for at least 30 patients for each group (15).

3. Results

Two hundred women were divided into pregnant women with gestational diabetes (n=35) and healthy pregnant women (n=165). Table 1 shows descriptive statistics of study parameters in women. The mean age and BMI of women were 31.6 ± 7.89 and BMI 25.01±2.05, respectively.

Table 1. Descriptive statistics of study parameters in women

	Study parameters	Median (range) Mean ± SD or n (%)
Maternal characteristics	Age(years)	32 (19-44) 31.6±7.89
	BMI (kg/m2)	32 (18-30) 25.01±2.05
Laboratory values	HbA1c(%)	5.24 (4.51-6.2) 5.27±0.23
	FPG	83 (69-135) 84.23±7.7
	Plasma glucose at 60-minute time (mg/dL)	145 (13-252) 145.8±36.93
	Plasma glucose at 120-minute time (mg/dL)	113 (13-223) 117.59±30.95
	Vitamin D (ng/ml)	25.65 (5-48) 23.85±10.02
Age groups	18-21	47(23.5)
	22-35	60(30)
	36-40	57(28.5)
	>40	36(18)
Cigarette	Yes	18(9)
	No	182(91)
Vitamin D	Deficiency	23(11.5)
	Insufficient	69(34.5)
	Sufficient	108(54)
Insulin	Yes	2(1)
	No	198(99)
GDM	Yes	35(17.5)
	No	165(82.5)

BMI: Body mass index, SD: standard deviation, HbA1c: Hemoglobin A1c, FPG: fasting plasma glucose, GDM: gestational diabetes mellitus

Table 2 compares GDM positive as a case group and healthy women as a control group on age and Vitamin D values.

Table 2. Comparison of case and control groups regarding age and vitamin D

Study parameters	GDM positive (n=35) median (range) mean ± SD	Healthy (n=165) median (range) mean ± SD	*p*
Age (years)	32 (19-44) 30.89±8.46	16 (6-31.1) 16.19±5.59	0.521*
Vitamin D (ng/ml)	16 (6-33.1) 16.18±5.59	31 (5-48 25.48±10.01	<0.001*
HbA1c (%)	5.5 (5.1-6.2) 5.52±0.25	5.2 (4.51-5.67) 5.21±0.18	<0.001*
FPG	90 (79-135) 92.37±11.2	82 (69-97) 82.5±5.37	<0.001*
Plasma glucose at 60-minute time (mg/dL)	197 (127-252) 196.97±25.57	139 (13-215) 134.95±29.02	<0.001*
Plasma glucose at 120-minute time (mg/dL)	159 (83-223) 157.63±37.42	112 (13-174) 109.09±21.39	<0.001*

*A Mann-Whitney U test

As stated in Table 2, a statistically significant association was found between the case and control regarding age (p>0.05).

As stated in Table 2, a statistically significant association was found between the case and control regarding Vitamin D (p<0.05). The serum vitamin D was significantly higher in the healthy group (Mean=16.18 vs. 25.48). There was a significant difference between the two groups in terms of HbA1c, FPG, and plasma glucose at 60 and 120 minutes (p<0.05).

Fig. 1 shows the serum vitamin D levels in women with gestational diabetes and healthy women.

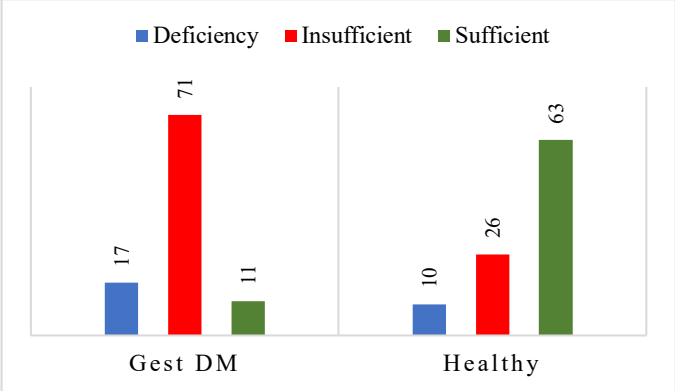

Fig. 1. The serum vitamin D levels in women with gestational diabetes and healthy women [Values in the figure are in percentages (%)]

In the GDM group, the frequency for the serum 25-hydroxyvitamin D (vitamin D) <12 ng/ml interval is 6 (17.1%). The frequency for vitamin D (12-20 ng/ml) and vitamin D (>20 ng/ml) was 25 (71.4%) and 4 (11.4%), respectively. In the control group, the frequency for the serum 25-hydroxyvitamin D (vitamin D) <12 ng/ml interval is 17 (10.3%). The frequency for vitamin D (12-20 ng/ml) and vitamin D (>20 ng/ml) was 44 (26.7%), and 104 (63%), respectively.

As stated in Table 3, a statistically significant association was found between age and the serum vitamin D level in the case group (p<0.05). As stated in Table 3, a statistically significant association was found between age and the serum vitamin D level in the control group (p<0.05).

Table 3. Comparison of serum vitamin D level according to age groups in women with gestational diabetes and healthy women

Study parameters		**GDM positive** (n=35) Median (range) Mean±SD	***p***	**Healthy** (n=165) Median (range) Mean±SD	***p***
Age groups	18-21	16.75 (14-19) 16.57±1.62	0.002	32 (24.8-41) 31.98±3.05	<0.001
	22-35	18.5 (14.6-31.1) 21.43±6.66		33.2 (24.8-48) 34.07±3.78	
	36-40	14 (6-22) 13.7±5.17		15 (5-23) 15.43±3.68	
	>40	12 (9-14) 11.83±2.04		14.5 (5-34.2) 18.62±10.61	

Age groups were tested by a Kruskal Wallis U test

Fig. 2 shows the serum vitamin D levels in age groups in the case and control groups. The mean of Vitamin D levels in each age group is presented in this figure.

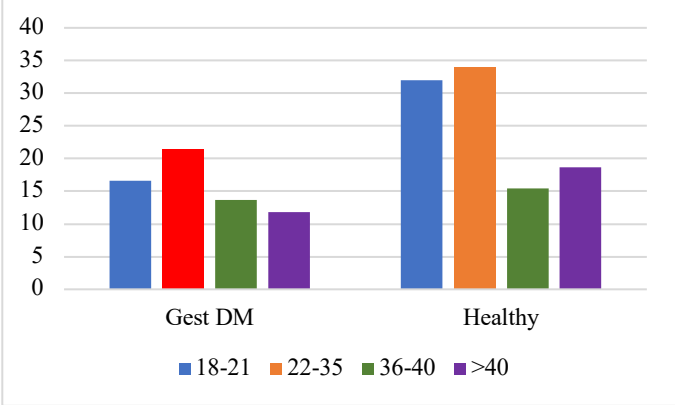

Fig. 2. The serum vitamin D levels in age groups in two groups

4. Discussion

The present study compared the relationship between vitamin D serum levels and GDM. There was a significant association between Vitamin D levels and GDM. The percentage of women whose vitamin D was within the acceptable range in pregnant women with diabetes was 11% compared to 63% of healthy pregnant women. There was a significant association between GDM and healthy groups in terms of HbA1c, FPG, and plasma glucose at 60 and 120 minutes and the age of women. Age groups the difference in vitamin D levels in healthy women in all age groups are more significant than in women. However, this difference decreases in women older than 36 years. Healthy women also face a decrease in vitamin D levels with age.

Previous studies have shown the ability of vitamin D in glucose homeostasis by regulating plasma calcium levels, which causes the regulation and secretion of insulin (16). Also, vitamin D improves the sensitivity of insulin target cells such as fat, liver, and skeletal muscles to insulin(17). Although the role of vitamin D in the pathogenesis of type 1 and type 2 diabetes has been proven, its role in GDM is still debated. We reviewed the literature and found that many studies have compared vitamin D levels and GDM. Some studies (8, 18-20) have documented no significant differences between vitamin D levels and GDM, and some studies (21-28) have indicated that vitamin D levels and GDM association were statistically significant. Milajerdi et al. (29) and Wang et al. (30), in two systematic reviews, reported a significant association between vitamin D deficiency and an increased risk of GDM.

Muthukrishnan et al. (18) found that serum Vitamin D levels were significantly higher in the control (45.8 ng/ml) versus the GDM group (24.7 ng/ml). Mosavat et al. (8) found that low maternal 25 (OH)-vitamin D in the second trimester of pregnancy is associated with GDM. The results of this study reported BMI, race, and ethnicity as influential factors in this association. Rajput et al. (19) said that severe vitamin D deficiency was significantly higher in GDM women, 44% versus 20% of healthy women. A meta-analysis by Zhang et al. (20) found that vitamin D deficiency increases GDM and

the use of supplements during pregnancy helps reduce the risk of GDM. Further studies showed that vitamin D deficiency was not only a problem in mothers, but in a 9-year follow-up, it was shown that the children of these mothers showed more resistance to insulin and were, therefore, at a higher risk for diabetes(31).

Ismail et al. (22) reported no significant difference in the mean vitamin D concentration between GDM and normal pregnancies(14.43±5.27 versus 15.45±5.29). This study believes that the lower average vitamin D in GDM is outdoor activity and ethnicity in the third trimester of pregnancy. Jafarzadeh et al. (21) found that vitamin D levels in the first and second trimesters of pregnancy were similar in GDM and healthy women. Makgoba et al. (23) reported no association between the development of GDM and low vitamin D. Lou et al. (24) found no association between maternal vitamin D levels and GDM. Keller et al. (25) found that women whose diet is enriched with vitamin D have a lower risk of GDM. Although this finding was insignificant, it was close to significance (p=0.0.8). Farrant et al. (26) reported the relationship between vitamin D concentration, age, and BMI, and there was no increase in the risk of GDM. Park et al. (27) reported no relationship between the serum level of vitamin D in the first and second trimesters and GDM and fetal growth. Baker et al. (28) confirmed the absence of such a relationship.

GDM is a multifactorial disease. Therefore, despite many studies in this field, regional, economic, racial, ethnic, and lifestyle differences are the most critical factors that have caused the findings of studies to contradict each other. On the other hand, the design and sample size of many studies differ, which can be another reason for the differences in the results.

One of the strengths of this study is the statistical population with sufficient size. However, considering the multifactorial nature of gestational diabetes, the study participants should be evaluated in terms of lifestyle and diet.

As a result, there is a significant relationship between vitamin D and GDM. In most studies that did not report a meaningful relationship, vitamin D levels are still lower in diabetic women. It is necessary to replace vitamin D with proper nutrition and supplements. Age does not affect GDM due to vitamin D deficiency. However, the effect of vitamin D on GDM has yet to be proven. It requires more studies focusing on the characteristics such as lifestyle, ethnicity, and diets of the studied women.

Ethical statement

The Ethics Committee of Medipol University Non-Interventional Clinical Research Ethics Committee approved this retrospective study (Date:13.9.2022 Decision no:789). All procedures were carried out by the ethical rules and the principles of the Declaration of Helsinki.

Conflict of interest

The authors have no conflicts of interest to declare.

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None to declare.

Authors' contributions

Concept: A.Ş.K., Design: A.Ş.K., Data Collection or Processing: A.Ş.K., Analysis or Interpretation: A.Ş.K., Literature Search: A.Ş.K., Writing: A.Ş.K.

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Coping strategies, anxiety, depression, and quality of life in rheumatoid arthritis patients

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Abstract

The purpose of this study was to evaluate the effect of Rheumatoid Arthritis (RA) disease, which is known to have adverse psychological effects on depression, anxiety, coping, and quality of life. The study included 59 RA patients and 56 healthy individuals (control group). Sociodemographic data form, Beck Anxiety Scale, Beck Depression Scale, COPE (coping) scale, and RA Quality-of-Life Questionnaire were used to collect data. The groups were compared according to the results. The RA patients were observed to have higher levels of depression and anxiety compared to the control group ($p<0.001$, all). The RA patients were found to have moderate depression and mild anxiety. The RA group had significantly lower problem-focused coping scores ($p=0.002$) than the control group. It was found that as the RA patients' anxiety and pain levels increased, so did their level of depression. Moreover, as their depression, anxiety, and pain levels increased, their quality of life decreased. It was found that RA patients had higher depression and anxiety levels compared to the control group. It was observed that the RA patients' level of depression increased with increasing anxiety and pain, while their quality of life decreased with increasing levels of depression, anxiety, and pain. We consider that psychiatric approaches can contribute to quality-of-life in patients with RA.

Keywords: rheumatoid arthritis, anxiety, depression, quality of life.

1. Introduction

Rheumatoid arthritis (RA) is a chronic connective tissue disease with stiffness, pain, and deformities in joints, which can affect other body systems, and includes periods of exacerbation and recovery (1). The incidence of RA is about 0.5-1% (2). Its etiology is unknown; in addition to hereditary factors, it is suggested that mental and social factors also play a role in the emergence and course of the disease (3). RA is a health problem affecting the quality of life (4). RA causes impairment in both the physical and psychosocial lives of individuals (1). Symptoms may result in restriction of daily activities, decreased work efficiency, social isolation, and dependence on others (3).

Coping attitudes include specific psychological and behavioral efforts used to struggle with the adverse effects of stressors (5). Psychiatric disorders such as depression and anxiety can occur in patients with RA. Pain, physical disability, and loss of social activities can play a role individually or together, leading to mood disorders (6, 7). It has been reported that 14.5-42.0% of RA patients have depression; in those with depression, the disease has more severe symptoms and functional impairments, and anxiety is as common as depression (1, 2, 6). The uncertainty of the course of the disease, the functional disorders it causes, and the pain and social isolation that develops due to the infection may predispose to the development of both anxiety and depression in RA (6-8).

In this study, we aimed to compare the RA patients with a healthy control group in terms of coping, depression, and anxiety.

2. Materials and Methods

This cross-sectional case-control study conducted between August and October 2020 included 59 RA patients and 56 healthy individuals. Sociodemographic data form, Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Coping with Stress Attitudes scale (COPE) scale, and Rheumatoid Arthritis Quality of Life Questionnaire (RAQoL) were used to collect data, and the groups were compared according to the results.

The socio-demographic data form includes the following information: age, gender, educational status, marital status, occupation, family history of RA, psychiatric history, and

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duration of RA. BDI is used to assess depression levels. The higher the total score, the more severe the depression (9, 10). BAI is a self-assessment scale developed to determine the frequency of anxiety symptoms experienced by individuals (11, 12). The COPE scale was designed to determine the coping strategies used in stressful situations. It consists of 60 items in 15 subscales answered (13, 14). The RAQoL form is a quality-of-life questionnaire explicitly developed for RA. The patients answer the questionnaire with 30 questions on their own in the form of yes/no. The higher the score, the lower the quality of life (15, 16). Visual Analogue Scale (VAS), pain severity is scored between 0 (no pain) and 10 points (worst pain imaginable) (17).

Inclusion criteria: RA patients over the age of 18, with cognitive functions sufficient to answer the questions, and the control group were included in the study. Exclusion criteria: Individuals with cognitive impairment preventing them from answering the questions, those who did not answer the questionnaire completely, and those with missing sociodemographic data were excluded from the study (Fig. 1).

In the statistical data analysis, International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) software package (v.22.0; SPSS Inc., Armonk, NY, USA) was used. Based on the data distribution in the comparison between groups, the One-way ANOVA test was used for normally distributed values in non-categorical data and the Mann-Whitney U test for non-parametric data. Categorical data were compared using the Chi-square test. Pearson Correlation analysis was used to analyze the relationship between scale scores. The statistical significance was set at p <0.05.

This study was conducted by Helsinki Principles, and ethical approval was taken from the local ethics committee (Decision Number: 2020/328, 27.08.2020).

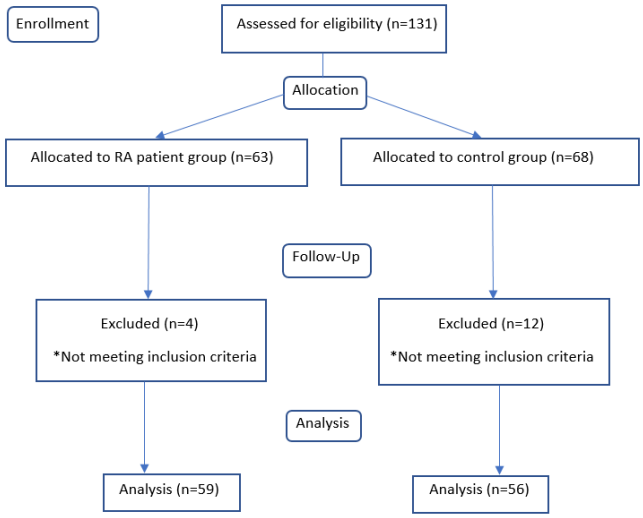

Fig. 1. Flow diagram of the study

3. Results

Our study included 59 RA patients and 56 healthy controls. The mean age of the RA patients was 57.7±12.4 years, and 38 (%64.4) of them were women. The mean age of the control group was 59.5±12.3 years, and 29 (%51.8) of them were women. In the RA patients, the rates of being a primary school graduate (p<0.001), being a housewife (p=0.006), having a psychiatric illness (p=0.010), and having a family history of RA (p<0.001) were statistically significantly higher compared to the control group (Table 1).

There was a statistically significant difference between the RA patients and the control group in terms of BDI (p<0.001) and BAI scores (p<0.001). It was observed that the RA patients had moderate depression and mild anxiety. The mean RAQoL score was 15.0±4.8, and the mean VAS score was 7.4±2.1 in the patients with RA (Table 2).

Table 1. Sociodemographic and clinical characteristics of RA patients and control groups

Variables		All participants (n=115)	RA patients (n=59)	Control groups (n=56)	p
Age (year), (mean±sd)		58.5±12.3	57.7±12.4	59.5±12.2	0.441
Gender, *n* (%)	Female	67 (58.3)	38 (64.4)	29 (51.8)	0.118
	Male	48 (41.7)	21 (35.6)	27 (48.2)	
Marriage status, *n* (%)	Single	7 (6.1)	4 (5.4)	3 (6.8)	0.864
	Married	108 (93.9)	55 (94.6)	56 (93.2)	
Education Status, *n* (%)	Literate	16 (13.9)	10 (16.9)	6 (10.7)	<0.001
	Primary school	46 (40.0)	38 (64.4)	8 (14.3)	
	Middle school	16 (13.9)	9 (15.3)	7 (12.5)	
	High school	16 (13.9)	2 (3.4)	14 (25.0)	
	University	21 (18.3)	0 (0)	21 (37.5)	
Occupation, *n* (%)	Housewife	44 (38.3)	25 (42.4)	19 (33.9)	0.006
	Officer	16 (13.9)	2 (3.4)	14 (25.0)	
	Worker	7 (7.1)	5 (8.5)	2 (3.6)	
	Self-employment	10 (8.7)	4 (6.8)	6 (10.7)	
	Retired	38 (33.0)	23 (39.0)	15 (26.8)	
Psychiatric history, *n* (%)	Yes	10 (8.7)	9 (15.3)	1 (1.8)	0.010
	No	105 (91.3)	50 (84.7)	55 (98.2)	
RA history of the family, *n* (%)	Yes	17 (14.7)	14 (23.7)	3 (5.3)	<0.001
	No	98 (85.3)	45 (76.3)	53 (94.7)	
Duration of RA (year), (mean ± sd)		-	11.5 ± 5.6	-	-

p, chi square test; *n*, number; sd, standard deviation; RA, Rheumatoid Arthritis

Table 2. Comparison of BDI, BAI, COPE, VAS, and RAQoL scores of RA patients and control group

Scales	RA Patients (mean±sd)	Control Groups (mean±sd)	*p*
BDI	17.4±9.7	7.0±5.5	<0.001
BAI	14.9±9.1	6.8±6.9	<0.001
COPE	154.4±27.3	154.9±14.4	0.895
RAQoL	15.0±4.8	-	-
VAS	7.4±2.1	-	-

p, independent samples test; sd, standard deviation; RA, Rheumatoid Arthritis; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; COPE, Coping with Stress Attitudes scale; RAQoL, Rheumatoid Arthritis Quality of Life questionnaire; VAS, Visual Analogue Scale

The problem-focused coping scores (p=0.002) were significantly lower, and the non-functional coping scores (p=0.007) were significantly higher in the RA group than in the control group. When the COPE subscales of the groups were compared, active coping (p<0.001), planning (p<0.001), positive reinterpretation and development (p=0.014), acceptance (p=0.032), focusing on the problem and revealing emotions (p=0.028), problem-focused coping (p=0.002) scores were found to be statistically significantly lower while joking (p=0.002), behavioral disengagement (p=0.004), substance abuse (p=0.006), denial (p=0.001), mental ignoring (p<0.001), and functionally focused coping (p=0.007) scores were found to be statistically significantly higher in the RA group compared to the control group (Table 3).

According to the results of the correlation analysis between COPE, BDI, BAI, RAQoL, VAS, and disease duration in RA patients, there was a positive correlation between BDI and BAI (p<0.001) and VAS (p=0.046), and between RAQoL and BDI (p=0.040), BAI (p=0.039), and VAS (p<0.001) (Table 4)

Table 3. Comparison of Groups According to COPE Coping Sub-Scale Scores

COPE points	RA Patients (mean±sd)	Control Groups (mean±sd)	*p*
COPE-1 (active coping)	11.8±2.4	13.6±1.5	<0.001
COPE-2 (stalling)	10.0±2.4	10.2±1.5	0.600
COPE-3 (planning)	11.2±2.7	13.1±2.2	<0.001
COPE-4 (using beneficial social support)	11.8±2.8	12.3±1.7	0.292
COPE-5 (suppressing other activities)	10.6±2.4	11.3±2.3	0.198
COPE-6 (positive re-interpretation and development)	12.8±2.2	13.7±1.7	0.014
COPE-7 (religious coping)	14.1±2.7	13.4±3.5	0.266
COPE-8 (joking)	8.0±3.7	6.3±1.7	0.002
COPE-9 (using emotional, and social support)	12.1±2.5	11.5±2.8	0.321
COPE-10 (acceptance)	10.1±3.0	11.2±2.5	0.032
COPE-11 (cognitive disengagement)	7.4±3.0	6.0±2.0	0.004
COPE-12 (substance use)	5.7±3.5	4.3±0.8	0.006
COPE-13 (denial)	8.4±3.4	6.6±2.6	0.001
COPE-14 (mental disengagement)	10.2±2.8	8.2±2.2	<0.001
COPE-15 (focusing on the problem and showing emotion)	10.6±2.5	11.7±2.5	0.028
COPE-P (Problem-focused coping)	55.4±10.4	60.8±6.9	0.002
COPE-E (Emotion-focused coping)	57.2±11.0	56.3±7.5	0.614
COPE-F (Function-focused coping)	42.4±11.0	37.9±5.2	0.007
COPE-T (Total coping points)	154.4±27.3	154.9±14.4	0.895

p, independent samples test; sd, standard deviation; RA, Rheumatoid Arthritis; COPE, Coping with Stress Attitudes scale

Table 4. Correlation analysis between COPE, BDI, BAI, RAQoL, VAS, and duration of illness in RA patients

Variables		COPE	BDI	BAI	RAQoL
COPE	***r***	-	-0.018	-0.016	0.131
	95% CI	-	-0.289, 0.268	-0.286, 0.270	-0.128, 0.421
	p	-	0.893	0.940	0.321
BDI	***r***	-0.018	-	0.994	0.269
	95% CI	-0.289, 0.268	-	0.990, 0.996	0.036, 0.504
	p	0.893	-	<0.001	0.040
BAI	***r***	-0.016	0.994	-	0.268
	95% CI	-0.286, 0.270	0.990, 0.996	-	0.025, 0.509
	p	0.940	<0.001	-	0.039
RAQoL	***r***	0.131	0.269	0.268	-
	95% CI	-0.128, 0.421	0.036, 0.504	0.025, 0.509	-
	p	0.321	0.040	0.039	-
VAS	***r***	0.078	0.261	0.251	0.799
	95% CI	-0.211, 0.390	0.064, 0.450	0.053, 0.436	0.709, 0.887
	p	0.557	0.046	0.055	<0.001
Duration of illness	***r***	-0.144	-0.126	-0.159	-0.067
	95% CI	-0.380, 0.055	-0.354, 0.117	-0.386, 0.078	-0.315, 0.182
	p	0.275	0.342	0.230	0.616

p-value, Pearson Partial Correlation Test; *r,* Correlation Coefficient; CI, Confidence Interval; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; COPE, Coping with Stress Attitudes scale; RAQoL, Rheumatoid Arthritis Quality of Life questionnaire; VAS, Visual Analogue Scale

4. Discussion

In our study, higher levels of anxiety and depression were observed in the RA patients compared to the control group, but no difference was observed in their total coping scores. The RA patients were found to have moderate depression and mild anxiety. RA can negatively affect patients in all areas, also leading to psychiatric disorders. In the study of Altan et al., it was revealed that the frequency of depression and anxiety in people with RA was higher than in the healthy control group (6). Frank et al. reported that 42.0% of the patients with RA had depression, and those with depression had more severe symptoms and functional disruptions (18). Zahura et al. found that depressive symptoms increase the perception of arthritis pain in RA. Therefore, it is possible to state that recognizing depression in RA patients may play an essential role in treating the disease (19). El-Miedany et al. reported that, in RA patients, anxiety was as frequent as depression. The researchers have suggested that anxiety may be a precursor of future depression and correlate with social stress (20).

In our study, the problem-focused coping scores were lower, and the non-functional coping scores ($p=0.007$) were higher in the RA group than in the control group. It was found that the RA patients had poor problem-focused and active coping skills; in coping with stress, they could not plan and interpret positively and were unable to accept, focus on the problem, and reveal their emotions. In our study, the majority of the RA patients were middle-aged women. In RA patients, the rates of being a primary school graduate, being a housewife, having a psychiatric history, and having a family history of RA were higher compared to the control group. In previous studies, it has been reported that hereditary factors play a role in the occurrence and course of RA (3). The RA patients were found to cope with a functional focus, neglect behaviorally, joke, use substances, deny, and neglect mentally. Individuals exposed to chronic stress such as RA may be inadequate in some subgroups to cope with this condition, but they can start the whole adjustment process. In the study groups of Bendtsen et al., the most frequently mentioned coping strategies were found to be related to the problem (21). In the study of Mıstık et al., a positive relationship was found between the severity of pain (VAS) and religious coping scores, in which the RA patients most frequently used emotion-focused coping attitudes (2). Harmful defense mechanisms such as self-blame or denial have also been observed to accompany depression in patients with RA. In addition to these social factors, RA can lead to psychiatric disorders by affecting the immune system. It is known that acute or chronic stress can cause anxiety by disrupting the body's defense mechanisms (6).

Altınkesen reported that the severity of pain in the late period affects the quality of life related to physical health in patients with RA. There is a strong positive correlation between functional status and quality of life. He reported that the limitation of movement in the distal joints, especially in the hand and wrist joints, prevented their daily life activities related to the skill and performance in their professions (22). Despite the advanced treatment options required for chronic use and available in selected patients, RA may cause deformation and muscle atrophy in small, medium, and large joints (23). Our study found that the level of depression increased with increasing anxiety and pain levels. It was observed that the quality of life decreased with increasing levels of depression, anxiety, and pain.

In RA, stressors can initiate pathophysiological changes or accelerate the course of these changes (3). RA causes joint deformities, widespread pain, dependence on others, and limitations in daily functions. Even if physical limitations and addiction do not develop in RA patients, they live with these fears. These fears lead to a change in one's body perception and depression by finding oneself worthless and inadequate (24). Studies have found that pain, dependence, and limitation of movement are the most significant stressors in RA patients (25). In the study of Aslan et al., it was shown that there was a relationship between disease-related characteristics and depression and anxiety in women with RA (1). In the study of Altan et al., it was found that depression and anxiety were generally associated with the disease activation parameters such as pain and swollen and sensitive joints (6). Murphy et al. reported a significant relationship between depression and VAS pain scores (26). Although there is evidence that disability causes depression in RA (27), there is also evidence that depression may cause disability (28). It has been reported that psychiatric morbidity significantly affects the overall course of the disease, the perception of pain, and the quality of life in RA patients (6). It can be interpreted as an expected result that these patients who have more joint deformities and pain and can perform fewer daily activities find themselves less attractive are less satisfied with their bodies. This makes the person more prone to depression (29).

In our study, there was no correlation between the disease duration and scale scores. Sharpe et al. found that the patients' mean depression score gradually increased over two years in which they followed up on early RA cases. However, interestingly, they found that the score decreased in the final evaluation, and they explained this with the hypothesis that the patients might have adapted to their disease (30).

One of the limitations of our study is that it is single-centered. Future studies should be carried out in multiple centers with more participants. The strength of our research lies in that it is a prospective study that compares RA patients with normal individuals in terms of anxiety, depression, coping, and quality of life.

This study found that problem-focused coping skills were low, and the levels of depression and anxiety were high in patients with RA. It was found that the level of depression increased with increasing anxiety and pain intensity. It was observed that the quality of life decreased with increasing

depression, anxiety, and pain intensity. We consider that psychiatric approaches can contribute to the quality of life in patients with RA.

Ethical Statement

This study was conducted by Helsinki Principles, and ethical approval was taken from the local ethics committee (Decision Number: 2020/328, 27.08.2020).

Conflict of interest

None to declare.

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None to declare.

Authors' contributions

Concept: H.İ., F.İ., Design: H.İ., F.İ., Data Collection or Processing: H.İ., F.İ., Analysis or Interpretation: H.İ., F.İ., Literature Search: H.İ., F.İ., Writing: H.İ., F.İ.

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Investigation of relationship between nesfatin's, chemerin's, apelin's levels and insulin resistance and metabolic syndrome in Polycystic Ovary Syndrome patients

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common gynecological endocrinopathies. In the literature, many studies support the involvement of IR (insulin resistance) and some adipokines in the pathophysiology of PCOS. In our study, we aimed to investigate the relationships of apelin, chemerin, nesfatin, IR, and MS (metabolic syndrome) to help clarify the pathophysiology of PCOS. We included 120 people in our prospective cohort study. We divided these 120 cases into three equal groups: a control group of healthy participants and two case groups of participants suffering from PCOS with a BMI below 30 and above 30. We conducted this study in a tertiary hospital between January 2014 and July 2014. We investigated the study groups in terms of demographic information, biochemical values, hormonal parameters, apelin, chemerin, and nesfatin values, and the relationships between MS and IR. Apelin, chemerin, and nesfatin values were 0.233±0.027 ng/dl, 232.79±221.87 ng/dl, and 2.11±1.27 ng/dl in the control group. These values were 0.304±0.093 ng/dl, 313.57±379.04 ng/dl, and 0.85±0.91 ng/dl in the nonobese PCOS patient group, respectively. The same parameters were 0.304±0.093 ng/dl, 344.31±386.23 ng/dl, and 0.74±1.42 ng/dl in the obese PCOS patient group. The HOMA-IR values of the groups were 1.79 ± 0.68, 3.61 ± 5.45, and 4.58 ± 4.39, respectively. The distribution of cases diagnosed with MS within the groups was 0%, 10%, and 17.5%, respectively. We compared all these statistically significant results ($p<0.005$). The fact that blood nesfatin levels are significantly lower and apelin and chemerin levels are significantly higher in PCOS patients suggests that the synthesis and secretion of these adipokines are effective in the mechanism of the disease. The development of MS. Comprehensive prospective studies are needed to clarify this situation.

Keywords: apelin, chemerin, HOMA-IR, metabolic syndrome, nesfatin, PCOS

1. Introduction

Reliable publications report that although PCOS is quite common, it is seen in 5-10% of women of reproductive age and 3% of adolescents (1). For diagnosis, at least two Rotterdam criteria must be met, including oligo-anovulation, clinical or biochemical signs of hyperandrogenism, and ultrasonographic polycystic appearance in the ovaries (2). In the long term, patients suffering from PCOS are at increased risk for hypertension, infertility, recurrent spontaneous abortion, Type II DM (diabetes mellitus), dyslipidemia, coronary artery disease, endometrial hyperplasia, and cancer. Therefore, gynecologists and endocrinologists should investigate the physiopathology and the treatment of this syndrome with a multidisciplinary approach. However, the presence of many factors in the development of the syndrome and the heterogeneity in its presentation makes it difficult to understand its pathophysiology (3) fully.

Plasma apelin level increases significantly in obesity concerning hyperinsulinemia and insulin resistance (4). In this context, it might play a role in the pathophysiology of PCOS. Chemerin increases insulin resistance by preventing insulin-mediated glucose uptake in muscle cells (5). Chemerin is associated with central obesity, IR, hyperglycemia, dyslipidemia, HT, and prothrombotic and proinflammatory processes (6) It may play a role in the etiopathophysiology of PCOS due to its involvement in glucose uptake and being an adipokine. In line with the knowledge that nesfatin may be associated with glucose metabolism, insulin resistance and obesity development mechanisms (7, 8), it comes to mind that it may also be associated with the mechanism of PCOS formation. The etiopathogenesis of MS is multifactorial (9). It has been revealed that the basis of MS is a disorder in the response of tissues to insulin, that pancreatic β cells secrete

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more insulin than expected due to IR, and as a result, hyperinsulinemia develops (9). HT, dyslipidemia, obesity, DM, and atherosclerotic vascular disease are also components of MS due to IR and hyperinsulinemia (10). In line with all this information, we wondered how much MS and PCOS are related.

Although there are some hypotheses about the pathophysiology of PCOS when we review the literature, there still needs to be a clear consensus. We argue that some adipokines contribute to the formation of PCOS and that the discovery of the precise location of these molecules in human physiology may explain the mechanisms of PCOS formation. Regarding this subject, which needs all kinds of scientific studies to explain its pathophysiology, we aimed to explain the relationship between nesfatin, chemerin, apelin values, and MS and IR in PCOS patients.

2. Materials and methods

Our study was designed as a case-control study. It consists of 120 cases, including 80 patients aged 18-43 years and 40 healthy participants, diagnosed with PCOS according to the Rotterdam criteria. We conducted the study between January 2014 and July 2014 at Yüzüncü Yıl University, Faculty of Medicine, Department of Obstetrics and Gynecology. We informed the volunteers about the study before participating and obtained written consent from those who agreed to participate. Before the study, we received ethics committee approval from the Ethics Committee of the Yüzüncü Yıl university with the decision dated 30.01.2014 and numbered 05.

We recorded the patients' age, heights, and weights and calculated their body mass index (BMI). We evaluated the patients for hirsutism using the Ferriman-Gallwey scoring system. In the blood we collected on the third day of their menstrual cycles from all the cases participating in the study, we looked at luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), total testosterone, sex hormone binding globulin (SHBG), progesterone values. We measured low-density lipid (LDL), high-density lipid (HDL), triglyceride (TG), total cholesterol, fasting serum glucose, and insulin levels in the same blood samples of the subjects. We calculated the HOMA-IR index [Clear blood glucose (mg/dl) X fasting insulin level (µU/ml) / 405] using fasting blood glucose and fasting insulin levels. We also calculated the LH/FSH ratios. Our study used the MS diagnostic criteria of the Turkish Endocrinology Metabolism Society MS Study Group (11). We measured hormone parameters such as LH, FSH, E2, total testosterone, progesterone, SHBG, and fasting insulin using the immuno chemiluminescence method (Roche-Hitachi Modular Analytics E-170, USA). We measured fasting glucose, cholesterol, HDL, LDL, and TG from biochemical parameters by spectrophotometric method (Olympus AU 600 Tokyo / Japan). On the third day of menstruation, we collected 10 cc of blood from the antecubital vein between 08:00 and 10:00 in the morning, following a 10-hour night fast. We examined biochemical and hormonal parameters at Yüzüncü Yıl University, Faculty of Medicine, Department of Biochemistry. We rotated the separated 4 cc of the blood samples in a centrifuge at 5,000 rpm for 5 minutes, separated the serum into 2 cc epandorfs, and stored them at -80 0C until we checked the levels of Apelin, Chemerin, and Nesfatin in this serum. We measured Apelin, Chemerin, and Nesfatin levels using a micro-ELISA kit (Bio-Tek Instruments inc. Miroquant Cal / USA).

We used Siemens Acuson Antares™ USA brand device for ultrasonography of the patients. We used Siemens Acuson CH6-2 5.71 MHz abdominal probe USA for a suprapubic pelvic ultrasound and Siemens 47 Acuson EC9-4 6.15 MHz transvaginal probe USA for a transvaginal ultrasound.

2.1. Inclusion criteria

Participants between 18-43, diagnosed with PCOS, and whose BMI was determined were included in the case groups. For the control group, participants with regular menstrual cycles in the same age range and a BMI below 30 were included in the study.

2.2. Exclusion criteria

We excluded those suffering from diseases such as thyroid disease, prolactinoma, Cushing's disease, significant depression, DM (diabetes mellitus), HT (Hypertension), Dyslipidemia, CAD (coronary artery disease), late-onset adrenal hyperplasia, kidney and liver defect. In addition, we excluded those who use drugs that may affect sex hormone and carbohydrate metabolism.

2.3. Statistical analysis

We performed a One-Way Analysis of Variance (ANOVA) to compare group means in terms of continuous variables. We used Duncan's test to identify different groups following the analysis of variance. We separately calculated the Pearson correlation coefficients for the groups to determine the relationship between these variables.

We used SPSS for Windows 24.0 (SPSS Inc., Chicago, IL, USA) for the analyses. We presented the data as mean, standard deviation, and ratio and considered them statistically significant when the P value was less than 0.05.

3. Results

In our study, we found apelin values to be 0.233±0.027 ng/dl in the control group, 0.304±0.093 ng/dl in the nonobese PCOS patient group, and 0.311±0.042 ng/dl in the obese PCOS patient group. Apelin values were statistically significantly lower in the control group (p=0.0001). Chemerin levels, one of the main subjects of our study, were 232.79±221.87 ng/dl in the control group, 313.57±379.04 ng/dl in the nonobese PCOS patient group, and 344.31±386.23 ng/dl in the obese PCOS patient group. These differences between chemerin values were statistically significant (p=0.0001). Nesfatin values, another central theme of our study, were 2.11±1.27 ng/dl in the control group, 0.85±0.91 ng/dl in the nonobese PCOS patient

group, and 0.74±1.42 ng/dl in the obese PCOS patient group. These results were also statistically significant (p=0.0001). In our study, MS rates, one of the main lines of our research, were 0% in the control group, 10% in the nonobese PCOS patient group, and 17.5% in the obese PCOS patient group. This difference between the groups was statistically significant (p=0.001). We calculated the HOMA-IR values of the groups as 1.79±0.68 in the control group, 3.61±5.45 in the nonobese PCOS patient group, and 4.58±4.39 in the obese PCOS patient group, and the difference between these values was statistically significant (p=0.009) (Table 1).

Table 1. Demographic characteristics, hormonal and biochemical parameters in Control, Nonobese PCOS, and Obese PCOS patient groups

	Control n:40	Non-obese PCOS n:40	Obese PCOS n:40	P
Age (years)	27.5±7.84	24.63±5.04	26.75±5.35	0.104
BMI (kg/m^2)	23.33±2.62	22.4±2.51	31.74±2.06	0.001*
FGS	1.63±2.25	10.3±6.73	12±6.50	0.001*
Waist circumference (cm)	69.38±8.74	72.73±10.44	104.78±13.51	0.0001*
MS	0 (%0)	4 (%10)	7 (%17.5)	0.001*
FBG (mg/dl)	87.30±12	91.95±12.95	98.23±12.9	0.001*
Fasting insulin (mU/ml)	9.13±2.88	16.15±20.11	20.13±18.1	0.008*
Systolic BP (mmHg)	119±22	118±18	123±23	0.089
Diastolic BP (mmHg)	72±13	76±14	82±13	0.066
HOMA-IR	1.79±0.68	3.61±5.45	4.58±4.39	0.009*
HDL (mg/dl)	44.33±5.63	52.73±13.59	42.95±9.81	0.001*
LDL (mg/dl)	89.4±27.89	101.08±27.89	97.78±22.22	0.147
Cholesterol (mg/dl)	155.78±28.1	173.03±39.6	171.43±28.83	0.036*
Triglyceride (mg/dl)	96.85±38.02	96.25±53.54	143.15±113.21	0.008*
FSH (mIU/ml)	4.98±1.11	4.2±1.58	4.62±1.42	0.046*
LH (mIU/ml)	4.64±2.46	10.78±6.06	13.41±4.96	0.001*
Estradiol (pg/ml)	50.18±25.41	52.08±21.62	52.83±16.84	0.852
T. testosterone (ng/dl)	1.62±0.49	2.54±0.75	2.68±0.63	0.001*
SHBG (nmol/L)	99.17±30.99	39.51±39.1	24.82±10.52	0.001*
LH/FSH	0.94±0.55	2.67±1.21	3.11±1.33	0.001*
Nesfatin (ng/ml)	2.11±1.27	0.85±0.91	0.74±1.42	0.0001*
Chemerin (ng/ml)	232.79±221.87	313.57±379.04	344.31±386.23	0.0001*
Apelin (ng/ml)	0.233±0.027	0.304±0.093	0.311±0.042	0.0001*

One-way ANOVA *p<0.05, BMI: body mass index, FGS: Ferriman Galvey Score, HOMA-IR: Homeostatic Model Assessment insulin resistance, FBG: fasting blood glucose

We performed a Pearson correlation analysis between apelin, chemerin, and nesfatin values, metabolic syndrome, and insulin resistance in the obese PCOS patient group. We found a statistically significant negative correlation between apelin, chemerin, and nesfatin values and waist circumference (r=-0.398, p=0.006), (r=-0.282, p=0.039), (r=-0.368, p=0.01), (Table 2). there was also a statistically significant positive correlation between apelin, chemerin, and nesfatin values in the obese PCOS patient group (r=0.793, p<0.0001), (r=0.854, p<0.0001), (Table 2). Again, there was a statistically significant positive correlation between the chemerin and nesfatin values of the same group (r=0.596, p<0.0001) (Table 2).

Table 2. Correlation table between apelin, nesfatin, chemerin, IR, and MS criteria in the obese PCOS patient group

		Glucose	Insulin	HOMA -IR	Systolic BP	Diastolic BP	TG	HDL	BMI	WC	Apelin	Chemerin	Nesfatin
Apelin	r	-0.092	-0.050	-0.058	-0.088	-0.088	-0.155	0.016	-0.185	-0.398**	1	0.793**	0.854**
	p	0.285	0.380	0.361	0.295	0.294	0.169	0.460	0.126	0.006		0.0001	0.0001
Chemerin	r	-0.074	-0.059	-0.065	-0.082	-0.083	-0.164	0.088	-0.226	-0.282*	0.793**	1	0.596**
	p	0.325	0.359	0.344	0.308	0.306	0.156	0.295	0.080	0.039	0.0001		0.0001
Nesfatin	r	-0.105	-0.172	-0.182	-0.050	-0.050	-0.169	0.004	-0.236	-0.368**	0.854**	0.596**	1
	p	0.259	0.144	0.131	0.381	0.380	0.148	0.491	0.071	0.010	0.0001	0.0001	

WC: Waist Circumference; Pearson correlation test, **<0,01, *<0,05

There was a statistically significant negative correlation between apelin, chemerin, and nesfatin values and waist circumference in the nonobese PCOS patient group (r=-0.321, p=0.022), (r=-0.417, p=0.004), (r=-0.329, p=0.019), (Table 3). We found a positive correlation between apelin, chemerin, and nesfatin values in this group. This correlation was statistically significant (r=0.655, p<0.0001), (r=0.610, p<0.0001), (Table 3). Again, a statistically significant positive correlation was observed between chemerin and nesfatin values in the same group (r=0.546, p<0.0001) (Table 3).

Table 3. Correlation table between apelin, nesfatin, chemerin, IR, and MS criteria in the non-obese PCOS patient group

		Glucose	Insulin	HOMA -IR	Systolic BP	Diastolic BP	TG	HDL	BMI	WC	Apelin	Chemerin	Nesfatin
Apelin	r	0.035	0.009	0.015	0.000	-0.003	-0.131	-0.057	-0.187	-0.321*	1	0.655**	0.610**
	p	0.416	0.477	0.464	0.500	0.493	0.209	0.364	0.124	0.022		0.0001	0.000
Chemerin	r	0.163	-0.011	-0.011	-0.003	-0.008	-0.119	-0.204	-0.156	-0.417**	0.655**	1	0.546**
	p	0.158	0.474	0.474	0.492	0.480	0.233	0.103	0.169	0.004	0.0001		0.0001
Nesfatin	r	-0.001	0.094	0.068	0.005	-0.007	-0.034	0.008	-0.236	-0.329*	0.610**	0.546**	1
	p	0.499	0.283	0.339	0.489	0.482	0.418	0.481	0.071	0.019	0.0001	0.0001	

WC: Waist Circumference; Pearson correlation test, **<0,01, *<0,05

Our study found a statistically significant positive correlation between apelin values and diastolic blood pressure in the control group (r=0.329, p=0.019) (Table 4). In the same group, we found a negative correlation between chemerin values and TG values and a positive correlation between nesfatin values (r=-0.270, p=0.046) (r=0.864, p<0.0001) (Table 4). Again, this group had a positive correlation between nesfatin values and fasting blood glucose values. This correlation was statistically significant (r=0.265, p=0.049) (Table 4).

Table 4. Correlation table between apelin, nesfatin, chemerin, IR, and MS criteria in the control group

		Glucose	Insulin	HOMA -IR	Systolic BP	Diastolic BP	TG	HDL	BMI	WC	Apelin	Chemerin	Nesfatin
Apelin	r	0.071	-0.037	0.019	-0.007	0.329*	0.029	0.017	-0.130	-0.182	1	0.004	0.200
	p	0.333	0.410	0.454	0.482	0.019	0.428	0.459	0.212	0.131		0.491	0.108
Chemerin	r	0.229	-0.078	0.018	-0.205	0.032	-0.270*	0.197	-0.152	-0.046	0.004	1	0.864**
	p	0.078	0.316	0.456	0.102	0.421	0.046	0.112	0.175	0.390	0.491		0.0001
Nesfatin	r	0.265*	-0.048	0.055	-0.254	0.049	-0.134	0.196	-0.110	-0.076	0.200	0.864**	1
	p	0.049	0.384	0.367	0.057	0.381	0.205	0.112	0.250	0.320	0.108	0.0001	

WC: Waist Circumference; Pearson correlation test, **<0,01, *<0,05

According to the design of our study, there was a statistically significant difference between the groups' BMI, FGS, waist circumference, LH/FSH ratio, SHBG, total testosterone, LH, FSH, fasting insulin, TG, cholesterol, HDL, FBG values (p<0.05).

4. Discussion

Approximately 10-30% of PCOS patients have overt IR (12). Consistent with the literature, we found that IR was high in subjects suffering from PCOS, regardless of BMI. There is impaired glucose tolerance at a rate of 30-35% in PCOS patients (13). In our study, fasting blood glucose values were high in PCOS patient groups, which supports the literature. Also, insulin levels were higher in PCOS patient groups (12, 13).

It has been shown that the plasma concentration of apelin is two times higher in overweight individuals and five times higher in morbidly obese compared to nonobese individuals (14, 15). In our study, we found apelin values higher in patient groups which is parallel with the literature.

When we searched the literature, it was understood that there were inconsistencies between the apelin levels of the control and patient groups. In a study by Altınkaya et al., apelin levels were lower in PCOS patients compared to the control group, unlike our study (16). In some studies, no statistical difference was found between the apelin values among the groups (17). Studies on more extensive series are needed to clarify this situation. There was a statistically significant positive correlation between apelin values and diastolic blood pressure, one of the MS components. As far as we understood from the literature, we attributed this situation to the positive inotropic effect of apelin (18). Apelin values were negatively correlated with waist circumference in obese and nonobese PCOS patients. This situation contradicts the existing information in the literature when we consider the relationship between apelin and obesity. We attributed this to the limited number of cases in our study.

In conclusion, plasma apelin levels were higher in PCOS patients than in the healthy control group in our study. When we evaluate this situation in a cause-effect relationship, it may be caused by obesity, increased adiposity, changes in adipocytokine levels, impaired LH/FSH interaction, IR, hypothalamic-pituitary axis effects, or local paracrine and endocrine behaviors. Depending on the metabolic changes that occur in PCOS, it can also develop with a compensatory mechanism. Further studies are needed to elucidate this mechanism.

Contrary to our study, in some studies, nesfatin values were higher in-patient groups (19, 20). We think that this difference between various studies may be caused by different variables such as the ethnic structures, diets, and lifestyles of the study groups. This problem can be solved by conducting comprehensive studies. One study found a positive correlation between nesfatin and BMI and HOMA-IR (19). Another study conducted in the same year found a negative correlation between BMI, fasting blood sugar, insulin, HOMA-IR, and nesfatin (20). While a positive correlation was observed between nesfatin and HOMA-IR in one publication, a negative correlation was observed in another (19, 21). We found a negative correlation between nesfatin values and waist circumferences in obese and nonobese patient groups. However, no study in the literature shows a clear relationship

between waist circumference and nesfatin values. Deniz et al. found a negative correlation between BMI values and nesfatin levels (20). Based on this information, this finding is indirectly supported by the literature. Although we found a positive correlation between nesfatin and fasting blood sugar in the control group, Deniz et al. found a negative correlation between FBG and nesfatin levels (20). However, while they discovered this negative correlation in the patient group, we found a positive correlation in our study in the healthy control group. Although we encountered a similar situation when we examined the literature, this can be explained by the increase in nesfatin values, which have an antihyperglycemic effect in healthy individuals to lower blood sugar. In a study conducted by Hiroyuki S. et al., it was reported that subcutaneous nesfatin replacement could be used to treat obesity in the future (22). Considering that nearly half of PCOS patients are obese, it comes to mind that obesity and even PCOS can be prevented with possible nesfatin replacement therapy in the future. Therefore, it can be used as a new treatment method that reduces IR. It is conceivable that both ligand and receptor components of the nesfatin signaling system can be found in ovarian tissue and that this new molecule may have potential regulatory roles in physiological and pathological conditions in the ovary. The decrease in nesfatin levels in women with PCOS may play a role in developing PCOS via the hypothalamo-pituitary-gonadal axis. In order to explain this situation more clearly, studies with extensive cases are needed.

It has been reported that the blood concentration of chemerin is high in obese individuals. It has been reported that BMI, TG level and blood pressure are associated with chemerin levels, and chemerin affects the pathogenesis of DM and MS complications (23). Chemerin values detected in our study were statistically significantly higher in PCOS patient groups, similar to those of Ademoğlu et al., conducted in 2014 (24). Chemerin values were negatively correlated with TG values of the control group, while they were negatively correlated with waist circumferences of the obese and nonobese PCOS patient groups. When we searched the literature, we could not find any information about waist circumferences, while TG and chemerin were positively correlated in some studies in PCOS patient groups (24). However, since a positive correlation was found with BMI in the publications we reviewed, we think there may be an indirect positive correlation with waist circumference. The negative correlation of TG and chemerin values in healthy individuals in our study supports the literature indirectly. We found a positive correlation between chemerin and nesfatin values in the control group. We could not find any information about this correlation in the literature. The working principles of both adipokines can explain this situation. In addition, when we reviewed the literature extensively, we tried to find a study investigating the relationship between nesfatin, chemerin, and apelin in patients with PCOS. This study found a statistically significant positive correlation between nesfatin, apelin, and chemerin in the obese PCOS patient group. We hope to inspire new studies to be planned in the future and shed light on the pathophysiology of PCOS.

Blood lipid levels in women with PCOS differ from women with regular menstrual cycles. The first researchers to investigate this situation were Wild et al. (25). MS is a fatal endocrinopathy that begins with IR and combines with abdominal obesity, glucose intolerance, or systemic disorders such as DM, dyslipidemia, HT, and coronary artery disease. It has been suggested that MS patients have an increased risk of cardiovascular disease and diabetes, as well as an increased risk of PCOS, non-alcoholic fatty liver disease, gallstones, asthma, sleep disorders, and some types of cancer (26). All over the world, the nutritional habits and sedentary lifestyles brought about by modern urban life have increased the prevalence of obesity and diabetes. Parallel to this situation, an increase in obesity and DM, a significant increase was observed in the number of patients with MS (27). HOMA-IR, fasting insulin, fasting blood glucose, HDL, total cholesterol, and TG values were statistically significantly higher in the patient groups compared to all groups. The number of MS cases in the patient groups was naturally higher in the obese PCOS group. We did not find a case in the control group that met the diagnostic criteria for MS. These results support the information in the literature and show that the number of MS increases in PCOS patients (27).

In line with all this information, it is understood that PCOS patients also face a problem, such as MS, that increases mortality and morbidity. In the long run, PCOS increases the likelihood of exposure to many chronic diseases. In this context, to reduce the morbidity and mortality of patients, people suffering from PCOS should pay attention to their diet and stay away from a sedentary life. Most importantly, the etiopathophysiology of PCOS should be understood as soon as possible, and appropriate treatments should be discovered in this way.

The low levels of nesfatin in the patient groups in our study bring to mind the idea that nesfatin can be used as a drug in obesity and PCOS patients. High levels of chemerin and apelin in patient groups suggest that they play a role in the pathophysiology of insulin resistance and PCOS. We think that they cause MS's development, especially in PCOS cases. More comprehensive studies are needed to elucidate all these relationships. We found that the frequency of metabolic syndrome increased in obesity and PCOS. This situation shows us that PCOS and obesity can indirectly progress to mortality and morbidity. Initiating treatment as soon as the syndrome is detected in cases with PCOS will reduce the mortality and morbidity of women in this group due to these chronic diseases. In this context, PCOS patients should be treated conservatively, medically, and additionally with close follow-up periods.

Ethical Statement

The volunteers were informed about the study before participating, and written consent was obtained from those who agreed to take part. Ethical approval from the Ethics Committee of Yüzüncü Yıl University was received before the study, with the decision dated 30.01.2014 and numbered 05.

Conflict of interest

The authors reported no potential conflict of interest.

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Nothing to declare.

Authors' contributions

Concept: O.U., Z.K., Design: O.U., O.K., Z.K., Data Collection or Processing: O.U., O.K., Z.K., Analysis or Interpretation: O.U., O.K., Z.K., Literature Search: O.K., Z.K., Writing: O.K. Z.K.

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Research Article

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A comparison of refraction measurements obtained by plusoptix A09 and autorefractometer in adult

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Abstract

To compare the refraction values obtained with the Plusoptix A09 photorefractometer and Topcon autorefractometer in adults. 230 eyes of 115 patients who had no eye disease other than refractive error were included in the study. Refraction measurements were made with the Plusoptix A09 photorefractometer and Topcon KR-8100P autorefractometer devices. Measurements were taken three times, and the averages were recorded. Median spherical, cylindrical, spherical equivalent, and cylindrical axis measurements obtained with both devices were statistically compared. Interdevice compatibility was evaluated with intraclass correlation coefficient (ICC) and Spearman correlation analysis. The median age of 115 patients was 37 years (range, 20-67); 50 (43.5%) were female, and 65 (56.5%) were male. The median spherical value obtained with Plusoptix A09 photorefractometer 0.50 D (range, -4.00-(3.25)), median cylindrical value -0.50 D (range, -2.00-(0.00)), spherical equivalent median 0.38 D (range, -4.00- (3.00)), the J0 power median was 0 (range, -1.00-(1.00)), and the J45 power median was 0 (range, -0.49-(0.37)). The median of the spherical value obtained by autorefractometer is 0.0 D (range, -3.75-(2.00)), the median of the cylindrical value is -0.50 D (range, -2.00-(0)), the median of the spherical equivalent is -0.25 D (range, -3.75- (1.75)), the J0 power median was 0 (range, -0.87-(0.98)), and the J45 power median was 0 (range, -0.59-(0.50)). There was a significant difference between spherical, cylindrical, and spherical equivalent measurements between devices. There was no significant difference between J0 and J45 measurements. Besides being used in childhood, the Plusoptix A09 photorefractometer can also be used as a fast and easy refraction measurement method, especially in physically or mentally handicapped adults with adjustment problems.

Keywords: Plusoptix A09, photorefractometer, refractive errors, autorefractometer

1. Introduction

Refractive errors are the leading cause of correctable visual impairment worldwide (1-2). Accurate measurement and treatment of refractive errors are essential in detecting and preventing amblyopia in children and eliminating asthenopic complaints in children and adults. Autorefractometers for refraction measurements have been widely used since the 1970s. (3)

Cycloplegic retinoscopy is the gold standard for detecting refractive errors. However, the fact that it requires experience and takes a long time limits its use (4). Measurement with photorefraction is a method developed for screening purposes; it is especially used to detect refractive errors in children and mentally and physically handicapped people. It has conveniences such as simultaneously taking measurements in both eyes and not having to contact the patient's head anywhere (5-7).

Plusoptix A09 photorefractor (Plusoptix GmbH, Nuernberg, Germany) is a non-invasive measurement instrument developed for children that measures rapid refraction from both eyes, pupil diameter, and interpupillary distance (8).

This study aimed to compare the measurements made with Plusoptix A09 in the adult population with the measurements made with the autorefractometer (Topcon desktop autorefractometer).

2. Materials and Methods

This prospective study was conducted at Hitit University's Erol Olçok training and research hospital in accordance with the Helsinki Declaration after obtaining written consent from patients and approval from the local ethics committee (2022-82). The study included 230 eyes from 115 patients who came to the department of ophthalmology for a refractive error examination.

Those who had ophthalmic surgery, ocular trauma, strabismus, nystagmus, cataract, corneal and retinal disorders were excluded from the study. Patients with systemic disease (excluding hypertension), using topical drugs, wearing contact lenses, or having refraction outside the measurement ranges of

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the Plusoptix A09 device (-7 D / +5 D) were not included.

Measurements were taken with the Plusoptix A09 photorefractometer (Plusoptix GmbH, Nuernberg, Germany) device in a dark room approximately 1 meter from the patient. There is a smiley face on the camera on the device, and after pressing the start button, the smiley face automatically lights up and makes sounds that will attract the patient's attention. The device is moved back and forth until green circles around the pupils appear on the screen. Results are seen on the monitor and saved. This device can take measurements between -7.0 D and +5.0 D spherical and cylindrical values in 0.25 Diopter (D) increments. If the spherical equivalent is outside this range, the measured value is only displayed as 'Hypermetropia' or 'Myopia.'

All patients' refraction measurements were taken first with the Topcon KR-8100P autorefractometer (Topcon Corporation, Tokyo, Japan) and then with the Plusoptix A09 photorefractometer. The measurements were taken three times, and the averages were recorded. All patients underwent a detailed eye examination, including anterior and posterior segments. All measurements and examinations were performed by the same ophthalmologist (MD).

Median spherical, cylindrical, and spherical equivalent measurements obtained with both devices were recorded. Cylindrical axis measurements were statistically compared as Jackson cross-cylinder power values (J0 and J45). Spherical equivalent (SE)= Spherical + Cylinder/2 was calculated. J0= [(-Cylinder/2)* cos(2*axis)] for Jackson cross cylinder 0^0 and 45^0 axes, respectively; It was calculated using the formulas J45= [(-Cylinder/2)* sin(2*axis)].

2.1. Statistical analysis

In the data evaluation, compliance with the normal distribution was examined with the Kolmogorov-Smirnov test. Wilcoxon test was used to compare the measurement values according to the devices. Spearman correlation test and intraclass correlation coefficient (ICC) were used to evaluate the agreement between the measurements of the devices. For statistical significance, $p<0.05$ was considered significant. IBM SPSS V22 package program was used in all statistical analyzes.

3. Results

The median age of 115 patients included in the study was 37 years (range, 20-67); 50 (43.5%) were female, and 65 (56.5%) were male. The median spherical value obtained with Plusoptix A09 photorefractometer 0.50 D [range: -4.00-(3.25)], median cylindrical value -0.50 D [range: -2.00-(0.00)], SE median 0.38 D [range: -4.00- (3.00)], J0 power median was 0 [range: -1.00-(1.00)] and J45 power median was 0 [range: -0.49-(0.37)]. The median spherical value obtained with Topcon autorefractometer 0 D [range: -3.75-(2.00)], median cylindrical value -0.50 D [range: -2.00-(0.00)], SE median -0.25 D [range: -3.75- (1.75)], the J0 power median was 0 [range: -0.87-(0.98)] and the J45 power median was 0 [range: -0.59-(0.50)].

Table 1. Comprasion of measurements obtained with Topcon autorefractometer and Plusoptix A09 photorefractometer

	Autorefractometer			Plusoptix A09			
	median	min	max	median	min	max	p
S (D)	0.00	-3.75	2.00	0.50	-4.00	3.25	**<0.01**
C (D)	-0.50	-2.00	0.00	-0.50	-2.00	0.00	**<0.01**
SE (D)	-0.25	-3.75	1.75	0.38	-4.00	3.00	**<0.01**
J0	0.00	-0.87	0.98	0.00	-1.00	1.00	0.05
J45	0.00	-0.59	0.50	0.00	-0.49	0.37	0.58

S: spherical, C: cylindrical, SE: sferical equivalent, J0: Jackson cross-cylinder power at 0º , J45: Jackson cross-cylinder power at 45º , D: diopter, min: minimum, max: maximum

There was a statistically significant difference in spherical, cylindrical, and spherical equivalent measurements between the two devices ($p<0.05$). There was no significant difference in J0 and J45 power measurements (respectively, $p=0.05$, $p=0.58$) (Table 1).

Table 2. Comparison of the measurements of the two devices with the Spearman correlation and Intraclass correlation coefficients (ICC) of the measurements between the devices

Autorefractometer- Plusoptix	ρ	ICC	95% CI
S (D)	0.769	0.921	0.898 to 0.939
C (D)	0.716	0.862	0.821 to 0.894
SE (D)	0.777	0.928	0.906 to 0.944
J0	0.876	0.939	0.921 to 0.953
J45	0.732	0.862	0.822 to 0.894

S: spherical, C: cylindrical, SE: spherical equivalent, J0: Jackson cross-cylinder power at 0º, J45: Jackson cross-cylinder power at 45º, ρ: Spearman correlation coefficient, ICC: Intraclass corelation coefficient, 95% CI: 95 % confidence interval

Table 2 shows the correlation of measurements between devices. Spearman correlation coefficient values between autorefractometer and Plusoptix A09 for spherical, cylindrical, SE, J0, and J45 power values are positive and significant (respectively, ρ=0.769, ρ=0.716, ρ=0.777, ρ=0.876, ρ=0.732, for all p<0.001) relationship was found. Intraclass correlation coefficients (ICC) values were spherical, cylindrical, spherical equivalent, J0, and J45 power values (respectively, 0.921, 0.862, 0.928, 0.939, 0.862, for all p<0.001). A strong positive correlation existed between all measurements made with the autorefractometer and Plusoptix A09. Figure 1 shows the compatibility of both devices with the Blant Altman graph (Fig. 1).

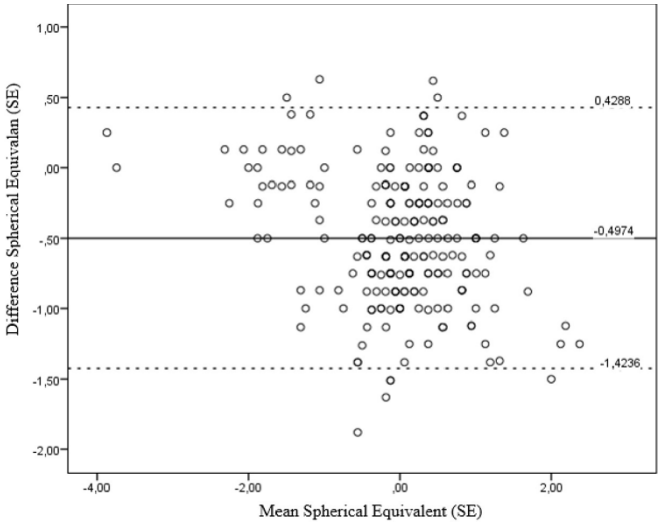

Fig. 1. Bland–Altman plots illustrating the differences and means of SE values obtained with the autorefractometer and Plusoptix photorefractometer

4. Discussion

In this study, we compared Plusoptix A09 and Topcon autorefractometer measurements in adults; Although there was a significant difference between spherical, cylindrical, and SE measurements, there was no significant difference between J0 and J45 power values. It was determined that the Plusoptix A09 device measured the median spherical and SE values more hyperopia than the autorefractometer.

Allen et al., in their study on 50 adults aged 16-61 years, found that the values measured by photorefractometer (Powerrefraktor; Plusoptix, Hillsboro Beach, FL) were 0.32 D more hyperopic than the values measured by Nidek autorefractometer (Nidek AR600-A, Nidek, Japan) (7). Demirel et al., in their study of 127 adults with a mean age of 33.3 years and 110 children with a mean age of 8.06 years, found the measurements taken with Plusoptix S08 0.25 D more myopic than the measurements taken with the Topcon autorefractometer. In addition, they showed that adults had an average of 0.50 D more hyperopia (8). Abrahamsson et al. measured 150 children between 6 months and five years with a photorefractometer (Powerrefractor, Reutlingen, Germany) and autorefractometer (Topcon, RM A2000, Mondal, Sweden). As a result, they found that the photorefractometer measures 0.42 D more hyperopia (6). Arıcı et al., in their study of 21 children with an average age of 9.95 years and 24 adults with an average age of 23.46 years, took the photorefractometer Plusoptix S08 and autorefractometer (Potec PRK-6000, Daejeon, Korea) measurements. And mean spherical values of 0.49 D in children and 0.63 D in adults were found to be hyperopic (9). In their study, Acar et al. took measurements with Plusoptix A09 and an autorefractometer, which included 272 adults with a mean age of 38.85 years. They found the measurements taken by the photorefractometer to be 0.72 D more hyperopic (10).

In our study, similar to the above studies, we found that the median spherical value was 0.54 D, and the median SE was 0.63 D more hyperopic measurement in adults in the measurements taken with Plusoptix A09. This was attributed to the fact that the photorefractometer stimulates accommodation less and is less affected by accommodation (11,12).

In their studies on cylindrical values, Arıcı et al. measured the photorefractometer Plusoptix S08 and autorefractometer (Potec PRK-6000, Daejeon, Korea) in their study. They found no significant difference between the two groups in terms of cylindrical values and cylindrical axis (9). Güler et al. showed no significant difference between cylindrical and cylindrical axis values in a study in which they compared 25 adults with a mean age of 30.01 years and 25 pediatric patients with a mean age of 11.08 years (12). In their study with 64 patients aged 2-19 years, Kıyak Yılmaz et al. showed no difference between cylindrical power and cylindrical axis measurements, similar to other studies (13). Anayol et al. found no difference between the groups in terms of cylindrical power in measurements taken with a photorefractometer and autorefractometer. Still, they found a statistically significant difference in Jackson cross-cylinder measurements at 0-degree axis (14).

In our study, unlike the studies above, there was a significant difference between the two devices in terms of cylindrical values (p<0.01). However, there was no difference between the median values. Cylindrical axis measurements were evaluated by converting them to J0 and J45 power values, and there was no significant difference between the two devices (p=0.05, p=0.58, respectively).

Photorefraction measurements have advantages such as being used in infants, children, and maladjusted patients, lack of physical contact, and fast and binocular measurement. In addition, measurements cannot be taken with the Plusoptix A09 device in cases where the pupil diameter is 3 mm below and 8 mm above and when the refraction is outside the limits of -7.0 D and +5.0 D.

One of the limitations of our study is the inability to compare the gold standard cycloplegic refraction measurements. However, the fact that Plusoptix A09 could not measure very small and very large pupils led us to obtain measurements without cycloplegia. Secondly, since healthy

adult individuals were evaluated in our study, we do not know how effective it is in adults with physical or mental disabilities.

As a result, although there was a significant difference between spherical, cylindrical, and SE measurements in the measurements made with both devices, it was observed that there was a strong positive correlation between the measurements. Considering that it measures more hyperopia, the Plusoptix A09 photorefractometer can be used as a fast and easy-to-apply refraction measurement method in adults with compliance problems, physically or mentally handicapped, as well as in children.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: M.D., K.Y., E.K., Design: M.D., K.Y., E.K., Data Collection or Processing: M.D., E.K., Analysis or Interpretation: M.D., K.Y., Literature Search: M.D., E.K., Writing: M.D., Y.K.

Ethical Statement

Approval was obtained from Hitit University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 14/09/2022 and the number of ethical committee decisions is 2022-82.

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Research Article

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Concurrent expression of immunhistochemical parameters in breast cancer patients; clinical implications and consistency with Bloom-Richardson system

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Abstract

We sought to determine prognostic importance of expression p53, c-erbB-2 (also known HER2/neu or HER2), estrogen receptor and progesterone receptor in breast cancer patients by investigating their relationship with histopathological and clinical parameters. We also investigated whether different parameters other than Bloom-Richardson grading system might be used in classification of breast cancer patients on the basis of concurrent expression of immunhistochemical parameters. Seventy-one invasive ductal carcinoma patients were included. We studied immunhistochemical parameters including, estrogen receptor, progesteron receptor, p53 and c-erbB-2. Specimens that were archived in pathology department were re-assessed to determine necrosis, lymph and blood vessel invasion, perineural invasion, peritumoral inflamatory reaction characteristics. Bloom-Richardson grading system was applied for each specimen. Multivariate discriminant analysis was performed to test the relationship between Bloom-Richardson system and immunhistochemical parameters. Mean age was 50.79 ± 11.92. Forty-eight patients (67.6%) were estrogen receptor positive, 34 (47.9%) were progesterone receptor positive, 38 (53.5%) were p53 positive and 46 (64.8%) were c-erbB-2 positive. Necrosis was less common and peritumoral inflammatory reaction was more common among estrogen receptor positive patients. According to the discriminant analysis, 52.1% of patients with concurrent expression of ER, PR, p53 and c-erbB-2 were correctly classified according to overall Bloom-Richardson grade, 49.3% were correctly classified according to nuclear pleomorphism score and 77.5% were correctly classified according to mitotic count. Prognostic classification of patients could be done on the basis of mitotic characteristics of the tumor. Further study is warranted to establish the standard threshold for mitotic count for breast tumors of different types.

Keywords: breast cancer, immunhistochemical markers, Bloom-Richardson grading system, mitotic caunt

1. Introduction

Breast cancer is the most common cancer among women worldwide and the second most common cause of cancer-related deaths, following lung cancer. About 1.000.000 new cases are detected per year. Although widespread mammography usage enabled the early detection of most lesions, no significant decrease in mortality has been achieved (1). A precise determination of populations at risk for developing breast cancer is almost impossible since the disease has multifactorial pathogenesis (2, 3). Status of hormone receptors, proliferative activity, inactivation of tumor suppressor genes, and overexpression of oncogenes are the prognostic factors that are interrelated with each other (4, 5) in disease pathogenesis.

In this study, we sought to determine the prognostic importance of expressions of tumor suppressor gene p53, a protooncogene c-erbB-2, and the status of estrogen and progesterone receptors in breast cancer patients by investigating their relationship with histopathological and clinical parameters. We also investigated whether different parameters other than the Bloom-Richardson grading system might be used to classify breast cancer patients based on the concurrent expression of immunohistochemical parameters.

2. Materials and Methods

2.1. Study design and patients

A retrospective archive study was performed in the radiation oncology and pathology departments of Ondokuz Mayıs University Hospital. Patients who received medical care in the radiation oncology department for the treatment of breast carcinoma between 1999 and 2003 were detected. Of these, 71 patients who were diagnosed, based on a histopathologic study, with invasive ductal carcinoma and those in whom we studied immunohistochemical parameters including estrogen receptor (ER), progesterone receptor (PR), p53 and c-erbB-2 were considered eligible for the study. Patient demographics, clinical data, preoperative mammographs, and TNM stages were recorded for analysis. Hemotoxyilen-eosin stained specimens that were stored in the pathology archive were re-assessed by the investigators to determine necrosis, lymph and blood vessel invasion, perineural invasion, and peritumoral inflammatory reaction characteristics. Bloom-Richardson grading system was applied for each specimen. All histomorphological assessments were performed using a Leica

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HMLB45 microscope. The microscope had an area diameter of 0.50 mm. Scoring for the mitotic count was as follows; 0-7 mitoses: 1 point, 8-14 mitoses: 2 points, 15 and more mitoses: 3 points. The number of metastatic lymph nodes, tumor diameter in metastatic nodes, the status of perinodal infiltration, and preoperative mammographs were used to perform TNM staging.

2.2. Re-assessment of specimens

The archived specimens were re-assessed for ER, PR, p53, and c-erbB-2 independently from pathology reports. The percentage of expressing cells for ER, PR, and p53 was determined by counting 300 tumor cells under x40 magnification. Positive staining <10% was considered as (-) staining, and >10% was considered as (+) staining. C-erbB-2 positivity was defined as membrane staining. According to the staining pattern, when no staining was observed, or membrane staining was present in <10% of tumor cells, it was defined as (-); when there was faint membrane staining in >10% of tumor cells and only part of the membrane was stained, it was defined as (+); when there was weak to moderate complete membrane staining in >10% of tumor cells, it was defined as (++); when there was strong complete membrane staining in 10% of tumor cells, it was defined as (+++). These categories were dichotomized as follows; (-) and (+) cases were defined as (-), and (++) and (+++) cases were defined as (+).

2.3. Statistical analysis

All statistical analyses were performed using SPSS (SPSS, Inc. Chicago, IL. USA) packaged software. Visual histograms and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) were used to determine normal distribution. Continuous variables were defined by the mean ± standard deviations. ROC analysis was used for testing the sensitivity and specificity of the 10% threshold for the expression of immunohistochemical parameters. The chi-square test was used to test the relationship between immunohistochemical parameters and menopause status, tumor size, lymph node involvement, grade, staging systems, and histological features. Pearson correlation was used to test the relationship between age and immunohistochemical parameters. A p-value less than 0.05 was considered as statistical significance. Multivariate discriminant analysis was performed to test the relationship between the Bloom-Richardson system and immunohistochemical parameters. Cases were divided into two categories, whether the mitotic count was less or greater than 13 mitoses per 10 high-power fields.

3. Results

The mean age was 50.79 ± 11.92 (ranging from 27 to 76). Forty-eight patients (67.6%) were ER positive, 34 patients (47.9%) were PR positive, 38 patients (53.5%) were p53 positive, and 46 patients (64.8%) were c-erbB-2 positive. Out of 71 patients, 61 underwent modified radical mastectomy (MRM) + axillary lymph node dissection (ALND), 5 underwent simple mastectomy + axillary sampling, 3 underwent quadrantectomy + axillary sampling, and 2 patients underwent lumpectomy + axillary sampling. Breast cancer is located on the left side in 36 patients (51%) and on the right side in the remaining. There was no significant correlation between age and expression of ER (r=0.082, p=0.497), PR (r=-0.099, p=0.413), p53 (r=-0.14, p= 0.245) ve c-erbB-2 (r=-0.210, p=0.079).

Table 1. Distribution of menopause status, tumor size groups, axillary lymph node metastasis and tumor characteristics among cases with positive expression of imunohistochemistry parameters.

Variable	ER positive (n=48/71)	PR positive (n=34/71)	p53 positive (n=38/71)	c-erbB-2 positive (n=46/71)
Menopause status				
Premenopause (n=29)	21 (72)	18 (62)	16 (55)	20 (69)
Postmenopause (n=42)	27 (64)	16 (38)	22 (52)	26 (62)
P value	0.607	0.057	1.000	0.618
Tumor size				
<2 cm (n=9)	7 (78)	4 (44)	4 (44)	5 (56)
2-5cm (n=46)	32 (70)	23 (50)	24 (52)	28 (61)
>5 cm (n=16)	9 (56)	7 (44)	10 (63)	13 (81)
P value	0.485	0.889	0.654	0.280
Axillary lymph node metastasis				
0 (n=26)	14 (54)	9 (35)	13 (50)	16 (62)
1-3 (n=22)	18 (82)	14 (64)	13 (59)	14 (64)
4-9 (n=16)	12 (75)	9 (56)	9 (56)	11 (69)
10 ve ↑(n=7)	4 (57)	2 (29)	3 (43)	5 (71)
Perineural invasion (12/71)				
Yes	9 (75)	5 (42)	9 (75)	6 (50)
No	3 (25)	7 (48)	2 (25)	6 (50)
P value	0.739	0.756	0.123	0.322
Blood vessel invasion (5/71)				
Yes	5 (100)	3 (60)	1 (20)	3 (60)
No	0 (0)	2 (40)	4 (80)	2 (40)
P value	0.167	0.665	0.176	1.000
Lymphatic invasion (11/71)				
Yes	9 (82)	7 (64)	7 (64)	7 (64)
No	2 (18)	4 (36)	4 (36)	4 (36)

P value	0.484	0.332	0.527	1.000
Necrosis (20/71)				
Yes	7 (35)	7 (35)	11 (55)	13 (65)
No	13 (65)	13 (65)	9 (45)	7 (35)
P value	**0.001**	0.197	1.000	1.000
Peritumoral inflammatory reaction (40/71)				
Yes	23 (58)	16 (40)	23 (58)	30 (75)
No	17 (42)	24 (60)	17 (42)	10 (25)
P value	**0.045**	0.156	0.480	0.049

Menopause status, increasing tumor size and the number of axillary lymph node metastases, perineural invasion, blood vessel invasion, lymphatic invasion, necrosis, and peritumoral inflammatory reactions were not significantly associated with expression of any markers, except necrosis was less common, and the inflammatory peritumoral response was more common among ER positive patients (Table 1).

Table 2. Distribution of Bloom Richardson grades among cases with positive expression of imunhistochemistry parameters

Bloom Richardson Grading System	ER positive (n=48/71)	PR positive (n=34/71)	p53 positive (n=38/71)	c-erbB-2 positive(n=46/71)
Grades				
I (n=11)	43.7 ± 27%	20.4 ± 74%	10.6 ± 21%	26.3 ± 30%
II (n=45)	47.6 ± 34%	29.1 ± 32%	23.2 ± 29%	47.4 ± 38%
III (n=15)	10.67 ± 25%	10.1 ± 22%	31.2 ± 36%	73.3 ± 25%
p values	0.001	0.094	0.227	p=0.004

According to the Bloom-Richardson grading, 11 patients (16%) were grade 1, 45 patients (63%) were grade 2, and 15 patients (21%) were grade 3. The rate of ER expression was significantly decreasing (p=0.001), and c-erbB-2 expression was significantly increasing with increasing tumor grade (p=0.030). The distribution of expression percentages among three different grades is given in table 2. According to the discriminant analysis, 52.1% of patients with concurrent expression of ER, PR, p53, and c-erbB-2 were correctly classified according to overall Bloom-Richardson grade (table 3), 49.3% were correctly classified according to nuclear pleomorphism score, and 77.5% were correctly classified according to mitotic count where cut-off value for the mitotic count was taken as 13 mitotic figures seen in 10 high power fields (table 4).

Table 3. Assessment of Bloom Richardson grade and discriminant grade

Bloom-Richard Grade	Discriminant grade			Total
	Grade I	Grade II	Grade III	
Grade I	**8 (72.7%)**	1 (9.1%)	2 (18.2%)	11
Grade II	18 (40%)	**18 (40%)**	9 (20%)	45
Grade III	2 (13.3%)	2 (13.3%)	**11 (73.4%)**	15

According to the discriminant analysis, 52.1% of patients with concurrent expression of ER, PR, p53 and c-erbB-2 were correctly classified according to overall Bloom-Richardson grade

Table 4. Assessment of mitotic count and discriminant grade

Mitotic grade	Discriminant grade		Total
	13 and lower	14 and higher	
13 and lower	**36 (75%)**	12 (25%)	48
14 and higher	4 (17%)	**19 (83%)**	23

According to the discriminant analysis, 77.5% of patients with concurrent expression of ER, PR, p53 and c-erbB-2 were correctly classified according to mitotic count grade

4. Discussion

Most studies investigated the relationship between clinical prognosis and expression of immunohistochemistry and prognosis markers, including tumor grade, DNA ploidy, S-phase analysis, and microscopic microvessel intensity, in order to explain and predict the clinical progress of breast cancer (5-8). According to the American Pathologist Consensus Statement in 1999, tumor size, histological grade, histological type, and hormone receptor status were considered the most useful categories in the clinical progress and management of breast cancer patients (9).

In studies where prognostic and survival effects of ER and PR status were investigated, it was suggested that the status of these hormone receptors was not adequate alone to predict the prognosis and early relapse and determine which patients would benefit from endocrine therapy (10). Chia et al. (9) found in their 10-year follow-up study of 1187 patients with non-metastatic breast cancer that 5-year survival was better in patients with ER expression and those with unknown receptor status than in those without ER expression, whereas 10-year survival was similar between groups. The incidence of ER expression was reported to range from 55 to 72%, whereas the

incidence of PR expression was reported to range from 33 to 70% (11). Our findings regarding ER (67.6%) and PR (47.6%) expressions seem compatible with those previously reported.

Overexpression of p53 and c-erbB-2 are known to be associated with the aggressive clinical course (2, 7, 12-17). Gretarsdottir et al. (13) found in their series of 193 breast cancer patients that patients with p53 expression tended to have a slightly worse prognosis, and these tumors were more resistant to therapy than those without p53 expression. In immunohistochemical studies, it was found that 16 to 58% of breast tumors had positive p53 expression (13, 18), whereas other studies reported different rates for positive p53 expression (5-7). Characteristics of the study group, type of antibody used, and subjective differences in assessment of positivity were reported as the potential causes of the inconsistency in results that were reported from different studies (19). Our finding that 53.5% of patients had p53 expression seems compatible with those previously reported.

Samur et al. (16) were the first who describe that c-erbB-2 expression was a poor prognostic factor for survival. Studies have also shown that c-erbB-2 overexpression was associated with an increased mortality risk (7). A scale that ranged between negative and 3+ (excessively positive) has been described for c-erbB-2 immunohistochemical staining (16). Excessive expression was reported to be ranging from 10-30% (2-4, 7, 14, and 16). Compatible with these findings, in our study, c-erbB-2 expression was found at 64.8%, and excessive expression was found at 50.7%.

The relationship between patient age and menopausal status and immunohistochemistry markers have been evaluated in various studies. Zavagno et al. (20) found in their series of 1226 breast cancer patients that tumors of patients under 40 years of age had more aggressive behavior than those of patients over 75 years of age. Rodrigues et al. (15) reported that younger patients had higher ER, PR, and p53 expressions, whereas there was no significant association between age and c-erbB-2 expression. Samur et al. (16) reported in a series of 169 patients that there was no significant association between age and c-erbB-2 expression. We found no significant correlation between age and expression of any immunohistochemistry marker.

Mc Guive et al. (22) found that ER expression was associated with a longer duration of disease-free survival in stage II postmenopausal patients. In general, ER expression was reported to be lower in premenopausal women (1). Samur et al. (16) found no significant association between menopause status and c-erbB-2 expression. We found no significant relationship between menopause status and the expression of any immunohistochemistry marker.

Most studies reported that tumor size was an important prognostic factor in patients without lymph node metastasis (9). Chia et al. (9) reported that tumor size and grade might be important determinants for predicting the clinical course and individualizing the therapy. One study of 767 patients who did not have lymph node metastasis and were not receiving systemic adjuvant chemotherapy reported that 27% of patients with tumors >10 cm had disease relapse. We found no significant correlation between tumor size and the expression of any of the immunohistochemistry markers. This may be due to differences in patient populations.

Patients who do not have axillary lymph node metastasis have a better prognosis, and lymph node status has been suggested to be the most important factor in predicting disease-free survival (5, 14, and 21). Although ER, PR, p53, and c-erbB-2 expressions have been considered as important predictors of outcome, their significance is controversial in patients who do not have lymph node metastasis. Reed et al. (14) reported that p53 and c-erbB-2 expressions were associated with worse prognosis in this group of patients. So et al. (21) found that the expression of p53 and c-erbB-2 did not have prognostic value in this group of patients.

Axillary lymph node status and immunohistochemical markers expression relation were also evaluated in various studies. Gretarsdottir et al. (13) found no difference between patients with and without lymph node metastasis regarding the presence of the p53 mutation. Ilhan et al. (23) reported that they couldn't find an association between nodal involvement and molecular subtypes of breast cancer. Consistent with those findings, we found no significant association between ER, PR, or p53 expression and axillary lymph node status.

In a series of 1500 women, Jafarimojarrad et al. (24) evaluated various conventional prognostic factors, including; ER, PR, p53, Cathepsin D, c-erbB-2, bcl-2, Ki-67, and p21 expression and they also assessed invasion parameters including; perineural, blood vessel and lymphatic invasion. Among these parameters, the only significant association was between ER expression and the presence of perineural invasion, whereas blood vessel invasion and lymphatic invasion were not associated with any conventional parameters. This is an interesting finding since ER expression has not been known to be associated with worse prognostic features. We found no significant association between perineural invasion and any immunohistochemistry markers. Also, consistent with these findings, we found no significant association between blood vessel or lymphatic invasion and ER, PR p53, or c-erbB-2 expression.

Some studies reported that increasing peritumoral inflammatory cell infiltration was associated with decreasing hormone receptor expression (25, 11). Another study reported that c-erbB-2 overexpression was also associated with this feature. We found that both ER expression and c-erbB-2 expression were significantly associated with peritumoral inflammatory cell infiltration. Fisher et al. (26) reported that ER expression was higher in tumors without necrosis. We found a significant association between ER expression and

tumor necrosis, whereas there was no significant association between tumor necrosis and expression of PR, p53, or c-erbB-2.

Bloom-Richardson grading is the most commonly used histologic scale worldwide (3, 25). Inter-observer variability is an important problem in applying this system. Technical factors, such as the fixation method, also influence its reliability (11, 25). It may also be related to the fact that tubal formation and nuclear properties are subjective features that are used in grading tumors. Another important point is that this system classifies most patients in grade II (25, 27). This large category is also poorly reproducible (27).

Reed et al. (14) reported in their study of 613 patients that histological tumor grade and tumor diameter were the most important prognostic factors in patients without lymph node metastasis. They found that increasing tumor grade was associated with decreasing ER and PR expressions and increasing p53 and c-erbB-2 expressions. They also reported that they obtained better prognostic groups when they stratified the histological groups as grade I, II, or III in survival analysis. In our study majority of patients were grade II (63%). We sought to determine whether the accumulation in group II was about the biological properties of the tumor or whether it was due to the incapability of the grading system to discriminate the cases truthfully. Therefore we performed a discriminant analysis to achieve a more sophisticated prediction of tumor grade based on the concurrent expression of ER, PR p53, and c-erbB2. We found that Blood-Richardson grade II showed the lowest consistency (40%) with discriminant classification. This finding supports the fact that various prognostic factors are incompatible with each other in Bloom-Richardson grade II cases.

The discriminant analysis we performed based on concurrent expression of ER, PR, p53, and c-erbB2 revealed that discriminant classes showed 52.1% compatibility with Bloom-Richardson grades. When we performed the analysis based on nuclear scores, we found that the compatibility was lower (49.3%).

Mitotic count is an important feature in the Bloom-Richardson scoring system (28). Chang et al. reported that this is a good single parameter for survival prediction (29). Buhmeida et al. (30) reported that the mean cut-off values of the mitotic activity index (mitotic figures/10 hpf) and the standardized mitotic index (mitotic figures/mm²) could be applied to distinguish breast cancer patients into groups with favorable and less favorable prognosis. Parham et al. found that grading or indexing by the presence or absence of mitotic activity or necrosis better predicted the clinical course than the Bloom-Richardson system (25). Baak et al. found that mitotic activity was a strong prognostic factor in patients without lymph node metastasis when the cut-off level for the mitotic activity index was set at 10 (25). Our study performed a discriminant analysis based on the concurrent expression of ER, PR p53, and c-erbB2, with a cut-off level for the mitotic count set at 13. We found that discriminant classes were 77.5% compatible with Bloom-Richardson grades.

Since concurrent expression of ER, PR p53, and c-erbB2 was highly compatible with grading by mitotic count, we suggest that prognostic classification of patients could be done based on the mitotic characteristics of the tumor. Further study may be justified to establish the standard threshold for the mitotic count for breast tumors of different types that allows performing a dichotomous classification

Conflict of interest

The authors declared no conflict of interest.

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The study was conducted at Ondokuz Mayıs University department of pathology

Authors' contributions

Concept: D.Y., F.K., Design: D.Y., F.K., Data Collection or Processing: D.Y., F.K., Analysis or Interpretation: D.Y., F.K., Literature Search: D.Y., Writing: D.Y.

Ethical Statement

Ethics committee approval is not required for this study.

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Evaluation of gender awareness among medical students in İstanbul

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Abstract

Our study aimed to evaluate the gender awareness in medicine of students in a medical school in Istanbul. The population of this descriptive study consists of 1523 medical students studying at a state university in Istanbul in the 2020-2021 academic year. The study took place between February and March of 2021. The study was conducted with 484 participants. The data were collected using a questionnaire for sociodemographic features and the Nijmegen Gender Awareness in Medicine Scale (N-GAMS) filled out by the participants. Statistically, $p<0.05$ was considered as the level of significance. Ethics committee approval was obtained from the relevant institution before the study.Most of the participants were female (n=319, 65.9%) and the participants' mean age was 21.8±2.1 years. Multivariate tests showed differences in gender role ideology towards patients and doctors according to the gender of the participants and the working situation of their mothers. Also, significant sex-related differences were found in gender sensitivity. As compared to others, male students or those whose mothers were unemployed held slightly more gender stereotypes towards patients and doctors.As shown in our results, the participants were stereotyped in gender role ideology and suboptimal gender sensitivity. Gender stereotypes can be prevented through gender-sensitive medical education. More studies, particularly qualitative studies, are needed on this subject to examine the students' gender awareness in medicine and perception levels related to their sociodemographic characteristics in more detail and to determine the measures that can be taken to solve the problem.

Keywords: gender awareness, gender sensibility, medical students, gender bias

1. Introduction

Biological sex explains the origin of men and women's different behaviors, with their biological characteristics determined by different chromosomes, reproductive organs, and hormones (1). The concept of gender refers to the socially and culturally constructed differences between women and men. Gender and role behaviors are learned and reinforced according to the cultural norms and values of the society. Thus, different social responsibilities are attributed to men and women in society (2). Gender awareness in medicine means that physicians have the knowledge and skills to recognize gender as a primary determinant of health and disease and to include it in their daily practice (3). Therefore, gender awareness targets better health conditions for men and women. Gender bias is widespread in medicine and healthcare and is one of the main drivers of health-related inequalities. Lack of gender awareness among physicians can lead to two fundamentally different gender biases. The first is the gender stereotype, the difference in the treatment applied without clinical justification by considering the gender difference between the patients (4-9). As an example of a common stereotype, physicians are more likely to interpret the symptoms of male patients organically and the symptoms of female patients psychosocially, and female patients are considered for less referral or further investigation than men with similar symptoms, and this raises questions about unequal care (10-13). The second gender bias, called gender blindness, is doctors' clinical failure to recognize or overlook differences between men and women. Male participants are predominantly represented in the treatment studies of many diseases, especially in the treatment studies of cardiovascular diseases, and the data obtained, and the treatment methods are also applied to women (14). It is necessary to know the approach and point of view of medical students toward this frequently encountered attitude. Studies point to the need for gender perspectives in medical education to determine the most accurate healthcare services for both men and women and to increase gender awareness among future doctors. For this purpose, raising gender awareness among medical students before graduation will not only break gender biases, but will also provide patient care above standards (3,8,15).

Our study aimed to evaluate the gender awareness in medicine among medical students in Istanbul using a valid and reliable scale in Turkish. At the end of our study, we aim to increase gender awareness in medicine in medical students, who are future doctors, and to provide suggestions in order to gain an objective perspective in the process of the development of medical doctor identity.

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2. Materials and Methods

2.1. Study population

This descriptive study was carried out at a public university in Anatolian side in Istanbul province in Turkey. The study took place between January and February of 2021. The total population of our study consisted of 1523 medical students. The sampling calculation was made by accepting alpha error level 5%, sample power 80% and design effect 1.5. It was aimed to reach minimum 461 people. Each class was accepted as a cluster and it was planned to take participants from each cluster according to the number of students (proportional to the population). The sampling was made using the haphazard sampling method (which can be considered as one of the limitations of our study). In total, 71 students from 1st-grade students, 75 students from 2nd-grade students, 96 students from 3rd-grade students, 55 students from 4th-grade students, 100 students from 5th-grade students, and 87 from 6th-grade students were reached. A total of 484 students participated in our study. Inclusion criteria were medical students who study at the concerned public university and individuals who gave informed consent.

2.2. Measures

Research data were collected using a questionnaire created to determine the participants' sociodemographic characteristics and “Nijmegen Gender Awareness in Medicine Scale (N-GAMS)” that assesses the gender awareness of the participants.

N-GAMS has been developed and validated at Radboud University in the Netherlands, by Verdonk et al, 2007 (3). The Turkish validity and reliability study of the scale was conducted by Akşehirli et al. at Erciyes University in 2019 (16). The Turkish version of the “Nijmegen gender awareness in medicine scale” was gathered into three factors, as in the original scale. These three subscales contain statements that students have to assess using a 5-point Likert scale (ranging from 1 “not agree at all” to 5 “totally agree”). Some statements have reverse meaning; therefore, an adjustment of reverse scoring statements was made.

The gender sensitivity (GS) subscale has eight statements, which explore the students’ general opinion of gender and sex in healthcare (e.g."Because male and female are not the same, physicians must treat everyone differently"). The gender role ideology, which is assessed towards patients (GRIP) score, includes eight statements that specifically relate to stereotypes about male or female patients and their communication regarding health problems (e.g."Female patients complain more about their health because they need more attention than male patients"). The Gender role ideology toward doctors (GRID) score, has ten statements, which explore students’ stereotypes towards doctors and their practice (e.g."Male physicians are more hasty in their examinations than female physicians."). A higher score in the GS statements means a higher gender sensibility. On the GRIP and GRID scales, high score implies more gender-stereotyping opinions.

The variables examined as sociodemographic variables in the research were gender, age, class, family type, perceived income level, , education status of the parents, and working status of the parents. The gender bias of the participants, which is the dependent variable of the study, was evaluated using the N-GAMS scale.

2.3. Statistical analysis

Descriptive data in the study were presented with means, standard deviation values and frequency tables. The percentages and frequency distributions of the answers given to each question were calculated. For the statistical analysis of the data, the Mann-Whitney U test and Kruskal Wallis variance analysis were used to compare continuous variables that did not fit the normal distribution. Visual (histogram) and analytical (Kolmogorov-Smirnov) methods were used to assess the suitability of variables to a normal distribution. The relationship of variables with significant correlation in univariate analyzes with subscales of N-GAMS (GS, GRID, GRIP) was evaluated with multivariate analysis since the outcome variables are interval variables, and a multivariate linear regression model was used for this. In this study, $p<0.05$ was considered as statistically significant.

3. Results

A total of 484 participants were included in the study. The sociodemographic characteristics of the participants are presented in Table 1. Most of the participants were female (65.9%) and the participants' mean age was 21.8±2.1 years. Also, 90.7% of the participants had an elementary family, and more than half of the participants (59.5%) reported their income level as moderate income. As for their mothers’ and fathers' educational status, 52.3% of the participants' mothers and 66.9 % of the participants' fathers were faculty/college graduates or had a Master's degree or Ph.D. While 45.7% of the participants' mothers were unemployed, the proportion of those whose fathers were unemployed was only 4.3%.

In Table 2, the scores of the participants in the GS subscale are compared according to their sociodemographic characteristics. Males (Median: 23.0, IQR: 8.0) compared to females (Median: 21.0, IQR: 9.0); those with high income (Median: 23.0, IQR: 8.0) compared to those with moderate income (Median: 21.0, IQR: 8.0) or low income (Median: 21.0, IQR: 12.0); and also the participants whose fathers were faculty/college graduates (Median: 22.0, IQR: 9.0) or had a Master's degree or Ph.D (Median: 22.0, IQR: 7.0) compared to those whose fathers were primary school graduates or lower (Median: 20.0, IQR: 8.0); were found to have statistically significantly higher gender sensitivities($p<0.0.5$). (There was no statistically significant difference between the participants those with moderate income or low income and the participants whose fathers were faculty/college graduates or had a Master's degree or Ph.D)

As far as the scores participants got from the GS subscale were compared no statistically significant differences were

detected within the class, family type, education status of the mother, working status of the mother, and working status of the father (p>0.0.5).

Table 1. Sociodemographic characteristics of the participants

Variables		n	%
Gender	Female	319	65.9
	Male	165	34.1
Class	1st-grade	71	14.7
	2nd-grade	75	15.5
	3rd-grade	96	19.8
	4th-grade	55	11.3
	5th-grade	100	20.7
	6th-grade	87	18.0
Family type	Elementary family	439	90.7
	Extended family	45	9.3
Perceived income level	High income	176	36.4
	Moderate income	288	59.5
	Low income	20	4.1
Educational Status of mother	Primary School or below	136	28.1
	High School or Equivalent	95	19.6
	Faculty/College	208	43.0
	Master's/PhD	45	9.3
Educational Status of father	Primary School or below	66	13.6
	High School or Equivalent	94	19.4
	Faculty/College	262	54.2
	Master's/PhD	62	12.8
Working status of mother	Unemployed	221	45.7
	Self employed	33	6.8
	Public sector	118	24.4
	Private sector	36	7.4
	Retired	76	15.7
Working status of father	Unemployed	21	4.3
	Self employed	94	19.4
	Public sector	136	28.1
	Private sector	99	20.5
	Retired	134	27.7
Total		484	100.0

Participants' mean score on the GRIP scale (19.21±7.07) was higher than their score on the GRID scale (17.03±6.25). The gender role ideology, towards patients (GRIP) score of participants were compared according to their sociodemographic characteristics in Table 3. Males (Median: 22.0, IQR: 11.0) compared to females (Median: 18.0, IQR: 11.0); those with low income (Median: 24.0, IQR: 9.0) compared to those with high income (Median: 18.0, IQR: 11.0) or moderate income (Median 19.0, IQR: 12.0); those whose mothers were primary school graduates or less (Median: 22.5, IQR: 10.0) compared to those whose mothers were university graduates (Median: 17.0, IQR: 11.0), and also those whose mothers were unemployed (Median: 21.0, IQR: 11.0) compared to those whose mothers were retired (Median: 16.5, IQR: 12.0) or public sector employees (Median: 17.0, IQR: 10) were found to display statistically significant differences (p<0.0.5). (There was no statistically significant difference between the participants those with high income or moderate income and the participants whose mothers were high school/equivalent or faculty/college graduates or had a Master's degree or Ph.D and also those whose mothers were retired or private or public sector employees)

Table 2. The gender sensitivity (GS) scores of participants

Variables		Median	IQR	p
Gender	Female	21.0	9.0	**p=0.01***
	Male	23.0	8.0	
Class	Preclinic	22.0	8.0	p=0.81*
	Clinic	22.0	9.0	
Family type	Elementary family	22.0	8.0	p=0.09*
	Extended family	20.0	10.0	
Perceived income level	High income	23.0	8.0	**p=0.04****
	Moderate income	21.0	8.0	
	Low income	21.0	12.0	
Educational Status of mother	Primary School or below	21.0	10.0	p=0.24**
	High School or Equivalent	22.0	8.0	
	Faculty/College	22.0	7.0	
	Master's/PhD	22.0	11.0	
Educational Status of father	Primary School or below	20.0	8.0	**p=0.03****
	High School or Equivalent	21.0	7.0	
	Faculty/College	22.0	9.0	
	Master's/PhD	22.0	7.0	
Working status of mother	Unemployed	21.0	8.0	p=0.35**
	Self employed	22.0	10.0	
	Public sector	22.0	7.0	
	Private sector	20.5	11.0	
	Retired	22.0	8.0	
Working status of father	Unemployed	21.0	9.0	p=0.12**
	Self employed	22.0	9.0	
	Public sector	22.0	9.0	
	Private sector	22.0	9.0	
	Retired	21.5	9.0	

*Mann-Whitney U test **Kruskal-Wallis test

There was no statistically significant difference between the class, family type, the region where they lived for the longest time, education status of the father and working status of the father and the scores they got from the GRIP subscale (p>0.05). (Table 3)

The Gender role ideology toward doctors (GRID) score of participants were compared according to their sociodemographic characteristics are examined in Table 4. Males (Median: 17.0, IQR: 12.0) compared to females (Median: 15.0, IQR: 9.0); those whose mothers had primary education or less (Median: 17.0, IQR: 11.0) compared to

others; those whose mothers were unemployed (Median: 17.0, IQR: 11.0) compared to those whose mothers were public sector employees (Median: 15.0, IQR: 8.0) or retired (Median: 13.5, IQR: 8.0) revealed that they had statistically significantly more stereotypic perspective towards doctors and their practices in the gender role ideology evaluated for doctors (p<0.0.5). (There was no statistically significant difference between the participants whose mothers were high school/equivalent or faculty/college graduates or had a Master's/ Ph.D degree and also those whose mothers were retired or private or public sector employees)

Table 3. The gender role ideology, which is assessed towards patients (GRIP) score of participants

Variables		Median	IQR	p
Gender	Female	18.0	11.0	**p<0.001***
	Male	22.0	11.0	
Class	Preclinic	18.0	11.0	p=0.20*
	Clinic	20.0	12.0	
Family type	Elementary family	19.0	11.0	p=0.28*
	Extended family	21.0	9.0	
Perceived income level	High income	18.0	11.0	**p=0.01****
	Moderate income	19.0	12.0	
	Low income	24.0	9.0	
Educational Status of mother	Primary School or below	22.5	10.0	**p<0.001****
	High School or Equivalent	18.0	11.0	
	Faculty/College	17.0	11.0	
	Master's/PhD	19.0	11.0	
Educational status of father	Primary School or below	20.0	12.0	p=0.47**
	High School or Equivalent	20.0	12.0	
	Faculty/College	18.5	11.0	
	Master's/PhD	18.5	10.0	
Working status of mother	Unemployed	21.0	11.0	**p<0.001****
	Self employed	20.0	10.0	
	Public sector	17.0	10.0	
	Private sector	18.5	13.0	
	Retired	16.5	12.0	
Working status of father	Unemployed	23.0	13.0	p=0.26**
	Self employed	18.0	12.0	
	Public sector	18.5	11.0	
	Private sector	20.0	12.0	
	Retired	19.0	11.0	

*Mann-Whitney U test **Kruskal-Wallis test

There was no statistically significant difference detected between the class, family type, perceived income level, , education status of the father, and working status of the father as far as scores they got from the GRID subscale were compared in Table 4 (p>0.05).

Table 4. The Gender role ideology toward doctors (GRID) score of participants

Variables		Median	IQR	p
Gender	Female	15.0	9.0	**p=0.007***
	Male	17.0	12.0	
Class	Preclinic	16.0	9.0	p=0.35*
	Clinic	15.5	10.0	
Family type	Elementary family	16.0	10.0	p=0.23*
	Extended family	17.0	10.0	
Perceived income level	High income	16.0	9.0	p=0.15**
	Moderate income	16.0	10.0	
	Low income	20.0	8.0	
Educational Status of mother	Primary School or below	17.0	11.0	**p=0.02****
	High School or Equivalent	15.0	10.0	
	Faculty/College	15.5	9.0	
	Master's/PhD	15.0	10.0	
Educational status of father	Primary School or below	16.0	11.0	p=0.34**
	High School or Equivalent	17.0	11.0	
	Faculty/College	16.0	9.0	
	Master's/PhD	15.0	9.0	
Working status of mother	Unemployed	17.0	11.0	**p=0.006****
	Self employed	16.0	7.0	
	Public sector	15.0	8.0	
	Private sector	14.5	9.0	
	Retired	13.5	8.0	
Working status of father	Unemployed	18.0	9.0	p=0.62**
	Self employed	15.5	10.0	
	Public sector	16.0	10.0	
	Private sector	16.0	7.0	
	Retired	15.0	10.0	

*Mann-Whitney U test **Kruskal-Wallis test

Multivariate tests showed differences in gender role ideology towards patients and doctors according to the gender of the participants and the working status of their mothers. Also, significant sex-related differences were found in gender sensitivity as well (B=1.540; p= 0.007). As compared to others, male students (B=2.252; p=0.001) or those whose mothers were unemployed (B= -1.439; p=0.034) held slightly more

gender stereotypes towards patients. As compared to others, male students (B=1.918; p=0.001) or those whose mothers were unemployed (B=1.975; p=0.002) held slightly more gender stereotypes towards doctors (Table 5).

Table 5. Multivariate linear regression results of N-GAMS subscales

GS	B	Sd.	Beta	t	p
Constant	19.262	0.939		20.521	<0.001
Gender(reference category: male)	·0	·4	·3	3	**)7**
Perceived income level (reference category: high income)	1.048	0.568	0.085	1.844	0.066
Educational status of father (reference category: primary school or below)	1.077	0.593	0.085	1.818	0.070
Family type (reference category: elementary family)	1.026	0.940	0.059	1.283	0.200
R^2=0.027, F=4.414, p=0.002					

GRIP	B	Sd.	Beta	t	p
Constant	17.547	0.531		33.073	<0.001
Gender (reference category: male)	2.252	0.654	0.151	3.444	**0.001**
Perceived income(reference category: low income)	2.521	1.598	0.071	1.578	0.115
Educational status of mother (reference category: primary school or below)	1.409	0.796	0.090	1.770	0.077
Working status of mother (reference category: unemployed)	1.820	0.711	0.128	2.559	**0.011**
R^2=0.074, F=8.666, p<0.001					

GRID	B	Sd.	Beta	t	p
Constant	15.176	0.426		35.664	<0.001
Gender (reference category: male)	1.918	0.583	0.145	3.291	**0.001**
Educational status of mother (reference category: primary school or below)	0.855	0.709	0.061	1.206	0.228
Working status of mother (reference category: unemployed)	1.975	0.633	0.157	3.120	**0.002**
Perceived income (reference category: low income)	1.383	1.424	0.044	0.971	0.332
R^2=0.058, F=8.419, p<0.001					

4. Discussion

A total of 484 participants were included in the study. Most of the participants were female (65.9%) and the participants' mean age was 21.8±2.1 years. According to results of our research; male students, those with high income and also the participants whose fathers were faculty/college graduates or had a Master's degree or Ph.D. were found to have statistically significantly higher gender sensitivities. As compared to others,males, those with low income, those whose mothers were primary school graduates or less, and also those whose mothers were unemployed held slightly more gender stereotypes towards patients or doctors.

As in other similar studies on this subject, in our research, the difference between female and male participation rates is striking. We think that this difference is due to female participants having more interest and curiosity in the research subject than male participants. We estimate that the females participated at a higher rate because they are more exposed to the adverse effects of low gender awareness in society.

Our study found statistically significant differences that males have a more gender-stereotypical perspective than females in the N-GAMS GRIP and GRID subscales. Similarly, in the studies conducted by Rrustemi et al. (17) and Andersson et al. (18), it was observed that male had a more stereotypical perspective in the GRIP subscale. Similar results have been found in other studies comparing stereotyped attitudes based on gender in male and female (19, 20). Previous research has shown that male students are less knowledgeable about gender issues and, at the same time, more skeptical than female students about applying gender issues in education (21, 22). The reason why male are more accepting of gender stereotypes may be that such stereotypes are generally more positive towards male (20).

In the studies conducted by Rrustemi et al. (17) and Andersson et al. (18), it was found that gender sensitivity increases with age, and gender stereotypes perspectives in the GRIP and GRID subscales decrease with age. Similarly, in the study conducted by Morais et al.(23), it was found that there was a negative correlation between the class and gender sensitivity, and a positive correlation between the class and gender role ideology towards patients and towards doctors. However, in our study, no significant difference was found between the gender sensitivity and stereotypes with students' grade level.

In the study conducted by Andersson et al., it was observed that the participants whose mothers had a medium-high education level were more opposed to the stereotypical perspective (18). Similarly, in a study Aylaz et al. conducted to determine university students' views on gender role, it was determined that high education level of the mother decreased students' gender role discrimination (24). However, in this study, no significant relationship was found between father education and attitude towards gender roles. Also, Pınar et al.

reported that in the group with the most stereotypical perspective, there were those with a low maternal education level (25). In our study, in line with the literature, it was found that the education levels of the mothers are essential in terms of gender attitudes and that the participants whose mothers have a lower education level have a more gender-stereotypical perspective in the GRIP and GRID subscales. In addition, contrary to the study of Ayaz et al., in our study, it was found that the participants whose fathers had a university or higher education level are more gender sensitive (24).

Our study found that the students whose mothers are unemployed have a more stereotypical perspective than the others. Similarly, in the study that Ongen conducted with university students using the "Gender Roles Attitude Scale", it was determined that students whose mothers were working adopted egalitarian roles regarding gender roles (26). Pınar et al.(25), in a study they conducted to determine gender attitudes in a university student dormitory, found that the mother's working status had a positive effect on students' gender awareness. We see mainly that the mothers' working status affects students' attitude towards gender roles. The reason for this may be the difference in the roles of the mother and father in the child's upbringing process in our society. We can say that the mothers have a more significant share in the child's upbringing and the establishment of social values and norms. In our study, we see that the fathers' educational status and working status are less effective than the education and employment status of the mothers.

According to our findings, those with a high income are more gender sensitive and have a more stereotypical perspective on the gender role ideology assessed toward patients than those with a middle or low income. Similarly, in the study of Varol et al., it was found that students with moderate or low income have a more stereotypical perspective (15). In the study of Pınar et al., it was stated that the group with low-income level was the group with the most stereotypical perspective (25).

Although social concepts about males and females were the basis of attitudes towards both patients and doctors, we found that students' gender stereotypes towards patients were more pronounced than stereotypes towards doctors, similar to the study of Andersson et al. (18).

Gender awareness in medicine is a concept that can be improved by training and increasing general awareness, especially with the training given to future medical students and healthcare professionals on this subject. It is necessary to increase gender awareness in medicine among future doctors and ensure equal opportunities for each individual. The inclusion of gender roles and gender equality training in the medical school curriculum is necessary to enable the mechanisms for inequality to be examined in detail, train more sensitive doctors, and give students a more egalitarian perspective. Eliminating the differences in students' gender equality perception level and raising students' gender equality perception can increase the number of gender-sensitive physicians and make a significant contribution to improving the quality of healthcare services received by individuals. We suggest that gender issues should be included in medical education to focus on gender attitudes. The goal of education about gender is to make students interested and aware of the significance of gender in medical work. Previous studies suggests that students become more positive and engaged as they learn more and become accustomed to gender debates (21, 22).

Events such as symposiums and conferences can also be organized to raise gender awareness. If such stereotypical thoughts are not shared and discussed, students will not be able to see their own attitudes and have less chance to discuss them, and consequently, the impact of education will be less.

If we look at the limitations of our study, first of all, the N-GAMS questionnaire has some difficulties. The scale is based on formulated negative stereotypes to which participants are asked to react. The use of negative stereotypes may have induced a social desirability response bias. Furthermore, having done it in a limited medical school student population may not reflect all medical school students. Also, females participated in our study at a higher rate than males and male students were underrepresented. We think that this difference can be due to the female participants being more interested in and curious about the research subject than the male participants. This situation may have caused the average scores of the scales to be more biased. Thus, we cannot exclude a selection bias. While the N-GAMS scale can be used to compare many students, qualitative researches can give greater depth and further explain the social discourse and reasoning underlying students' results.

As shown in our results, the participants were stereotyped in gender role ideology and had suboptimal gender sensitivity. Gender stereotypes can be prevented through gender-sensitive medical education. More studies particularly qualitative studies are needed on this subject to examine the students' gender awareness, and perception levels related to their sociodemographic characteristics in more detail and to determine the measures that can be taken to solve the problem.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: M.K.A., S.D., S.H., M.K. Design: M.K.A., S.D., S.H., M.K. Data Collection or Processing: M.K.A., C.D., I.C.,

D.E., I.K. Analysis or Interpretation: M.K.A., S.D., C.D., I.C., D.E., I.K. Literature Search: M.K.A., C.D., I.C., D.E., I.K. Writing: M.K.A., S.D., C.D., I.C., D.E., I.K.

Ethical Statement

Approval was obtained from Marmara University Ethics Committee, the study started. The ethics committee decision date is 05/02/2021 and the number of ethical committee decisions is 09.2021.160.

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Retrospective analysis of bacterial agents in central nervous system infections and antibiotic resistance: A nine years evaluation at a university hospital

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Abstract

The follow-up of bacterial agents and antibiotic resistance profiles that cause central nervous system (CNS) infections has guided the choice of life-saving empirical treatment against these infections. This study aimed to evaluate the bacterial agents isolated from cerebrospinal fluid (CSF) samples and their antibiotic susceptibility for nine years with the suspicion of CNS infection. We retrospectively analyzed the results of the patients sent CSF samples for bacteriological diagnosis with the suspicion of CNS infection between August 2012 and April 2021. The isolated bacterial species were identified using conventional methods, biochemical tests, and various commercial identification systems. Microorganisms were isolated in 121 (14.85%) of the 815 CSF samples examined. The most commonly isolated agents are methicillin-resistant coagulase-negative *Staphylococci* (n: 59; 48.76%) among Gram-positive and *Klebsiella* spp. (n: 12; 9.92%) among Gram-negative. The follow-up of bacteria isolated from CNS infections, which can progress rapidly and cause serious complications, and the changes in antibiotic susceptibility over the years are known as a guide in regulating appropriate treatment. Our results may contribute to the selection of antibiotics that can be used especially in cases where urgent empirical treatment is required.

Keywords: antimicrobial resistance, bacterial agents, central nervous system, infection, cerebrospinal fluid

1. Introduction

Central nervous system (CNS) infections such as meningitis, encephalitis, and brain abscesses are associated with high mortality and morbidity; for this reason, prompt diagnosis and effective treatment are crucial. CNS infections, which can be caused by various microorganisms such as viruses, bacteria, parasites, and fungi, can progress faster than other infectious diseases and can cause permanent damage or death in a short time (1). Bacterial pathogens are more common among the causative agents of CNS infections (2).

Most CNS infections are community-acquired, but they may develop related to health care due to trauma, use of cerebrospinal fluid shunts, and external drains in some patients. In community-acquired bacterial CNS infections, organisms commonly isolated include *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae* (3). However, frequently detected agents may change over the years depending on risk factors such as age, geographical differences, seasonal changes, vaccination status, genetic structure, and socioeconomic conditions (4-6).

Accurate diagnosis of bacterial agents and monitoring of antibiotic resistance patterns over the years are life-saving and guide empirical treatment selection. In other words, empirical treatment depends on regional patterns of antibiotic resistance of common pathogens. This study aimed to evaluate the bacterial agents isolated from cerebrospinal fluid (CSF) samples and their antibiotic susceptibility for nine years with the suspicion of CNS infection.

2. Materials and Methods

2.1. Study Design and Patients

We retrospectively analyzed the results of the patients who were sent CSF samples for bacteriological diagnosis with the suspicion of CNS infection between August 2012 and April 2021. In the CSF samples sent to the laboratory at different times, the same bacteria on the same patient and with the same antibiotic sensitivity were accepted as only one isolate. Other isolates were not evaluated in this study.

2.2. Culture conditions and antibiotic susceptibility tests

All CSF samples admitted to the laboratory were evaluated after inoculation on 5% sheep blood agar, chocolate agar, and MacConkey agar media and incubated for 18-36 hours at 37°C. The identification of microorganisms from specimens that have shown growth detected by conventional methods was achieved by analyzing colony structure, growth characteristics, and conducting biochemical tests. In addition, all CSF samples sent to the laboratory were inoculated into automatic blood culture system bottles (BACTEC BD, United States). Gram staining and subculture passages were performed to identify the agent from the blood culture bottle with a positive signal.

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Antibiotic susceptibility tests of isolated bacteria on Mueller-Hinton agar were performed with the Kirby-Bauer disc diffusion method using commercial antibiotic discs (Oxoid, England) according to Clinical Laboratory Standards Institute (CLSI) (before 2016) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (after 2016) criteria (7, 8).

Phoenix (BD, United States) and MALDI-TOF MS (Bruker, Germany) commercial identification and antibiotic susceptibility systems were also used in doubtful identifications.

2.3. Statistical Analyses

The patient data was evaluated with IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA) package program and Microsoft Excel version 2013. Descriptive statistics were used to define the age of the patients, and frequencies (n) and percentages (%) were used to describe microbiological culture test results.

3. Results

Microorganisms were isolated in 121 (14.85%) of the 815 CSF samples examined. Sixty-eight (56.2%) were isolated from male, and 53 (43.8%) were isolated from female patients. These patients' ages ranged from 0 to 76 years, and the mean age and SD were 23.92 ± 26.21 years.

The most commonly isolated agents are methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) (n:59; 48.76%) among Gram-positive and *Klebsiella* spp. (n:12; 9.92%) among Gram-negative (Table 1).

Table 1. Distribution of bacteria (n:121) isolated from CSF samples

Bacteria	Number (n)	%
Gram positive		
Coagulase-negative staphylococci	59	48.76
Staphylococcus aureus	9	7.44
Streptococcus spp.	9	7.44
Enterococcus spp.	6	4.96
Gram negative		
Klebsiella spp.	12	9.92
Acinetobacter spp.	10	8.26
Pseudomonas spp.	6	4.96
Enterobacter spp.	5	4.13
Escherichia coli	2	1.65
Serratia marcescens	1	0.83
Stenotrophomonas maltophilia	1	0.83
Chryseobacterium indologenes	1	0.83

The distribution of isolated microorganisms by years is given in Fig. 1. Table 2 presents the distribution of culture positivity in CSF samples based on the clinics to which they were sent for laboratory analysis. An examination of the requesting clinics regarding culture positivity revealed that most of the samples were sent from the Neurosurgery clinic (n:31; 25.62%), followed by the Pediatrics and Anesthesia and Reanimation clinics (Table 2).

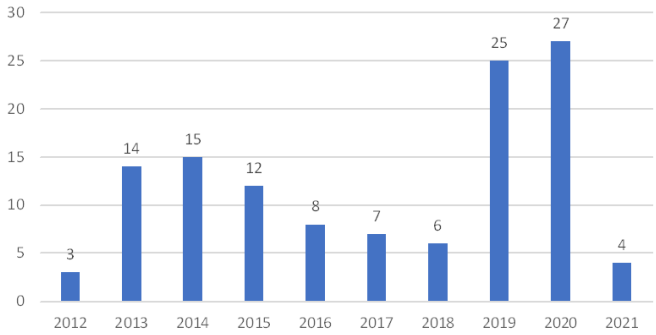

Fig.1. Distribution of isolated bacteria years

Table 2. Distribution of CSF samples with growth in culture according to clinics

Clinics	n	%
Neurosurgery	31	25,62
Pediatrics	29	23,97
Anesthesia and Reanimation	23	19,01
Neurology	14	11,57
Newborn	13	10,74
Infectious Diseases and Clinical Microbiology	3	2,48
Radiology	3	2,48
Others*	5	4,13

* Orthopedics and Traumatology, Medical Oncology, Emergency Medicine

It was determined that 99 of the 121 patients with culture positivity in CSF samples were found samples from Turkish citizens, 12 were Libyan citizens, 3 were Iraqi citizens, 2 were Azerbaijani citizens, 2 were Moldovan citizens, and one of these CSF samples taken from Georgia, Kazakhstan, and Russian citizens. Antibiotic susceptibilities of isolated strains are given in Table 3.

Table 3. Antibiotic resistance rates of isolated bacteria from CSF samples

Bacteria	Antibiotic resistance* (%)														
	DA	AN	GN	TOB	LZD	AMP	TZP	AMC	CAZ	FEP	MEM	IMP	CIP	LEV	SXT
Coagulase-negative staphylococci (59)	43.4	17.9	47.6	57.6	2.7								64.7	62.7	37.5
Klebsiella spp. (12)		25	83.3			100	70	70		90	41.7	36.4	63.6	54.5	0
Acinetobacter baumannii complex (10)		40	60	30			60		80	60	60	60	60	50	0
Staphylococcus aureus (9)	11.1	33.3	22.2	33.3		44.4							22.2	0	0
Streptococcus spp. (9)	11.1					33.3				11.1					
Enterococcus spp. (6)			50		0	16.7							100	100	
Pseudomonas spp. (6)		0	16.7	0			16.7		16.7	0	40	20	20	20	
Enterobacter spp. (5)		0	20			100	0	80	0	0	0	0	20	0	0
Escherichia coli (2)		0	0			50	0	0	0	0	0	0	0	0	0
Serratia marcescens (1)		0	0			100		100							0
Stenotrophomonas maltophilia (1)														0	
Chryseobacterium indologenes (1)		100	0	100			100		100	100	100	100	0	0	0

* DA: clindamycin; AN: amikacin; GN: gentamicin; TOB: tobramycin; LZD: linezolid; AMP: ampicillin; PRP: piperacillin-tazobactam; AMC: amoxicillin-clavulanic acid; CAZ: ceftazidime; FEP: cefepime; MEM: meropenem; IMP: imipenem; CIP: ciprofloxacin; LEV: levofloxacin; SXT: trimethoprim-sulfamethoxazole

4. Discussion

Despite the development of antibacterial agents and modern microbiological methods used in the differential diagnosis of the agents, bacterial CNS infections are serious infections that cause morbidity and mortality worldwide (1, 9). These life-threatening infections require immediate antimicrobial therapy, prompt identification of the pathogen, and early initiation of appropriate antimicrobial therapy are essential to improve clinical outcomes (10, 11).

Significant differences exist in the spectrum of pathogens isolated from cerebrospinal fluid cultures in developed and developing countries. While in developed countries, Group B streptococci (GBS), *Escherichia coli,* and *Listeria monocytogenes* were generally found as important organisms; there were bacteria such as *Klebsiella* spp., *E. coli,* and *Streptococcus pneumoniae* as well as Group B streptococci in developing countries (12).

In our study, the most common agents were methicillin-resistant coagulase-negative staphylococci among Gram-positive bacteria and *Klebsiella* spp. among Gram-negative bacteria. These were followed by *Acinetobacter baumannii* complex, *Staphylococcus aureus,* and *Streptococcus* spp., respectively.

In the study conducted by Durand et al. (13) with 493 cases of acute bacterial meningitis in adults in the USA, the rate of hospital-acquired infection was 40%. Except for *H. influenzae,* Gram-negative bacteria have been reported to cause 33% of hospital-acquired meningitis. Frequent use of invasive medical supplies and contamination of respiratory support equipment are factors that increase the likelihood of nosocomial infections (14).

Klebsiella spp. and *Acinetobacter baumannii* complex, with the extraordinary spreading ability of the most common pathogens isolated from CSF samples, leads to mainly considering nosocomial infections. The high number of Gram-negative bacteria among the causative agents indicates that hospital-acquired infections are more common than community-acquired infections. In addition, when the distribution of CSF samples with culture positivity according to clinics was examined, Neurosurgery ranks first, followed by Pediatrics and Anesthesia, respectively. It can be predicted that the higher number of microorganisms reproducing from CSF in these units may be due to the gradual increase in invasive interventions and the prolongation of hospital stays with the developing technology.

Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommended that specific vaccines against *S. pneumoniae, Neisseria meningitidis*, and

Haemophilus influenzae type b be added to national immunization programs; thus, a decrease in cases caused by these microorganisms has been observed (15-18). In our study, *N. meningitidis* and *H. influenzae* could not be isolated in any case during the nine-year period, and *S. pneumoniae* was isolated in only one case. This finding is consistent with studies conducted in developed countries where meningitis vaccines are administered (19, 20). We think this situation may be due to the introduction of *H. influenzae* type b and conjugated pneumococcal vaccines into routine vaccination in our country after 2006.

In some reports conducted in our country and worldwide, the detection rates of bacterial agents in CSF were between 5.3% and 31.5% (13, 14, 21-23). Our study isolated bacteria in 121 (14.85%) of 815 CSF samples. This rate may vary depending on the development level of the countries, age, the way the agent is taken, routine vaccination programs, geographical location, and treatment or operation.

The distribution of isolated microorganisms varies from year to year. The lowest distribution by year appears in 2012 and 2021. The examined periods included short times such as 4 months in 2012 and 3 months in 2021, which may explain why the lowest distribution of isolated microorganisms occurred during these years. However, no specific reason for the increase in the number of microorganisms isolated in 2019 and 2020 has been identified, and it is considered a spontaneous increase.

In conclusion, the data obtained from this study showed that the most common agents isolated from CSF samples are Coagulase Negative Staphylococci, *Klebsiella* spp., and *Acinetobacter* spp. We believe the periodic determination of the microorganisms causing CNS infection and their resistance rates may be significant in controlling nosocomial infections and guiding empirical treatment regulation. Therefore, we think that routinely analyzing collected clinical laboratory data in monitoring antimicrobial resistance will benefit the review of treatment guidelines.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.I.T., Design: A.I.T., S.S., Data Collection or Processing: A.I.T., M.D., Analysis or Interpretation: A.I.T., S.S., M.D., Literature Search: S.S., Writing: S.S., M.D.

Ethical Statement

Ethics committee approval is not required for this study.

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Maternal and perinatal outcomes of sars-cov-2 infection in unvaccinated and vaccinated pregnant patients

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Abstract

The objective of this study is to evaluate maternal and fetal outcomes of Covid-19 in vaccinated compared with unvaccinated pregnant patients. The study included 244 pregnant patients with COVID-19 infection confirmed by a positive SARS-CoV-2 polymerized chain reaction (PCR) test. Two hundred-four patients were not unvaccinated, and 40 patients were vaccinated. The clinical, biochemical, and radiological results and maternofetal outcomes of the pregnant patients were recorded. All the data were evaluated statistically. The pregnant patients included in the study had a mean age of 31.4 in the vaccinated group and 28.3 years in the unvaccinated group. Comorbidities were determined in 25% of the patients, and the most common symptom was fever in 65.5% of cases. Sore throat, loss of taste sensation, and loss of smell sensation were detected significantly more in the unvaccinated group ($p<0.01$). Most of the patients were in third-trimester pregnancy in both groups. No patient in the vaccinated group needed intensive care due to covid. A significant difference was found between the two groups in terms of neonatal intensive care requirements.This study determined that intensive care requirement is increased for both mother and newborn, covid symptoms could be prolonged, and hospital stay is longer in the unvaccinated group. Considering these data, the covid-19 vaccine should be offered to all pregnant women.

Keywords: COVID-19, vaccine, pregnancy, cesarean, intensive care

1. Introduction

The severe acute respiratory syndrome coronavirus 2 virus (SARS-CoV-2), an agent of viral pneumonia, has resulted in a global pandemic (1). The disease caused by this virus has been named coronavirus 2019 (COVID-19), yet no effective treatment has been found (2). Different disease conditions of COVID-19 are seen in different populations. Therefore, prioritization for inpatient treatment can be made by determining different risk groups (3). Pregnancy is accepted worldwide as a risk factor for COVID-19 (4). Although pregnant women were excluded from the first trials of COVID-19 vaccines, the urgent need to protect this group, vaccines were offered to them before clinical trials were completed (5). However, current data presented the safety and effectiveness of vaccines in pregnant women as well (6).

Throughout pregnancy, biochemical, physiological, and immunological changes occur (7). Therefore, almost all diseases require different treatment strategies from the non-pregnant population (1). Previous studies have shown no difference in mortality between pregnant and non-pregnant women (8). However, when the same groups were compared in terms of the length of stay in the hospital, pregnant women had a more extended stay in the hospital than non-pregnant women (9). The effects of COVID-19 infection in pregnancy are also seen in the fetus (9).

Previous studies have shown vertical transfer of the SARS-CoV-2 virus (10). Although various studies have been conducted to date, reports of the effects of COVID-19 on pregnant patients are still limited. The aim of this study was to analyze COVID-19 in vaccinated versus unvaccinated pregnant patients and to compare clinical and biochemical results and fetal effects.

2. Materials and Methods

This single-center study included pregnant patients diagnosed with COVID-19 who presented at the Adana City Training and Research Hospital between February 2020 and June 2022. The study was approved by the Institutional Review Board (decision no:1191/301220) and the Turkish Ministry of Health (T01-21-35) and carried out in accordance with the Helsinki Declaration principles.

Given the high frequency of COVID-19 infection in Turkey and the fact that our institution is one of the major pandemic hospitals in the area, every pregnant woman who applied was given a COVID-19 PCR test. A SARS-CoV-2 PCR test was performed on nasopharyngeal and oropharyngeal smear samples to diagnose COVID-19 infection (9). The patients'

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demographic, laboratory, and follow-up characteristics were obtained. Patients with SARS-COV-2 infection were the subject of the study, and hence, patients with negative PCR results were excluded. If women with severe COVID-19 disease were at or near term, delivery was considered for increasing oxygen requirements. The patients were divided into two groups, group 1: vaccinated and group 2: unvaccinated. The vaccinated group included patients with at least one dose vaccinated with Covid 19 vaccine before or during pregnancy.

Emergency cesarean sections were performed on patients who required intubation or whose oxygen saturation did not increase after intensive oxygen treatment. COVID-19 patients who were asymptomatic and non-severe were monitored for the development of severe illness, and self-isolation was indicated. The timing of the patients' deliveries was determined by obstetric/medical indications. If there is no additional indication for cesarean section and there is no emergency, the patients were delivered by vaginal delivery.

Age, number of pregnancies, history of miscarriages, type of delivery of previous pregnancies, gravida, parity, pregnancy complications, pregnancy outcome, comorbidities, smoking status, length of hospital stay, need for intensive care, gestational age at birth, and continuing COVID-19 symptoms at 1 and 6 weeks after the birth were all recorded for each patient. The presence of COVID-19, birth weight, neonatal intensive care requirement, and APGAR scores at 1 and 5 minutes were all recorded for the neonates. All the patients were treated according to the national COVID-19 guidelines. According to vaccination status, patients were compared.

2.1. Statistical Analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, IL, USA). The variables were tested using visual and analytical methods (Shapiro–Wilk's test) to determine whether they were normally distributed or not. Comparative analyses were made using Fisher's exact test for categorical variables, the independent samples t-test for normally distributed continuous variables, and the Mann–Whitney U test for abnormal distributions. Descriptive analyses were presented using mean ± SD, and propriety data were shown as median and min–max values. A p-value of <0.05 was considered significant.

3. Results

Two hundred forty-four pregnant women with a positive SARS-CoV-2 PCR test were followed up during the study period. The demographic and clinical characteristics of the pregnant women in the study are shown in Table 1. The mean age of the patients was 28.3 years (range 18-43 years), BMI was calculated as a mean of 26.7 (range 22-35), and the mean gestational age at the time of diagnosis was 36.6 weeks. A total of 200 (98%) cases were diagnosed in the third trimester. Median gravida was two, parity was one, and the median number of living children was one in two groups. The median length of hospital stays of the patients admitted for treatment was 11(± 10.2) days for the unvaccinated group and 4±8.4 for the vaccinated group. The duration was found to be significantly shorter in the vaccinated group (p<0.01).

Table 1. The demographic and clinical characteristics of pregnant women with COVID-19 infection

Parameter	Vaccinated (n:40)	Unvaccinated (n:204)	p-value
Age (years)	31.4± 4.6	28.3 ± 5.9	<0.01
BMI (kg/m^2)	27.2±4	26.7 ± 2.3	0.35
Gestational age at the time of Covid-19 diagnosis (weeks)	36.2±2.9	36.6±3.8	0.54
Gravidity	2±3.2	2 ± 3.1	0.38
Parity	1±3,9	1± 4.5	0.42
Number of living child	1±3.2	1± 3.4	0.37
Previous miscarriages	1±2.3	1±2.4	0.62
Time from COVID-19 diagnosis to delivery (days)	10± 1.4	7± 82.3	0.14
Length of hospital stay (days)	4± 8.4	11±24.2	<0.01
Smoker (current/history)	6 (15.6)	54(26.4)	0.27
Admission to hospital			
Emergency Department	14(35.2)	100(49.3)	0.13
Covid-19 Outpatient Clinics	12(30.1)	52(25.4)	0.62
Obstetric Outpatient Clinics	16(40.0)	50(24.5)	0.54
COVID-19 case in the family	22 (55)	117(57.3)	0,61
Covid 19 infection Findings on Chest X-Ray	2(5)	23(11.2)	0.23
Covid 19 infection Findings on Pulmonary CT	1 (2,5)	17(8,3)	**0.02**
Comorbid disease			
Hypertension	1(2.5)	16(7.8)	<0.01
Diabetes Mellitus	1(2.5)	5(2.4)	0.21
Thyroid Gland Diseases	0(0)	2(0.9)	NA
Pulmonary Diseases	3(7.5)	3(1.4)	0.067
Liver Diseases	0(0)	1(0.4)	NA
Symptom			

Fever	12 (30)	111 (54)	0.236
Cough	5(12.5)	75(36.7)	0.728
Sore Throat	2(5)	26(12.7)	<0.01
Loss of taste sensation	1(2.5)	14(6.8)	<0.01
Loss of smell sensation	2(5)	20(10.2)	<0.01
Tiredness	7(17.5)	37(18.1)	0.652
Loss of appetite	16(40)	57(27.9)	0.523
Tiredness continuing in the 6th week postpartum	12(30)	59(29)	0.865

Abbreviations: BMI: body mass index, CT: computed tomography, Data are mean standard deviation or n (%) unless otherwise specified Values are given as a number (percentage), mean ± standard deviation (range), or median (interquartile range, range).

There was no difference between the groups in terms of BMI, gravida, parity, time from Covid -19 diagnosis to delivery, smoking status, and the unit to which they applied to the hospital (emergency department, Covid-19 outpatient Clinics, Obstetric Outpatient Clinics). Seventy-five percent (n:184) of the pregnant women in the study population were a non-smoker. In 114 (46,7 %) of patients, admission to the hospital occurred through the emergency department. In order to determine the origin of COVID-19 transmission, it is necessary to identify the vectors that could have carried it; the patients were questioned about the existence of positive family members; 57.3% (n=117) of the unvaccinated group and 55% (n=22) of the vaccinated group had a positive family history (p: 0.61).

A total of 41 patients refused all methods of radiological imaging. Radiological imaging revealed no lung findings on Chest X-ray (CXR) examination of 72,9% (n:178) of the patients. Pulmonary computed tomography was not performed in 61.0% (n:149) of patients. Findings were determined in 7.3 percent of individuals to whom tomography was performed (n:18).

All the pregnant patients were clinically evaluated in respect of comorbid diseases. Comorbidities were identified in 25% (n:51) of patients, and the most common condition was hypertension. Laboratory tests were performed for all the patients for diagnostic and treatment purposes. Twelve (5.8%) patients had anemia, and 4 (1.9%) had thrombocytopenia. The most common hematological abnormality was neutrophilia. Only 8 (4.5%) patients had elevated liver enzymes; one was HELLP syndrome. The blood levels of urea and creatinine were within the normal limits, except for three patients who died owing to multiorgan failure. C-reactive protein was found to be increased in 87% of the patients. Procalcitonin levels were similarly high. In addition, no significant difference was found between the groups in terms of laboratory tests. The symptoms seen in pregnant patients during COVID-19 infection are shown in Table 1. There was no difference between the groups in terms of symptoms. Nasal oxygen was administered to 36.9% of the patients, and 2.9% received mechanical ventilator support in the unvaccinated group.

Table 2. Pregnancy characteristics and maternal, fetal outcomes of patients with COVİD 19 infection

	Vaccinated (n:40)	**Unvaccinated (n:204)**	**P value**
Trimester of the pregnancy at the time of diagnosis	**n (%)**	**n (%)**	
1st trimester	0	1(0.4)	0.456
2nd trimester	4(10)	3(1.4)	
3rd trimester	36(90)	200(98)	
Cesarean indication	**n (%)**	**n (%)**	
History of previous cesarean	8(20)	70(51.9)	0.742
Maternal-related problems	1(2.5)	26(19.3)	0.264
Cephalopelvic disproportion	1(2.5)	3	0.438
Fetal distress	1(2.5)	36(17.6)	0.842
Delivery type	**n (%)**	**n (%)**	
Vaginal birth	29(72.5)	37(33)	0.082
Cesarean section delivery	11(27.5)	136(67)	
Pregnancy outcome	**n (%)**	**n (%)**	
Abortus	0	1(0.5)	0.475
Premature birth	1(2.5)	37(18.2)	
Stillbirth	0	1(0.5)	
Term live birth	39(97.5)	165(81.3)	
Pregnancy complications	n (%)	n (%)	
Intrahepatic cholestasis of pregnancy	0	2(1.1)	0.658
Pre-eclampsia	0	8(3.9)	
Placenta previa	0	3(1.4)	
HELLP Syndrome	0	1(2)	
No pregnancy complications	40(100)	191(93.6)	
Nasal Oxygen	8(20)	73(36.9)	0.261
Admitted to the intensive care unit	0	13(5.3)	NA
Mortality due to Covid-19 infection	0	4(1.6)	NA

Data are mean standard deviation or n (%) unless otherwise specified Values are given as a number (percentage), mean ± standard deviation (range), or median (interquartile range, range).

Pregnancy characteristics and maternal and fetal outcomes of patients with COVID-19 infection were summarized in table 2. Ninety-six percent (n:236) of the patients were in the third trimester at the time of diagnosis. There was no difference between the two groups regarding the delivery type, cesarean indications, pregnancy prognosis, and pregnancy complications. In the unvaccinated group, 13(5.3%) patients needed intensive care. Mortality due to COVID-19 was in 4 (1.6%) patients.

The median birthweight of the newborns was 3200 gr (range, 620-4900) in the unvaccinated group and 2940 (2100-3190) in the vaccinated group. The median 1-min APGAR score was 8 (range, 0-9), and the median 5-min APGAR score was 9 (range, 0-10). Nutrition of the newborns was started with maternal breastfeeding in 63.5% (n:155) of cases. A SARS-CoV-2 PCR test was applied to all the infants; in the unvaccinated group, 5.4% (n = 11) were found to have a positive result. There were no newborns with positive test results in the vaccinated group. The number of cases admitted to the neonatal intensive care unit was found to be higher in the unvaccinated group by 14.9% (n:29). Neonatal outcomes of newborns are shown in Table 3.

Table 3. Neonatal outcomes of newborns

	Vaccinated (n=40)	**Unvaccinated(n=204)**	**P value**
Parameter	Min-Max	Min-Max	
Birthweight of term newborn (gr)	2100-3190.0	620-4900	NA
1-min Apgar	4-8	1-9	
5-min Apgar	8-9	3-9	
Requirement for neonatal intensive care	**n(%)**	**n(%)**	
Admitted to intensive care unit	1(2.5)	29(14.9)	<0.01
Intensive care not required	39(97.5)	166(85.1)	0.647
COVID-19 positivity in the newborn	**n(%)**	**n(%)**	
Not tested	0	11(5.4)	NA
Negative	40(100)	192(94.6)	
Positive	0	1(0.49)	

Data are mean standard deviation or n (%) unless otherwise specified Values are given as a number (percentage), mean ± standard deviation (range), or median (interquartile range, range).

4. Discussion

The physiological and immunological changes that occur in women during pregnancy lead to complications related to respiratory tract infections, maternal and fetal mortality, or increased morbidity (11, 12). COVID-19 infection in pregnant women leads to higher mortality rates and more negative outcomes associated with the disease course than the general population (13, 14). In a recent meta-analysis of 111 papers, researchers found that SARS-CoV-2 infection significantly increased the risks of premature delivery, pre-eclampsia, stillbirth, neonatal mortality, and maternal mortality compared to uninfected pregnant patients (15). Therefore, the protection of pregnant women against COVID-19 is of great importance in preventing adverse outcomes of the disease (15).

Vaccinating pregnant women against COVID-19 is crucial because of the increased risk of poor outcomes following SARS-CoV-2 infection during pregnancy. In a population study with many cases, it was emphasized that the vaccination acceptance rate of pregnant women was less than the normal population (16). Consistent with this information, our study was conducted on 244 pregnant women with SARS-CoV-2 PCR test positivity, and during the study, the number of vaccinated pregnant women was only 40. This finding demonstrates that the vaccination acceptance rate among pregnant women is relatively low.

Examining the research that evaluates the progression of the infection reveals the following: The cumulative incidence of infection in vaccinated women was significantly lower than in unvaccinated women (17). The hospitalization rate was found to be higher in vaccinated pregnant women than in unvaccinated pregnant women, and the severe covid rate was reported more in unvaccinated pregnant women (5). In our study, consistent with these data, the length of hospital stay was found to be significantly higher in the unvaccinated group.

The results obtained in the current study are similar to the most commonly seen COVID-19 symptoms defined in the literature. According to data from the Centre for Disease Control (CDC) in the USA, the most common symptoms of COVID-19 patients were cough (84%) (18). Aktaş et al. determined fever (70%) most often in the first presentations at the hospital of pregnant women with COVID-19 and reported that the findings of persistent dry cough (34%) and weakness (13%) were seen less often (19, 20). Similarly, in the current study, the most frequently seen symptom was fever at the rate of 65.5%, followed by cough (39.4) and myalgia (12.8%). There was no difference between the groups in terms of these symptoms.

In a surveillance report of pregnant and non-pregnant women with laboratory-confirmed COVID-19 published by the CDC in the USA, it was stated that there was a higher probability of admission to the Intensive Care Unit for mechanical ventilation (0.5% vs.0.3%) for pregnant women compared to non-pregnant women (1.5% vs. 0.9%) (21). In our study, 13 patients in the unvaccinated group were admitted to the intensive care unit. None of the patients in the vaccinated group were admitted to the intensive care unit. However, it should be noted that our study's number of vaccinated patients

is very low. Therefore, based on these data alone, it may not be possible to say that the vaccine reduces the need for intensive care. However, similar results were found in many comprehensive studies (18-21).

Previous studies have reported high rates of obstetric complications such as premature birth and cesarean section delivery in women infected with SARS-CoV-2 (22). Blakeway et al. reported similar stillbirth rates, fetal abnormalities, postpartum hemorrhage, cesarean delivery, small for gestational age, and high maternal dependency unit or ICU or NICU admission rates in vaccinated and unvaccinated pregnant women in their study (19). Morgan et al. reported in their comprehensive study with 10,092 cases that severe coronavirus disease was higher in the unvaccinated group (5). According to the World Perinatal Medical Association COVID-19 Working Group data, 26.3% of pregnancies affected by COVID-19 infection result in premature birth and 4.1% in perinatal death (23). In the current study, 18.2% of the cases resulted in premature birth and 0.5% in stillbirth. According to this data, cesarean section delivery was performed in 54.2% of pregnant women with COVID-19, and this rate was 67% in the current study. The study group report stated that 27.5% of the newborns received intensive care support (24), whereas this rate in the current study was 14.9%.

The main limitation of the study is the small sample size. In addition, the fact that it is a retrospective design study is a disadvantage. However, its major value is that it provides comparison data on a highly significant topic. This issue will become clearer as the number of immunized pregnant women increases if clinicians are provided with more actionable information about vaccination. On this topic, prospective studies with more cases are required.

As efforts continue to improve vaccine acceptance, obstetricians and gynecologists should inform pregnant women of the potential benefits of the SARS-CoV-2 vaccine in preventing serious or critical illnesses.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: S.S., C.A.,S.B., Design: S.S., S.A., Data Collection or Processing: M.A.N, C.A., Analysis or Interpretation: S.S., S.A., R.N., Ş.G.G., Literature Search: S.S., C.A., Writing: S.S., C.A., S.B.

Ethical Statement

The study was approved by the Institutional Review Board (decision no:1191/301220) and the Turkish Ministry of Health (T01-21-35), and it was carried out in accordance with the Helsinki Declaration principles.

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Morphology and mineral composition of dentine of teeth with a wedge-shaped defect

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Abstract

Wedge-shaped defects (WSD) are a common pathology with multi-factorial etiology. The study aimed to evaluate the features of the morphological structure and mineral composition of teeth dentine with a WSD to determine their possible relationship with potential etiological factors. Ten maxillary and mandibular human teeth with a WSD extracted for orthodontic purposes and their longitudinal sections were studied using a JSM-6490 LV focused beam electron microscope (scanning) with a system of energy-dispersive X-ray microanalysis INCA Penta FETx3. We studied the mineral composition of dentine in the incisal region (tubercle), surface junction forming a WSD and in 150 μm from it. We identified a percentage of the weight amounts of some elements in the dentine (carbon, oxygen, calcium, phosphorus, sodium, magnesium, sulfur, chlorine, zinc, potassium, and aluminum). The dentine of the teeth with a WSD was characterized by heterogeneity of the morphological structure, which depended on the topography, and the characteristics of the pathological process; it was combined with the changes in the mineral composition. The most pronounced differences in the ultrastructure of dentine were revealed in the area of the surface junction forming the defect, and they were found at a distance of 150 μm from it. We determined a significant difference in the amount of chemical elements in dentine at the surface junction forming the defect and at a distance of 150 μm from it; in the area of the incisal region (tubercle) (with wear facets and without them), $p \leq 0.05$. It is impossible to identify one etiological factor responsible for the occurrence of a WSD of the teeth. We think their treatment and prevention will be more effective when we understand the morphological features of hard dental tissues' structure and mineral composition.

Keywords: chemical elements, dentine, morphology, non-carious cervical lesions, scanning electron microscope

1. Introduction

The high prevalence of non-carious cervical lesions (NCCL) of teeth (60.2% (1), 88.1% (2)) explains a comprehensive study of the issues of their etiopathogenesis. Some authors described their morphology and cross-section shapes (3-6). Recent studies show that enamel is a highly substituted crystalline apatite, but dentine apatite may play a more critical role in regulating ion exchange as well as mineral crystallinity (7). The characteristics of the morphological structure and chemical composition of hard dental tissues describe complicated processes associated with the conditions caused by age and pathology in the body and identify their development (8). Dentine presents physiological exchanges of trace elements after mineralization, and some factors can influence its concentration (9), including cervical pathology. According to Stănuși et al. (10), microcracks precede the onset of non-carious cervical pathology. Microcrack presence disrupts chemical bonds between hydroxyapatite crystals and follow-up penetration of water and other molecules, making a tooth more vulnerable to dissolution, chemical erosion, or abrasive factors (11). But the ideas about the structure of enamel and dentine change over time in some respects, which encourages further research.

Wedge-shaped defects (WSD) are a common form of NCCL (1, 5, 6). Their occurrence increases with the age of patients (1, 2), affecting the microstructure, hardness, and chemical characteristics of dentine (12). According to Levrini et al. (4), a WSD results from abfraction and has a characteristic macroscopic morphology with many ultrastructural features (3). Igarashi et al. (6) consider their occurrence most likely caused by wear of friction and microstructural loss by stress. The size and depth of the defect are proportional to the intensity and frequency of the applied force (4). In our opinion, the study of a WSD with the help of an electron microscope (scanning) with a system of energy-dispersive X-ray microanalysis makes it possible to assess the peculiarities of the morphology and study the mineral composition (3-5) that is primary in etiopathogenesis. The analysis of the mineral composition of the dentine of the teeth with a WSD was presented in previously published papers (13, 14). Unlike intact samples, there were less sodium, chlorine, and calcium (in the incisal edge [tubercle] and cervical region), sodium, and magnesium (at the equator) detected, $p \leq 0.05$. The dentine of the teeth with a WSD contained more oxygen and sulfur (in the incisal edge (tubercle)), phosphorus and zinc (at the equator), carbon and potassium (in the cervical region), $p \leq 0.05$. Understanding how chemicals affect dentine apatite structure is of great clinical importance (7). According to Femiano et al. (11), local factors can play only a secondary role. So, it is urgent to identify the characteristics of the morphological structure and distribution of dentine chemical elements of the teeth with a WSD.

The study aimed to evaluate the features of the

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morphological structure and mineral composition of teeth dentine with a WSD to determine their possible relationship with potential etiological factors (abrasion, attrition, erosion, abfraction).

2. Materials and Methods

Using a JSM-6490 LV focused beam electron microscope (scanning) with a system of energy-dispersive X-ray microanalysis INCA Penta FETx3 (OXFORD Instruments, England) there were examined ten maxillary and mandibular extracted teeth with a WSD and their longitudinal sections based on previously described method (13). A detailed medical history was collected from the patients whose teeth were examined. A practicing dentist examined these patients. We completed teeth check-ups of patients with no systemic diseases, didn't take regular medications, or had no specific dietary habits. No orthopedic constructions, amalgam fillings, or extracted teeth were in the oral cavity. The teeth were extracted for some orthodontic indications. We studied the morphological structure of the teeth with the help of x10…x5000 magnification (14). To calculate local mass fractions of chemical elements, we used the peak-to-background ratio method, taking into account matrix corrections for atomic number, fluorescence, and absorption, measured in normal mass percentage (normal mass%). The chemical composition of dentine (in a total of 103 areas) in the incisal region (tubercle), surface junction forming a WSD, and in 150 μm from it was studied. We identified it as a percentage of the weight amounts of carbon, oxygen, calcium, phosphorus, sodium, magnesium, sulfur, chlorine, zinc, potassium, and aluminum. Dentine was examined at approximately the same distance from the enamel-dentine border. The research was performed at the base of the Donetsk Institute of Physics and Technology of the National Academy of Sciences of Ukraine.

Statistical analysis was carried out with the help of the Statistica 12.0 computer program (3BA94C4ED07A). We used the G*Power program to calculate the sample size. Student's T-test was used to assess the reliability of obtained results. The differences were believed to be statistically significant at $p \leq 0.05$. There were averaged replication measurements in one sample before carrying out statistical analysis.

3. Results

At the macroscopic level (at magnification up to x20), the lesion focus had a wedge shape, precise contours, and uneven edges. It was located in the cervical region of the vestibular surface of only coronal part of the tooth (Fig. 1) or spread to the root part (Fig. 2). Noteworthy are the numerous cracks that penetrated the enamel and dentine in the lesion focus (Fig. 2B). Cement lesions with exposed dentine were seen in the area of the lower (near-gingival) surface (Fig. 3). At the mesoscopic level (at magnification of x100-5000) the dentine surface looked uneven, crater areas were identified in some samples (Fig. 4). Most of the samples had occlusal/incisal wear facets which had an uneven surface and numerous cracks (Fig. 5). Disruptions of the structure of the intertubular substance in the form of cracks and usuras were revealed (Fig. 6). The dentinal tubules were irregular or elongated, with irregular contours (Fig. 7). Organic material was detected on the dentine surface in a deep WSD (Fig. 8), partial or complete obliteration of the lumen of the dentinal tubules was observed depending on the area of the study (Fig. 9). Partial obliteration was more often identified on the upper (coronal) and lower (near-gingival) surfaces forming the defect (Fig. 9A). Complete obliteration was characteristic of the dentine area that was located at the surface junction forming a WSD (Fig. 9B). In the same area foci of demineralization were more often detected where it was rather difficult to identify the structural elements of dentine (Fig. 10). Dentine acquired a dense structure only at a distance of more than 150 μm from the defect.

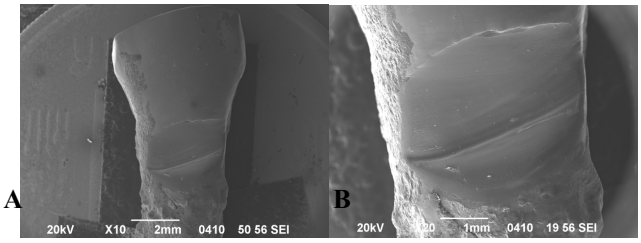

Fig. 1. WSD in the cervical region of the tooth A. Magnification x10 B. Magnification x20

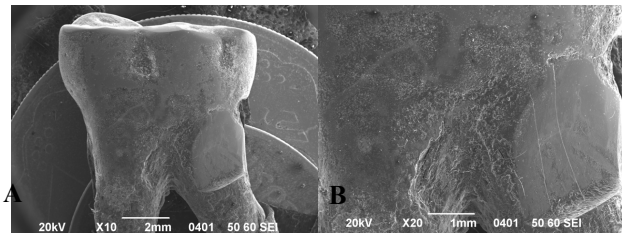

Fig. 2. WSD in the region of the distal root of the mandibular molar A. Magnification x10 B. Magnification x20

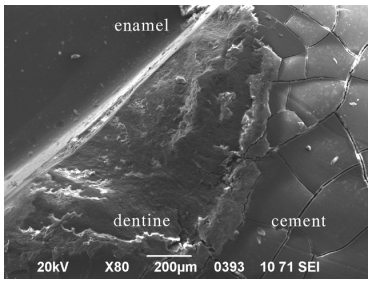

Fig. 3. Cement damage with exposed dentine in the area of the lower (near-gingival) surface of a WSD (magnification x80)

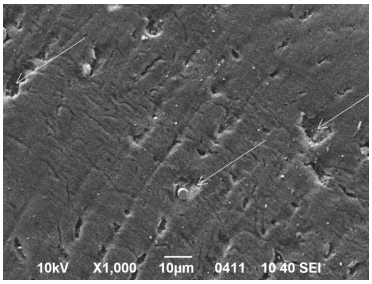

Fig. 4. 'Cratered' features on the dentine surface (magnification x1000) (arrowed)

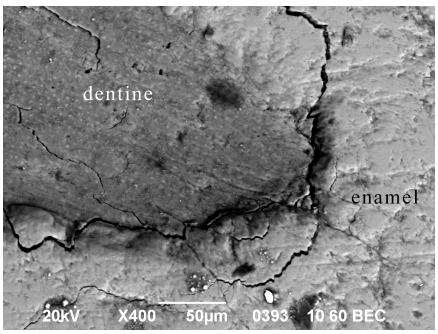

Fig. 5. Surface of wear facet (magnification x400)

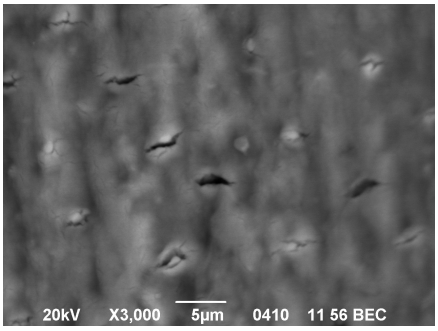

Fig. 6. Changes in the structure of the intertubular substance (magnification x3000)

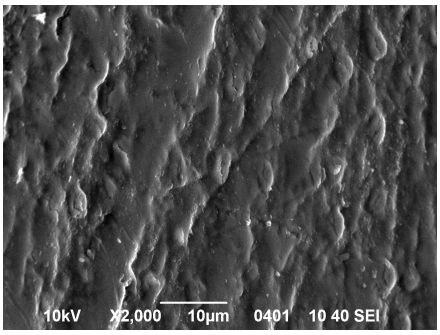

Fig. 7. Dentinal tubules in the area of a WSD (magnification x2000)

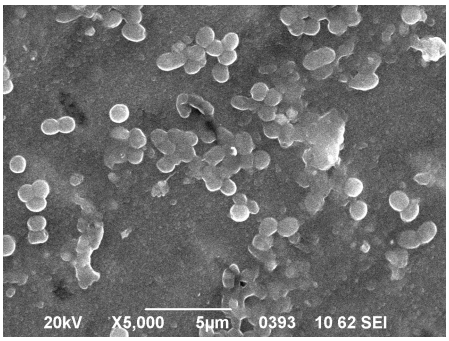

Fig. 8. Organic material on the dentine surface (magnification x5000)

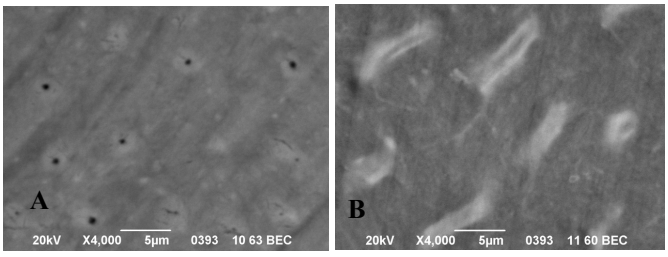

Fig. 9. Obliteration of the lumen of the dentinal tubules in a WSD (magnification x4000) A. Partial obliteration of dentinal tubules B. Complete obliteration of dentinal tubules

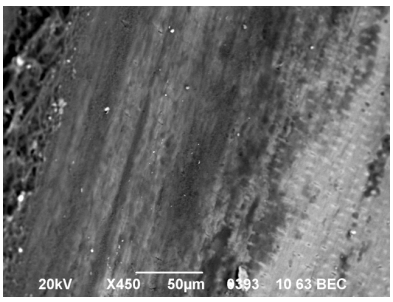

Fig. 10. Dentine at the surface junction forming a WSD (magnification x450)

A disruption of the dentine-enamel bonding with the formation of micro-crevices at the dentine-enamel junction was determined on thin longitudinal sections (Fig. 11). The enamel penetrated the dentine in some specimens (Fig. 12). The sections revealed internal microcracks that passed from enamel to dentine (Fig. 13). In dentine with occlusal/incisal wear facets there were identified the areas of sclerotic dentine where the areas of partial and complete obliteration of the lumen of the dentinal tubules alternated which had the wrong direction or were completely absent (Fig. 14).

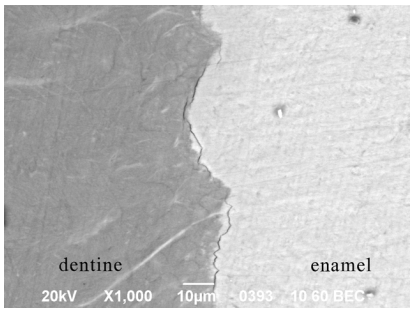

Fig. 11. Microcrevices at the dentine-enamel junction, longitudinal section (magnification x1000)

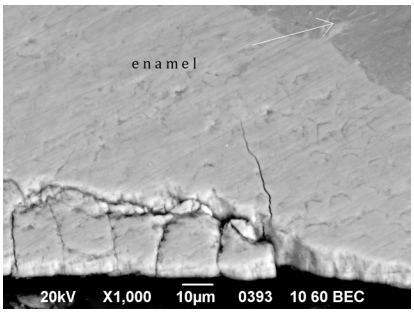

Fig. 12. Penetration of enamel into dentine, longitudinal section (magnification x1000)

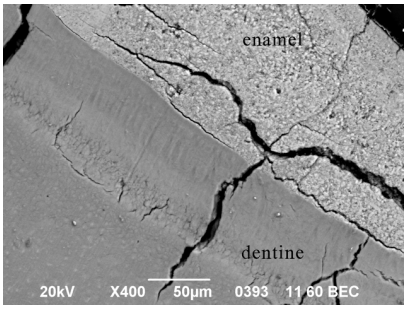

Fig. 13. Microcracks in enamel and dentine, longitudinal section (magnification x400)

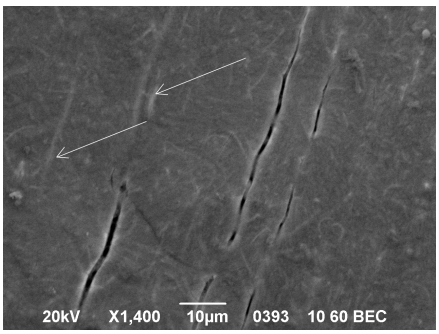

Fig. 14. Sclerotic dentine area, longitudinal section (magnification x1400) (arrowed)

The mineral composition of dentine was determined in the area of the surface junction forming a WSD, where the greatest morphological changes were detected and its comparison with dentine at a distance of 150 µm from the defect (Table 1). Significant differences were obtained in carbon, oxygen, magnesium, phosphorus, and zinc content depending on the study area, p≤0.05. In the area of the surface junction forming a WSD, there was less oxygen, magnesium, phosphorus, zinc, and more carbon, p≤0.05.

Table 1. Mineral composition of dentine in the area of the surface junction forming a WSD and at a distance of 150 µm from it

Chemical element (normal mass %)	Dentine at the surface junction forming a WSD (X ±m)	Dentine in 150 µm from the surface junction forming a WSD (X ±m)	P
C	32.47±9.3678	25.09±2.1645	<0.0001*
O	33.76±4.0247	36.16±2.0153	0.0041*
Na	0.34±0.1465	0.39±0.1152	0.1948
Mg	0.19±0.1628	0.42±0.1751	<0.0001*
Al	0.03±0.0427	0.04±0.0547	0.7138
P	11.34±2.6162	13.55±0.5866	<0.0001*
S	0.17±0.1903	0.17±0.1726	0.8683
Cl	0.11±0.1234	0.09±0.0665	0.0885
K	0.05±0.0659	0.02±0.0333	0.1021
Ca	21.44±5.7701	23.89±0.6777	0.4010
Zn	0.15±0.1694	0.29±0.2966	0.0126*

* Statistically significant

Table 2. Mineral composition of dentine in the area of incisal region (tubercle) and teeth with a WSD

Chemical element (normal mass %)	Dentine with occlusal/incisal wear facets	Clinically intact dentine	P
C	38.36±0.8508	27.52±1.5732	<0.0001*
O	29.24±0.5804	36.54±0.6683	<0.0001*
Na	0.39±0.0612	0.36±0.0644	0.3698
Mg	0.14±0.0438	0.44±0.0817	0.0000*
Al	0.02±0.0120	0.02±0.0232	0.6909
P	11.17±0.2656	12.46±0.5450	<0.0001*
S	0.11±0.0275	0.10±0.0501	0.6103
Cl	0.14±0.0424	0.09±0.0414	0.0668
K	0.10±0.0330	0.02±0.0228	<0.0001*
Ca	20.14±0.5114	22.46±1.0749	<0.0001*
Zn	0.21±0.1144	0.05±0.0572	<0.0001*

* Statistically significant

A comparative analysis of the mineral composition of dentine in the region of the incisal region (tubercle) was also carried out (Table 2). Significant differences were found in the carbon, oxygen, magnesium, phosphorus, potassium, calcium, and zinc content, p≤0.05. Dentine with occlusal/incisal wear facets differed in a smaller amount of calcium, magnesium, phosphorus, and oxygen and a large amount of carbon, potassium, and zinc, p≤0.05.

4. Discussion

The morphological features of the enamel of the teeth with a WSD were described in previously published papers (15). According to Michael et al. (3) and Hayashi et al. (16), short periods of activity and longer periods of stability can be distinguished in the development of NCCL. The active phase is characterized by smooth defect surfaces and clear contours. During it, open dentinal tubules are identified morphologically, while hyperesthesia symptoms are detected clinically (4). The period of stability corresponds to closed dentinal tubules due to the formation of sclerotic dentine with increased density (4). Therefore, depending on the stage of the pathological process, the number of exposed dentinal tubules can vary (3). Probably, it explains the different degrees of obliteration of the lumens of the dentinal tubules developing as a defense mechanism of the body in the specimens with a WSD (17). Chistyakova et Petrouk (18) observed obliteration of the dentinal tubules along the entire length of exposed dentine. According to Daley et al. (19), the differences in the diameter of open and sclerotic tubules at different dentin levels are associated with intratubular dentine deposition. Yan et al. (20) explain the disturbances in intratubular mineralization by an imbalance of calcium and phosphorus ions.

"Dead tracts" were revealed in the dentine of teeth with NCCL by Walter et al. in 88% of the cases (62% were directly in the lesion focus). Sclerosed dentine was observed in the lesion focus of the teeth in 48% (5). According to Abou Neel et al. (21), secondary and tertiary dentine formation is associated with its ability to regenerate. The research results of Tkachenko et al. (8) indicate the presence of hypermineralized dentine in the affected area with stenosis and obliteration of the lumen of the dentinal tubules. The researchers found the greatest changes in the dentine morphology of the teeth with a WSD at the surface junction, forming the defect. The changes spread at a distance up to 150 µm from it. According to the point of view of Guimarães et al. (22), it is connected with higher stress levels focused at the lesion zenith and in the entire cervical region of the tooth (23).

The areas of demineralization were identified in the teeth where the depth of the defect was within the dentine contributing to the formation of NCCL at an early stage, according to Nascimento et al. (24). This is due to the water content in the dentine, through which the diffusion of acid to the mineral components is carried out (25). During demineralization, calcium is released (21), which we observed in the teeth with a WSD (13). Replacing calcium with other chemical elements increases the vulnerability of hard tissues to the effects of acids and their solubility (21). The subsequent

loss of dentine minerals and occlusal attrition contributes to the further development of the pathology and can be considered an etiological factor (26). The revealed significant differences in the amount of sodium in all studied areas of teeth indicate its important role in maintaining the normal state of dentine (27). The significantly higher content of sulfur in the area of the incisal edge (tubercle) and potassium in the cervical region ($p \leq 0.05$) (14) is probably due to the presence of wear facets (2) and microcracks in the enamel (15) of the teeth with NCCl. According to Fernández-Escudero et al. (9), the increase in the concentration of sulfur and potassium in coronal dentine is age-related and does not depend on sexual identity. The development of wear facets in combination with microcracks in teeth with a WSD confirms that occlusal stress is the main etiological factor and the root cause of the development of this NCCL form (11). Badavannavar et al. (25) consider the presence of pathological wear and microcracks to be a manifestation of abfraction. According to Worawongvasu (28), abrasion and erosion are combined etiological factors in the formation of NCCL. Significant differences in the amount of magnesium and zinc indicate their influence on the morphology, crystallinity, and solubility of dentine apatite (7).

The dentine surface of some samples, like the enamel (15), had crater areas and organic material, which agreed with the data (3, 4). The development of crater areas is associated with the effect of corrosion factors (acid attack), or it is considered a sign of abfraction. The presence of organic material indirectly indicates the roughness of the dentine surface. Numerous cracks in the lesion focus of the teeth with a WSD were described in the publications as the most common in this form of NCCL and as a result of abrasion or abfraction (3, 4). The cracks in the dentine did not have a clear direction associated with the peculiarities of its structural organization (29) or multi-factorial etiology (3). It is possible that they may be an artifact (3).

Longitudinal sections revealed internal microcracks with numerous branches passing from enamel to dentine. Similar results were described by Stănuși et al. (10). But dentine is able to withstand significant loads and perform its functions even in the presence of a large number of cracks and significant damage. The penetration of enamel into dentine revealed in the study in some areas of the dentine-enamel junction was called "enamel bridges," which are involved in the maturation of enamel and dentine (12).

In conclusion, obtained results made it possible to clarify the microstructure of dentine in the wedge-shaped form of NCCL, and determine the features of its mineral composition. But it is impossible to identify one etiological factor responsible for the occurrence of a WSD of the teeth. They are probably an external manifestation of the combination of abrasion, attrition, erosion, and abfraction. In our opinion, the treatment and prevention of a WSD will be more effective when understanding the morphological features of hard dental tissues' structure and chemical composition, which justifies further research.

Conflict of interest

The authors declared no conflict of interest.

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No funding was used for the study.

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None to declare.

Authors' contributions

Concept: I.I.Z., Design: I.I.Z., Data Collection or Processing: I.I.Z., Analysis or Interpretation: I.I.Z., Literature Search: I.I.Z., Writing: I.I.Z.

Ethical Statement

The study was carried out based on the principles of the WMA Declaration of Helsinki Ethical Principles for Medical Research Involving Human Objects as amended in 2013, Order No. 690 of the Ministry of Health of Ukraine (dated September 23, 2009) and approved by the Bioethics Commission of Donetsk National Medical University (No 43, dated January 21, 2021). Before the study, written informed consent was taken from all the participants.

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COVID-19 disease and vaccine status in tuberculosis patients in Sakarya

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Abstract

Tuberculosis was the leading cause of death worldwide before COVID-19. The present study aimed to examine the cases of COVID-19 disease and vaccination against COVID-19 in patients receiving tuberculosis treatment in Sakarya. In this descriptive study, 132 people aged 18 years and over, registered in Sakarya Tuberculosis Dispensary and continuing treatment in 2021, were included. Cases of COVID-19, hospitalization, intensive care unit status and vaccination status against COVID-19 were recorded. Statistical significance level was accepted as $p<0.05$. The mean age of the patients was 49.8±20.6 years. 65.2% of the patients were male (n=86). It has been determined that 21.2% (n=28) of the patients have been diagnosed with COVID-19. No statistically significant difference was observed between the patients' gender and history of COVID-19 infection with mortality ($p>0.05$). There was no statistically significant difference between the ages of groups with and without COVID-19 ($p>0.05$). Age was statistically significantly different in the surviving and deceased groups ($p<0.05$). There was a statistically significant difference between the vaccination status of the patients and mortality ($p<0.05$).People being treated for tuberculosis are at risk as long as the danger of COVID-19 continues. More emphasis should be placed on the importance of vaccination against COVID-19, especially in elderly individuals receiving tuberculosis treatment.

Keywords: COVID-19, tuberculosis, vaccination, mortality

1. Introduction

COVID-19, caused by SARS-COV-2, is a highly contagious acute viral disease. On the other hand, tuberculosis is a chronic bacterial infection, and the most common form is pulmonary tuberculosis. They present with similar symptoms, such as cough, fever, and respiratory distress (1). As of November 16, 2022, the COVID-19 pandemic has been reported to have caused 632 million confirmed cases and 6.5 million deaths worldwide (2). It was revealed that tuberculosis was the leading cause of death worldwide before COVID-19, resulting in the death of approximately 1.5 million people per year (3). Immune status, which makes people susceptible to tuberculosis, may also be a risk factor for COVID-19 infection (4). According to historical evidence, higher mortality is observed in patients with concomitant tuberculosis infection with respiratory viruses (5). Findings indicating a more severe course of COVID-19 in tuberculosis patients have been presented in some studies (6-8) According to a meta-analysis, the prevalence of tuberculosis was between 0.4% and 4.4% among COVID-19 patients. In addition, the prevalence of tuberculosis in severe COVID-19 cases was higher as well (9) People with chronic diseases such as hypertension, diabetes mellitus, lung cancer, and chronic obstructive pulmonary disease are also at higher risk of death and hospitalization associated with COVID-19 and tuberculosis (10,11).

The burden brought by COVID-19 and tuberculosis on the health system is very high and causes many years of life to be lost (12). Available data are insufficient to understand the potential impact of COVID-19 on treatment outcomes of tuberculosis patients (13,14). With the development of effective vaccines against COVID-19, deaths have decreased, but mortality may still be high in unvaccinated individuals and those with underlying chronic diseases (15,16). Due to the fact that the COVID-19 pandemic has not yet lost its impact worldwide and we are likely to experience a new phase of the epidemic as a result of new variants that may occur at any moment, there is a need to examine the current status of COVID-19 and the vaccination status of current tuberculosis patients. The present study aimed to examine the cases of COVID-19 disease and vaccination against COVID-19 in patients receiving tuberculosis treatment in Sakarya.

2. Materials and Methods

The research was designed as a descriptive study. One hundred thirty-two patients aged 18 and over who were registered in Sakarya Adapazarı Tuberculosis Dispensary, diagnosed with active pulmonary tuberculosis in 2021, and whose treatment was ongoing were included in the study.

According to the Tuberculosis Diagnosis and Treatment guide published by the Ministry of Health of the Republic of Turkey, the diagnosis of pulmonary tuberculosis is made bacteriologically in three methods: (17)

1. Smear-positive pulmonary tuberculosis

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2. Smear-negative pulmonary tuberculosis

3. Molecular test positive pulmonary tuberculosis

SARS-COV-2 positivity is detected by PCR in the combined nose and throat swab obtained as a result of the physician's decision from the people who applied to the hospital with any complaint.

After the approval of the ethics committee, patient records were obtained from the tuberculosis dispensary. The information of the patients about COVID-19 was taken from the digital database. Vaccine data were also questioned from the vaccine tracking system application of the Ministry of Health. In the study, the information of the people who received active pulmonary tuberculosis treatment in 2021 was examined on June 30, 2022. Cases of COVID-19, hospitalization, intensive care unit (ICU) status and vaccination status against COVID-19 were recorded. There are three types of vaccines used against COVID-19 in Turkey at the time of the research. These are Sinovac (CoronaVac), Pfizer/ BioNTech (BNT162b2), and Turkovac (ERUCoV - VAC) vaccines. At the time of our study, it was observed that the Turkovac vaccine was not administered to any patient since it was new. Thus, it will not be mentioned in the rest of the study. The vaccination status of the patients was categorized as follows, considering the guidelines of the Turkish Ministry of Health (18). The first group consisted of patients who had not been vaccinated at all or who had not received two doses of the BNT162b2 vaccine and had less than three doses in total. The patients in the second group were those who did not have two doses of the BNT162b2 vaccine but had at least three doses of the vaccine in total. The third group was those who had at least two doses of the BNT162b2 vaccine.

SPSS 22.0 program was used in the statistical analysis of the data. In descriptive statistics, frequency and percentages were used for qualitative data, and mean, standard deviation, and median (minimum; maximum) values for quantitative data. Pearson Chi-square, Yates corrected Chi-square, Fisher's Exact Test, and Student's t-test performed the statistical analyses. The statistical significance level was accepted as $p<0.05$.

3. Results

One hundred thirty-two patients were included in the study. The mean age of the patients was 49.8±20.6 years. 65.2% of the patients were male (n=86). In addition to pulmonary tuberculosis, 6.1% (n=8) of the patients also had extrapulmonary tuberculosis disease. 8.3% (n=11) of the patients applied for recurrence.

It was determined that 21.2% of the patients were diagnosed with COVID-19 after the date they started to receive tuberculosis treatment. 28.6% (n=8) of those who had the disease were treated in the hospital ward, and 21.4% (n=6) were treated in the intensive care unit. The median days of the patients receiving treatment in the ward were 1.5 (min:1 max:10), and the median days that the patients treated in the intensive care unit received treatment was 10 (min:5 max:21). It was observed that 10.6% (n=14) of the patients died during the follow-up. 41.7% (n=55) of the patients had at least two doses (BNT162b2) vaccine. 79.2% (n=84) of those who had at least one dose of vaccine had at least one dose of vaccine in the last six months. (Table 1)

Table 1. Distribution of Disease and Vaccination Status, Sakarya, 2022

		Frequency (Percent)
COVID-19 Status (n=132)		
	Have-had	28 (21.2)
	Have not had	104 (78.8)
Hospitalization Status (n=28)		
	Those with service admission	8 (28.6)
	Those without service admission	20 (71.4)
Hospitalization in ICU (n=28)		
	Treated in ICU	6 (21.4)
	Not treated in ICU	22 (78.6)
Death Status (n=132)		
	Deceased	14 (10.6)
	Survivors	118 (89.4)
Vaccination Status (n=132)		
*	Group 1	52 (39.4)
	Group 2	25 (18.9)
	Group 3	55 (41.7)
Vaccination Status in the Last Six Months (n=106)		
	Non- vaccinated	22 (20.8)
	Vaccinated	84 (79.2)

*Group descriptions are specified in the methods section.

Table 2. Investigation of the Reasons Affecting the Patients' Status of Passing COVID-19, Sakarya, 2022

		COVID-19 Status		
		Not have Had n (%)	Have Had n (%)	* p
Gender	Male	73 (84.9)	13 (15.1)	**0.034** [y]
	Female	31 (67.4)	15 (32.6)	
Vaccination Status	Group 1	39 (75.0)	13 (25.0)	0.637
	Group 2	21 (84.0)	4 (26.0)	
	Group 3	44 (80.0)	11 (20.0)	

Row percentage is presented * Pearson chi-square test was performed. [y]: Chi-square test with Yates correction was performed. Group descriptions are specified in the methods section.

No statistically significant difference was observed between the vaccination status of the patients and their COVID-19 exposure (p=0.637). There was a statistically

significant difference between gender and COVID-19 transmission status (p=0.034). (Table 2)

No statistically significant difference was observed between the patients' gender (p=0.242) and history of COVID-19 infection (p=0.076) with mortality. There was a statistically significant difference between the vaccination status of the patients and mortality (p=0.013). It was determined that the effect of vaccination status on survival was caused by the difference between group 3 and both group 2 and group 1. No difference was found between group 1 and group 2. It was revealed that 35.7% (n=5) of the deceased died during active COVID-19 disease. When the vaccination status of these five patients was examined, four had no vaccine against COVID-19. (Table 3)

Table 3. Investigation of the Causes Affecting the Survival of the Patients, Sakarya, 2022

		Death Status		
		Survivors n (%)	**Deceased n (%)**	* p
Gender	Male	79 (91.9)	7 (8.1)	0.242 [f]
	Female	39 (84.8)	7 (15.2)	
Vaccination Status	Group 1	42 (80.8)	10 (19.2)	**0.013**
	Group 2	22 (88.0)	3 (12.0)	
	Group 3	54 (98.2)	1 (1.8)	
COVID-19 Status	Have had	96 (92.3)	8 (7.7)	0.076 [f]
	Have not had	22 (78.6)	6 (21.4)	

Row percentage is presented * Pearson chi-square test was performed. [f]: Fisher's Exact Test was performed. Group descriptions are specified in the methods section.

There was no statistically significant difference between the ages of groups with and without COVID-19 (p=0.843). Age was statistically significantly different in the surviving and deceased groups (p<0.001). (Table 4).

Table 4. Examining the Relationship between Patient Age and COVID-19 and Mortality Status, Sakarya, 2022

	Age	***p**
	Mean ± Standard Deviation	
COVID-19 Status (n=132)		0.843
Have not had	50.0 ± 20.1	
Have had	49.1 ± 22.6	
Survive Status (n=132)		**0.001**
Survivors	46.9 ± 19.3	
Deceased	73.8 ± 14.9	

*Student's T-test was performed.

4. Discussion

In the examined period, it was observed that 21.2% of the patients had COVID-19 infection. Considering the factors affecting the status of being COVID-19-positive, a significant difference was determined only in terms of gender. The incidence of COVID-19 was higher in females. In a qualitative study conducted in Turkey, people with active pulmonary tuberculosis were interviewed, and it was revealed that 28.6% had COVID-19 (19). In a database review performed with a method similar to this study, patients who received tuberculosis treatment in the last five years were examined, and 11.2% had COVID-19 (20).

The present study revealed that 28.6% of the patients were admitted to the hospital ward and 21.4% to the ICU. In one study, the hospitalization rate for COVID-19 was reported as 12% (21). In a study examining 654 patients in Turkey, the rate of ICU admission due to COVID-19 was 7.5% (22). In our example, it has been demonstrated that patients who had recently started tuberculosis treatment had higher rates of hospitalization and ICU admission.

The COVID-19 mortality rate in patients with tuberculosis was 17.8%. In a study conducted in Turkey in the early period when there was no COVID-19 vaccine yet, mortality in the coexistence of tuberculosis and COVID-19 was found to be 6% (23). According to the latest reports of the World Health Organization, as of November 2022, the overall mortality rate of COVID-19 is 1.04% (2). According to the data of the Ministry of Health in Turkey, the overall mortality rate is 0.6% (24). Based on this data, it can be considered that active tuberculosis patients are more likely to die if they have COVID-19 than other COVID-19 patients.

In our study, a significant difference was observed between the deceased and surviving groups regarding age. While the mean age of the deceased group was 73.8±14.9 years, it was 46.9±19.3 years in the survivors. A study performed in California reported that the mean age of those who died in the group with tuberculosis and COVID-19 was significantly different from the group that survived, and the median age of those who died was 81 (25). In a meta-analysis study, it was reported that age affected death in 89 COVID-19 patients with tuberculosis (26).

The present study demonstrated a significant difference between the vaccination statuses against COVID-19 in the deceased and surviving groups. It was observed that those who had at least two doses (BNT162b2) vaccine died less frequently than those in other groups. Only 41.7% of our patients had at least two doses (BNT162b2) vaccine. 39.4% of the patients were either never vaccinated or had at most one dose of any vaccine. As of November 2022 in Turkey, 85.6% of the population received at least two doses of any vaccine (27). This rate was 76.3% for Sakarya province (27). In tuberculosis patients, a very risky group for COVID-19, these rates were even below the general population. A qualitative study performed in Turkey reported that almost all of the 14 tuberculosis patients were vaccinated, and 71% were vaccinated by the (BNT162b2) vaccine (19).

People being treated for tuberculosis are at risk as long as the danger of COVID-19 continues. When combined, the mortality risk of both tuberculosis and COVID-19 causes catastrophic clinical outcomes. More emphasis should be placed on the importance of vaccination against COVID-19, especially in elderly individuals receiving tuberculosis treatment. We believe that more research will be needed on how often booster vaccines should be given to these disadvantaged groups.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: E.A.G, S.T.Ç, A.Ö Design: E.A.G, S.T.Ç, İ.O Data Collection or Processing: E.A.G, N.A, F.A.K, Z.D.M, Ş.T Analysis or Interpretation: E.A.G, S.T.Ç, N.A, İ.O, F.A.K Z.D.M, Ş.T Literature Search: E.A.G, S.T.Ç, A.Ö Writing: E.A.G, S.T.Ç, Z.D.M

Ethical Statement

Ethics committee approval for the study was obtained from Sakarya University Faculty of Medicine Non-Invasive Research Ethics Committee with the application file number 154 on June 02, 2022.

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The efficiency of ergonomics, active break and stretching exercise program in office workers with chronic neck and back pain

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Abstract

The prevalence of chronic neck, shoulder and back pain in office workers is increasing rapidly. Chronic musculoskeletal pain has great socioeconomic impact as it is associated with reduced work performance and poor quality of life. The purpose of this study is to evaluate the influence of ergonomic modification, active rest, and stretching exercise programs on office workers' neck, upper back and shoulder pain. In our study, 74 patients aged between 22-50 years working in the office at the computer were evaluated. Brochures including ergonomic modification, active rest and stretching exercise program were given to the patients and necessary information was given. Visual Analogue Scale (VAS) was used to evaluate neck, shoulder and back pain and neck disability index (NDI) was used to evaluate functional outcomes. Evaluations were performed before treatment and at the first and third months after treatment. The VAS value, which was 5.39 ± 0.62 before the treatment, was 2.86 ± 1.40 at the first month after the treatment and 2.66 ± 1.15 at the third month after the treatment, and this improvement in the VAS score was statistically significant ($p<0.01$). While the NDI score was 21.91 ± 1.40 before the treatment, it was calculated as 12.77 ± 5.39 in the first month after the treatment and 12.02 ± 4.93 in the third month after the treatment, and this improvement in the NDI score was statistically significant ($p<0.01$). In conclusion, neck, shoulder and upper back pain can be reduced in patients working in the office environment with the help of ergonomics, stretching, and taking active breaks as much as working conditions allow.

Keywords: office workers, pain, neck, stretching, ergonomics, active breaks

1. Introduction

Today, desk-office workers have remained sedentary for extended periods due to computer usage. The repetitive strain of the musculoskeletal system related to poor-ergonomic equipment is called work-related musculoskeletal disorders (1-4). The prevalence of chronic neck, upper back, and shoulder pain experienced by office workers, has been increasing rapidly (5).It is reported that the prevalence of neck and shoulder pain is %55 and %38, respectively, in office workers (6). Chronic neck, back, and shoulder pain have a substantial socioeconomic impact because of associated with decreased job performance and poor life quality (7). Approximately the %56 of the reports received are due to diseases that are work-related musculoskeletal disorders (8). In the treatment of these patients, treatment methods such as exercises (9,10) manual therapy, massage, ergonomics, and group therapies are used (11). In addition, steps have been taken to prevent neck, shoulder and upper back pain or prevent its recurrence in recent years.

Although there have been many studies evaluating the effectiveness of methods including ergonomic modification, rest breaks and exercise, conflicting results have been reported. For example, in a randomized controlled study, Shariat et al. (12) compared ergonomic modification, exercise, ergonomic modification combined with exercise, and control groups with the Cornell Musculoskeletal Disorders Questionnaire, and they found significant improvement in all three treatment groups compared to baseline values and control group. however, they reported that there was no significant difference between the treatment groups.

In 2007 and 2013 reviews, the effectiveness of conservative interventions for the treatment of arm, neck or shoulder-related complaints in office workers was evaluated and it was reported that exercise had a similar effect on pain reduction, recovery, and sick leave when compared to the control group (13,14). However, this evidence was gathered from poor-quality studies.

The purpose of this study is to evaluate the influence of ergonomic modification, active rest, and stretching exercise programs on office workers' neck, upper back and shoulder pain.

2. Material and Methods

74 patients (60 female, 14 male) with neck, shoulder, and upper back pain, who applied to our polyclinic between 01/07/2021 and 30/11/2021, were included in our study. Inclusion criteria: A full-time office worker aged 22-50, with at least five years of work experience, with Visual Analog Scale (VAS) ⩾5/10cm moderate to severe neck, shoulder, or back pain for over three

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months. The exclusion criteria were determined as follows: VAS <5/10cm, pregnancy, history of trauma or surgery in the neck region, cervical radiculopathy, congenital spine anomaly, rheumatic disease, and tumor. Demographic characteristics such as age, gender, body mass index, sitting time/day, computer use time/day, comorbidities, history of muscle injury, and regular use of medicines like analgesics were recorded.

The patients were given exercise brochures to ensure that exercises were done correctly, and how to do each movement was shown by the specialist physician. The exercise program included neck, shoulder, trunk, upper back stretching as well as shoulder rolling exercises with ten repetitions of each exercise. It was recommended to be done in two sessions a day, five days a week, through four weeks (3).

For ergonomic intervention, brochures were given to the patients about the optimal height of the chair and desk, sitting posture, the distance and level between the eyes and the monitor, the position of the arm and elbow while holding the mouse, and they were asked to adjust their working position according to their workplace (15).

Each patient was demanded to take active breaks that they could adjust according to their working conditions, and it was recommended not to sit in a chair during breaks. It was stated that the frequency and duration of the breaks could be adjusted between 30 seconds and 15 minutes per break and 3-20 times per workday.

The primary outcome was the pain score measured using the VAS (16). For the severity of the pain, according to the VAS, the "no pain" is usually graded as 0 points, while the "worst pain imaginable" is 10 points. The Neck Disability Index (NDI) was used in the secondary outcomes to assess neck function and quality of life (17). Evaluations were made in the 1st and 3rd months after the treatment, at the first polyclinic treatment. Adjusting to the exercise program was determined by the frequency of exercise reported by the participants. Statistical method: Statistical analysis was performed by using the IBM SPSS Statistics 22 software (IBM Corp., Armonk, NY, USA). Means and standard deviations were used for baseline patient characteristics, and percentages were used for categorical variables. Paired T-test was used for pair wise comparisons of VAS and NDI scores before and after treatment, and change over time was evaluated by repeated analysis of variance analysis. The P <0.05 was considered to be statistically significant.

3. Results

The study consisted of 74 participants, 60 of which were women while 14 of which were men. The average age of the patients was 39.4 (between 22-50) years, and the average symptom duration was 17.9 (5-39) months. The average time spent on computers was 6.1 hours a day (2-8 hours) and % 35.1 of the patients had to use analgesics due to neck and back pain (Table 1).

Table 1. Demographic data of the patients included in the study

Age (year)	39.4 ± 8.0
Female sex, n (%)	60 (81)
BMI	25.6 ± 8.1
Computer usage duration (hours/day)	6.1 ± 1.9
Symptom duration (months)	17.9 ± 8.7
Regular analgesic usage, n (%)	26 (35.1)

BMI: Body Mass Index

While the pre-treatment VAS value was 5.39 ± 0.62, it was calculated as 2.86 ± 1.40 in the first month after the treatment and 2.66 ± 1.15 in the third month after the treatment. This improvement in the VAS score was found to be statistically significant in the first month after the treatment (p<0.01), and it continued to progress in the third month after treatment. While the pre-treatment NDI score was 21.91 ± 1.40, it was calculated as 12.77 ± 5.39 in the first month of post-treatment and 12.02 ± 4.93 in the third month of post-treatment. This improvement in NDI score was statistically significant in the first month of post-treatment (p<0.01) and continued to progress in the third month of treatment (Table 2).

Table 2. Change in VAS and NDI values before and after treatment.

	Pre-treatment	Post-treatment (1st month)	Post-treatment (3rd month)	p
VAS	5.39 ± 0.62	2.86 ± 1.40	2.66 ± 1.15	<0.01
NDI	21.91 ± 1.40	12.77 ± 5.39	12.02±4.93	<0.01

4. Discussion

The most important finding of this study is that stretching exercises, arranging ergonomics and taking active breaks as long as workplace conditions allow have positive effects on reducing neck, shoulder and back pain in patients who work at a computer for a long time. The results of this study support other studies in the literature. For instance, Irmak et al.[18] in a randomized controlled study with 39 desk-worker patients, reported that a 10-week exercise scheme reduced pain perception in office workers. Tunwattanapong et al. (3) gave 96 patients with neck pain an informative brochure showing the correct position and ergonomics to be applied during daily work. In addition to the treatment group, they performed neck and shoulder stretching exercises five days a week and twice a day for four weeks. They reported that exercise reduced pain, improved neck functions and increased quality of life in office workers with chronic neck and shoulder pain.

Many studies emphasize that strengthening exercises to the shoulder, arm, and hand muscles can relieve neck or shoulder pain in office workers (19-22).Johnston et al. (23) in a randomized controlled study with 740 patients, through 12 weeks, the group which was given exercise and the group which was promoted health but not given exercise group are compared after ergonomics training, given to the patients and

at the 12th week, it was reported that there had been neck pain relief in the exercise group (p=0.02), however, they reported that the improvement in pain intensity could not be sustained in both groups at 12 months. The better combination to gain the maximum benefit from the exercises is to combine stretching exercises with the acute to sub-acute phase of musculoskeletal pain, followed by strengthening exercises with pain reduction (3). In this study, we did not recommend strengthening exercises to patients; we only recommended stretching exercises because our study aimed to treat acute and sub-acute neck and back pain periods.

Many studies show that ergonomics and breaks are adequate to prevent pain in office workers who suffer from the treatment of musculoskeletal pain (24-27). Akkarakittichoke et al. (24) reported that active breaks and ergonomic interventions shortened recovery time and reduced recurrence of neck and low back pain in 193 high-risk office workers. Breaks allow for a reduction in computer exposure time and muscle relaxation. A field research (28) on office sitting posture and sedentary time demonstrated that, on average, employees sit for extended periods without taking a break. Still, with a schedule reminding them to take a break, it was highly adaptable to take short breaks every hour. We did not apply for a standardized break program in our study. We proposed a program in which the patients determine the break frequency and duration in accordance with their working hours at the workplace. A decrease in the frequency of musculoskeletal pain, discomfort, and reporting has been reported owing to ergonomics (29). There is also evidence that ergonomic office interventions effectively reduce costs associated with musculoskeletal disorders and can increase employee productivity (30). A systematic review showed moderate-quality evidence of no benefit from isolated ergonomic interventions for pain reduction (31). Hence, we suppose that ergonomic interventions should not be isolated; instead, they should be combined with active break and exercise schemes.

There is some restrictions in our study. The first is the absence of a control group in our study. Another of which is the short tracking period. We cannot know whether the effect of the applied treatment will be maintained for a lengthy period. Another restriction is that the exercise program is carried out, the frequency and duration of active breaks are not standardized, and the assessment is according to the patient's statement.

In conclusion, neck, shoulder and upper back pain can be reduced in patients working in the office environment with the help of ergonomics, stretching, and taking active breaks as much as working conditions allow.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: O.Ç., Design: O.Ç., Data Collection or Processing: O.Ç., Analysis or Interpretation: O.Ç., Literature Search: O.Ç., Writing: O.Ç.

Ethical Statement

Approval was obtained from İstanbul Medipol University Non-Invasive Clinical Research Ethics Committee, the study started. The ethics committee decision date is 16/02/2023 and the number of ethical committee decisions is 190.

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Research Article

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Utility of preoperative neutrophil–lymphocyte and platelet–lymphocyte ratios in differential diagnosis of benign, borderline, and malignant ovarian tumors

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Abstract

To investigate the utility of preoperative neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) in the differential diagnosis of benign, borderline, and malignant ovarian tumors. This retrospective study was conducted on patients with adnexal masses who underwent surgical resection at Haseki Training and Research Hospital between September 2008 and September 2015. Sociodemographic characteristics, histopathological results and laboratory parameters were obtained from medical reports. Hematological parameters and calculated NLR and PLR were analyzed between tumor groups using IBM SPSS statistics version 22. 381 patients with a mean age of 40.81 (age range: 13-83 years) were included. Of those, 293 patients (76.91%) had benign ovarian tumors, 18 (4.72%) had borderline, and 70 (18.37%) had malignant ovarian tumors. The mean NLR was 2.318 ± 2.29 and the mean PLR was 134.35 ± 59 in benign ovarian tumors, the mean NLR was 4.27±5.23 and the mean PLR was 165.06±98.72 in borderline ovarian tumors, the mean NLR was 4.08±4.37 and the mean PLR was 194.72±114.85 in malign ovarian tumors. The NLR and PLR were significantly higher in malignant than benign or borderline ovarian tumors (all p= 0.0001). The diagnostic cut-off value of NLR for differentiating between benign or borderline and malignant tumors was 1.17, whereas that of PLR for distinguishing between benign/borderline and malignancy was 87.94.The present study indicated that NLR and PLR were significantly higher in ovarian cancers than in benign or borderline ovarian masses. Preoperative NLR and PLR values may help distinguish malignant from benign or borderline ovarian tumors.

Keywords: ovary tumor, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR)

1. Introduction

Ovarian cancer was the third most common gynecologic cancer with the highest mortality rate (1). The high mortality rate of ovarian cancer is attributed to its asymptomatic nature, the lack of early detection and surveillance methods, and the delayed onset of symptoms. In addition to these factors, the lack of sensitive and specific markers for the early detection of ovarian cancer results in the majority of cases being detected at an advanced stage (2, 3).

Several studies reported that inflammation plays a major role in the development of a variety of cancers by various mechanisms, including up-regulation of cytokines and inflammatory mediators, inhibiting apoptosis, triggering angiogenesis, and stimulating DNA damage (4). In addition, systemic inflammatory response mediators inhibit immune function by increasing leukocytes, neutrophils, and platelets and decreasing lymphocytes. Furthermore, platelet count may increase due to malignant disease.

Preoperative inflammatory markers, namely neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) may help to differentiate malignant from benign or borderline ovarian tumors in the preoperative period. NLR and PLR are noninvasive, easily measured, and cost-effective markers. They have been used as predictive markers or prognostic factors for a variety of cancers (5). Although the pathophysiological mechanisms underlying inflammation and oncogenesis have not yet been fully understood, the development of new biomarkers that predict cancer continues to attract attention. Therefore, we aimed to assess the utility of preoperative NLR and PLR levels to differentiate malignant from benign or borderline ovarian tumors.

2. Materials and Methods

This retrospective study included 381 patients who underwent surgery for a suspected adnexal mass between September 2008 and September 2015 in the Obstetrics and Gynecology Clinic of the Haseki Training and Research Hospital (Approval number: 299). The study protocol was approved by the Research Ethics Committee. The patient's demographic and clinical characteristics, including age, preoperative hematological parameters, and final histopathological results

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were reviewed from the hospital's electronic medical records. According to the final histopathological results, patients were divided into three groups: benign, borderline, and malignant. Preoperative complete blood count parameters were recorded and evaluated for each group, including white blood cell count, neutrophil count, lymphocyte count, monocyte count, NLR, platelet count, and PLR.

Patients with pre-existing local or systemic infection, chronic diseases, blood transfusions in the last three months, other malignancies, thrombolytic drugs, and those diagnosed with para tubal cyst, para ovarian cyst, leiomyoma, and tuba ovarian abscess were excluded from the study.

2.1. Statistical Analyses

The data were analyzed using IBM SPSS statistics version 22 (IBM, Armonk, NY, USA) with a significance of 0.05. Categorical data were presented as numbers (n) and percentages (%), while quantitative data were presented as means and standard deviations. Hematological parameters, geometric means for NLR and PLR, and 95% confidence intervals were compared according to age classification and tumor pathology using one-way analysis of variance (ANOVA). The optimal cut-off value was determined via ROC curve analysis. To determine the optimal cut-off value for the NLR and PLR, we found a cut-off level that maximizes Youden's J statistic. The area under the curve (AUC), specificity, and sensitivity were calculated. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 381 patients with a mean age of 40.81 (13-83) years were included in the study. The final histopathological reports revealed that 293 patients (76.91%) had benign ovarian tumors, 18 (4.72%) had borderline, and 70 (18.37%) had malignant ovarian tumors. The mean age of patients with benign, borderline, and malignant tumors was 37.76, 50.72, and 51.02, respectively.

The histopathological characteristics of patients with benign ovarian tumors (n=293) were as follows: serous cystadenoma (n=95), mature cystic teratoma (n=79), endometrioma (n=56), mucinous cystadenoma (n=28), corpus luteum (n=14), serous cystadenofibrom (n=7), fibroma-fibrothecoma (n=7), struma ovarii (n=2), and benign Brenner tumor (n = 1).

The histopathological characteristics of patients with malignant ovarian tumors (n=70) were as follows: serous papillary carcinoma (n=39), mucinous carcinoma (n=13), endometrioid carcinoma (n=4), Kruckenberg tumor (n=3), granulosa cell tumor (n=3), and dysgerminoma (n=2). Borderline pathologies (n = 18) included mucinous borderline tumors (n = 11) and serous borderline tumors (n=7).

Patients with benign ovarian tumors were significantly younger than those with borderline and malignant tumors (p <0.001).

In benign ovarian tumors, the mean neutrophil/lymphocyte ratio (NLR) was found to be 2.318 ± 2.29 (0.05-20.62) and the mean PLR was 134.35 ± 59 (0.93-588.33), in borderline ovarian tumors mean NLR was 4.27±5.23 (0.79-17.03) and mean PLR was 165.06±98.72 (51.85±391.53), in malignant ovarian tumors mean NLR count was 4.08 ± 4.37 (1-28.29) and mean PLR count was 194.72 ± 114.85 (56.32-623.44).

There was no statistically significant difference between age groups and NLR and PLR of patients (p= 0.93, 0.95, respectively). The geometric means of NLR and PLR by age groups are shown in Table 1.

Table 1. Comparison of geometric mean neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) by age groups

Age	n	NLR	PLR
<45	235	2.06 (1.92-2.25)	127.91 (117.98-136.73)
45-54	75	2.06 (1.76-2.43)	134.74 (122.07-149.69)
55-64	38	2.15 (1.79-2.60)	124.08 (92.90-154.31)
≥65	33	2.38 (2.04-2.88)	133.25 (112.97-159.93)
Total	381	2.10 (1.97-2.24)	129.29 (121.39-136.36)
ANOVA p		0.93	0.95

Table 2. Appropriate cut-off value, sensitivity and specificity for differentiating benign or borderline and malignant ovarian tumor using ROC curve analysis

	Cut-off value	AUC	95% CI	P-value	Sensitivity (%)	Specificity (%)
NLR (Benign or borderline vs malignancy)	1.17	0.66	0.59-0.73	<0.001	91	13
PLR (Benign or borderline vs malignancy)	87.94	0.64	0.57-0.71	<0.001	90	16

Abbreviations; ROC: Receiver operating characteristic, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, vs: versus, AUC: area under the curve; CI: confidence interval

Patients with benign, borderline, and malignant ovarian tumors were evaluated using ROC curve analysis (Table 2 and Fig. 1). The diagnostic cut-off value, sensitivity, and specificity for NLR and PLR were calculated. The diagnostic cut-off value that maximized Youden's J statistic of NLR and PLR was used for differentiating. The diagnostic cut-off value of NLR (AUC=0.66, p<0.001) for differentiating between malign and benign/borderline ovarian tumors was 1.17, with a sensitivity of 91% and a specificity of 13% (Fig. 1A). The diagnostic cut-off value of PLR (AUC= 0.64, p<0.001) for differentiating between malign and benign/borderline ovarian tumors was 87.94, with a sensitivity of 90% and specificity of 16% (Fig. 1B).

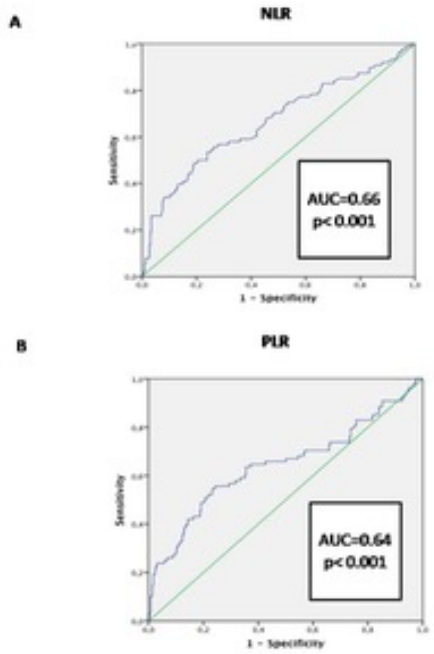

Fig 1: Receiver operating characteristic (ROC) curves analysis of neutrophil-lymphocyte ratio (NLR) (A) and platelet-lymphocyte ratio (PLR) (B) in patients with benign or borderline versus malignant ovarian tumor

4. Discussion

The present study evaluated the association of preoperative NLR and PLR between benign, borderline and malignant ovarian tumors. The study indicated that preoperative NLR and PLR values were significantly higher in malignant ovarian tumors than those in the benign or borderline ovarian tumor group. The diagnostic cut-off value of NLR in predicting malignant ovarian tumors was set at 1.17, with a sensitivity of 91% and a specificity of 13%. The cut-off value for the PLR values was also calculated to be 87.94, with a sensitivity of 90% and specificity of 16% for distinguishing malignant ovarian tumors from benign or borderline ovarian tumors.

Recently, it has been found that patients with malignant tumors have high CRP values, neutrophilia, and lymphocytopenia secondary to the inflammatory response (6). Leukocytosis and neutrophilia have been shown to be readily available prognostic factors in non-small cell lung cancer, cervical cancer, and endometrial cancer. Furthermore, an elevated leukocyte count was associated with poor survival (7). Research has shown neutrophilia may be a critical predictive factor for oncologic outcomes in patients with persistent or recurrent cervical cancer (8). A systematic review and meta-analysis by Ahmed Ebu-Zaid et al. in 2021 found that preoperative leukocytosis correlates with unfavorable pathological and survival outcomes in endometrium cancer (9). It has also been shown that preoperative leukocytosis was related to an increased risk of recurrence and mortality in patients with non-endometrioid endometrial adenocarcinoma (10). A meta-analysis conducted by Chen et al. demonstrated that NLR was an available predictor of overall survival and progression-free survival for patients with ovarian cancer (11). Prior research reported that NLR and PLR elevation was associated with poor survival in numerous cancers (12).

Systemic inflammation plays a crucial role in oncogenesis and cancer progression. In a number of studies, biomarkers of the systemic inflammatory response, NLR and PLR, were found to predict ovarian tumor characteristics. In response to inflammation, increased neutrophil, platelet, and relatively decreased lymphocyte counts can be observed, which results in elevated NLR and PLR. Previous research has established that NLR and PLR could benefit diagnosis and prognosis prediction. A study conducted by Polat et al. demonstrated that the preoperative NLR and PLR of patients with malignant ovarian tumors were significantly higher than those of patients with benign tumors. They suggest that NLR and PLR might help predict malignant ovarian cancers (13). Cho et al. also concluded that increased NLR and PLR levels served as a quick, easy, and cost-effective method for discriminating between malignant tumors and benign masses of the ovary (14). The current study confirmed that high NLR and PLR could be used to differentiate between malignant and benign or borderline tumors. In view of all that has been mentioned so far, NLR and PLR can be used in guiding clinicians in the management of a wide range of cancers.

Elevated NLR and PLR have been associated with adverse oncological outcomes as well as reflecting advanced stage and more aggressive disease (15,16). Moreover, a recent study found that NLR could be a feasible, acceptable, and efficacious biomarker in the evaluation of sensitivity to chemotherapy in advanced serous ovarian cancer (17).

The limitations of this study include that we were unable to compare parameters according to the histological subtype of benign and malignant masses. However, statistical significance would have been difficult to establish due to the relatively small number of cases in each subtype. Another possible limitation of the study was a retrospective design. Lastly, the main findings of this study were not externally validated, and further well-designed studies in an independent cohort are needed. Notwithstanding these limitations, we could evaluate patients with borderline tumors as a separate group. We hope

that the use of novel models incorporating variables such as age, CA125, ultrasound findings, and NLR and PLR values may contribute to the early detection of ovarian masses. Future studies should be conducted with larger sample sizes to discover additional markers and incorporate them into clinical practice.

In conclusion, NLR and PLR were significantly higher in ovarian cancers than in benign or borderline ovarian masses. NLR and PLR values seem to be useful inflammatory markers in predicting malignant ovarian tumors preoperatively.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: S.S.K., G.Y., D.S.A., Design: S.S.K., E.K., R.B.T., Data Collection or Processing: S.S.K., D.S.A., Analysis or Interpretation: E.K., R.B.T., P.B.İ, P.Y., G.Y., E.M., Literature Search: S.S.K., E.K., D.S.A., P.Y., Writing: S.S.K., E.K., G.Y., E.M., R.B.T., P.B.İ.

Ethical Statement

Approval was obtained from İstanbul Haseki Training and Research Hospital Ethics Committee, the study started. The ethics committee decision date is 19/12/2018 and the number of ethical committee protocol number is 299.

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Research Article

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Frontal sinus repair after bifrontal craniotomy: a single center experience

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Abstract

In this study, we analyzed the patients who underwent surgery using their adipose tissue, which is a practical and economical approach to the reconstructing the frontal sinus during bifrontal craniotomy. The study aimed to evaluate the economic feasibility and safety of this classical technique to repair the frontal sinus and reduce complications.Patients who were underwent bifrontal craniotomy for anterior skull base tumors and underwent intraoperative frontal sinus repair between January 1, 2016 and January 1, 2022 were retrospectively evaluated. Demographic characteristics of the patients (age, gender), duration of hospitalization, etiological causes, post operation complications, management of complications, and follow-up periods were analyzed.A total of 25 patients were included in the study. Of the patients, 11 were male (46%) and 14 were female (54%). The median age of the patients was 59 (49.5–66) years. The median duration of hospitalization and follow-up period were 7 (6–8.5) days and 27 (17–48) months, respectively. Cerebrospinal fluid leakage developed as a complication in only one patient (4%) in the postoperative period. We applied the classical technique of using the patient's own adipose tissue for repair after frontal sinus opening that occurs in bifrontal craniotomies for anterior skull base tumors. This is a practical, effective, and economical method that causes less harm to patients.

Keywords: bifrontal craniotomy, frontal sinus, reconstruction, rinore

1. Introduction

Bifrontal craniotomy is an effective surgical method for treating tumors in the sellar region, and in anterior skull base and anterior cerebral artery aneurysms (1-5). However, it may be necessary in this technique to enter the frontal sinuses and pass the lower border of the craniotomy through the orbital rim or slightly lower (6). Performing the procedure toward the frontal sinus can cause leakage of cerebrospinal fluid and a consequent and frequent infection (5,7-9). To prevent these complications that occur due to the opening of the frontal sinus, the connection of the frontal sinus with the intracranial space must be closed.

Different methods have been tried by neurosurgeons to block the connection between the intracranial space and the frontal sinus. Few methods aim to close this area with autologous tissue such as the patient's own adipose tissue, muscle tissue, or fascia. Synthetic materials such as polymethyl, methacrylate, hydroxyapatite cement, or bone wax have also been used for the same technique (1,10-15). Patients' own tissue may be preferred due to its availability and low chances of rejection by the body. Synthetic materials are expensive, unlikely to be absorbed, and can cause inflammation and infection.

In this study, we analyzed patients who underwent surgery in which their adipose tissue was used, which is a practical and economical approach to reconstruct the frontal sinus. Results from 34 patients showed that the classical technique is an economical and safe method to repair the frontal sinus and reduce complications.

2. Materials and Methods

Patients who underwent bifrontal craniotomy for anterior skull base tumors and underwent frontal sinus repair between January 1, 2016 and January 1, 2022 at the Kahramanmaraş Sütçü İmam University Neurosurgery Clinic were retrospectively evaluated. The study received approval from the local ethics committee. (Date June 21, 2022; Session Number: 2022/21; Decision No.: 04). A total of 25 patients who met the study criteria were included. Of these patients, adipose graft from the patient was used for frontal sinus repair. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Common demographic information (age, gender), duration of hospitalization, etiologic causes, types of postoperative complications, complication management, and follow-up periods of the patients were analyzed. Statistical Package for Social Sciences v20.0 was used for statistical analyses. Categorical data were presented as number (n) and percentage (%), while numerical data were presented as median (1st and 3rd quartiles).

2.1. Surgical technique

The operation was performed under general anesthesia with the

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patient in the supine position. A coronal skin incision was made at the hairline, and the skin flap and periosteum were lifted. The bone flap was removed by opening a temporal burr hole and performing a craniotomy in lower border up to the orbital rim. Next, the frontal sinus was opened. The mucosal membrane of the frontal sinus was exposed, immediately disinfected using povidone-iodine, and sealed with bone wax to prevent air embolism. The sagittal sinus was ligated as anteriorly as possible and cut. After the tumor was removed, an adipose graft was taken from under the patient's scalp tissue for frontal sinus repair during closure. The bone wax in the frontal sinus was removed and the adipose graft was placed in the frontal sinus. Furthermore, the connection between the frontal sinus and the intracranial distance was closed. Next, the dura, bone, and skin flaps were closed in accordance with the procedure. During postoperative follow-up, 1 g of ceftriaxone 2 × 1 was administered to the patients for 5 days. Patients were discharged when their general condition improved.

3. Results

All patients included in the study were operated on through surgery to treat olfactory groove tumors (100%). Of the patients, 11 were male (46%) and 14 were female (54%). The median age of the patients was 59 (49.5–66) years. The median duration of hospitalization and follow-up period were 7 (6–8.5) days and 27 (17–48) months, respectively. Cerebrospinal fluid leakage developed as a complication in one (4%) patient in the postoperative period. In this patient, complication was observed at the 48th hour after the surgery. The patient also developed a fever at the postoperative 72nd hour. In this patient, the leak disappeared on day 4 using fluid restriction and antibiotherapy. The demographic data of the patients according to their gender and presence of complications are presented in Table 1.

Table 1. Comparison of demographic data, duration of hospitalization, follow-up period, and complications of patients according to their gender

Parameter		Sex		Total
		Male	Female	
Rhinorrhea (n%)	Yes	0 (0%)	2 (8%)	2 (8%)
	No	11 (44%)	12 (48%)	23 (92%)
Total		11 (44%)	14 (56%)	25 (100%)
Additional procedure (n%)	Yes	0 (0%)	2 (8%)	2 (8%)
	No	11 (44%)	12 (48%)	23 (92%)
Total		11 (44%)	14 (56%)	25 (100%)
Age (year) (median)(1st and 3rd quartile)		59 (55 – 66)	58.5 (44.75 – 66.5)	
Hospitalisation (day) (median)(1st and 3rd quartile)		6 (6 – 8)	7 (5.75 – 9.25)	
Follow-up period (month) (median)(1st and 3rd quartile)		25 (18 – 52)	32.07 ± 19.48 (5 – 65)	

4. Discussion

Bifrontal craniotomy is one of the most commonly used surgical methods in neurosurgical practice. The bifrontal approach provides excellent access to the pathology area. However, since a larger craniotomy area is used with a bifrontal craniotomy, the bone graft removed also brings some problems (16,17). The opening of the frontal sinus during this procedure is inevitable in most cases. The border of the craniotomy extends to the frontal sinus and then the frontal sinus is exposed. If frontal sinus repair is not performed correctly, subsequent complications will most commonly originate from the frontal sinus(7,11,18).

Complications originating from the frontal sinus are difficult to manage and increase morbidity. The most important of these complications are cerebrospinal fluid (CSF) leakage and frontal sinus infections. CSF leakage is a critical complication for the patient and clinician to deal with after surgery(19,20). Meningitis and intracranial abscesses may develop after these complications. This may result in rapid deterioration in the patient's general condition and neurologic examination (21). This can lead to a prolonged hospital stay and life-threatening consequences. Therefore, frontal sinus repair is as important as the surgery performed and should be managed well(22).

When repairing the frontal sinus opened by bifrontal craniotomy, the frontal sinus mucosa must first be removed for obliteration, and the debris must be scraped from the frontal sinus wall. The frontal sinus must then be obliterated. This must be done meticulously. Otherwise, the mucosa may become polyferous and subsequently cause various complications, such as mucocele abscess. These are also problems that increase morbidity. After carefully removing the mucosa, the frontal sinus can be repaired by filling it with different materials(16,17).

Different materials and techniques have been reported for frontal sinus repair. Among the most commonly used methods is the use of the patient's own tissues such as fat or muscle(23-26). Synthetic materials such as polymethyl methacrylate, hydroxyapatite cement and its derivatives, or bone wax have been used for frontal sinus repair (27-31). These materials are preferred for their antibacterial properties and easy availability. These methods are generally more expensive than the traditional technique of using the patient's own tissues and do not always provide adequate closure in terms of sinus repair(32).

In addition, they do not prevent infections adequately, on the contrary, they may increase infection rates (33). Bone wax may lead to inflammation and wound healing problems (29,34).

The use of the patient's own tissues for frontal sinus repair has long been a preferred method (34,35). Some authors have reported significant decreases in infection rates after the use of adipose grafts (36).

Some of the adipose tissue used with this technique eventually vascularizes and prevents the development of

infection, while some of it forms fibrous tissue and closes the connection between the intracranial area and the frontal sinus (37-39). Because of these benefits, using the patient's own tissue has become very popular among surgeons (40).

In our center, we use the patient's own adipose tissue, which is the classical technique, for repair after frontal sinus opening in bifrontal craniotomies performed for anterior skull base tumors. It has been reported that the adipose graft can be taken from the patient's abdominal tissue or scalp tissue. In our center, we use scalp tissue. It is possible to remove enough adipose tissue from both locations. The reason why we take adipose grafts from under the scalp is that the scalp tissue opened for bifrontal craniotomy is of sufficient size and width in all patients, allowing us to take the required amount and size of adipose graft. This eliminates the need to take an adipose graft from the abdomen with a second incision. Thus, we do not impose an additional surgical burden due to a second incision.

This method is a practical, effective and low-cost method that causes less harm to patients.

Out of 25 patients who underwent the procedure in our clinic, only one patient had cerebrospinal fluid leakage. Cerebrospinal fluid leakage stopped on the 4th postoperative day without the need for additional surgical procedure and there was no additional deficit in the patient. Therefore, it was concluded that this method, which is more effective and economical compared to using different exogenous materials, was successful.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: E.C.K., K.Z.Y., Design: E.C.K., Data Collection or Processing: E.C.K., K.Z.Y., Analysis or Interpretation: E.C.K., Literature Search: E.C.K., Writing: E.C.K.

Ethical Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was approved by the Clinical Studies Bioethics Committee of the Medical Faculty of Kahramanmaras Sutcu Imam University (Date June 21, 2022; Session Number: 2022/21; Decision No: 04).

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The effect of onion juice on menstrual disorder and hormonal parameters in patients with polycystic ovary syndrome

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Abstract

Our aim in this study was to objectively observe the effect of onion juice on menstrual disorders (irregular menstruation, dysmenorrhea, amenorrhea, menorrhagia, hypermenorrhea, and hypomenorrhea) in patients with *polycystic ovary syndrome* (PCOS). A method that affects these women's quality of life without side effects has an essential effect on infertility as a global problem. We conducted a prospective randomized analysis of age-matched and body mass index (BMI)-matched women between April 2019 and March 2020. The study population included 64 reproductive-aged women with PCOS. Data collection by questionnaire and blood samples was done before and after 15 days of onion juice use. There was a statistically significant association between before and after onion juice use in terms of high density lipoprotein (HDL), low density lipoprotein (LDL), and triglyceride (TG) in case group ($p<0.05$). There was a statistically significant association between before and after onion juice use in terms of irregular menstruation, dysmenorrhea, amenorrhea, and menorrhagia ($p<0.05$) in case group. According to the findings, menstrual disorders decreased after 15 days of onion juice use. As a result, onion juice can be used to reduce the complications of PCOS in women. Improvement in dysmenorrhea and amenorrhea has essential effects on the health of women with PCOS.

Keywords: Polycystic ovary syndrome, irregular menstruation, menstrual disorders, onion juice

1. Introduction

Polycystic ovary syndrome (PCOS) is premenopausal women's most common endocrine disease (1,2). In these women, androgen levels are? is higher than normal, and their ovaries are full of micro follicles that have turned into cysts (3,4). Women with PCOS are usually overweight or obese and experience irregular menstruation (5,6). Diet and exercise are the best ways to treat these patients (7,8). However, there are different opinions about the extent of the impact and how it has been the subject of much research(9,10). The current research has investigated the effect of a diet including onion juice on the clinical and laboratory symptoms of PCOS women.

There are different health implications for the prevalent menstrual disorders (irregular menstruation, dysmenorrhea, hypomenorrhea, menorrhagia, or hypermenorrhea and premenstrual symptoms) as health indicators in women. There is a varying irregular menstruation prevalence from 5% to 35.6%, depending on the occupation, age, and residence country (11). PCOS is the most common cause of menstrual irregularities causing female infertility. It is the most prevalent cause of female infertility because it is very difficult to predict the day of ovulation in these women (12). There are several treatments now, but there is an association between them and moderate to serious side effects. Regular menstruation resulting from the presentation of more effective methods with fewer side effects increases the chance of pregnancy in women.

Oral contraceptive (OC) drugs are used effectively to regulate menstruation. Today, OCs are the first preferred drug group in the patient group with PCOS and menstrual irregularity (13). Venous thromboembolism is the most common vascular complication in women using OCs. The probability of embolism increases 2-4 times in OCs users compared to non-users (14,15). In addition, many studies have reported that OCs cause cervical cancer, especially in positive human papilloma virus (HPV) patients (16,17). OCs, which are widely used as a menstrual regulator in patients with PCOS, have many side effects, which create difficulties in their use in practice (18). Especially patients who have menstrual irregularity and also want pregnancy seek an alternative way (6). The fact that some patient groups did not want to use chemical drugs, resorted to traditional and complementary medicine, our retrospective observation of satisfaction in patients using onion juice, and the fact that no previous study has been done on this subject in the literature encouraged us to conduct this study (19).

The focus of PCOS management is usually on lifestyle changes (diet and exercise) to alleviate symptoms and reduce the related risk of cardiovascular disease and type 2 diabetes (20,21).

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We aimed to compare the menstrual-reducing and menstrual-regulating effects of onion juice in patients with PCOS and its effect on hormones and some biochemical parameters.

2. Material and Method

Thirty-one women were included in the control group, and thirty-three women who consumed brown onion juice for 15 days were included in the case group. A prospective randomized comparison of the anti-menstrual and menstrual-regulating effects of brown onion juice and its effect on blood hormones and some biochemical parameters in PCOS patients was made. In this study, brown onion juice was given to the case group for 15 days. After 15 days, menstrual disorders and laboratory parameters were studied for six months.

All subjects participated in the study voluntarily. It was planned that half of the patients will drink the standardized onion juice prepared in our Bezmialem University Phytotherapy Center twice a day (2 cups of 200 ml in total) on an empty stomach for 15 days, and they will stop curing in case of menstruation any day they drink the brown onion juice. The other half of the patients is our control group, and it is planned to drink the placebo (sugar water with added onion flavor suitable for food) prepared by our Bezmialem University Phytotherapy Center in the same way. In this way, after the patients complete 15 days, the two groups will be compared in a randomized, controlled manner. During the 6-month period after the patients drink onion juice, their menstrual patterns will be compared. The protocol for preparing brown onion juice involves boiling it with water at a ratio of 1:10, including the onion shells, and then diluting the cooled extract with water to the required volume. The prepared brown onion juice will be standardized in terms of the marker components quercetin and quercetin glycosides, and all patients will be provided with the same product. While preparing the placebo product, the onion flavor compatible with the food will be diluted 1:1000 with 1% dextrose water.

The inclusion criteria were as follows: 1) 18-40 years old. The exclusion criteria were as follows: 1) known chronic disease, 2) over 40 years of age, 3) pregnant women, 4) having gastritis, reflux, or onion allergy, and 5) women with the lactation period.

We utilized the G-Power 3.1 program to calculate the example size. The two groups' total mean was calculated based on the Mann-Whitney test with a power of 80%, an effect size of 50%, and beta/alpha ratio of 1 for at least 30 patients for each group (22).

2.1. Statistical Analysis

The Kolmogorov-Smirnov test was conducted to check the normality, and the nonparametric tests were performed given the groups' non-normality before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, including age, body mass index (BMI), fasting blood sugar (FBS), follicle-stimulating hormone (FSH), luteinizing hormone (LH), Estradiol (E2), Prolactin (PRL), thyroid-stimulating hormone (TSH), Free T4 (FT4), HDL, LDL, total cholesterol, triglyceride, total testosterone, 17-OH Progesterone, *sex hormone-binding globulin (*SHBG),Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and dehydroepiandrosterone sulfate (DHEAS). The Mann-Whitney U test was deployed to examine the difference between the case and control groups for abnormal continuous variables. An independent t-test was deployed to examine the difference of normal variables. Chi-square tests were applied to describe the relationship between proportions of categorical variables such as pregnancy results, ongoing pregnancy rate, and abortion rate. SPSS v24 was employed for statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

3. Results

This study included sixty-four-year old (26.42±2.48) and BMI (22.61±1.66) women. Table 1 shows that descriptive statistics of maternal characteristics and laboratory parameters.

Table 1. Descriptive statistics of study parameters in women (n=64)

	Study parameters	median (range) mean ± SD
Maternal characteristics	Age	26(23-35)26.42±2.48
	BMI	23(18.4-26.1)22.61±1.66
Laboratory values	FBS	84.5(68-98)84.56±6.41
	FSH	4.3(1.2-8.4)4.38±1.48
	LH	6.95(2.2-11.2)6.96±2.06
	E2	50(24-68)50.44±10.58
	PRL	16(8.48-35.1)17.17±5.23
	TSH	1.835(0.8-5.2)2±0.84
	Free T4	0.58(0.23-1.12)0.59±0.19
	HDL	66(38-89)65.44±10.5
	LDL	97.5(56-185)106.92±30.95
	Total Cholesterol	166(120-285)175.72±41.53
	Triglyceride	55(0.21-89)40.43±37.78
	Total Testosterone	0.645(0.18-158)45.61±52.47
	17OH Progesterone	0.3205(0-1.77)0.38±0.23
	SHBG	39(23.8-124)42.5±18.54
	HOMA-IR	2.42(0.58-5.3)2.39±1.08
	DHEAS	254.5(129-430)262.84±73.96

SD, standard deviation. BMI, body mass index; FBS, fasting blood sugar; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, Estradiol; PRL, prolactin; TSH, thyroid-stimulating hormone; FT4, Free T4; HDL,high density lipoprotein ; LDL,low density lipoprotin ; SHBG, *sex hormone-binding globulin* ; DHEAS, dehydroepiandrosterone sulfate.

Table 2 shows the comparison of the control group on maternal characteristics and laboratory values. In the present investigation, we compared laboratory parameters between case and control groups and evaluated the ability of those parameters to distinguish between groups.

Table 2. Comparison of laboratory parameters in two time periods in control group

Study parameters	Before the study (n=33) median (range) mean ± SD	After 15 days (n=33) median (range) mean ± SD	*p*-value
FBS	83(75-94)84.92±5.24	86(68-98)85.53±5.98	0.742**
FSH	3.7(2.5-7.1)4.28±1.28	4.7(1.2-8.4)4.49±1.69	0.465**
LH	7.1(2.75-11.23)6.71±1.76	6.7(2.35-10.97)6.59±2.2	0.242*
E2	46(24-66)48.96±10.98	51(33-67)50.03±10.18	0.277*
PRL	16(12-34.1)17.22±5.12	17.6(8.48-23)17.14±4.99	0.749*
TSH	1.52(0.79-4.9)2.18±0.87	2.21(0.78-3.15)2.21±0.71	0.514*
Free T4	0.59(0.24-1.14)0.59±0.17	0.53(0.22-0.91)0.57±0.18	0.490**
HDL	64(37-79)66.99±9.97	68(49-87)67.99±9.93	0.523*
LDL	113(75-187)117.12±28.58	87(55-159)115.71±28.98	0.654*
Total Cholesterol	169(121-279)185.97±47.19	164(125-239)183.15±29.85	0.234**
Triglyceride	80(51-88)73.99±11.98	0.5(0.21-57)74.08±13.73	0.754*
Free Testosterone	0.35(0.17-0.79)90.38±0.14	96(0.36-158)93.74±33.76	0.746**
17OH Progesterone	0.34(0.2-1.76)0.39±0.28	0.32(0-0.74)0.36±0.15	0.966*
SHBG	39.2(24.7-123)46.91±24.96	37(32-82)47.55±24.54	0.789**
HOMA-IR	2.47(0.76-5.3)2.35±1.08	2.14(0.58-4.5)2.22±1.07	0.585*
DHEAS	253.2(128-431)271.37±81.19	258(174-389)275.03±62.69	0.214*

M, Mean; N, number of subjects; FBS, fasting blood sugar; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, Estradiol; PRL, prolactin; TSH, thyroid-stimulating hormone; FT4, Free T4; HDL,high density lipoprotein ; LDL,low density lipoprotin ; SHBG, *sex hormone-binding globulin* ; DHEAS, dehydroepiandrosterone sulfate * Mann-Whitney U test; ** Independent t-test

Table 3. Comparison of laboratory parameters in two time periods in case group

Study parameters	Before the study (n=31) median (range) mean ± SD	After 15 days (n=31) median (range) mean ± SD	
FBS	83(72-96)83.42±6.36	86(68-98)85.77±6.33	0.144**
FSH	3.8(2.5-7.1)4.28±1.28	4.7(1.2-8.4)4.49±1.69	0.595*
LH	7.2(2.98-11.2)7.31±1.89	6.5(2.2-11.2)6.59±2.2	0.162*
E2	47(24-67)48.94±11.68	52(34-68)52.03±9.18	0.242**
PRL	16(11-35.1)17.12±5.2	17.6(8.48-23)17.23±5.34	0.925*
TSH	1.56(0.8-5.2)1.79±0.9	2.23(0.8-3.5)2.22±0.73	**0.008***
Free T4	0.62(0.25-1.12)0.63±0.19	0.54(0.23-0.95)0.55±0.19	0.090**
HDL	65(38-80)61.64±10.06	69(50-89)69.49±9.53	**0.005***
LDL	112(76-185)116.42±29.77	88(56-163)96.81±29.34	**0.007***
Total Cholesterol	177(120-285)186.21±48.91	165(123-245)164.55±28.65	0.036**
Triglyceride	81(52-89)74.58±12.27	0.5(0.21-57)4.08±13.73	**<0.001***
Free Testosterone	0.36(0.18-0.82)0.4±0.15	96(0.36-158)93.74±33.76	**<0.001***
17OH Progesterone	0.35(0.1-1.77)0.4±0.29	0.32(0-0.74)0.36±0.15	0.866*
SHBG	39.1(23.8-124)46.21±25.06	38(32-52)38.55±4.54	0.329*
HOMA-IR	2.47(0.76-5.3)2.55±1.08	2.14(0.58-4.5)2.22±1.07	0.235**
DHEAS	253.2(129-430)251.38±82.49	258(174-389)275.03±62.69	0.204**

M, Mean; N, number of subjects; FBS, fasting blood sugar; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, Estradiol; PRL, prolactin; TSH, thyroid-stimulating hormone; FT4, Free T4; HDL,high density lipoprotein ; LDL,low density lipoprotin ; SHBG, *sex hormone-binding globulin* ; DHEAS, dehydroepiandrosterone sulfate * Mann-Whitney U test; ** Independent t-test

There was not a statistically significant association between two periods of time in terms of FBS, FSH, LH, E2, PRL, TSH, Free T4, HDL, LDL, Total Cholesterol, Triglyceride, Free Testosterone, 17OH Progesterone, SHBG, HOMA-IR, and DHEAS in the control group (p<0.05).

As stated in Table 3, a Mann-Whitney test did not find a statistically significant association between before and after the consumption of onion juice in the case group in regard to Age, BMI, FSH, LH, PRL, TSH, 17OH Progesterone, and SHBG (p>0.05). Independent t-test did not find a statistically significant association between before and after the consumption of onion juice in the case group in terms of FBS, E2, Free T4, total cholesterol, HOMA-IR, and DHEAS (p>0.05). The Mann-Whitney test found a statistically significant association between before and after the consumption of onion juice in the case group in regard to HDL, LDL, triglyceride, and total testosterone (p<0.05). The serum HDL and free testosterone levels were significantly higher in case group before the consumption of onion juice. The serum LDL and triglyceride levels were significantly higher in the case group before the consumption of onion juice.

As stated in Table 4, a chi-square test found a statistically significant association between the treatment rate of dysmenorrhea and the consumption of onion juice (p<0.05). There was a statistically significant association between the treatment rate of menorrhagia and the consumption of onion juice (p<0.05). There was a statistically significant association between the treatment rate of hypermenorrhea and the consumption of onion juice (p<0.05). There was not a statistically significant association between the treatment rate of hypomenorrhea and the consumption of onion juice (p>0.05). Onion juice's impact on the menstrual cycle was observed in this study based on the results represented in table 4.

Table 4. The relationship between menstruation problems between case and control groups

Variables		Case(n=31) n(%)	Control(n=33) n(%)	p
Menstruation occurred after the 15-day treatment period	Yes	27 (81.2)	8 (25.8)	**<0.001***
	No	6 (18.8)	23 (74.2)	
The dysmenorrhea	Yes	5 (15.1)	26 (83.9)	**<0.001***
	No	28 (84.9)	5 (16.1)	
4 or more amenorrhea in 6 months	Yes	13 (39.4)	25 (80.6)	**<0.001***
	No	20 (60.6)	6 (19.4)	
The menorrhagia	Yes	4 (12.1)	17 (54.8)	**<0.001***
	No	29 (87.9)	14 (45.2)	
The hypermenorrhea	Yes	15 (45.5)	24 (75.9)	**0.009***
	No	18 (54.5)	7 (24.1)	
The hypomenorrhea	Yes	14 (42.4)	18 (58)	0.211*
	No	19 (57.6)	13 (42)	

*A Chi-square test

4. Discussion

The current study examined the possible effects of onion juice on menstruation problems and laboratory parameters in women with PCOS. In our study, the menstruation occurrence (81.2 % vs. 25.8%), dysmenorrhea (15.1% vs. 83.9%), amenorrhea frequency (39.4% vs. 80.6%), menorrhagia (12.1% vs. 54.8%), and hypermenorrhea (45.5% vs. 75.9%) were significantly different between the case and control groups, respectively. In dysmenorrhea as the primary variable, 84.9% of women have reported treatment for dysmenorrhea. In women who used onion juice, four or more amenorrheas in 6 months occurred much less frequently (39.4% vs. 80.6%). Only 12% of women in the case group have faced menorrhagia.

Hypermenorrhea and menorrhagia are directly related to an increase in menstrual flow (23). Hypermenorrhea is less common after onion juice consumption than in the control group (45.5% vs. 75.9%). The hypomenorrhea was nearly the same in both groups. This study showed statistically significant differences after the consumption of onion juice in HDL, LDL, triglyceride, and free testosterone parameters. Changes in these laboratory parameters cause menstrual-reducing and menstrual-regulating effects.

Dysmenorrhea is a considerable health-related problem in women of reproductive age (24). Bajalan et al. (25) reported the effect of food groups and eating habits on this problem. Based on the study's findings, the use of onion juice can be very effective in improving this pain and can be suggested as an effective treatment. Except for hypomenorrhea, other menstrual disorders demonstrated a positive effect of onion consumption. The frequency of amenorrhea has decreased by half after consuming onion juice, which is important considering the importance of amenorrhea in the reproductive system.

Nutrition and exercise are two non-drug treatments to

relieve the symptoms of the disease (20). It has been proven that lifestyle modifications, particularly exercise and diet, have effectively managed the symptoms and reduced the risk factors associated with PCOS (26). According to many people, this has a higher effect than medication. Several studies have been conducted on the effect of exercise and diet on obese women with PCOS and found that weight loss has led to favorable results (20,21,27). In the current study, the main difference from previous studies is that the effect of onion juice is unrelated to weight loss. This treatment can be effective regardless of weight.

Krouni et al. (28) reported a reduction in BMI, free testosterone and LH levels and an increase in SHBG after low calorie diets. The high-calorie diet leads to reversible hormonal disorders and menopause (29). Medicinal plants of natural origin are also known as low-calorie foods, and medicinal plants have been shown to help alleviate PCOS complications (30). Wang et al. (31) identified Cinnamon's function as an adjunctive therapy to treat PCOS. The effect of Cinnamon on metabolic dysfunction and menstrual cycle in PCOS women was studied in another research as a randomized controlled trial on 45 women. Oral cinnamon supplements were administered. The luteal phase, menstrual cycle, and progesterone levels were all followed. The menstrual cycle was enhanced through Cinnamon supplementation, which was found to contribute to the treatment of PCOS. Jelodar et al. (32) studied flax seeds' effect on PCOS patients' ovarian morphology, concluding that ovarian volume decreased, the number of follicles in the ovaries increased, and menstrual cycle duration improved through flax seed supplementation. On the contrary, the research found that body weight, blood sugar levels, or hirsutism did not improve.

The positive effects of different types of onions on diabetes (33), hypertension (33), liver and kidney injury (34), and sperm (35) were the primary motivation to conduct this study. The onion in the Middle East and Asian countries is widely known to be used in traditional medicine and food.

However, there are few studies about the effect of onion juice on women. Lee et al. (36) did the only similar study. In this study, the Welsh onion was administered to letrozole-treated rats for two weeks for treatment. Regarding serum hormonal levels, onion extract therapy positively affected serum estrogen levels and LH/FSH ratio. LH and FSH are necessary for ovulation, and PCOS patients often have an increased LH/FSH ratio two to threefold, causing ovulation disruption. Based on the findings, ovarian cysts and follicular growth are normalized by the onion extract. The steroid hormone-related receptors in the letrozole-induced PCOS rat model showed mRNA expression restoration after being treated with onion extract. Onion extract treatment altered and relieved ovarian function. Contrary to this study, our results did not affect the LH and FSH parameters of onion juices.

The main limitation of this research is its small sample size. The sample size was limited to 64 patients. Based on our knowledge, the present study is the first time onion juices were used to treat menstrual disorders. In this study, the unpleasant side effects of PCOS, such as irregular menstruation, were improved using onion juice. Most of the studies on the effect of exercise and nutrition on PCOS seek to reduce the complications of this disease by losing weight. Providing a successful method for treating the disease without side effects is one of the most important strengths of this study.

In this study, brown onion juice was used. This type of onion is chosen because of its abundance in the country and easier access. The future study is aimed to investigate the effects of different types of onion. For future studies, it is recommended to check the length of different treatments (one month, two months and three months) on the effect of onion juice.

This study revealed that onion juice can be used to reduce the complications of PCOS in women. The availability of onion juice can be a valuable opportunity to popularize this type of treatment. Additional investigations are required to explore the causal relationship between onion juice and menstrual disorders.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Authors' contributions

Concept: A.Ş.K., Design: A.Ş.K., Data Collection or Processing: A.Ş.K., Analysis or Interpretation: A.Ş.K., Literature Search: A.Ş.K., Writing: A.Ş.K.

Ethical Statement

The Ethics Committee of Bezmialem University Hospital approved this prospective randomized study. (Date:6.3.2019 Decision No:5/26). Sixty four women participated in this study between April 2019 and March 2020. All procedures were carried out in accordance with the ethical rules and the principles of the declaration of Helsinki.

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Examining the correlation between fear of COVID-19, sexual functions, depression, and anxiety in the women who underwent COVID-19

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Abstract

This study aimed to examine the correlation between fear of COVID-19, sexual functions, depression, and anxiety in women who have undergone COVID-19. Seventy-five volunteer women who have experienced COVID-19 were included in this study. The sociodemographic characteristics of the participants were obtained using a demographic information form. The Fear of COVID-19 Scale, the Female Sexual Function Index (FSFI), the Female Sexual Distress Scale-Revised (FSDS-Revised), the Patient Health Questionnaire (PHQ-4), and the Global Pelvic Symptom Bother Questionnaire (GPFBQ) were used for assessments. Significant, positive correlations were found between the Fear of COVID-19 Scale scores with the FSFI and GPFBQ scores ($p=0.015$, $p=0.005$), and significant, positive, and moderate correlations were found between the FSDS-Revised scores with PHQ-4-Anxiety and Depression, PHQ-4-Total, and GPFBQ scores ($p<0.001$, $p=0.002$). The findings of this study demonstrate a correlation between the fear of COVID-19 and sexual function, as well as pelvic floor dysfunction in women who have undergone COVID-19. Moreover, a connection was identified between sexual function and sexual distress, anxiety, and depression, as well as between pelvic floor dysfunctions and anxiety and depression in women affected by COVID-19. Consequently, it is suggested that pelvic floor and sexual functions, along with psycho-social states, may be adversely impacted in women infected with COVID-19.

Keywords: COVID-19, sexual dysfunction, depression, anxiety.

1. Introduction

Throughout history, the world has witnessed various pandemics that have significantly impacted the lives of millions of individuals. Despite advancements in technology and medical research, the emergence of new pathogens continues to pose a threat to the global health system (1-3). In December 2019, a novel virus first appeared in Wuhan, China, and was initially referred to as the New Coronavirus (2019-nCoV). However, due to its close resemblance to SARS-CoV, the World Health Organization renamed the disease SARS-CoV-2 on February 11, 2020, and declared it a pandemic on March 11, 2020 (4,5).

While clinical symptoms typically manifest on the 4th or 5th day following exposure to the infection, studies have indicated that the incubation period may extend up to 14 days (2,6). Despite the majority of infected patients being asymptomatic or experiencing mild symptoms that do not require hospitalization, the overall number of severe cases and fatality rates remain significant (7). COVID-19 is characterized by common symptoms such as fever, cough, dyspnea, and fatigue and is primarily transmitted through direct contact or respiratory droplets, as supported by available evidence. The risk of infection is substantially increased when exposed to droplets expelled through coughing or sneezing, particularly within a one-meter distance from an individual exhibiting symptoms (8).

When evaluating the symptoms of COVID-19, it becomes evident that cough is a common manifestation. In populations with a higher prevalence of chronic cough, it serves as a significant mode of infection and is associated with an increased incidence of urinary and fecal incontinence, pelvic organ prolapses, and pelvic floor dysfunction. The relationship between pelvic floor dysfunction and cough is attributed to recurrent microtrauma in the pelvic floor due to intra-abdominal pressure (9-11). A previous study involving patients with Chronic Obstructive Pulmonary Disease revealed a frequent occurrence of incontinence, with the severity of incontinence directly proportional to the increased intra-abdominal pressure during coughing (12).

Although urinary incontinence itself may not be life-threatening, it has been suggested to adversely impact the psychological and social well-being of both men and women (13-15). Stress incontinence, as defined by the International Society of Urogynecology and the International Continence Society, refers to urinary incontinence that occurs during coughing, sneezing, or physical exertion (16). Despite the increased likelihood of stress incontinence due to the frequent occurrence of cough in COVID-19, there is a scarcity of studies examining this specific association.

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The potential of COVID-19 to affect various bodily systems to differing degrees gives rise to numerous physiological complications. Disabilities not only have a negative impact on an individual's social life during the illness but also persist even after they become medically stable (11). It is widely recognized that the disruption of social life due to COVID-19, along with reduced income and a highly stressful lifestyle, can affect women's sexual desire and frequency of sexual intercourse. Sexual health encompasses the fulfillment of everyone's sexual rights and encompasses physical, emotional, mental, and social well-being. However, strict adherence to social distancing measures and the contagious nature of the disease during the pandemic have adversely affected sexual health (17,18).

While studies conducted during the pandemic have explored the effects on sexual desire, frequency of sexual intercourse, conception, and the use of contraception, we have not come across any literature that evaluates sexual functioning and stress incontinence related to cough in individuals with a medical history of COVID-19. We believe that some underestimated symptoms may include stress urinary incontinence and affected sexual functions in women experiencing cough due to COVID-19. Therefore, the objective of our study is to examine the correlation between fear of COVID-19, sexual functions, depression, and anxiety in women who have undergone COVID-19.

2. Materials and Methods

This cross-sectional study involved 75 women and aimed to examine the correlation between fear of COVID-19, sexual functions, depression, and anxiety in women who had previously contracted COVID-19. Participants were recruited from various regions in Türkiye through announcements made on social media platforms such as Facebook, Instagram, WhatsApp, etc. Prior to the commencement of the study, participants were required to provide informed consent, confirming their voluntary participation, and were informed about the study's objectives. Exclusion criteria included pregnancy, recent childbirth, and gynecological surgery within the past six months. Questionnaires intended for the participants were administered electronically via email or text message using Microsoft Forms. A power analysis was conducted to determine the sample size, with a margin of error set at 0.10±0.15 and a confidence interval of 80%, resulting in a required sample size of 75 individuals. The study received the necessary permissions from the Department of Physiotherapy and Rehabilitation in the Faculty of Health Sciences at Izmir Democracy University and from the Ministry of Health. The study protocol was approved by the ethics committee of Izmir Democracy University (Decision No: 2022/03-16).

As data collection tools, we utilized an information form that included personal information such as name, surname, telephone number, and smoking status, as well as demographic and clinical information including age, obstetric history, gestational week, and educational status. Additionally, standardized questionnaires, including the Fear of COVID-19 Scale, the Female Sexual Function Index (FSFI), the Female Sexual Distress Scale-Revised (FSDS-Revised), the Patient Health Questionnaire (PHQ-4), and the Global Pelvic Symptom Bother Questionnaire (GPFBQ) were utilized.

The Fear of COVID-19 Scale, developed by Ahorsu et al. in 2020, was designed to assess fear levels associated with COVID-19. It utilizes a five-point Likert-type scale, ranging from 1 (I totally disagree) to 5 (I totally agree), and consists of 7 items representing a single factor. The scale's minimum score is 7, while the maximum score is 35. Higher scores on the scale indicate a greater fear of COVID-19. The Turkish validity and reliability of the scale were established by Artan et al. through a process that involved reviewing scales related to various fear parameters, conducting participant interviews, and seeking expert evaluations (19,20).

The Female Sexual Function Index (FSFI) is a Likert-type scale consisting of 19 items. FSFI's validity and reliability study was developed by Rosen et al. It assesses sexual dysfunction. It encompasses six sub-dimensions, namely sexual desire (questions 1 and 2), arousal (questions 3, 4, 5, and 6), lubrication (questions 7, 8, 9, and 10), orgasm (questions 11, 12, and 13), satisfaction (questions 14, 15, and 16), and pain (questions 17, 18, and 19). Each question evaluates the sexual function of women within the past four weeks. The scale provides a range of raw scores from 4 (lowest) to 95 (highest). After multiplying the coefficients, the total score ranges from 2 (lowest) to 36 (highest). The optimal cut-off value for women is considered to be 26.55, with scores above 26.5 indicating normal sexual function, while scores below 26.5 suggest sexual dysfunction. The Turkish validity and reliability study of the FSFI was conducted by Aygin and Aslan in 2005 (21,22).

The Female Sexual Distress Scale-Revised (FSDS-Revised), developed by Derogatis et al., is designed to assess sexually-related personal distress in women. The scale was adapted to Turkish by Aydın et al., who also conducted the validity and reliability study. Comprising 13 items, the FSDS-Revised evaluates various aspects of distress related to sexual activity in women. Participants respond to the items using a 4-point Likert-type scale, with choices ranging from never (0) to rarely (1), sometimes (2), often (3), or always (4). The minimum score that can be obtained on the FSDS-Revised is 0, while the maximum score is 52. Higher scores indicate higher levels of sexual distress. In the Turkish version of the scale, a cut-off point of ≥11 is accepted to determine the presence of sexually-related personal distress in women (23,24).

The Patient Health Questionnaire (PHQ-4), developed by Kroenke et al. in 2009, is a Likert-type scale that assesses depression and anxiety. The Turkish validity and reliability of this questionnaire, consisting of two sub-dimensions,

depression and anxiety, were established by Demirci and Ekşi in 2018. To calculate the anxiety sub-dimension score, items 1 and 2 in the questionnaire are summed. The depression sub-dimension score is calculated based on items 3 and 4. The PHQ-4 total score ranges from 0 to 12. A score of 0-2 indicates no psychological stress, 3-5 indicates mild psychological stress, 6-8 indicates moderate psychological stress, and 9-12 indicates severe psychological stress (25,26).

The Global Pelvic Floor Symptom Bother Questionnaire (GPFBQ), developed by Peterson et al. in 2010, assesses various dysfunctions related to the pelvic floor, including a sudden urge to urinate, dyspareunia, stress and urge urinary incontinence, voiding difficulty, frequent urination, pelvic organ prolapse, obstructive defecation, and anal incontinence. The questionnaire consists of 9 questions. Participants respond to the questions by indicating "yes" or "no" (0), depending on the presence or absence of the symptom. If the response is "yes," they rate their discomfort on a scale ranging from "not at all" (1) to "a lot" (5). The total score ranges from 0 to 45; the mean score is calculated and multiplied by 20 to obtain a score between 0 and 100. A higher score on the questionnaire indicates greater dysfunction. The Turkish validity and reliability analysis of the questionnaire was conducted by Doğan (27,28).

2.1. Statistical analysis

The statistical analysis was conducted using the SPSS 15.0 program. Descriptive statistics, including numbers, frequencies, means ± standard deviation, and medians (minimum-maximum), were calculated for the obtained measurements and presented in tables. The normal distribution of numerical variables in each group was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. As the values, except for the Fear of COVID-19 Scale scores, did not follow a normal distribution, Spearman Correlation Analysis was performed to examine the relationships between variables. The strength of the correlations was determined as strong ($r_s \geq 0.70$), moderate ($r_s = 0.40\text{-}0.69$), and weak ($r_s \leq 0.39$) based on the results of this analysis (29). The statistical significance level for the study was set at $p<0.05$.

3. Results

A total of 75 women, with a mean age of 37.88±13.094 years, were included in the study. Table 1 presents the demographic characteristics of the participating women. The median age was 36 years (range: 19-73), the mean height was 1.63±0.059 meters, the median body weight was 64 kg (range: 45-132), and the median body mass index (BMI) was 24.22 kg/m^2 (range: 18.08-49.08) (Table 1).

Table 2 presents the correlations between the scale scores of the participants. The results of the analyses revealed several statistically significant correlations. There were statistically significant, positive, and weak correlations between the Fear of COVID-19 Scale scores with the FSFI and GPFBQ scores (p=0.015, p=0.005). Furthermore, a statistically significant, positive, and weak correlation was found between the FSFI and FSDS-Revised scores (p=0.003). Additionally, statistically significant correlations were observed between the FSDS-Revised scores and the PHQ-4-Anxiety, PHQ-4-Depression, PHQ-4-Total, and GPFBQ scores (p<0.001, p=0.002). Notably, statistically significant correlations were found between the PHQ-4-Anxiety scores with the PHQ-4-Depression, PHQ-4-Total scores, and GPFBQ scores (p≤0.001). Moreover, statistically significant correlations were observed between the PHQ-4-Depression scores with the PHQ-4-Total scores and GPFBQ scores (p<0.001, p=0.038). Lastly, a statistically significant, positive, and weak correlation was found between the PHQ-4-Total and GPFBQ scores (p=0.006).

Table 1. Descriptive characteristics of individuals

Descriptive Characteristics	Individuals (n=75)
	x±SD / median (min-max)
Age (year)	36 (19-73)
Height (m)	1.63±0.059
Weight (kg)	64 (45-132)
Body mass index (kg/m^2)	24.22 (18.08-49.08)
Time after COVID-19 disease (month)	3 (0.10-19)
Working status (n; %)	
Working	52; 69.4%
Not working	23; 30.6%
Education level (n; %)	
Primary school	2; 2.7%
High school	14; 18.7%
Higher education	59; 78,7%
Marital status (n; %)	
Single	21; 28%
Married	54; 72%
Cough condition during COVID-19 disease (n; %)	
Yes	58; 77.3%
No	17; 22.7%
Urinary incontinence during COVID-19 disease (n; %)	
Yes	11; 14.7%
No	64; 85.3%

m: meter, kg: kilogram, n: frequency, %: percentage, x±SD: mean±standard deviation, min: minimum, max: maximum.

Table 2. The relationship between the scale scores of the individuals

Individuals (n=75)		Fear of COVID-19 Scale	Female Sexual Function Index	Female Sexual Distress Scale-Revised	Patient Health Questionnaire-4-Anxiety	Patient Health Questionnaire-4-Depression	Patient Health Questionnaire-4-Total	Global Pelvic Symptom Bother Questionnaire
1. Fear of COVID-19 Scale	**r_s**	-	**0.279**	0.219	0.150	0.086	0.132	**0.320**
	p		**0.015***	0.059	0.200	0.465	0.259	**0.005**
2. Female Sexual Function Index	**r_s**	-	-	**0.335**	0.201	0.092	0.140	0.170
	p			**0.003***	0.083	0.434	0.230	0.145
3. Female Sexual Distress Scale-Revised	**r_s**	-	-	-	**0.495**	**0.350**	**0.428**	**0.508**
	p				**<0.001***	**0.002***	**<0.001***	**<0.001***
4. Patient Health Questionnaire-4-Anxiety	**r_s**	-	-	-	-	**0.828**	**0.947**	**0.361**
	p					**<0.001***	**<0.001***	**0.001***
5. Patient Health Questionnaire-4-Depression	**r_s**	-	-	-	-	-	**0.959**	**0.240**
	p						**<0.001***	**0.038***
6. Patient Health Questionnaire-4-Total	**r_s**	-	-	-	-	-	-	**0.312**
	p							**0.006***
7. Global Pelvic Symptom Bother Questionnaire	**r_s**	-	-	-	-	-	-	-
	p							

n: frequency, r_s: Spearman correlation coefficient, *p<0,05

4. Discussion

The present study revealed significant correlations between fear of COVID-19 and sexual function as well as pelvic floor dysfunction. Additionally, correlations were found between sexual function and sexual distress, anxiety, and depression; and between pelvic floor dysfunctions and anxiety and depression in women infected with COVID-19. These findings suggest that the pelvic floor and sexual functions, along with the psycho-social states, may be affected in women who have contracted COVID-19.

The anal and urethral sphincters are innervated by the pudendal nerve. Inflammation or demyelination of the pudendal nerve, which may occur due to COVID-19, can lead to bladder and bowel incontinence (30). Furthermore, existing literature reports an increased frequency of urination in individuals with COVID-19, and there are studies exploring telemedicine applications for patients with urinary incontinence issues (1,31). The incidence of urinary incontinence is higher in populations with a higher prevalence of chronic cough and increases with age and obesity (32). Frequent and high levels of intra-abdominal pressure due to coughing can lead to recurrent microtrauma in the pelvic floor. Although there is limited evidence, COVID-19, characterized by symptoms such as shortness of breath and recurrent cough, may contribute to pelvic floor insufficiency and consequently result in urinary incontinence (11).

In our study, 77.3% of the participants reported having a cough during their illness, while 14.7% of them reported experiencing urinary incontinence due to COVID-19, which is consistent with the existing literature. Coughing is known to be a significant trigger for stress urinary incontinence, and considering that most participants reported cough-related complaints, we believe that coughing may have contributed to the development of incontinence in these individuals.

Although we anticipated a higher incidence of urinary incontinence during COVID-19, we speculate that the relatively young age and normal body mass index of the participants had a protective effect against urinary dysfunction. In our assessment using the GPFBQ, we specifically inquired about urinary incontinence in the past month, and the prevalence was determined to be 25%. We hypothesize that both the early and late symptoms documented in the literature following COVID-19, as well as other comorbidities experienced by the participants, could potentially contribute to the occurrence of urinary incontinence in the later stages of the disease.

Various measures, such as mandatory lockdowns, were implemented during the pandemic to mitigate the spread of COVID-19. While these measures have been effective in curbing the transmission of the virus, they have also had significant repercussions, including the cessation of social gatherings, the closure of businesses, and widespread financial hardships resulting from job losses (33). The challenges brought about by COVID-19 have not only posed physical health problems but have also given rise to a psycho-social crisis (34). Previous studies investigating the impact of disasters on mental health have demonstrated that large-scale traumatic events accompanied by economic and social consequences are associated with an increased mental burden (33). The pandemic has not only heightened public health concerns but has also contributed to the emergence of significant psychological distress, including avoidance behaviors and post-traumatic stress disorder (35).

In recent years, social isolation and imposing restrictions on personal freedoms have significantly altered people's attitudes toward life and health-related behaviors. The losses incurred due to COVID-19 have sparked a heightened awareness of mortality and existential anxiety (36). The pandemic's profound disruptions to daily life, apprehension about personal and loved ones' health, and overall uncertainty about the future contribute to stress factors. The response to stress can vary based on gender, with women often being the most affected during humanitarian crises such as pandemics, wars, or natural disasters (33,34,37). Women bear a heavier physical and psycho-social burden than men due to the demands of multiple social roles, including childcare, elderly care, occupational and academic responsibilities, household chores, and other obligations (38). With the closure of schools due to quarantine measures, it is anticipated that the workload for women has increased. A study reported a significant surge in violence against women during the lockdown period. The unequal societal norms and gender roles further illustrate that women are affected by the pandemic to a different extent than men (34). Additionally, factors such as socioeconomic vulnerability, tobacco use, exposure to interpersonal violence, social exclusion, and excessive exposure to information can contribute to a heightened perception of fear during the pandemic (38).

In the COVID-19 pandemic process, changes in social life through prolonged quarantine periods, increased anxiety and depression have brought together sexual dysfunction. Bhambhvani et al. (39) examined sexual function and frequency in women, and it was reported that while there was no change in the frequency of sexual function, there was an increase in sexual dysfunction. In the studies, moderate and high levels of fear of COVID-19, which may contribute to increased stress burden and mental distress, were reported, and in a descriptive study conducted on 400 married couples, it was found that fear of COVID-19 harmed the sexual functions of married women and could reduce the quality of life in married individuals (40,41). Despite the fact that sexual dysfunction is not listed as the primary complaint by the individuals who underwent COVID-19, it has been observed as a worsening problem in women during the pandemic period (42). It is thought that COVID-19 infection causes similar inflammatory changes in the pelvic organs as well as its effects on the body. In addition, existing pelvic floor dysfunctions have become more evident in the pandemic due to the limitation of physical activity in daily life and other reasons (42). In a study conducted by Carlin et al. (43), it was found that the COVID-19 pandemic significantly increased the dysfunction in women who had pelvic floor dysfunction. In our study, the change in the women's sexual functions due to COVID-19 was evaluated with the FSFI, which included six sub-parameters. It was observed that the infection of the participants caused a decrease in their sexual functions, and women reported moderate levels of pelvic floor dysfunction, according to the GPFBQ.

Sexual health requires not only women's physical well-being but also their emotional and mental well-being. While the increased fear due to COVID-19 may cause physiological and hormonal changes in the body, the fear of catching COVID-19 may negatively affect a person's sexual functions.

In the current study, we found that there were positive correlations between the fear of COVID-19, sexual functions, pelvic floor dysfunctions, and psychological influences of the participants. We consider that such states as recurrent cough, dyspnea, decrease in physical activity, fear, and anxiety may be effective in revealing their pelvic floor dysfunctions.

During any contagious disease pandemic, the psychological reactions of society play an important role in both shaping the spread of the disease and the emergence of emotional distress and social disorder during and after the pandemic (44). In particular, the couples who experienced more conflict due to living together all the time during the pandemic reported engaging in less intimate relationships and sexual intercourse with their spouses (45). In a study, half of the sample reported a decrease in the frequency of sexual intercourse during the quarantine period due to two possible reasons: distress due to quarantine and inability to reach a preferred sexual partner. Another study conducted on COVID-19 concluded that social distancing negatively affected sexual

activity (46). In the literature, it has been documented that adequate sexual activity is positively associated with psychological well-being, and it affects sexual health in a positive way (47). In a systematic review of 34 articles from 18 countries, the issues ranging from cognitive, emotional, and personality factors affecting sexual life during the pandemic to factors such as relationships, bringing up children, and employment status during this period were discussed, and it was found that women experienced more sexual problems than men during the COVID-19 pandemic, including low sexual desire and low sexual satisfaction. In most of the studies, it was concluded that while decreases in the frequency of sexual intercourse occurred during the pandemic, high levels of stress could also be a major contributor to sexual dysfunction (17).

In our study, it was found that the participants reported mild levels of stress. Literature suggests that emotions are related to sexual problems. Therefore, we believe that the stress reported by the participants may be related to sexual dysfunction and sexual distress. While sexual activity has positive effects on psychological well-being and sexual health, the presence of emotional distress, such as stress and anxiety, may lead to a decrease in sexual intercourse frequency.

As post-COVID-19 patients may be more prone to anxiety and post-traumatic stress disorder-type symptoms, it is important for physiotherapists to assess their patients' mental health concerns. Anxiety can increase the risk of urgent and frequent urination and place them at higher risk for constipation by overloading the patient's sympathetic nervous system. In addition, anxiety can lead to excessive activity and pain in the pelvic floor by causing a chronic gripping pattern in the pelvic floor muscles (11). Although patients with pelvic floor dysfunction are not under a life-threatening risk, it is well-known that they face limitations in their daily lives, which negatively affect their quality of life, including social, emotional, and sexual well-being (43).

In the results of our study, it was observed that there was mild anxiety and depression in the participants, and there was a correlation with GPFBQ. We also consider that the reason for the mild course of anxiety and depression due to COVID-19 may be owing to the fact that the participants did not experience severe symptoms when they were infected and/or due to their demographic characteristics.

As a result of our study, we believe that the pelvic floor, sexual functions, and psycho-social state may be affected in women who have undergone COVID-19. Microtraumas that may occur due to increased intra-abdominal pressure and the restriction of physical activity in daily life may play a significant role in the emergence of pelvic floor dysfunction.

While dysfunction in the pelvic floor negatively affects both sexual activity and psychological well-being in women, such psychological influences as anxiety and depression can also decrease sexual functions in women. Together with the fact that sexual function and pelvic floor problems are among the issues that women have difficulty talking about in our society, its correlation with the psycho-social state is also supported by the literature. Even if they are not included in the main symptoms of COVID-19, these dysfunctions are very important in human life, especially in the individual's quality of life. For this reason, there is a need for educational programs and more research in order to be able to ensure public awareness.

There were some limitations in our study. Since the current study included women in Turkey, the participants could not be evaluated face-to-face. Instead, they were evaluated on the Internet through Microsoft Forms. Another limitation was that the chronic diseases of the participants were not questioned. In addition, the absence of a control group in the present study was another limitation.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: Ö.Ç.Ö, B.Y., K.Ö., Design: Ö.Ç.Ö, B.Y., K.Ö., Data Collection or Processing: Ö.Ç.Ö, B.Y., K.Ö., Analysis or Interpretation: Ö.Ç.Ö, B.Y., K.Ö., Literature Search: Ö.Ç.Ö, B.Y., K.Ö., Writing: Ö.Ç.Ö, B.Y., K.Ö.,

Ethical Statement

Approval was obtained from Izmir Democracy University Non-Invasive Clinical Research Ethics Committee, the study started. The ethics committee decision date is 23/02/2022 and the number of ethical committee decisions is 2022/03-16.

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A new viewpoint of Eagle syndrome: The effect of SP medial angulation on symptomatology

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Abstract

Eagle syndrome is an uncommon condition triggered by head movements caused by a long styloid process (SP) or calcification of the stylohyoid ligament, and characterized by symptoms such as sore throat, pain at the root of the tongue, earache, and odynophagia or globus. The aim of this study was to examine the effects of medial angulation of the SP on symptomatology. The study included 49 patients diagnosed with long SP and followed up between January 2018 and January 2020 in the Ear, Nose, and Throat Clinic of Samsun Training and Research Hospital. The medial angle between the SP and the virtual line drawn vertically from the temporal bone attachment point of the SP was measured and recorded. Evaluation was made of 49 patients, comprising 23 (46%) males and 26 (54%) females in the age range of 19-65 years. The SP length ranged from 25mm to 44mm. The presence of at least one symptom was determined in 24 (48%) patients; in 11 (45%) males, and 13 (55%) females. A significant positive correlation was determined between right medial angulation and symptom severity in patients with globus (p:0.01, r:0.5). It should be kept in mind that, in addition to SP length, SP medial angulation could be an additional factor affecting the formation and severity of symptoms in patients with Eagle syndrome.

Keywords: Eagle, styloid process, angulation, severity, globus

1. Introduction

The styloid process (SP) and stylohyoid ligament are thin cylindrical structures, which develop embryologically from the second branchial arch, and are seen inferior and anterior in the lower section of the temporal bone. It is important as muscles and ligaments that play a role in chewing and swallowing are attached to this structure and there is proximity to important anatomic structures (1, 2). The SP generally ossifies between the ages of 5-8 years. However, different degrees of ossification can occur in the stylohyoid ligament and this can cause the development of long SP with calcium deposition at the SP tip (3). Although SP length varies according to individual and ethnic characteristics, normal length has been reported to be 25 mm, and length greater than this is referred to as long SP (4, 5).

Eagle syndrome, first defined by the American otolaryngologist, Watt W. Eagle, is an uncommon condition triggered by head movements caused by calcification of a long SP or stylohyoid ligament, and is characterized by symptoms such as sore throat, pain at the root of the tongue, earache, and odynophagia or globus (6, 7). The reported incidence is 4%, but 4-10% of those with long SP are seen to be symptomatic (3, 8). Opinions have emerged that in addition to SP length at this low rate, medial or anterior angulation could play a role in symptom development, and thus the presence of symptoms with angulation has become a matter of debate. To the best of our knowledge, there is no study in the English literature which has shown a relationship between symptom intensity and the SP angle. The aim of this study was to examine the relationship of SP length and angulation with the presence of symptoms and symptom severity in cases with long SP, and to discuss this in the light of current literature.

2. Material and Methods

The study included 49 patients diagnosed with long SP and followed up between January 2018 and January 2020 in the Ear, Nose, and Throat Clinic of Samsun Training and Research Hospital. Patients were excluded from the study if they were aged <18 years, had any chronic disease, a history of cervical surgery, or any psychiatric disease. ENT examinations and endoscopic nasopharynx and larynx inspections, a complete blood count and routine biochemical analysis including thyroid function tests, were performed on all patients and any patients with any abnormality detected were excluded from the study.

The tomography images of the patients were examined and the medial angle between the SP and the virtual line drawn vertically from the temporal bone attachment point of the SP was measured and recorded (Fig. 1). Ossified and calcified stylohyoid ligaments were included in the measurements, and the SP length was measured and recorded (Fig. 2).

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Fig. 1. Measurement of the SP medial angle: the angle between the SP and the virtual line drawn vertically from the temporal bone attachment point of the SP

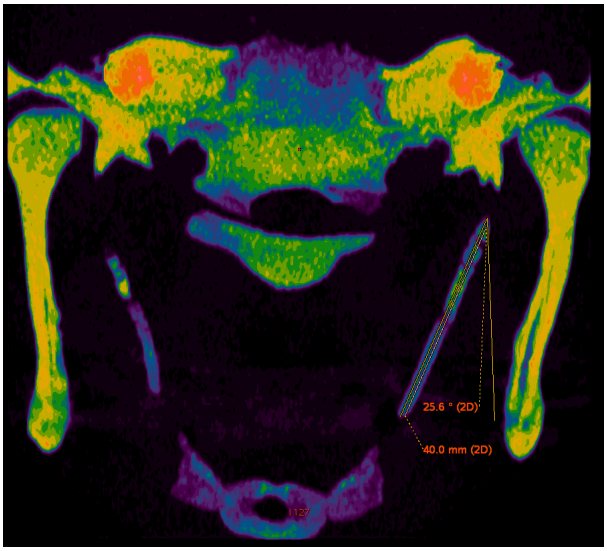

Fig. 2. SP length measurement

Ther complaints of the patients (earache, sore throat, globus sensation, pain triggered by neck movements) were recorded. The severity of the symptoms was evaluated subjectively using a Visual Analog Scale (VAS) (Fig. 3). The patients were instructed to mark the severity of their symptoms on a line numbered from 0 to 10, where 0 represents no problem and 10, intolerable.

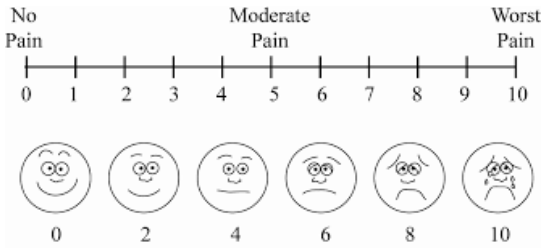

Fig. 3. Visual Analog Scale (VAS): subjective scoring used for the measurement of symptom severity

The patients were separated into two groups according to the presence of symptoms: Group 1 with patients with symptoms, and Group 2 with patients with no symptoms.

2.1. Radiological Imaging

CT scans were obtained in the coronal plane (prone or hanging head position) with 1-mm slice thickness, 0.8-mm reconstruction increment, 16–18-cm FOV, 120 kV, 110 mA and 512×512 matrix size on a Somatom Plus Spiral CT scanner (Siemens AG, Erlangen, Germany). An average of 49 images were obtained from each patient. The images were reconstructed with a real-time 3D (RT3D) interactive volume-rendering module of a Workstation (3D Virtuoso CT/MR Workstation, Siemens AG, Germany), and 3D images were obtained using RT3D render.

2.2. Statistical Analysis

In statistical comparisons between groups, the t-test was used for continuous variables, and the Chi-square test for categorical variables. In all measurements, a value of $p < 0.05$ was considered statistically significant. All statistical analyses were conducted using SPSS 24.0 statistics software (IBM SPSS Statistics for Windows, NY, USA).

3. Results

Evaluation was made of 49 patients, comprising 23 (46%) males and 26 (54%) females in the age range of 19-65 years. The SP length ranged from 25mm to 44mm. The presence of at least one symptom was determined in 24 (48%) patients, 1 (45%) males and 13 (55%) females. The demographic data of the patients and the SP length angulations of the groups are shown in Table 1.

Table 1. The demographic data of patients and the SP length and angulation values of the groups

	Group 1 (symptom + N=24)	Group 2 (symptom- N= 25)	P value
	Mean ±SD	Mean ±SD	
Age	47.208±15.455	41.720±16.789	0.25
Right SP length	32.504±8.585	30.760±5.222	0.704
Left SP lenght	33.533±6.799	28.544±4.331	0.015
Right SP angulation	22.045±4.089	18.364±4.334	0.003
Left SP angulation	21.879±3.292	18.716±4.625	0.014

Mann-Whitney U test

The complaint of earache was reported by 22 (44%) patients. No significant correlation was determined between SP length, SP angulation and symptom severity in the patients with earache.

The complaint of sore throat was reported by 19 (38%) patients. No significant correlation was determined between SP length, SP angulation and symptom severity in the patients with sore throat.

Globus was present in 18 (36%) patients. A significant positive correlation was determined between right medial angulation and symptom severity in the patients with globus (p:0.01, r:0.5). No significant correlation was determined for the other parameters.

The complaint of pain triggered by neck movements was reported by 16 (32%) patients. A significant positive correlation was determined between this complaint and both right and left SP length (right-p:0.01, r:0.5; left-p:0.03, r:0.4). No significant correlation was determined between SP angulation and symptom severity.

4. Discussion

The styloid process provides attachment of the M. stylopharyngeus, M. stylohyoideus, and M. styloglossus, which play a critical role in chewing and swallowing, to the stylohyoid and stylomandibular ligaments (9). It is also in close proximity to important neurovascular structures such as the hypoglossal nerve, carotid artery, and internal jugular vein (10). Originating from pressure on the structures surrounding the long SP, symptoms emerge such as ipsilateral neck pain, and the feeling of a foreign body when swallowing (2, 11). The presence of a long SP together with symptoms, which was first described by Eagle, is now known as Eagle syndrome. Although the incidence shows great variability, it has been reported on average at 4%.

Earache is the most commonly reported complaint (12, 13). In the current study, earache was the most frequently seen complaint, followed by sore throat, globus, and pain triggered by neck movements.

There are studies in the literature showing that there is no correlation between age and gender with/and ? SP length and symptomatology (12, 14). In contrast, Pokharelve et al. reported that females have a longer SP than males (15). Bafaqeeh et al. also showed that the SP was longer in females than males and they were more symptomatic (2). In the current study, SP length and angulation were determined to be greater in females than males, and the presence and severity of symptoms were also greater in females.

In the literature, there are various classifications related to SP length and shape. Başekim et al. (16) presented a new classification named LAM (length, angulation, morphology) with the addition of angulation and morphological findings to the previous length and shape-based classifications for SP. In computed tomography (CT) examinations performed for various reasons, 7% of patients have been reported with SP length >4cm, and 77% with SP length 2-4cm. In all the patients in the current study diagnosed with long SP, the SP length was >2.5cm. In 7 (14.2%) patients, the SP length was measured bilaterally >4cm.

The diagnosis of Eagle syndrome is made with the help of radiological examination in addition to symptoms. However, the determination of SP length on direct radiographs is difficult because of the superimposition of anantomic structures. Therefore, 3D CT examination is the gold standard in the measurement of the SP and styloid complex axis, length, and angle (17, 18). It is recommended that CT images be taken in the axial and coronal planes.

Recent studies have shown that the presence of symptoms is associated more with SP angulation than SP length (15, 20). In particular, pain in the throat triggered by neck movements has been associated with irritation of the structures in the parapharyngeal area because of abnormal angulation rather than SP length (4, 20). Yavuz et al. found that the anteroposterior angle had a significant effect in symptomatic patients, whereas no significant difference was observed in mediolateral angulation (21). Some studies have shown that a decrease in medial angulation has a greater effect on the presence of clinical symptoms than anteroposterior angulation (15, 22, 23). Kent et al. reported that the proximity of the SP to the superior constrictor and glossopharyngeal nerve was more important than length and angulation in respect of symptoms (24). In the current study, the relationship was investigated between symptom presence and severity and the SP length and medial angulation. A significant positive correlation was determined between medial angulation and globus sensation, and there was not seen to be any significant correlation between other symptoms and angulation.

The feeling of symptoms unilaterally suggested that the two sides (right and left) of SP angulation could be different. In a study by Kosar et al. (26), a significant difference was determined between the right and left medial angulation in all the patients, whereas Nayak et al. (19) reported that medial angulation was less on the left side. In contrast, Onbaş et al. (27) determined no significant difference between the right and left sides in respect of medial angulation. In the current study, angulation on both sides was found to be greater in the patients with symptoms compared to the patients without symptoms.

The limitations of this study were the relatively low number of patients and the fact that symptoms were evaluated without determination of the side. Therefore, there is a need for further studies with a larger patient population to evaluate symptoms by determining the right and left sides.

In conclusion, it should be kept in mind that SP medial angulation, in addition to SP length, could be an additional factor affecting the formation of symptoms and symptom severity in patients with Eagle syndrome.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: N.F.T., Design: N.F.T., Data Collection or Processing: N.F.T., U.Y., Analysis or Interpretation: U.Y., Literature Search: U.Y., Writing: N.F.T., U.Y.

Ethical Statement

Approval was obtained from Samsun University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 15/06/2022 and the number of ethical committee decisions is 2022/3/7.

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The clinical features and concomitant diseases in preschool high myopia

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Abstract

The aim of this study was to summarize the clinical and demographic findings of preschool children presenting to the ophthalmology clinic with high myopia and to compare them with controls. A retrospective chart review was performed in patients with myopia of -6D or higher before the age of 4 years. The control group of similar age and sex was created from the patients presenting to the ophthalmology department for routine eye examination without any apparent complaint. Child and parent characteristics, concomitant ocular and systemic pathologies were recorded and analyzed. Seventy patients (35 cases with high myopia and 35 controls) were included in the study. Compared to controls, the cases in the study group had significantly higher rate of birth in spring (37.1% vs 8.6%), higher rate of strabismus (57.1% vs 14.3%) and paternal smoking (54.3% vs 37.1%), higher parental consanguinity (34.3% vs 11.4%) and higher rate of concomitant diseases (51.4% vs 17.1%). Birth season, paternal smoking and consanguinity were found to correlate with high myopia in children under the age of 4. High rates of ocular and systemic comorbidities should be considered in patients with high myopia at preschool age.

Keywords: high myopia, preschool myopia, strabismus, consanguinity, birth season

1. Introduction

Myopia is a disorder which can be corrected with glasses or contact lenses. However the rapid increase in prevalence in recent years and the increased risk of potentially blinding complications makes myopia a major public health concern (1). Myopia usually occurs due to the elongation of the axial length of the eye. Controlling the axial elongation during the eye development may prevent myopia and may result in normal vision. However routinely used optical correction just makes the object to focus on the macula and has no impact on the axial length. Understanding the biological basis for the myopia development and exploring the causes are the major target points for prevention. Recent studies have shown that the myopia is not just genetic, but has a heterogeneous etiology. Intensive near work, decreased amounts of time outdoors are found to be strong environmental factors for the myopia development and progression (2,3). Myopia prevalence is high in school age children and low in preschool children. Preschool myopia also differs from school age myopia in terms of risk factors and clinical course; however it is less discussed in the literatüre (4-6). Given that myopia is a risk factor for amblyopia and strabismus, the early detection and treatment of refractive errors in infancy is crucial. The aim of this study was to determine the clinical features and associated co-morbidities in preschool children with high myopia.

2. Materials and Methods

This study was approved by the Ethics Committee of the University and conducted in accordance with the Declaration of Helsinki. The charts of patients admitting to the Ondokuz Mayis University ophthalmology clinic between 2013 and 2021 were reviewed. The clinical records of children diagnosed with myopia before the age of 4 years were retrospectively analyzed. The patients with high myopia were included in the study. High myopia was defined as a spherical equivalent refraction of > -6D in cycloplegic refraction measurement in at least one eye. Age, gender, prematurity, mother's age at delivery, presence of any other ocular or systemic diseases, visual acuities, cycloplegic refractions, anterior and posterior segment findings were recorded. The parents were asked about time spent outdoors (sun exposure) and time spent in front of screen devices (mobile phones and tablets), as well as maternal and paternal myopia or history of smoking. The causes of high myopia were determined if present. The exclusion criteria were as follows: patients receiving the myopia diagnosis at or over 5years of age, infants with myopia <-6D, and patients with inadequate or missing data. The control group was chosen from patients presenting to the ophthalmology department for routine eye examination without any apparent complaint. The control group consisted of patients of similar age and sex with the study group.

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Cycloplegic refraction was obtained at each visit. Refractions were measured at least 30 minutes after the two consecutive administrations of 1% cyclopentolate 20 minutes apart, which is a routine procedure in the outpatient clinic. A handheld authorefractor Retinomax Plus (Nicon Inc., Japan) was used to obtain the refraction. However, all refractive values were rechecked with retinoscopy. Axial length measurement of the globe was performed in some patients.

The patients were analyzed in terms of risk factors such as maternal or paternal myopia, smoking, prematurity and birth season. The possible causes like treatment for retinopathy of prematurity (ROP), systemic diseases, microspherophakia were recorded. The patients without an eligible cause were evaluated as isolated high myopia or pathological myopia.

2.1. Statistical analysis

Data were analyzed with SPSS (Version 22 for Windows, SPSS Inc, Chicago, IL, USA).The Shapiro–Wilk test was applied to examine whether the measurements in the study were normally distributed. Normally distributed continuous variables were presented as mean ± standard deviation and compared between the study groups using independent t- test. Continuous variables that were not normally distributed were presented as median with the minimum and maximum values and statistically analyzed using the Mann-Whitney U test. Paired t tests or Wilcoxon tests were used to assess within-group changes over the intervention period. Categorical variables, presented in counts or as percentages, were analyzed using the chi-square or Fisher exact test when appropriate. P values lower than 0.05 were considered statistically significant.

3. Results

Seventy children were included in the study (35 high myopes and 35 controls). The refraction in the right and left eyes in the study group were -8.46±3.20 and -8.35±3.37 respectively. The refraction in the right and left eyes in the control group were +2.00±1.65 and +2.16±1.75 respectively. There was a significant difference between the groups($p<0.001$).

Table 1. Demographic factors of patients

	High myopia (n=35)	Control group (n=35)	p
Age (years)	1.64±1.09	1.76±1.29	0.673
Gender (F/M)	19/16	19/16	1
Maternal age at birth (years)	28.29±4.93	27.70±4.13	0.613
Follow-up time (months)	26.5±29.30	4.14±10.04	**<0.001**
Screen devices (hr/day)	0.74±1.11	1.10±2.49	**<0.001**
Sun exposure (hr/day)	2.48±2.06	1.49±1.67	**<0.001**

Nineteen (54.3%) females were present in both groups. The mean maternal age at birth, mean age of the patients at the diagnosis, gender, follow-up time, time spent with screen devices and time spent outdoors (sun exposure) are presented in Table 1. The age and gender of the patients and maternal age at delivery were similar in the study and the control groups ($p>0.05$). The study group had a higher rate of sun exposure, while the control group had a longer time spent in front of the screen devices ($p<0.001$).

The distribution of prematurity, maternal and paternal myopia/ smoking, season of birth, strabismus and consanguinity are given in Table 2. Paternal smoking and parental consanguinity were significantly higher in the study group compared to controls (p: 0.035, p: 0.023 respectively). The higher rate of birth was observed in spring for the study group (37.1%, p: 0.016). The rate of strabismus was higher in the study group with 13 cases of esotropia and 7 cases of exotropia (57.7%). In the control group, there was one case of esotropia, 3 cases of exotropia, and one case with oblique dysfunction ($p<0.001$).

Table 2. Clinical and parental features of patients

		High myopia (%)	Control group (%)	p
Premature delivery		6 (17.1)	4 (11.4)	0.495
ROP treatment		4 (11.4)	1 (2.9)	0.164
Birth season	Winter	5 (14.3)	9 (25.7)	0.23
	Spring	13 (37.1)	3 (8.6)	**0.004**
	Summer	14 (40.0)	14 (40.0)	1
	Autumn	3 (8.6)	9 (25.7)	0.057
Maternal myopia		6 (17.1)	10 (28.6)	0.424
Paternal myopia		3 (8.6)	8 (22.9)	0.168
Maternal smoking		2 (5.7)	4 (11.4)	0.508
Paternal smoking		19 (54.3)	13 (37.1)	**0.035**
Strabismus		20 (57.1)	5 (14.3)	**<0.001**
Consanguinity		12 (34.3)	4 (11.4)	**0.023**
Concomitant disease		18 (51.4)	6 (17.1)	**0.003**

Myopia was associated with ROP in 4, microspherophakia in 1, microcornea and foveal agenesis in 1, microcornea and chorioretinal coloboma in 2, congenital glaucoma in 1, persistant hyaloid artery on the disc in 1. Degenerative myopia was diagnosed in 4 (one unilateral and three bilateral cases), and isolated high myopia in 21 cases (two had myelinated nerve fibers).

Concomitant diseases were present in 18 cases (51.4%) with high myopia and included the followings: Sturge-Weber syndrome, atrial septal defect, patent foramen ovale, tetralogy of fallot, lung disease, asthma, iron deficiency anemia, developmental defects, cerebral palsy, mental retardation, hypospadias, enuresis, stuttering and partial trisomy 29 with congenital glaucoma, hypothyroidism, vesicoureteral reflux and incontinence. Concomitant diseases in the control group were present in 6 cases (17.1%) and included intracranial hemorrhage with ventriculoperitoneal shunt, asthma, epilepsy, developmental defect, hydrocephalus and hyperactivity.

4. Discussion

Cases with high myopia at preschool age presenting to the ophthalmology department may have various causes and concomitant ocular or systemic pathologies. Most studies in

the literature focus on high myopia at school age. In this study, we analyzed thirty-five preschool children with high myopia. Compared to the control group, high myopia cases were followed for a longer period, were mostly born in spring, and had a higher rate of paternal smoking, strabismus, parental consanguinity and concomitant disease.

Specific geographical distribution of myopia may show that some populations might be genetically more susceptible to the environmental risk factors. However, families share environments, as well as genes (2,7). We observed a higher rate of parental consanguinity in high myopia group (34.3% vs 11.4%). Although this finding supports the evidence of genetic susceptibility at an early age, we did not find a significant effect of paternal or maternal myopia. One possible explanation for this is that preschool high myopia may have a recessive inheritance pattern. Hence, consanguineous parents may have a higher risk of having a myopic child. Myopia in parents has been shown to be a risk factor for the development of myopia in children in several studies. However, most studies focused on patients at an older age, so, it is difficult to extrapolate their findings to our study (8-10). In a study screening for myopia it was found that parental myopia significantly increased the risk of myopia in 11- and 15-year old children, but not in 7-year olds (10). It may be considered that high myopia at an early age and myopia at school age may have different risk factors.

Myopia at school age has been shown to increase with near work and decreased outdoor activities (8-10). In contrast to this evidence, we obtained that high myopic patients spend more time outdoors and less time in front of mobile phones and tablets compared to controls. We don't know exactly if this is the cause or the result of myopia. If we take into account the longer duration of follow-up of the patients with high myopia in our study; we may attribute these findings to the attention of parents, who follow the recommendations of physicians on prevention of myopia. We routinely and strictly advise the patients with myopia to stay away from mobile phones and tablets, and discuss the beneficial effects of sun exposure with parents.

Compared to control group, the majority of our cases with myopia were born in spring. This might support the findings in the literature. Studies conducted on adults and newborns showed that those born during months with longer daylight hours are at higher risk of myopia (3,11). Many other environmental factors may influence the development and progression of myopia, as well as light exposure that may affect emmetropization, thus influencing refraction.

The history of paternal smoking was higher among the patients with high myopia. The insignificant result regarding maternal smoking may be attributed to the low rate of maternal smoking in both the study and the control groups. Some studies reported that parental smoking is associated with a lower prevalence of myopia, lower myopic refraction and shorter axial length in children. The possible mechanism of ocular growth affected by the nicotine was proposed (12,13). However, there are also studies in the literature reporting either no association between parental smoking and myopia or an increased risk of myopia in smoking mothers (14,15). The study involving the Singapore Chinese children at a very young age (6-72 months) reported an 11% of myopia prevalence (refraction at least -0.5D), and found no association of paternal smoking with refractive error and an inverse relation of refraction with maternal smoking. There are some differences between this study and ours, as we only included the patients with high myopia (at least -6.0D) with a different ethnicity.[14] Such diversity in the results reflects the multifactorial nature of myopia.

There were no patients diagnosed with staphyloma in our study. Hsiang HW et al. found that posterior staphyloma is not common in children but its prevalence increases with age (16). Retinal detachment was not observed in any of cases. However lifelong observation is suggested as the risk of blinding complications increases as time progresses.

There were significantly higher rate of concomitant diseases in patients with high myopia compared to the control group. The frequency of strabismus among high myopes was 4 times higher than that in the control group. This association is consistent with the findings in the literature. Zhang et al analyzed the prevalence of strabismus and its risk factors in school aged children in Hong Kong. They observed a significant positive association between strabismus and myopia of >-1.0D (OR 1.72, p:0.012) (17). A meta-analysis involving 23.541 children showed that myopia (generally over -0.50D or -1.0D) resulted in an increased risk of developing concomitant strabismus (OR: 3.22, 95% CI: 1.84–5.65, $P < 0.0001$), and even more increased risk of exotropia specifically (OR: 5.23, 95% CI: 2.26–12.09, $P = 0.0001$). Decreased accommodative effort in patients with myopia is postulated as the main cause of strabismus (18). High myopia accompanied with anomalies in the eye, brain, heart or genitourinary system may be a part of the neurodegenerative disorders requiring genetic evaluation (19). Hence, such patients should be referred for detailed pediatric and genetic evaluation.

The major limitation of this study is the limited number of patients. Also, factors such as time spent outdoors or in front of mobile phones depended solely on the response of the parents that may be inaccurate, especially if the parents are working.

In conclusion, this is a summary of demographic and clinical findings in children presenting with high myopia before the age of 4 years. Preschool high myopia differs from teenage myopia in terms of genetic and environmental factors. Its rate increases in cases born in spring and in consanguineous parents. It is associated with a higher incidence of strabismus. Children with myopia presenting to the outpatient clinic have an increased risk of concomitant ocular and systemic diseases.

Detailed history taking and thorough physical examination should be performed for early detection and treatment of the disease to avoid life-long complications. Further studies with larger sample size are needed to identify the exact risk factors and to analyze the progression of high myopia at a very early age.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: M.U., T.A., Design: M.U., T.A., Data Collection or Processing: M.U., T.A., Analysis or Interpretation: M.U., T.A., Literature Search: M.U., T.A., Writing: M.U., T.A.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 27/02/2020 and the number of ethical committee decisions is 2020/93.

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Increasing risk of silent aspiration in stroke patients

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Abstract

Dysphagia is a common complication of stroke that is often undiagnosed and leads to aspiration. This cross-sectional study aimed to detect the risk of silent aspiration during the swallowing process based on the location of the stroke lesion. A total of 48 stroke patients undergo a Fiberoptic Endoscopic Evaluation of Swallowing (FEES) examination to determine dysphagia profile based on penetration, standing secretion, residue, leakage, and silent aspiration. On the pre-swallowing assessment, there was an abnormal cough reflex and tongue movement weakness in 77.1% and 47.9% of patients. On the FEES assessment, penetration occurred in 72.9% of the patients and 100% of stroke patients with mixed lesions. Standing secretion occurred in 56.3% of the patients and 83.1% of stroke patients with brain stem lesions. Food residue occurred in 81.3% of patients and 100% of stroke patients with mixed lesions. Pre-swallowing leakage occurred in 91.7% of patients and 100% of stroke patients with mixed and brainstem lesions. Silent aspiration occurred in 29.2% of total patients and 60% of stroke patients with mixed lesions. The risk of silent aspiration in repeated strokes was five times higher than in first-stroke patients (P = 0.013). In conclusion, mixed lesions cause more severe oral and pharyngeal phase disorders as well as a higher risk of penetration and aspiration than other lesions.

Keywords: stroke, respiratory aspiration, deglutition disorders, swallowing, dysphagia

1. Introduction

Stroke is recognized as one of the world's five common causes of disability-adjusted life-years (DALY). It is associated with several medical complications that lead to high healthcare costs and prolonged hospital admissions (1, 2). In 2019, the Global Burden Disease (GBD) reported that stroke is the second-leading cause of death (11.6% of total deaths) and the third-leading cause of disability and death combined (5.7% of total DALYs). From 1990 to 2019, the incidence of strokes increased by 70%, the prevalence of stroke increased by 85%, deaths from stroke increased by 43%, and DALYs increased by 32%. The incidence and prevalence rates of stroke in young people significantly increase due to the increased metabolic risk factor for stroke, including elevated blood pressure, diabetes mellitus, and obesity among young people (3).

Patients with stroke are vulnerable to multiple complications (4). Dysphagia, defined as swallowing impairment, is a common complication in 33-73% of stroke patients (5). Most of the complications of stroke can recover within a week. However, dysphagia can persist for up to 6 months (4). Dysphagia is accompanied by stroke mortality and post-stroke complications, such as pneumonia, dehydration, malnutrition, and poor long-term outcome (6). Dysphagia is considered the main risk factor for pneumonia after stroke. Patients with dysphagia are three times more likely to have pneumonia (1). In addition, dysphagia also affects the quality of life, socialization, self-esteem, and increases healthcare-associated costs (7). Elderly stroke patients have more chance of having dysphagia due to the alterations in breathing coordination, swallowing, and reduced cough reflex.

Swallowing can easily be disrupted after stroke because it is a complex and fast neuromuscular mechanism that requires the coordination of more than 30 muscles, five cranial nerve, and several brain regions (7). Previous studies demonstrated the correlation between lesion location of stroke and post-stroke dysphagia. The brain stem (the pons and medulla) is the central control of swallowing (8). Damage to the brainstem structures is strongly suspected to be associated with dysphagia due to corticospinal fractures and superior longitudinal fasciculus connecting the swallowing centers in the cerebral cortex to the central pattern generator for deglutition (9).

The Flexible Endoscopic Evaluation of Swallowing (FEES) was proved to be a significant tool for assessing oropharyngeal dysphagia and aspiration severity. FEES is a comfortable, tolerable, and excellent procedure for assessing dysphagia as long as the focus is on the oropharyngeal structures and aspirations because the FEES directly visualizes these structures without the risk of radiation (10, 11).

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Despite its association with stroke mortality and other post-stroke complications, post-stroke dysphagia is often undiagnosed and under-addressed. Stroke patients may not be aware of liquid or food entering the airway, leading to aspiration. Therefore, this study aimed to detect the risk of silent aspiration during the swallowing process using FEES examination in stroke patients based on the location of the stroke lesion.

2. Material and Methods

This cross-sectional descriptive study determines the prevalence of silent aspiration and features of swallowing in stroke patients with brainstem and non-brainstem lesions. The study was conducted at the Integrated Dysphagia Clinic, Endoscopic Bronco-Esophagology Division, Otorhinolaryngology-Head and Neck Surgery Department, Faculty of Medicine Universitas Indonesia (FMUI)/ Cipto Mangunkusumo Hospital (RSCM), Jakarta, Indonesia. A consecutive sampling technique was used in this study.

The inclusion criteria included stroke patients without swallowing difficulty before having a stroke, who have the results of computed tomography (CT) or head magnetic resonance imaging (MRI) examinations, can come to the Dysphagia Clinic, can be positioned semi-sitting or sitting, and willingness to undergo FEES examination. The exclusion criteria included unconscious, uncooperative, patients with severe cognitive impairment, or patients with contraindications for FEES examination, such as unstable vital signs or bleeding disorders. This study examined FEES on 48 stroke patients who came directly or were sent from the neurology treatment and outpatient room. The patients will undergo history taking, general ear nose and throat (ENT) examination, preswallowing assessment to analyze the structure of the swallowing process, and FEES examination to assess five parameters of swallowing, such as penetration, standing secretion, residue, leakage, and silent aspiration, using five different consistencies of food (oatmeal, puree, crackers, gastric rice, and milk). The FEES examination was performed by an ENT specialist using Fiberoptic nasopharyngolaryngoscopy Olympus visera ENF type V. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corporation, Armonk, NY, USA). Differences between groups were considered statistically significant if the p-value of < 0.05.

3. Results

Of the 48 subjects, the largest gender is male, more than three times the number of female subjects, as shown in Table 1. The distribution of subjects based on age, which is divided into four age groups, is mostly found in the 50-59 years age group (22 subjects or 45.8%), followed by 18 subjects (37.5%) at age ≥60 years.

Based on the frequency or history of stroke, divided into two groups, the first-time stroke group (56.3%) was slightly more than the recurrent stroke group (43.7%). The distribution of subjects based on stroke onset was divided into two groups, and the group with an onset of less than one month (54.2%) was almost the same as the onset group of more than or equal to one month (45.8%).

Table 1. Characteristics of the subjects

Characteristics of research subjects		N (%)
Sex	Men	37 (77.1)
	Women	11 (23.9)
Age group	<40	3 (6.3)
	40-49	5 (10.4)
	50-59	22 (45.8)
	≥60	18 (37.5)
Stroke frequency	First	27 (56.3)
	Repeated	21 (43.7)
Stroke onset	< 1 month	26 (54.2)
	≥ 1 month	22 (45.8)
Stroke type	Hemorrhagic	9 (18.8)
	Ischemic / infarction	35 72.9)
	Hemorrhagic infarction/hemorrhagic transformation	3 (6.2)
	Computer tomography and MRI of the head normal	1 (2.1)
Location of the lesion	Brain stem	6 (12.5)
	Hemisphere/cortex and subcortical	37 (77.1)
	The mixture of brainstem and hemisphere	5 (10.4)
History/complaints of dysphagia	Elongated feeding time	24 (50)
	Hoarseness after eating/drinking	3 (6.3)
	Cough while swallowing	23 (47.9)
	Regurgitation	3 (6.3)
	Consistency of food consumed	
	• All consistency	32 (66.7)
	• Soft & fluid	14 (29.1)
	• Liquid	2 (4.2)
	History of pneumonia	5 (10.4)

Prolonged feeding time, followed by coughing while

swallowing, is the most common complaint of dysphagia. Regurgitation and hoarseness after eating or drinking was found in only three subjects (6.3%). Based on the consistency type of food consumed, most subjects (66.7%) still consumed all types of food, from solid to liquid. Due to medical instructions, two subjects (4.2%) consumed only liquid food via nasogastric tube (NGT).

From the results of computed tomography and MRI in Table 1, in general, the type of stroke in this study was ischemic stroke (72.9%) with a frequency of more than three times the hemorrhagic stroke (18.8%). Three subjects on the supporting examination showed a mixture of hemorrhagic and ischemic strokes. According to the location of the stroke lesion, as seen in the investigation, the location of most lesions was cortical or subcortical / hemisphere lesions (77.1%). One subject on investigation showed no stroke or normal lesions. Because the subject was clinically suspected as the cause of the brain stem lesion, it was included in the brainstem lesion group, but the type of stroke could not be determined as ischemic or hemorrhagic.

Table 2. FEES initial examination results (n = 48)

Characteristics of the subject	(%)
Drooling	4 (8.3)
Poor oral hygiene	19 (39.6)
Weakness in tongue movement	23 (47.9)
Weakness of the buccal muscles	15 (31.3)
Palate motion asymmetry	11 (22.9)
Weakness of closure of the velopharynx when swallowing	20 (41.7)
NGT	4 (8.3)
Vocal cords paralyze	14 (29.2)
Poor cough reflex	37 (77.1)
There is no swallowing motion on command	11 (22.9)

The initial FEES examination was carried out to evaluate the state of the structures involved in the swallowing process, and the evaluation results are shown in Table 2. The most common abnormality on this examination was abnormal cough reflex (77.1%). Furthermore, in sequence, it was followed by abnormalities of tongue movement weakness (47.9%), velopharyngeal weakness (41.7%), poor oral hygiene (39.6%), buccal muscle weakness (31.3%), vocal cord paresis (29.2%), there was no swallowing motion on orders/volunteers (22.9%), drooling (8.3%), and the use of NGT (8.3%).

The data in Table 3 shows that the mixed lesion group has the highest percentage of FEES parameters with the most (four parameters), namely preswallowing leakage, residue, penetration, and silent aspiration. Judging from the number of findings for each parameter, the hemispheric lesion group showed the highest number for all parameters. The highest number of these parameters is preswallowing leakage (33 subjects), and the lowest is silent aspiration (9 subjects).

Table 3. Distribution of FEES parameters according to the location of the brainstem, hemisphere, and mixed lesions

FEES parameter	Amount (percent of the total subjects, n = 48)	Lesions		
		Mixed (n=5)	Brain stem (n=6	Hemisphere (n=37)
Standing secretion	27 (56.3)	4 (80)	5 (83.3)	18 (48.6)
Preswallowing leakage	44 (91.7)	5 (100)	6 (100)	33 (94.3)
Residue	39 (81.3)	5 (100)	5 (83.3)	29 (82.9)
Penetration	35 (72.9)	5 (100)	4 (66.7)	26 (70.3)
Silent aspiration	14 (29.2)	3 (60)	2 (33.3)	9 (24.3)

Pearson Chi-Square test results on the relationship between the incidence of silent aspiration and stroke frequency in Table 4 showed a significant relationship with a significance level of p = 0.013 (OR=5,227; CI=1,336 – 20,450). The risk of silent aspiration in repeated strokes is five times greater than in the first stroke.

Table 4. Relationship between stroke frequency and silent aspiration

Frequency of stroke	Silent aspiration	
	Yes	No
Repeated	10 (71.4)	11 (33.4)
First	4 (28.6)	23 (67.4)

4. Discussion

The most predominant gender in our study is in accordance with a study by Wang et al. (12), which showed a higher incidence rate of stroke in men than women. The male predominance in stroke may be caused by the higher blood pressure in men than women, the higher proportion of alcoholic drinkers in men, and sex steroid hormones, which can alter vascular reactivity in the cerebral vasculature (12). However, a meta-analysis by Yang et al. (13) showed no gender differences in swallowing difficulties after stroke (13). The largest age group in our study is similar to a study by Setyopranoto et al. that found the highest prevalence rate of stroke in 50-59 years old (14). Age is one of the important factors affecting post-stroke dysphagia, which can be explained by a higher probability of advanced cranial nerve degeneration and abnormal swallowing reflex function in older patients (13). The most common type of stroke in our study is in accordance with the studies by Abdu et al. (15) and Salvadori et al. (16), which showed ischemic stroke as the most common type of stroke (15, 16).

Prolonged feeding time is the most common complaint by the patients in our study. Stroke can affect oral, pharyngeal, and esophageal transit time, leading to prolonged feeding time.

Stroke patients may have uncoordinated initiation of oral transport, slow chewing, delayed initiation of tongue movement, and tongue pumping, which can affect oral transit time (17, 18). It can be explained by several lesions involved in stroke. The left hemisphere stroke may lead to speech apraxia, further implicating oral movement regulation. The left superior and middle frontal gyrus lesions, basal ganglia, and a small portion of the insula are possibly associated with prolonged oral transit time. The frontal cortex affects oral phase delay due to its association with decision-making, planning complex cognitive behavior, and execution, which are required for swallowing. The insular lesion is also associated with oral phase prolongation because of its function as the primary integrative region for volitional swallowing (18). Stroke patients may also have reduced pharyngeal peristaltic and delayed swallowing reflexes, leading to prolonged pharyngeal transit time (17). Right-hemisphere stroke tends to impair the pharyngeal phase (19). However, a study by Han et al. (17) showed no significant hemispheric difference in the oropharyngeal transport time (17). Patients with stroke also have a significantly longer esophageal transit time than healthy adults (20).

In our study, the abnormal cough reflex is the most common abnormality in the initial FEES examination. Coughing and swallowing have similar muscles, nerves, and control mechanisms. Brain lesions associated with swallowing, such as the superior temporal gyrus and superior longitudinal fasciculus, overlapped with lesions involved in weak cough (21). Cough, produced voluntarily or reflexively, is important in airway protection (21, 22). Reflexive cough, controlled by the brainstem, is automatically generated by afferent activation. Voluntary cough, modulated by cerebral cortices, is a conscious activation of the respiratory muscles, including the diaphragm (21). Reflexive and voluntary coughs reduce after acute stroke (21, 22). Abnormally infrequent coughing may indicate impaired sensitivity of the cough reflex and reduced protection of the lower respiratory tract, putting patients at risk of developing pneumonia through silent aspiration (23). The second most common abnormality in the initial FEES examination in our study is tongue weakness. In swallowing, the tongue directs and squeezes the bolus to the pharynx by contact from the front of the hard palate backward, resulting in effective and safe transport to the pharyngeal cavity. If this oral phase is impaired, there will be an absence or delay of the sensory input transmission to the cortical areas for formed pharyngeal reflex response to protect against aspiration before swallowing (24).

Aspiration is a passage of liquid or food through and below the true vocal folds (25). It is common in stroke patients and increases the risk of chest infections (21). Aspiration is indicated by two or more symptoms, such as lack of gag reflex, dysphonia, voice quality change, inadequate cough, dysarthria, and cough after swallowing. Aspiration can also not be accompanied by symptoms, including cough, which is usually called silent aspiration (26). Of the patients who aspirated, approximately 67% have silent aspiration (27). The prevalence of silent aspiration obtained from our study was 29.2%. This is consistent with a study by Santos et al. (28) that stated the incidence of silent aspiration in stroke patients ranges from 28-52% (28). Silent aspiration increases the risk of pneumonia 1.3 times more than aspiration with clinical symptoms and 13 times more than healthy adults (26, 27). In our study, the mixed lesion is the most common lesion location found in patients with silent aspiration. A study by Garon et al. (25) showed that the prevalence rate of silent aspiration is 79.3% in brainstem stroke, 51% in right hemisphere stroke, and 40.3% in left hemisphere stroke (25). Brainstem and cortical regions of the brain control the sensory and motor airway protective mechanism. Patients with stroke have damage to these neural pathways, which can cause laryngeal motor or sensory loss that reduces the effectiveness of airway protection (27). Two main laryngeal mechanisms to prevent aspiration are glottis closure and material expulsion inhaled into the lungs (22). Prolonged pharyngeal transit time is also associated with aspiration in post-stroke patients (29). In addition, our study also found a significant association between the frequency of recurrent strokes and silent aspiration. To the best of our knowledge, this is the first study to report a higher risk of silent aspiration in recurrent strokes. The five times greater risk of silent aspiration in recurrent strokes should raise clinical precautions in managing recurrent strokes.

Our study found penetration in 72.9% of the subjects. Penetration can occur during, before, or after swallowing. Penetration before swallowing is caused by delayed swallowing reflex or glossopharyngeal closure impairment during the preparatory phase. Penetration during swallowing is caused by the insufficient larynx closure because of the vocal folds adduction failure or laryngeal elevation failure. Penetration after swallowing is caused by the static of food in the pharynx (30). Our study also found standing secretion in 56.3% of patients. A possible factor is the hyposensitivity of part of the hypopharynx, especially the vallecular region and the piriformis sinus. The findings of high preswallowing leakage (91.7%) also indicate a high problem of palatoglossal valve abnormalities which should prevent the leakage. The residue was also found to be relatively high (81.3%) in our study. The pharyngeal residue in stroke patients is considered to be associated with the impairment of several biomechanical oropharyngeal swallowing actions, such as pharyngeal contraction, oral propulsion, and pharyngoesophageal transition opening (31). The vallecular residue amount also correlates with the ability to cough (32).

Swallowing disorders, both the oral phase and the pharyngeal phase, are a problem that is commonly found in stroke patients, which is more common in men over 50 years of age. Based on the FEES examination in this study, the prevalence of silent aspiration was 29.2% in stroke patients. The risk of silent aspiration is five times higher in recurrent

strokes when compared to the first stroke. Mixed lesions (brainstem and hemispheres) cause more severe oral and pharyngeal phase disorders than other lesions and are at greater risk for penetration and aspiration.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: S.T., F.S., Design: S.T., D.M., F.S., Data Collection or Processing: S.T., D.M., F.S., Analysis or Interpretation: S.T., D.M., F.S., Literature Search: S.T., D.M., Writing: S.T., D.M.

Ethical Statement

This study was approved by the Committee of Medical Research Ethics in the Faculty of Medicine at Universitas Indonesia (No: 269/PT02.FK/ETIK/2008). The patients provided written and verbal informed consent before study participation.

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Need for long-term permanent pacemaker and its association with mortality in patients undergoing transcatheter aortic valve implantation

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Abstract

Despite two decades of experience and increased procedural success in Transcatheter Aortic Valve Implantation (TAVI), the need for permanent pacemaker implantation (PPI) is still a matter of debate. We aimed to investigate the need for PPI and the long-term impact of PPI on all-cause mortality in patients with TAVI. We included retrospectively All TAVI recipients between June 2016 and January 2021 admitted to our tertiary center. In-hospital data were retrieved from the institutional digital database and mortalities were recorded from the national E-Health application. The primary outcome was to determine the frequency of PPI requirements following TAVI. The median follow-up was 52 (12-72) months. PPI had been deemed necessary in 20 (15%) of 132 TAVI recipients. When examined according to the devices used for TAVI, PPI was necessary in 25% of Evolut R (Medtronic, CA, USA), 4% of Edwards Sapien (Edwards Lifesciences, CA, USA), 16% of Portico (Abbott Structural Heart, St Paul, MN, USA), 26% of Medtronic CoreValve (Medtronic, CA, USA), 20% of Myval THV (Meril Life Sciences, Gujarat, India), and in none of ACURATE neo™ (Boston Scientific, Marlborough, MA, USA) recipients. Mortality was similar among those with and without PPI requirements. Multiple regression revealed that hyperlipidemia and preoperative valvuloplasty significantly decreased risk for all-cause mortality, while higher CRP increased mortality risk. New-generation TAVI devices appear to decrease the need for PPI compared to older-generation devices, as reported in the literature. PPI was not associated with all-cause mortality at a median follow-up of 52 months in TAVI recipients.

Keywords: transcatheter aortic valve replacement, pacemaker, mortality, preoperative valvuloplasty

1. Introduction

Transcatheter aortic valve implantation (TAVI) was introduced in 2002 and has since been identified as a breakthrough development in interventional cardiology (1). Initially, TAVI was reserved for patients with severe, symptomatic aortic valve stenosis in whom conventional surgical aortic valve implantation (SAVR) would cause high risk. However, recent randomized, multi-center, prospective studies in patients with severe aortic stenosis indicate that, among patients deemed to be at low-risk for SAVR, the TAVI approach lowers the risks for death, stroke or re-hospitalization at one year of follow-up compared to conventional SAVR (2, 3). Current European Society of Cardiology (ESC) and European Association for Cardio-Thoracic Surgery (EACTS) guidelines recommend TAVI in older patients (≥75 years) or those who are unsuitable for or at high-risk for surgery (STS-PROM / EuroSCORE II >8%), while SAVR is recommended for (i) those older than >75 years with an STS-PROM / EuroSCORE II of <4%, or (ii) those unsuitable for transfemoral TAVI (4).

Despite two decades of experience and increased procedural success in TAVI, there is still debate concerning long-term valve durability, advantages/disadvantages relative to SAVR, risks for periprocedural stroke, and, ultimately, the need for permanent pacemaker implantation (PPI). As a result of the anatomical proximity, conduction abnormalities constitute a major complication of TAVI due to potential injuries to conductive sites. Left bundle branch block (LBBB) accounts for 10-30% of the conduction system abnormalities after TAVI (5). The frequency of PPI in TAVI recipients ranges between 4–24% (6). However, the incidence of PPI in recipients of new-generation prostheses is unclarified and there is limited data concerning clinical factors associated with the need for PPI.

This study aimed to investigate the frequency of PPI and the long-term impact of PPI requirement on mortality in TAVI recipients.

2. Material and Methods

All consecutive patients undergoing TAVI in our institute, which is a tertiary center, between June 2016 and January 2021 were examined retrospectively. All TAVI procedures were performed via the transfemoral approach, following the final decision made by the heart team. TAVI was not performed in patients with absolute contraindications (those with life expectancy less than 1 year, patients who were not expected to experience quality of life improvement due to comorbidities, and individuals with inadequate annulus size, thrombus in left ventricle or ascending aorta, active endocarditis, or increased risk of coronary ostium obstruction) and those with relative

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contraindications (obstructive coronary artery disease, hemodynamic instability, left ventricular ejection fraction of < 20 %) did not undergo TAVI. Additionally, subjects who had previously undergone pacemaker implantation for other reasons were excluded from the study. The study was approved by the Clinical Research Ethics Committee of Haseki Training and Research Hospital.

Data concerning demographic characteristics, comorbidities, blood type, aortic pathologies, previous surgical/non-surgical interventions (if any), pre- and post-procedural transthoracic echocardiography measurements, TAVI device size and type (brand), complications, intra-hospital PPI application and mortality were retrieved from the institutional digital database. Hyperlipidemia was defined as having a total cholesterol of >200 mg/dl or being on antihyperlipidemic medications. Also, laboratory results, including complete blood count, inflammatory indices (neutrophil-to-lymphocyte ratio; NLR, lymphocyte-to-MCV ratio; LMR, platelet-to-lymphocyte ratio; PLR, etc.), and biochemical measurements (renal function tests including glomerular filtration rate, liver function tests, lipid profile, etc.) were recorded. Data concerning mortality after discharge was obtained from the National E-Health application in which all personal health issues, including laboratory tests, imaging studies, interventions and mortality are recorded.

The primary outcome measure of this study was to address the frequency of PPI in subjects undergoing TAVI. The secondary outcome measure was identifying factors independently associated with long-term all-cause mortality.

2.1. Statistical analysis

All analyses were performed on SPSS v25 and were subject to the classical two-tailed $p < 0.05$ significance threshold (SPSS Inc., Chicago, IL, USA). We evaluated Q-Q and histogram plots to assess normal / non-normal distribution in continuous variables. Data concerning continuous variables were depicted with mean ± standard deviation in the presence of normal distribution, while median (1st quartile–3rd quartile) values were used for those with non-normal distribution. Absolute (n) and relative (%) frequency were used to depict categorical data. Comparison results for normally-distributed continuous variables were analyzed with the independent samples t-test. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Categorical variable distributions were compared with chi-square tests or Fisher's exact test. Multiple logistic regression analysis (forward conditional selection) was used to determine the best factors that could independently predict mortality.

3. Results

The median follow-up period was 52 (12-72) months. Twenty (15%) of the 132 TAVI recipients had required PPI. Subjects with and without PPI were similar with respect to age, sex, comorbidities, left ventricular function, presence of additional aortic regurgitation, preoperative valvuloplasty, and TAVI prosthesis and device type. Subjects requiring PPI had lower creatinine values [0.94 (0.75 - 1.19) mg/dl vs 0.76 (0.65 - 0.91) mg/dl, $p = 0.010$] and higher GFR [68 (52 - 84) vs 86 (64 - 92), $p = 0.022$] compared to those without PPI (Table 1).

Table 1. Summary of patient characteristics and laboratory measurements with regard to permanent pacemaker need

		Permanent pacemaker		
	Total (n=132)	**Yes (n=20)**	**No (n=112)**	**p**
Age	76 (70.5 - 82)	76.5 (71 - 82)	76 (70.5 - 82.5)	0.839
Sex				
Female	82 (62.1%)	13 (65.0%)	69 (61.6%)	0.970
Male	50 (37.9%)	7 (35.0%)	43 (38.4%)	
Race				
Domestic	126 (95.5%)	19 (95.0%)	107 (95.5%)	1.000
Immigrant	6 (4.5%)	1 (5.0%)	5 (4.5%)	
Comorbidities				
Hypertension	82 (62.1%)	12 (60.0%)	70 (62.5%)	1.000
Diabetes mellitus	51 (38.6%)	4 (20.0%)	47 (42.0%)	0.108
Hyperlipidemia	76 (57.6%)	9 (45.0%)	67 (59.8%)	0.322
COPD	24 (18.2%)	3 (15.0%)	21 (18.8%)	1.000
Cerebrovascular disease	16 (12.1%)	2 (10.0%)	14 (12.5%)	1.000
Malignancy	9 (6.8%)	0 (0.0%)	9 (8.0%)	0.354
Peripheral artery disease	8 (6.1%)	1 (5.0%)	7 (6.3%)	1.000
Coronary artery disease	39 (29.5%)	8 (40.0%)	31 (27.7%)	0.397
Blood group				
A	60 (45.5%)	9 (45.0%)	51 (45.5%)	0.073
B	16 (12.1%)	0 (0.0%)	16 (14.3%)	
O	47 (35.6%)	11 (55.0%)	36 (32.1%)	

AB	9 (6.8%)	0 (0.0%)	9 (8%)	
Rh group				
Negative	23 (17.4%)	5 (25.0%)	18 (16.1%)	0.343
Positive	109 (82.6%)	15 (75.0%)	94 (83.9%)	
LVEF				
≥ %50	91 (68.9%)	16 (80.0%)	75 (67.0%)	0.292
< %50 - ≥ %30	30 (22.7%)	4 (20.0%)	26 (23.2%)	
< %30	11 (8.3%)	0 (0.0%)	11 (9.8%)	
Aortic pathology				
Stenosis	119 (90.2%)	19 (95.0%)	100 (89.3%)	0.693
Regurgitation	2 (1.5%)	0 (0.0%)	2 (1.8%)	
Stenosis + Regurgitation	11 (8.3%)	1 (5.0%)	10 (8.9%)	
Other valvular pathology	18 (13.6%)	2 (10.0%)	16 (14.3%)	1.000
Preoperative valvuloplasty	67 (50.8%)	9 (45.0%)	58 (51.8%)	0.752
Glucose	117 (104 - 156.5)	118.5 (107 - 143.5)	116 (103.5 - 163)	0.962
Urea	42.30 (35.75 - 58.00)	39.20 (31.40 - 42.30)	43.55 (36.25 - 59.55)	**0.036**
Creatinine	0.91 (0.73 - 1.14)	0.76 (0.65 - 0.91)	0.94 (0.75 - 1.19)	**0.010**
GFR	69 (53 - 87)	86 (64 - 92)	68 (52 - 84)	**0.022**
Uric acid	6.66 ± 1.98	5.86 ± 1.70	6.81 ± 2.00	0.056
Calcium	9.06 ± 0.64	8.98 ± 0.61	9.07 ± 0.64	0.541
Total protein	64.4 (60.5 - 69)	66 (60.6 - 68.3)	64.4 (60.5 - 69.5)	0.941
Albumin	36.06 ± 4.59	36.48 ± 3.61	35.98 ± 4.76	0.659
Globulin	28.05 (26 - 32)	28.05 (24.15 - 31.5)	28.45 (26 - 32)	0.614
Total cholesterol	197.68 ± 51.85	197.06 ± 63.59	197.79 ± 49.76	0.956
HDL cholesterol	45.09 ± 11.78	44.89 ± 13.41	45.13 ± 11.53	0.936
LDL cholesterol	124.32 ± 43.63	128.89 ± 53.38	123.47 ± 41.85	0.631
Triglyceride	123 (91.5 - 163.5)	111 (95 - 140)	124 (91 - 168)	0.323
Hemoglobin	11.80 ± 1.63	12.09 ± 1.60	11.75 ± 1.64	0.394
Hematocrit	35.80 ± 4.41	36.49 ± 3.98	35.67 ± 4.49	0.451
Platelet ($x10^3$)	232.30 ± 70.47	242.25 ± 64.71	230.52 ± 71.57	0.495
MCV	84.31 ± 5.95	84.13 ± 6.55	84.34 ± 5.86	0.885
MPV	10.40 ± 1.04	10.41 ± 0.97	10.40 ± 1.06	0.957
MCHC	32.93 ± 1.42	33.09 ± 1.52	32.90 ± 1.40	0.584
WBC	7660 (6155 - 9770)	6870 (5970 - 9200)	7765 (6175 - 9930)	0.169
Lymphocyte	1715 (1300 - 2275)	1670 (1400 - 2085)	1760 (1275 - 2285)	0.975
Neutrophil	5000 (3715 - 6890)	4375 (3495 - 6135)	5220 (3845 - 7030)	0.184
Monocyte	525 (405 - 755)	465 (395 - 625)	560 (410 - 800)	0.177
Eosinophil	135 (70 - 230)	100 (55 - 225)	140 (70 - 230)	0.400
Basophile	30 (20 - 40)	30 (20 - 35)	30 (20 - 40)	0.754
Lymphocyte to MCV ratio	20.98 (15.03 - 26.95)	21.09 (16.02 - 24.63)	20.89 (14.55 - 27.46)	0.975
Neutrophil to MCV ratio	59.50 (44.79 - 84.38)	49.56 (40.24 - 70.76)	61.35 (45.76 - 85.27)	0.165
Platelet to lymphocyte ratio	128.76 (99.34 - 172.39)	137.51 (106.75 - 182.25)	128.76 (98.39 - 169.43)	0.564
Neutrophil to lymphocyte ratio	2.88 (2.05 - 4.19)	2.33 (1.97 - 3.80)	2.93 (2.06 - 4.28)	0.272
Monocyte to lymphocyte ratio	0.30 (0.22 - 0.49)	0.24 (0.21 - 0.38)	0.31 (0.22 - 0.50)	0.176
Eosinophil to lymphocyte ratio	0.07 (0.04 - 0.12)	0.06 (0.04 - 0.11)	0.07 (0.05 - 0.12)	0.429
Basophil to lymphocyte ratio	0.02 (0.01 - 0.02)	0.02 (0.01 - 0.02)	0.02 (0.01 - 0.02)	0.937
CRP	6.80 (2.95 - 16.30)	6.65 (3.25 - 16.95)	7.05 (2.81 - 16.30)	0.881
TAVI type				
Evolut R	55 (41.7%)	11 (55.0%)	44 (39.3%)	0.270
Edwards Sapien Balloon Expandable	26 (19.7%)	1 (5.0%)	25 (22.3%)	

Accurate Neo	12 (9.1%)	0 (0.0%)	12 (10.7%)	
Portico	18 (13.6%)	3 (15.0%)	15 (13.4%)	
Medtronic Corevalve	15 (11.4%)	4 (20.0%)	11 (9.8%)	
Mywall	5 (3.8%)	1 (5.0%)	4 (3.6%)	
None	1 (0.8%)	0 (0.0%)	1 (0.9%)	
TAVI device size, mm	27 (26 - 29)	28 (26 - 29)	26 (26 - 29)	0.820
Other complication	37 (28.0%)	5 (25.0%)	32 (28.6%)	0.954
Mortality	50 (37.9%)	7 (35.0%)	43 (38.4%)	0.970

Data are given as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables.

With respect to device type, PPI was required in 25% of patients with Evolut R (Medtronic, CA, USA), 4% of patients with Edwards Sapien (Edwards Lifesciences, CA, USA), 16% of patients with Portico (Abbott Structural Heart, St Paul, MN, USA), 26% of patients with Medtronic CoreValve (Medtronic, CA, USA), 20% of patients with Myval THV (Meril Life Sciences, Gujarat, India), and none of the patients with the ACURATE neo™ (Boston Scientific, Marlborough, MA, USA) device (Table 1). Device size was similar between patients with and without PPI.

The mortality rate was similar among patients who did and did not undergo PPI. Comparison of survivors and non-survivors revealed that non-survivors had higher age, creatinine, uric acid, NLR and CRP levels, while they had lower hemoglobin, total protein and albumin levels compared to survivors. Subjects with mortality had more frequently received the ACURATE neo™ (BostonScientific, MA, USA) and Portico™ (Abbott Vascular Solutions, CA, USA) devices (Table 2).

Table 2. Summary of patients characteristics and laboratory measurements with regard to mortality

	Status		
	Alive (n=82)	**Exitus (n=50)**	**p**
Age	74.5 (69 - 80)	78 (73 - 84)	**0.010**
Sex			
Female	51 (62.2%)	31 (62.0%)	1.000
Male	31 (37.8%)	19 (38.0%)	
Race			
Domestic	78 (95.1%)	48 (96.0%)	1.000
Immigrant	4 (4.9%)	2 (4.0%)	
Comorbidities			
Hypertension	57 (69.5%)	25 (50.0%)	**0.040**
Diabetes mellitus	34 (41.5%)	17 (34.0%)	0.503
Hyperlipidemia	57 (69.5%)	19 (38.0%)	**0.001**
COPD	14 (17.1%)	10 (20.0%)	0.849
Cerebrovascular disease	9 (11.0%)	7 (14.0%)	0.809
Malignancy	6 (7.3%)	3 (6.0%)	1.000
Peripheral artery disease	6 (7.3%)	2 (4.0%)	0.710
Coronary artery disease	29 (35.4%)	10 (20.0%)	0.093
Blood group			
A	38 (46.3%)	22 (44.0%)	0.720
B	9 (11.0%)	7 (14.0%)	
O	28 (34.1%)	19 (38.0%)	
AB	7 (8.5%)	2 (4.0%)	
Rh group			
Negative	18 (22.0%)	5 (10.0%)	0.129
Positive	64 (78.0%)	45 (90.0%)	
LVEF			
≥ %50	61 (74.4%)	30 (60.0%)	0.215
< %50 - ≥ %30	15 (18.3%)	15 (30.0%)	
< %30	6 (7.3%)	5 (10.0%)	

Aortic pathology			
Stenosis	75 (91.5%)	44 (88.0%)	
Regurgitation	1 (1.2%)	1 (2.0%)	0.805
Stenosis + Regurgitation	6 (7.3%)	5 (10.0%)	
Other valvular pathology	13 (15.9%)	5 (10.0%)	0.491
Preoperative valvuloplasty	49 (59.8%)	18 (36.0%)	**0.014**
Glucose	119.5 (103 - 153)	116 (104 - 175)	0.811
Urea	40.1 (32.3 - 54.6)	46.95 (39.9 - 64.8)	**0.004**
Creatinine	0.82 (0.70 - 1.11)	0.93 (0.80 - 1.19)	**0.026**
GFR	78 (55 - 90)	61 (51 - 77)	**0.004**
Uric acid	6.32 ± 1.77	7.25 ± 2.21	**0.011**
Calcium	9.12 ± 0.58	8.96 ± 0.72	0.185
Total protein	66 (61 - 72)	63.2 (60.2 - 66)	**0.026**
Albumin	37.00 ± 4.49	34.49 ± 4.38	**0.002**
Globulin	28 (25 - 32)	29 (26.1 - 32)	0.646
Total cholesterol	203.86 ± 48.39	185.16 ± 56.85	0.069
HDL cholesterol	46.15 ± 11.67	42.92 ± 11.87	0.166
LDL cholesterol	127.04 ± 40.46	118.82 ± 49.56	0.344
Triglyceride	131.5 (98 - 175)	110.5 (69 - 136)	**0.011**
Hemoglobin	12.05 ± 1.58	11.40 ± 1.66	**0.027**
Hematocrit	36.43 ± 4.40	34.76 ± 4.27	**0.035**
Platelet ($x10^3$)	224.44 ± 66.23	245.18 ± 75.84	0.101
MCV	84.53 ± 5.51	83.95 ± 6.64	0.592
MPV	10.32 ± 1.04	10.53 ± 1.04	0.271
MCHC	33.05 ± 1.38	32.73 ± 1.46	0.212
WBC	7500 (6140 - 9360)	8455 (6180 - 10390)	0.089
Lymphocyte	1775 (1330 - 2200)	1560 (1270 - 2490)	0.577
Neutrophil	4800 (3620 - 6240)	5625 (4200 - 7710)	**0.039**
Monocyte	560 (400 - 740)	520 (430 - 760)	0.899
Eosinophil	150 (80 - 230)	110 (50 - 230)	0.160
Basophile	30 (20 - 40)	30 (20 - 40)	0.824
Lymphocyte to MCV ratio	21.37 (15.62 - 26.76)	18.79 (13.97 - 30.17)	0.673
Neutrophil to MCV ratio	56.37 (42.61 - 76.81)	66.25 (46.51 - 92.22)	**0.033**
Platelet to lymphocyte ratio	121.70 (102.63 - 155.63)	140.44 (94.46 - 196.30)	0.168
Neutrophil to lymphocyte ratio	2.66 (1.98 - 3.75)	3.28 (2.31 - 5.12)	**0.035**
Monocyte to lymphocyte ratio	0.30 (0.22 - 0.39)	0.31 (0.21 - 0.58)	0.622
Eosinophil to lymphocyte ratio	0.08 (0.05 - 0.13)	0.07 (0.04 - 0.11)	0.236
Basophil to lymphocyte ratio	0.02 (0.01 - 0.02)	0.02 (0.01 - 0.02)	0.520
CRP	4.9 (2.4 - 11.1)	11.4 (6 - 24)	**0.002**
TAVI type			
Evolut R	30 (36.6%)	25 (50.0%)	
Edwards Sapien XT	14 (17.1%)	12 (24.0%)	
Accurate Neo	11 (13.4%)	1 (2.0%)	
Portico	15 (18.3%)	3 (6.0%)	**0.046**
Medtronic Corevalve	8 (9.8%)	7 (14.0%)	
Mywall	4 (4.9%)	1 (2.0%)	
None	0 (0.0%)	1 (2.0%)	
TAVI device size, mm	27 (26 - 29)	26 (26 - 29)	0.706
Permanent pacemaker	13 (15.9%)	7 (14.0%)	0.970

Other complication	25 (30.5%)	12 (24.0%)	0.545

Data are given as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution, and as frequency (percentage) for categorical variables.

We performed multiple logistic regression analyses to determine the factors independently associated with mortality. Patients with hyperlipidemia had a lower risk of death than those without (OR: 0.258, 95% CI: 0.117 - 0.569, p = 0.001). Preoperative valvuloplasty decreased the risk of death (OR: 0.358, 95% CI: 0.161 - 0.792, p = 0.011). Also, patients with higher CRP were found to have a higher risk of death (OR: 1.035, 95 % CI: 1.007 - 1.064, p = 0.014). Other variables included in the model, age (p = 0.269), hypertension (p = 0.789), urea (p = 0.175), creatinine (p = 0.678), GFR (p = 0.142), uric acid (p = 0.451), total protein (p = 0.226), albumin (p = 0.075), triglyceride (p = 0.089), hemoglobin (p = 0.059), hematocrit (p = 0.052), neutrophil (p = 0.362), neutrophil-to-MCV ratio (p = 0.229), NLR (p = 0.730) and TAVI type (p = 0.305) were found to be non-significant (Table 3).

Table 3. Significant predictive factors of the mortality, multiple logistic regression analysis

	Odds ratio	95.0% CI for Exp (β)	p
Hyperlipidemia	0.258	0.117 – 0.569	0.001
Preoperative valvuloplasty	0.358	0.161 – 0.792	0.011
C-reactive protein	1.035	1.007 – 1.064	0.014
Constant	1.317		0.461

Dependent variable: Mortality; Nagelkerke R^2=0.256; Correct prediction=68.2% CI: Confidence interval

4. Discussion

In our series, PPI was required in 15% of TAVI recipients, which is compatible with the literature. The mortality rate was similar in subjects with and without PPI. Higher age, creatinine, uric acid, NLR and CRP levels, and lower hemoglobin, total protein and albumin levels were noted in subjects with mortality. Blood urea nitrogen and creatinine were lower in subjects undergoing PPI. However, neither blood urea nitrogen nor creatinine were associated with mortality. Except for higher CRP levels, none of these parameters, including device type, were independently associated with a higher likelihood of mortality.

TAVI has become a highly reliable and safe therapeutic option for patients with severe aortic stenosis since its introduction in 2002 (7). TAVI was initially reserved for patients with severe aortic stenosis and high surgical risk; however, currently, TAVI represents the standard of care in treating severe aortic stenosis among patients older than 70 years of age. The two predominant device types used for TAVI are balloon-expandable and self-expandable valve systems. While self-expandable systems have the advantage of a larger effective orifice area and lower gradient, the likelihood of PPI is reported to be elevated with this kind of device compared to balloon-expandable devices (8, 9). New-onset conduction abnormalities are reported in 35% of patients undergoing TAVI, with LBBB being the most common type (10). Development of conduction abnormalities after TAVI or SAVR result from the proximity between the aortic valve and the conduction system of the heart. Another important point to note is that around 22% of patients undergoing TAVI develop atrioventricular block; however, these cases are demonstrated to resolve in about half of these patients within the first 24 hours (11).

A meta-analysis of 41 studies reported a PPI rate ranging between 2% and 51% following TAVI (12). The need for PPI was much more common in patients receiving the self-expanding Medtronic CoreValve (25-52.8%) device compared to subjects receiving the balloon-expandable Edwards Sapien/Sapien XT valve (5-7%). Latest-generation devices have reduced the need for PPI, as demonstrated by frequencies between 2.3% and 36.1% (6). This is illustrated by the PPI frequencies reported for different generations. For instance, with the Evolut R, the frequency (14.7-26.7%) is lower compared to the early generation CoreValve device (16.3–37.7%), but is higher than that of the new-generation Sapien 3 device (4-24%). The reported frequency of PPI with ACURATE neo is quite low, around 8.3%, as reported by the study of Möllmann et al., which included 1000 patients (13). In Portico devices, the corresponding frequencies have been reported as 21.9% and 27.7% in two different studies (14, 15). In a recent registry comparing the Myval device with alternative devices, a PPI frequency of 7.4% was described (16).

In our study, PPI was required in 25% of patients with Evolut R, 4% of patients with Edwards Sapien XT, 16% of those with Portico, 26% of those with Medtronic CoreValve, and 20% of those with Myval devices. None of the patients receiving ACURATE neo required PPI. The frequencies reported in our study are compatible with previous data except for the Myval and ACURATE neo devices; however, these devices were used in only 5 and 12 patients, respectively; thus, data is limited in this respect.

The prognostic impact of PPI following TAVI is another point of concern. Unfavorable consequences of PPI following TAVI have been reported in large-scale trials. Undergoing PPI after TAVI has been shown to be associated with a 31% increase in 1-year mortality (17). Additionally, another study by Sharobeem et al. showed that PPI during follow-up was associated with an increased risk of hospitalization for heart failure (18). A recent meta-analysis of 31 studies, including 51,069 TAVI recipients, demonstrated that PPI was associated with a risk of all-cause death and re-hospitalization for heart failure as measured at a mean follow-up duration of 22 months (19). However, in some studies, it was stated that there was no difference between these two groups in terms of mortality, and there is no definite consensus on this issue (20-23). Therefore, it is recommended to conduct more comprehensive studies evaluating the results of long-term follow-up in order to guide

the treatment approach in line with the results. Similar to the conflicting literature, our study also found no significant differences in mortality between patients with and without PPI who had been followed for a median of 52 months. Multiple logistic regression revealed that PPI did not significantly predict long-term mortality after TAVI.

Considering our results together with contemporary literature, we can feasibly suggest that new-generation TAVI devices have reduced PPI requirements. Additionally, our study found that PPI was not associated with all-cause mortality at a median follow-up of 52 months.

This study has some limitations to be mentioned. The retrospective design and extraction of post-discharge mortality data from the National E-Health application rather than outpatient clinic visits are among its primary limitations. Sample size can also be considered relatively small to reach clear conclusions regarding the relationships between new-generation devices and PPI and/or mortality, especially in devices used less frequently. Nevertheless, our relatively long follow-up period provides valuable data concerning the relationship between PPI and mortality.

In conclusion, our results revealed that PPI was required after 20 (15%) of the 132 TAVI procedures performed between June 2016 and January 2021 at our center. When taken together with the literature, our study shows that new-generation TAVI devices may be associated with reduced PPI requirements compared to older-generation devices. Having received a permanent pacemaker was not associated with mortality; however, higher CRP was associated with increased likelihood for all-cause mortality, while having received preoperative valvuloplasty was associated with decreased mortality likelihood among TAVI recipients who were followed up for a median of 52 months.

Conflict of interest

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None to declare.

Authors' contributions

Concept: O.O., M.M.C., Design: O.O., M.M.C., Data Collection or Processing: O.O., M.M.C., Analysis or Interpretation: O.O., M.M.C., Literature Search: O.O., M.M.C., Writing: O.O., M.M.C.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Clinical Research Ethics Committee of Haseki Training and Research Hospital (Decision no: 08-2022, Decision date: 19.01.2022).

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Peptic ulcer and its complications in Ancient Mesopotamian Cuneiform Texts

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Abstract

While the earliest known records on the digestive system were made by Hippocrates in 400 BC, the first description of peptic ulcer was made by Marcellus Donatus in 1586. However, when the cuneiform texts of Ancient Mesopotamia, which provide information on medical subjects, are examined, records of many diseases draw attention. Peptic ulcer is one of the important diseases that draws attention in the medical records of Ancient Mesopotamian societies, especially in the *Sakikku* series obtained from the Asurbanipal Library. This study is based on the information obtained by comparing the cuneiform documents with the current medical literature. The study aims to reveal that the findings of peptic ulcer disease were recorded earlier than Hippocrates. 10 of the 11 cuneiform texts analysed in this study provide information about epigastric pain, the most prominent symptom of peptic ulcer. Words meaning peptic ulcer are found in 6 texts (Sumerian A.GA.ZI 2, Akkadian *tugānu* 4) (54.5%). In 3 texts, death is stated as the prognosis if bloody vomiting continues. The term "gastroduodenal disease" in relation to the gastroduodenal resultant, where obstruction and bleeding are common, occurs in 5 texts. In 4 of them (*tugānu* 3, A.GA.ZI 1), peptic ulcer disease is mentioned (80%). In 4 texts in which "gastric outlet disease" and "peptic ulcer disease" are mentioned together, vomiting also accompanies the disease. Additionally, since the concrete symptoms of peptic ulcer could be identified, the cause of the disease was not attributed to abstract concepts such as the devil, evil spirit, and demon in the related texts.

Keywords: peptic ulcer, Ancient Mesopotamia, cuneiform, history of peptic ulcer, gastroenterology

1. Introduction

In Mesopotamia, located between the Euphrates and Tigris rivers, revolutionary developments emerged 6000 years ago. Many "firsts", especially in fields such as writing, law, urbanisation, and trade, have appeared in this region. Civilisations such as Sumer, Akkad, Babylon, and Assyria were founded in this area. The Sumerians invented writing, which marked the beginning of the historical period and the recording of humanity's verbal memory (1). Cuneiform texts containing medical records were also found as a result of excavations.

People have felt anxiety, fear, and anguish due to diseases they could not explain or overcome. In an effort to dispel the psychological effects of these diseases on people, they associated them with abstract concepts and rituals (2). These cuneiform medical documents contain abstract concepts such as evil spirits, devils, and demons. This is due to the close relationship between the polytheistic belief system that prevailed in ancient Mesopotamian societies and medicine. In this context, the priestly *āšipu*s, characterised as "white magicians" carried out activities in line with their duties after the training they received in the temples (3). From the texts, it can be determined that the *āšipu* were good observers and had important medical knowledge (4).

The *sakikku*, a continuation of the symptom-prognosis series, is a vital source of data on ancient Mesopotamian medicine that has survived to the present day. The *sakikku* series was written by Esagil-kin-apli, who lived in the Middle Assyrian Period and is described as the Hippocrates of the Ancient East. The series, dated to the beginning of the 1st millennium BC, consists of 40 cuneiform tablets containing approximately 3000 records and means "symptoms" (5, 6).

Around 1000 cuneiform tablets related to medicine were found in the library of Ashurbanipal (7). These documents were published in Assyrian Medical Texts (AMT) in 1923, Traité Akkadien de Diagnostics et Progostics Médicaux (TDP) by R. Labat in 1951, and Die Babylonishch-Assyrische Medizin (BAM) by F. Kocher in 1980 (8). Scurlock published the generally scattered medical documents by classifying them according to diseases and systems in 2005 and 2014 (7). Although there is information about peptic ulcers in these publications, no studies have evaluated them in light of current medical literature.

This study aims to show that the determinations of peptic ulcer disease were recorded in Ancient Mesopotamian texts long before Hippocrates.

2. Material and Methods

The main material evaluated within the scope of the study is

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the information obtained from 11 cuneiform tablets. The cuneiform texts were obtained from various sources, including The Sultantepe Tablets (STT), Assyrian Medical Texts (AMT), Die Babylonisch-Assyrische Medizin (BAM), The Diagnostic and Prognostic Series of Tablets (DPS), Traité Akkadien de Diagnostics et Pronostics Médicaux (TDP), and Scurlock (2005). The study was based on comparing the information in the cuneiform texts obtained from these sources and artifacts with current medical literature.

3. Results

Records of peptic ulcers have also been found in the cuneiform sources of Ancient Mesopotamia, which has ancient knowledge. In the cuneiform texts, the term "stomach" is mentioned as "TUN" in Sumerian and "*takāltum*" in Akkadian (9). Other words in the Sumerian dictionary that mean stomach, abdomen, and appetite are "MU-TUN, MU-TUN-NA" (9).

In the cuneiform texts providing information about Ancient Mesopotamian medicine, the general clinical findings of the patient are evaluated with an observant approach without mentioning the name of the disease. The texts conclude by commenting on the patient's prognosis after associating with the relevant god, evil spirit, or demon. However, the following text only contains symptoms and signs of the gastrointestinal system and does not include abstract concepts. The text states that the mouth of the stomach causes complaints of upper abdominal pain and echoing bitterness. "6.92 If the mouth of a person's stomach is sick (and) his upper abdomen (epigastrium) burns him, burns him hotly, stings him, and continually hurts him, ?? or DÚR.GIG, to cure him... (3,10)." The patient's condition was associated with the abstract concept in none of the 11 texts analysed.

Nowadays, the importance of dietary habits and food intake for peptic ulcer formation is recognised. In the following text, epigastric pain, mucus discharge, and loss of appetite are attributed to eating and drinking a substance called *kišpu*: "6.10 If his upper abdomen (epigastrium) continually has mucus, his epigastrium burns him hotly, he cannot sleep day or night, he loses his appetite for bread and water, (and) his flesh is tense, that person has eaten or drunk *kišpu*, to dispel it ... (3,11)." "Epigastric pain" was found in 10 out of 11 texts and was the most prominent symptom of peptic ulcer (Table 1).

In Mesopotamian medical texts, although the disease name is not mentioned in general, the words "A.GA.ZI" in Sumerian and "*tugānu*" in Akkadian in cuneiform texts are compatible with the clinical symptoms and findings of peptic ulcer disease (3). In the texts evaluated within the scope of the study, it was determined that the name of peptic ulcer disease was included in six texts, two of which were A.GA.ZI and four of which were *tugānu* expressions (Table 1). According to the current literature, upper abdominal pain and complaints of burning and pain are common symptoms of peptic ulcer (12). The word "A.GA.ZI" is mentioned in the text below with these symptoms. "6.97 [If] a person's [upper abdomen (epigastrium)?] ˹burns˺, [gives him a] ˹jabbing˺ pain, stings, and hurts him, that person is ˹sick˺ with A.[GA.ZI], to cure him (3,13)."

Table 1. Distribution of knowledge about peptic ulcer in cuneiform texts

Cuneiform text number	Epigastric burn	A.GA.ZI	Bloody Vomiting	Death Risk	Sick Mouth of Stomach	Tuganu	Vomits without Having Eaten	General semptoms *
6.10	+							+
6.97	+	+						
6.93	+		+	+				
6.94	+		+	+				
6.95	+		+	+				
6.103	+	+			+		sahhu +	
6.30	+				+	+	+	
6.98	+				+	+	+	
6.101					+	+	sahhu +	
6.99	+					+		
6.92	+				+			
Total (11)	10	2	3	3	5	4	4	1

*General Semptoms: Not appetite, sleeplessness, mucus

In the following texts, in addition to upper abdominal pain, intense or up to three days of bloody vomiting is considered to have a poor prognosis and may result in death (Table 1). In these texts, it can be seen that the *āšipu*s regarded persistent haemorrhagic vomiting in peptic ulcers as fatal. "6.93 If a burning pain is firmly established in his upper abdomen (epigastrium) and he continually produces dark blood, he will die (3,14). 6.94 If a needling pain or burning pain is firmly

established in his upper abdomen (epigastrium) and he excretes blood, he will die (3,14). 6.95 If a needling pain or burning pain is firmly established in his upper abdomen (epigastrium) and he vomits blood (and he has been sick) for two or three days, he will die (3,14)."

Bleeding, obstruction, and perforation, complications of peptic ulcer, are frequently observed in the region called the "gastric outlet" (12). The term "gastric outlet disease" is mentioned in five texts analysed in this study. In order to indicate the condition of peptic ulcer, the word "*tugānu*" is mentioned in three of these five texts, and the word "A.GA.ZI" is mentioned in one. However, the expression "gastric outlet disease" is also used (Table 1). One of these texts includes the following expression: "6.103 If a person eats bread and drinks beer and then continually produces saḫḫu, he incessantly vomits saḫḫu, he shows saḫḫu [and] black blood, he has constriction of the mouth of the stomach, and his upper abdomen (epigastrium) burns, burns hotly, stings, and hurts him, that person is sick with A.GA.ZI, to cure [him]..." (3,13). In addition, there are examples where the expressions "stomach outlet disease" or "stomach mouth disease" are used together with tugānu (stomach ulcer): "6.30 If *tugānu* (peptic ulcer; see below) afflicts a person, his upper abdomen (epigastrium) burns him hotly, (and) he vomits without having eaten (*gâšu*), that person has a sick mouth of the stomach, to cure him... (3,10). 6.98 If *tugānu* afflicts a person, his upper abdomen (epigastrium) burns him hotly, (and) he vomits without having eaten, that person has a sick mouth of the stomach, to cure him... (3,10). 6.101 If a person has *tugānu* (with) *saḫḫu*, that person has a sick mouth of the stomach, to cure him... (3,10)" exemplifies this situation.

It can be said that "gastric outlet disease" is seen as a complication of peptic ulcer in the texts. Additionally, texts 6.98 and 6.101 contain the terms nausea/vomiting, "*tugānu*", and "gastric outlet disease" together, while texts 6.101 and 6.103 contain the term "saḫḫu", meaning vomiting with a thick consistency (3). Thus, it is evident that all four texts in which the words *tugānu* or A.GA.ZI, which define peptic ulcer, are found together with the term "gastric outlet disease", include the symptom of vomiting. In these texts, vomiting probably occurred as a complication of gastric ulcer disease due to obstruction of the gastroduodenal resultant (gastric outlet disease).

Today, cholelithiasis is the first differential diagnosis for peptic ulcers due to the confusion of clinical symptoms. Peritonitis that develops due to the perforation of a gastric ulcer (*tugānu*) has symptoms of fever, vomiting with bile, and sharp pain in the upper abdomen (12). *Āšipu* naturally makes a differential diagnosis between *tugānu* (gastric ulcer) and *pašittu* (cholelithiasis), as seen in the following text: "6.99 If a person's upper abdomen (epigastrium) gnaws at them before they have eaten, they continually have internal fever, and when they belch, they vomit bile, that person is sick with *pašittu* (gall bladder disease) (or) *tugānu*, to cure him... (3,13)."

4. Discussion

The term ulcer refers to the loss of the mucous layer on the inner surface of hollow organs. In peptic ulcer disease, various organs, such as the distal oesophagus, stomach, and duodenum, may be affected. The most common form of peptic ulcer is the duodenal ulcer, frequently observed in the first 3-6 cm of the duodenum (15). Peptic ulcer develops histopathologically by the progression of defective erosions, deeper than 5 mm in diameter and smaller than 1 mm in diameter, on the gastric mucosa into deeper layers (16).

Gastric ulcer was historically first described by Marcellus Donatus in 1586. Through an examination performed on a patient who died acutely after intense sputum and vomiting, Donatus determined an ulcer in the pylorus part and the lower outlet hole of the stomach, leading to the patient's death. However, the first person to define peptic ulcer as a disease was Morgagni in 1737. A more detailed description of the symptoms and pathology of gastric ulcers was provided by Matthew Baillie in 1793. In 1817, Travers reported two cases of duodenal ulcer (17).

Apart from modern medical literature, there are also records from antiquity about gastric ulcers. According to Hippocrates, in 400 BC, the stomach's digestion process involves grinding and heating. On the other hand, Celsius recognised the presence of acid in the stomach in the 30s BC and recommended avoiding acidic foods if there was an ulcer in the stomach.

An inscription on one of the columns of the 4th century BC Temple of Aesculapius in Epidaurus, Greece, is believed to contain information about the first gastric ulcer surgery. The inscription reads, "A man with an ulcer in his stomach. He incubated and saw a vision; the god seemed to order his followers to seize and hold him, that he might incise his stomach. So he fled, but they caught and tied him to the doorknocker. Then Asklepios opened his stomach, cut out the ulcer, sewed him up again, and loosed his bonds. He went away whole, but the chamber was covered with his blood" (18).

However, the first definitive diagnosis of peptic ulcer disease could only be made in the 20th century. As a result of the autopsy examination of a mummy who died in 167 BC in China in 1975, it was determined that the person died due to disseminated intravascular coagulation (DIC), a disseminated coagulation disorder that developed after septic shock due to diffuse peritonitis resulting from perforation of the ulcer in the prepyloric region (18,19).

The general characteristic of cuneiform medical texts is evaluating the patient with an observant approach and listing symptoms and findings. At the end of the text, a prognosis is predicted by associating the patient's current condition with the evil spirit, god, or demon, which is thought to be the source of the related disease (6,7). However, this is not the case for the

cuneiform texts giving information about peptic ulcers in this study. This is probably because Ancient Mesopotamian healers had reached more concrete determinations about peptic ulcers.

Peptic ulcer disease is a common clinical condition observed symptomatically in approximately 10% of the population (12,20). It is unsurprising that peptic ulcer disease, frequently observed in today's society, was found in cuneiform texts belonging to Ancient Mesopotamian medicine, which has a history of thousands of years. Although it depends on many factors, especially dietary habits, the incidence of peptic ulcers in Ancient Mesopotamia is expected to be similar to today. However, we do not have the chance to learn these rates from texts.

The texts also described peptic ulcers and their fatal complications (7). Peptic ulcer haemorrhage is a very important complication despite the treatment methods developed today. Bleeding complications can be observed in half of the peptic ulcer patients, and 10% of the complications carry a risk of death (12). In Ancient Mesopotamia, where modern treatment methods were unavailable, bleeding likely had a mortal course. *Āšipu*s were concerned about the increased duration of peptic ulcer bleeding. The prolongation of the "bloody vomiting" symptom in the texts is associated with an increased expectation of death (Table 1).

"Gastroduodenal resultant" is the most common site of obstructions resulting from fibrosis of the ulcer (12). Before eradicating Helicobacter Pylori and current treatments such as antacids, 60% of peptic ulcer patients had gastric outlet obstruction complications (12). The fact that the words "*tugānu*" or "A.GA.ZI" were used together in four of the five texts analysed in this study shows the close relationship between "gastric outlet disease" and peptic ulcer (Table 1). The presence of these expressions, which require anatomical knowledge, in the texts suggests that the *Āšipu* knew the anatomy of the stomach and duodenum. However, autopsies are not yet known to have been performed in Ancient Mesopotamia. It is recorded that Hippocrates, Alkmeon, and Herophilos performed the first known autopsy in the 5th century BC (21). In our opinion, the presence of nausea and vomiting in cuneiform texts in which the expressions "A.GA.ZI" or "*tugānu*" and "gastric outlet disease" are mentioned should be considered obstructive symptoms. The addition of fever to gastrointestinal complaints indicates an infectious condition. Peritonitis caused by perforation of a peptic ulcer may be confused with the clinical picture of cholelithiasis. In text 6.99, the *Āšipu* seems to consider two important diseases related to these clinical presentations in the differential diagnosis.

In most texts related to peptic ulcer clinics, the Sumerian words "A.GA.ZI" and the Akkadian words "*tugānu*" were mentioned. Some of these texts also included clinical findings related to peptic ulcers. Epigastric pain and burning were the most prominent symptoms described in these texts. It was determined that complications of peptic ulcers, such as bleeding, obstruction, and perforation at the gastroduodenal resultant, were characterised as "gastric outlet disease" in cuneiform texts. Additionally, the texts stated that bloody vomiting associated with peptic ulcer bleeding is fatal if prolonged. Through research conducted for this study, it was determined that data on peptic ulcers was recorded in the cuneiform texts of Ancient Mesopotamia dating back to periods much earlier than Hippocrates.

Conflict of interest

The authors declared no conflict of interest.

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Authors’ contributions

Concept: B.D., Design: B.D., Data Collection or Processing: B.D., Analysis or Interpretation: B.D., Literature Search: B.D., Writing: B.D.

Ethical Statement

Ethics committee approval is not required fort his study.

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Stem cell therapy in knee osteoarthritis, a recent literature review

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Abstract

Cell-based therapy and novel approaches using mesenchymal stromal cells (MSCs) has been explored in recent years as a new regenerative therapy for knee osteoarthritis. MSCs are envisioned as the most extensively explored new therapeutic drugs in the treatment of cell-based osteoarthritis due to their ability to differentiate into chondrocytes and their immunomodulatory properties. Various procedures for cell selection and preparation have been described in studies on MSCs. This article focuses on a review of the available literature on MSC based cell therapy in primary knee osteoarthritis. Promising results have been obtained for cartilage repair in around thirty human studies. These results are like other cartilage repair methods, with good results in approximately 80% of the cases. However, the number of patients in stem cell studies is small and the follow-up period is very short. The new cartilage formed is a repair tissue and does not have the original cartilage structure. Therefore, there is not enough information about the long-term results of this treatment. It has not been shown to be superior to other cartilage repair methods. Orthopedic use of stem cells is still in its infancy. Although the results obtained are promising, they are not yet included in standard treatment protocols. Stem cell research continues all over the world, but the ideal application method is unknown. There is a need for further clinical studies with a larger patient populations and longer follow-up periods.

Keywords: cartilage, mesenchymal stromal cells, osteoarthritis, regenerative therapy, knee

1. Introduction

Osteoarthritis (OA) is a significant public health problem. While there is remarkable international variation in the prevalence and incidence due to OA, the burden is increasing in most countries (1). Because life expectancy is increasing as the population ages, OA is becoming a more significant component of the global disease burden. OA has been defined as a synovial disease affecting articular cartilage and bone for many years. The American Council of Rheumatology (ACR) defined OA in 1986. According to this definition, OA is a heterogeneous, progressive, complex joint disease that develops due to deterioration of articular cartilage integrity, and causes clinical and radiological findings, as well as changes in bone and joint (2). OA is the most common joint disease in the world. It is the most common cause of pain and loss of function in adults in Western societies (3).

The pathogenesis of OA is quite complex, and not only related to so-called "wear and tear" mechanical stress (4). Even today, it is still not fully explained. The situation is simple because trauma causes OA, but when the cause of primary OA is questioned, the situation becomes quite complex. Is OA a cause of joint failure, or is it a result of the structural condition of the joint? Many studies are carried out to find an answer to this question and develop new treatment methods.

OA's joint damage should be considered organ failure like kidney or heart failure. The products formed in anabolic and catabolic processes play a role in the pathophysiology of OA. At the end of this process, joint failure develops. For many years, the pathogenesis of OA was based on the thesis that cartilage degeneration develops due to long-term mechanical stress on the joint. Still, it is believed that OA is a very complex multifactorial disease, thanks to the developments in molecular biology (4, 5). The extracellular matrix is produced by chondrocytes, while fibroblast-like synoviocytes produce the synovial fluid. These two cells are essential in maintaining the joint's microenvironment (These two cells are critical to the joint's microenvironment (much more fluent))(6). There is a balance between the anabolic and catabolic processes in the joint's microenvironment. While growth factors, transforming growth factor (TGF-β) and chondrocytes repair the damage in cartilage tissue, matrix metalloproteinase (MMP)-1,3,13 and anti-aggregant enzymes-ADAM-4 and 5 prevent this (7). The balance between these anabolic and catabolic processes is disturbed in OA. The chronicity of synovial inflammation in the joint causes macrophages to accelerate the catabolic process, which causes the release of more pro-inflammatory cytokines (IL-1β, IL-6, Tumor necrosis factor-alpha (TNF-α)) (8). In addition to these processes, pathological changes in the subchondral bone, which can be seen in all stages of OA, play an essential role in pathogenesis. In this pathological process,

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radiological findings can be detected as osteophyte formation, subchondral sclerosis, and chondral damage. Cartilage damage is thought to originate from subchondral bone (9). A pro-inflammatory environment associated with damaged cartilage combined with mechanical stress increases the release of pro-inflammatory cytokines and mediators, thereby accelerating the degradation of the cartilage matrix (10). Although the disease may be due to genetic and epigenetic factors, gender, ethnicity, and age (cellular ageing, apoptosis, and lubricin), it is also associated with obesity and overweight, dietary factors, sedentary lifestyle, and sports injuries (11).

Knee osteoarthritis (KOA) is the most common subtype of OA (12). KOA is a significant cause of pain and musculoskeletal dysfunction worldwide (13). The incidence of joint pain is directly proportional to age (11). Pain in joint movements, effusion, crepitation, cyst in the popliteal region (Baker), instability due to ligament laxity, valgus or varus deformity, and limitation in walking are the clinical features of KOA.

Many agents are used in OA treatment and are being studied to be developed. Each of these agents has advantages and disadvantages. The treatment should be shaped by taking into consideration the patient's specific comorbidity. No treatment definitively cures OA, stops its progression, or slows it down. Therefore, it is necessary to develop new treatment methods. These treatment methods should stop or slow down the progression of OA and have no or minimal side effects. Further studies should be encouraged.

Cell-based therapy and novel approaches using mesenchymal stromal cells (MSCs) have recently been explored as a new regenerative therapy for knee osteoarthritis (10, 14, 15). The International Society for Cell & Gene Therapy refers to MSCs as a bulk population with unique secretory, immunomodulatory, and homing properties. The minimal criteria include being plastic adherent, expressing specific surface markers, and being capable of in vitro differentiation into adipocyte or chondrocyte (16). MSCs, which can be differentiated into various functional tissue cells, have demonstrated a superior ability to regenerate damaged cartilage as well as provide significant and clinically relevant pain relief (17, 18). Conventional surgical OA treatment (arthroscopic debridement, microfracture, autologous or allogeneic cartilage transplantation, chondrocyte transplantation) is mainly effective in symptomatic treatment and pain management. It cannot contribute to the regeneration of degenerated cartilage or the reduction of joint inflammation. The fibrous cartilage tissue obtained from these treatments is not the same as natural hyaline cartilage. MSCs are envisioned as the most extensively explored new therapeutic drugs in the treatment of cell-based OA due to their ability to differentiate into chondrocytes and their immunomodulatory properties (19). In animal and clinical studies, MSCs have been reported to hold therapeutic potential for cartilage, regeneration, including stabilization of cartilage metabolic activity and chondrogenic differentiation (20-22). Various procedures for cell selection and preparation have been described in studies on MSCs. The mechanism of MSCs for KOA has not yet been clearly demonstrated. This article focuses on a review of the available literature on MSC-based cell therapy in primary knee osteoarthritis.

2. Mesenchymal Stem Cells Derived Therapies

Stem cells are derived from perivascular cells called pericytes in the human body and are involved in tissue repair and healing. When a pericyte leaves the basal lamina of a blood vessel, it is exposed to the surrounding tissue environment and becomes an MSCs (23). These resting cells play a role in the repair process by transforming into cells such as cartilage, bone, muscle and fat tissue needed in the injury area when damage occurs in the body. There are two types of stem cells; embryonic stem cells and adult stem cells (24). Embryonic stem cells can be obtained from the placenta, amniotic fluid, and umbilical cord. Adult stem cells are found in all tissues and blood in the body, but they are very rare. These cells must be replicated to be effective in therapy. Bone marrow (BM) (25), trabecular bone (26), adipose tissue (AT) (22), synovial fluid (27) and peripheral blood (28) are the sources from which adult stem cells can be obtained for the treatment of KOA.

MSCs are multipotent adult stem cells that are abundant in a variety of tissue types (Fig. 1). Stem cells from different sources have different differentiation capacities, clinical benefits, and cultural characteristics (29). MSCs-based cell therapy is of great interest because of the multifunctionality of MSCs, including self-renewal, differentiation into specialized cells with multiple functions and many tissues, secretion of regenerative growth factors, and immunomodulation. Therefore, the chosen cell source is essential for successful outcomes in MSCs-based cell treatments, including bone marrow, fat, synovial fluid, and synovium. In the literature review, bone marrow-derived MSCs (BM-MSCs) may be the predominant source of cells, followed by adipose tissue-derived MSCs (AT-MSCs).

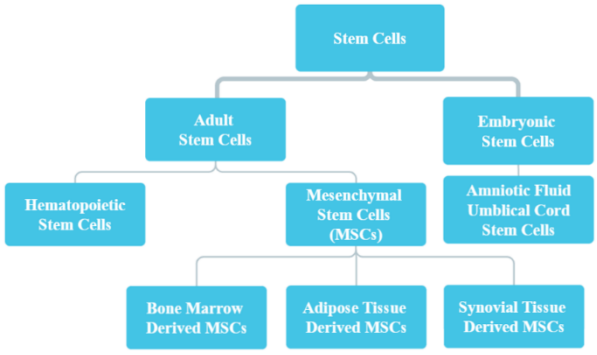

Fig. 1. MSCs are multipotent adult stem cells and highly present in multiple tissue types.

MSCs are an adult stem cell lineage and differ from pluripotent embryonic stem cells (ESCs) because they have a

more limited range of differentiation potential. ESCs can differentiate and self-renew in all cell types. Specific markers are available through cluster differentiation (CD) marker phenotypes of CD73, CD90, and CD105 that distinguish MSCs from other stem cell phenotypes (30). MSCs have multiple functionalities that include anti-inflammatory, immunomodulatory, and paracrine effects. MSCs can secrete several growth factors and anti-inflammatory proteins. The mechanisms of action of MSCs are very complex and beyond the scope of this review. For this reason, MSCs are preferred in the treatment of KOA because of their ability to differentiate directly into chondrocytes and their ability to optimize the catabolic and anabolic balance within the joint in the anabolic direction (31). It has been reported that MSC treatment effectively reduces pain and improves OA's clinical symptoms (32). This article will focus on orthopaedic applications of MSC therapy in primary knee osteoarthritis.

We emphasized the therapeutic potential of MSCs. However, there are many differences in current clinical practice (32). The lack of homogeneity in terms of treatment methods is thought-provoking. Patient selection, stem cell source selection, stem cell isolation methods, methods of applying stem cell therapy to the patient, the diversity of the place where stem cell therapy is applied, the diversity of treatments applied in addition to stem cell therapy, and the methods used to evaluate the success of stem cell therapy are not standardized. There is no standardization between studies in the literature. Therefore, there is a need to define a set of procedures to standardize MSC treatment modalities. This study aims to review the current MSC treatment methods and clinical studies used for KOA by reviewing the literature.

3. Patient Selection

Firstly, choose the appropriate origin of MSCs, then isolate MSCs from other cells, and inject the MSCs (Fig. 2). OA is a growing public health problem worldwide, affecting more than half of the population aged 65 and over. Since OA is a progressive disease, age is one of the most critical risk factors (11). In the literature, stem cell therapy was applied to patients between the ages of 18 and 75 who were diagnosed with OA symptomatically and radiologically (33). It is stated that ethnic and racial differences may affect pain and functional outcomes (34).

Existing comorbidities in patients can significantly affect the progression of OA. Various epidemiological studies have examined the prevalence of other chronic conditions in people with OA. It is estimated that 59% and/OR to? 87% of adults with OA have at least one other chronic illness, the most common being cardiovascular disease, diabetes mellitus, and hypertension (35, 36). Comorbidities reduce the quality of life and worsen joint functions in OA patients. The interaction between OA and comorbidities should be taken seriously, and patients with comorbidities should be carefully studied and excluded (33).

Fig. 2. The flow diagram of applying MSC-based therapy. First, choose the appropriate origin of MSCs, isolate MSCs from other cells, and inject the MSCs

4. Cell Selection and Isolation

Autologous or allogeneic MSC options can be used in treatment. Maybe the best treatment option is as there is a less immune reaction to autologous MSCs (22). The Autologous MSC option was preferred in many studies in which knee osteoarthritis was treated with stem cells (33). Adipose tissue and bone marrow are the most common sources of autologous MSC (14). It was seen in the literature review that bone marrow was the most frequently used MSC resource (14, 37, 38), and AT was the second most commonly used (21, 22). There are several sources of allogeneic MSCs. Allogeneic MSCs derived from adipose tissue or bone marrow can be obtained from other humans. Allogeneic MSCs from the placenta or umbilical cord can be donated with consent by a healthy mother just in time. In addition, various medicinal products are available as sources of allogeneic MSC. Administration of allogeneic MSCs may be more convenient than autologous MSCs because the process does not require invasive MSC collection and saves the time associated with waiting for cell expansion (39). A possible limitation of implanting allogeneic MSCs is host immune rejection; however, MSCs can be tolerated due to their immunomodulatory properties (39).

5. Adult Stem Cell Sources

5.1. Adipose Tissue Derived Mesenchymal Stem Cells

Adipose Tissue-Derived Mesenchymal Stem Cells (AT-MSCs) are MSCs thought to be isolated from pericytes, the stromal vascular fraction (SVF), located in the capillary and perivascular advent of large blood vessels in adipose tissue (40). Adipose tissue has been recognized as a potential source of autologous MSC due to its relative ease of harvest, the abundance of MSCs, and high chondrogenic potential compared to other sources such as bone marrow (21). AT-MSCs share features in morphology and phenotype with Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSCs) (41). Some literature publications comparing AT-MSCs with BM-MSCs indicate that AT-MSCs are present in higher numbers per unit of tissue volume, multiply faster in culture, and are less susceptible to ageing caused by culture expansion (42). AT-MSCs are not affected by the patient's age, sex, or

physiological condition (43). Because of these advantages, SVF and culture-augmented AT-MSCs are becoming more common. Adipose tissue is mostly taken from abdominal subcutaneous fat tissue by lipoaspiration.

5.2. Bone Marrow Derived Mesenchymal Stem Cells

BM-MSCs are mesenchymal stem cells isolated from bone marrow aspirate (BMA) or bone marrow concentrate (BMAC). Just as AT-MSCs are found in high volume in adipose tissue stroma, BM-MSCs are found in high volume in bone marrow spaces. It shares standard features with BM-MSCs and AT-MSCs (41). For example, pericyte origin, expression of common cell surface markers, gene expression profiles and differentiation potential are similar. Bone marrow is the gold standard for deriving MSCs for transplantation (44). BM-MSCs are a reliable source of MSCs, and such MSCs have superior osteogenic potency (38). MSCs should have plastic attachment and express CD105, CD73 and CD90 under standard culture conditions and also lack expression of CD45, CD34, CD14, CD11b, CD79a, CD19 and human leukocyte antigen isotype DR surface molecules (45). Differentiation of MSCs into osteoblasts, adipocytes, and chondroblasts should be confirmed in vitro. While these criteria will likely require modification, these minimum criteria will encourage a more uniform characterization of MSCs and facilitate data exchange between researchers. (46).

6. Culture

MSCs are multipotent adult stem cells and are highly present in multiple tissue types. Stem cells from different sources have different differentiation capacities, clinical benefits, and cultural characteristics (29). One of the characteristic features of MSCs is their ability to adhere to tissue culture plastic and generate colonies when plated at low densities (47). We know the ability of MSCs to undergo chondrogenic, osteogenic, and adipogenic differentiation.

MSCs grow from individual foci, or colonies from the microscopic view, and these colonies generated from progenitor cells have been called the colony-forming unit fibroblast (48). Fibroblasts express the same cell immunophenotypic markers, as well as the genes known to be expressed in stem cells, and were expressed in adipose and dermal stem cells OR stem cells in dermis. Fibroblasts can also differentiate into the three cell lineages mentioned above, adipocytes, osteocytes, and chondrocytes (49).

The differentiation of mesenchymal stem cells (MSCs) into cartilage-producing cells - chondrocytes is highly dependent on culture conditions. Mediators capable of promoting chondrogenesis, such as transforming growth factor-beta (TGF-β) have been described using simplified in vitro models (50). In a recent study by Yin et al., histological evaluation and gene expression analysis showed that BMSCs differentiated into mature chondrocytes after 21 days of culture without using exogenous growth factors (TGF-β) (51). Chondrogenesis can be performed in vitro in 2-dimensional or 3-dimensional culture systems. A 3-dimensional culture system allows cells to adapt to their natural morphology, facilitating more excellent/effective cell contact and interaction with the ECM. Also, the efficiency of chondrogenesis tends to be lower in the 2-dimensional culture system (52). Co-culture of platelet-rich plasma (PRP), MSCs and chondrocyte will promote chondrogenesis without hypertrophic and pathological responses (53).

7. Transplantation

Transplantation of MSCs is a promising strategy given the high proliferative capacity of MSCs and their potential to differentiate into cartilage-producing cells - chondrocytes. Transplantation is a process in which MSCs isolated or cultured on a supporting material such as collagen membranes or scaffolds are inserted and fixed directly into the lesion area of the cartilage. This method minimizes the dispersal of MSCs in the graft so that they can differentiate into chondrocytes in the cartilage. Koh et al. (2016) used a commercially available fibrin sealant product containing lyophilized human plasma fibrinogen and thrombin solution loaded with MSC suspension. When the two solutions were mixed, the adhesive instantly formed a gel, and the gel was implanted into the cartilage lesion surface under arthroscopic guidance (17). Akgun et al. (2015) planted MSCs on the surface of type I/III collagen membranes, then transplanted the membrane directly to the lesion site on subchondral bone and fixed cells using fibrinogen and thrombin (54).

Recently, paracrine effects of transplanted MSCs have received more attention than the differentiation of MSCs into chondrocytes. Donor MSCs were not preserved in the host tissue of patients who received MSC injections for different diseases after one year. Other studies have consistently reported that engraftment of transplanted MSCs in host tissues is not (not what? Not possible?) (10). The lack of engraftment may be partly due to the lack of transplantation technology and its less frequent use than injections.

8. Applying MSCs

Injection is the most common method used to introduce mesenchymal stem cells into the joint cavity. MSC and MSC-derived exosomes, chemokines and cytokines secreted by MSCs exert a paracrine effect within the joint that creates a regenerative environment by chondrogenesis, chondrocyte proliferation, reduction of apoptosis and regulation of catabolism (14).

Most substances, such as hyaluronic acid (55), platelet-rich plasma (55, 56), and saline (56), are known to improve OA symptoms on their own; therefore, it is essential to exclude background effects and carefully analyze clinical outcomes. Injection of MSCs may include single or multiple doses of different concentrations; however, the therapeutic effects of treatment with different doses and concentrations are controversial.

Although there are hundreds of experimental studies on cartilage repair and the alleviation of osteoarthritis symptoms, there are few human studies. Promising results have been obtained for cartilage repair in around thirty human studies. These results are similar to other cartilage repair methods, with good results in approximately 80% of the cases. However, the number of patients in stem cell studies is small, and the follow-up period is very short. The new cartilage formed is a repair tissue and does not have the original cartilage structure. Therefore, there is not enough information about the long-term results of this treatment. It is not superior to other cartilage repair methods. Orthopaedic use of stem cells is still in its infancy. Although the results are promising, they are not yet included in standard treatment protocols. Stem cell research is continuing worldwide, yet it is not known what the ideal application method is. There is a need for further clinical studies with larger patient populations and longer follow-up periods.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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Mechanisms and therapeutic opportunities in erectile dysfunction for advanced glycation end products (AGEs)

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Abstract

Diabetes mellitus (DM) is one of the world's most common diseases. Its impact on the male reproductive system is one of its key impacts. Up to 90% of diabetic men experience erectile dysfunction and decreased libido, which can result in infertility. Several researchers have investigated the negative impacts of reactive oxygen species and the subsequent development of oxidative stress that occurs due to DM. Non-enzymatic glycosylation products (AGEs) have been found in diabetic men's reproductive tracts. AGEs work by generating reactive oxygen species (ROS) or attaching to receptors on their own. The binding of AGE to the receptor (RAGE) has been demonstrated to play a role in physiological processes such as lung homeostasis, bone metabolism, neural systems, and the immune system. The human body has several defense against AGE accumulation, which are reduced in diabetic individuals. The situation can be improved by using some preventive measures, either by using oral drugs or natural therapeutic agents. Also, herbal medicine is gaining popularity in the market to treat various ailments. Because of the long cultural history of use and the present resurgent interest, using herbal treatments to manage male sexual dysfunction is beneficial.

Keywords: Diabetes, erectile dysfunction, male infertility, receptor for advanced glycation end products, reactive oxygen species

1. Introduction

Erectile dysfunction (ED), often known as impotence, is a condition in which a male experiences problems with his penile erection. Within 10 years of diagnosis, up to 50% of diabetic men suffer from ED (1). Fertility is also affected by a variety of factors like lifestyle factors, obesity, limited or absence of exercise, and lower urinary tract symptoms, and one of the most common of them is erectile dysfunction (2). In the United States, 30 million men suffer from ED, with a global prevalence incidence of 10-20% (3). The cavernosal and penile arterial smooth muscle walls play a significant role in the penile erectile tissue (4). Molecules involved in the erection pathway could be utilized as targets for the development of novel ED therapies (5). Diabetic-induced ED (DIED) is often resistant to PDE-5 inhibitor treatment (1).

During a long-term hyperglycaemic state in diabetes mellitus, glucose creates covalent adducts with plasma proteins, and this non-enzymatic process is known as glycation. Glycation of proteins and the production of advanced glycation end products (AGEs) are significant in the pathophysiology of diabetic complications such as retinopathy, nephropathy, neuropathy, and cardiomyopathy, as well as other diseases such as rheumatoid arthritis, osteoporosis, and aging (6). AGE levels in the testis, epididymis, and sperm are raised in hyperglycaemic conditions (7). Methylglyoxal (MG) is the precursor of AGEs and interferes with NO release (8, 9). Receptors of AGE (RAGE) present in the male reproductive system combine with reactive oxygen species, which initiate the damage of nuclear DNA (10).

AGEs show involvement in nitric oxide (NO) bioavailability, molecular pathways activated by receptors of AGEs, intercellular or intracellular deposition of insoluble complexes, and activity in cavernous tissue (11). In the penile tissues of diabetic patients, accumulation of AGEs (pentosidine) has been detected (12). Aminoguanidine prevents the formation of AGEs, in DIED (13). AGE production is known to trigger diabetes and is also connected to male infertility disruption (14). AGEs have been shown to limit testosterone production and secretion by Leydig cells by stimulating the generation of reactive oxygen species (ROS), which impacts male infertility (15). Intracavernosal vasoactive agent injections are the most effective treatment for DIED (16).

Natural products have been used since ancient times for the treatment of many diseases and illnesses. Glycation-induced ED can also be treated with natural products. By altering the NO/cGMP system, *Panax notoginseng* protects endothelial function in the penile corpus cavernosum (17). *Pausinystalia yohimbe* and *Epimedium grandiflorum* play a role as central sexual impulses as well as enhancing the release of NO from

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cavernosal endothelial cells (18). Therefore, in this review, we will talk about how glycation can affect erectile dysfunction and a therapeutic approach as well as natural products to minimize the effects. In this review, articles were identified in databases like PubMed, Google Scholar, Scopus, and Web of Science.

2. Erectile Dysfunction (ED)

Male infertility has a variety of causes, ranging from genetic mutations to lifestyle choices to medical illnesses (7). Some causes of male infertility are listed below in Fig. 1. The worldwide prevalence rate in hypogonadism is 9.6%, in retrograde 0.4-2%, in premature ejaculation 70%, and in ED 10-20% (19-21). Erectile dysfunction (ED) is the inability to achieve or maintain an erection sufficient to permit satisfactory sexual intercourse (22). ED has a huge negative influence on a man's quality of life, causing him to lose self-esteem and intimacy and experience anxiety and despair, all of which increase sexual dysfunction. The most important etiological factors of organic ED are metabolic syndrome, cardiovascular disease, and diabetes mellitus (23). Any process or disease that affects penile arteries or nerves, smooth muscle tissue, hormone levels, the corporal endothelium, or tunica albuginea can be a reasonable cause of ED (24).

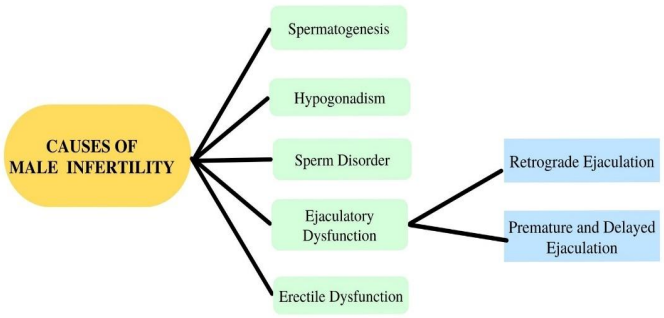

Fig. 1. Causes of male infertility

2.1. Risk factors of ED

Obesity, medications, heart disease, atherosclerotic disease, smoking, aging, diabetes, psychological stress, depression, vascular difficulties, and many more variables are linked to ED. Some of the psychogenic and organic factors responsible for ED are shown in Fig. 2. ED can also be caused by antihypertensive or antidepressant medications. In approximately 60% of men, diabetes mellitus is the cause of ED. 40% of diabetic males have hypogonadal symptoms (25). Controlling glycaemic and hypertension levels in diabetics is critical since these factors raise the risk of both microvascular and macrovascular problems, which might include ED (26). The timing of the onset should always be assessed. Gradual and progressive histories suggest an organic cause, whereas a sudden onset of complete ED in the absence of trauma or other obvious causes suggests a possible psychological cause (27).

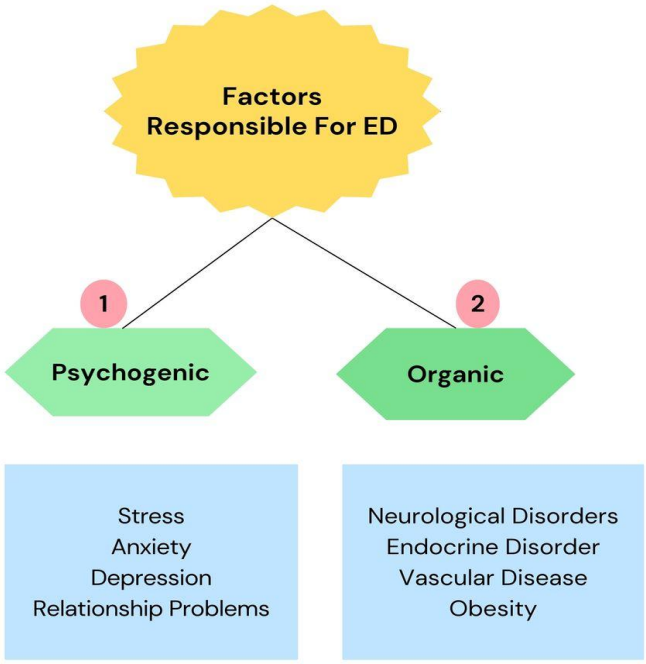

Fig. 2. Risk factors responsible for ED

2.2. Mechanism of Penile erection and ED

The integration of endocrine, vascular, psychologic, neurological, and local anatomical systems has been involved in the development of an erection (25). The non-adrenergic non-cholinergic nerve (NANC) neurotransmitter promotes vascular and cavernosal relaxation, increasing blood flow during erection (28). It is believed that the corpus cavernosum nerves and the corpus cavernosum sinusoidal endothelial cells of the penis release NO during erection; it is synthesized by neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS), respectively. For normal sexual performance, nNOS initiates penile erection by producing NO, and eNOS participates in sustained erection by producing NO (29).

Guanosine triphosphate (GTP) is converted to cyclic guanosine monophosphate (cGMP) due to the diffusible nature of NO by the enzyme guanylate cyclase in penile SM (30). The elevation in the cGMP level results in the activation of protein kinase G, which causes phosphorylation of ion channels, which triggers arterial and trabecular SM relaxation and a decrease in intracellular calcium and potassium (31, 32). The penis erection increases due to arterial blood flow increasing into the lacunar spaces of the penis due to vasodilation, intracavernosal pressure rising by a veno-occlusive mechanism, and entrapment of the blood in the corpora cavernosa. These events collectively result in increased intracavernosal pressure, which leads to a full penile erection, as shown in Fig. 3. The sinusoidal small vessels of the penis are sensitive to endothelial structural and functional changes; even minor modifications lead to erectile dysfunction (33). It has been established that penile nerve terminals and vascular endothelium produce insufficient amounts of NO, which results in an impaired erection or complete impotence (34).

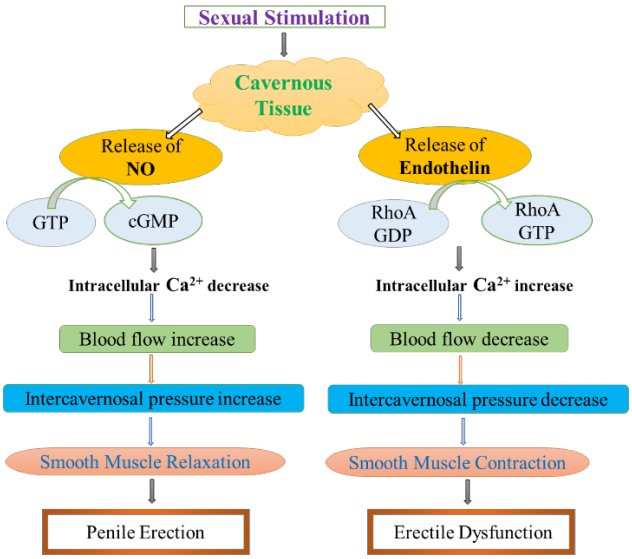

Fig. 3. Events involved in penile erection and its dysfunction

According to the researchers, ED in diabetics may be associated with an imbalance toward increased penile vasoconstriction as a result of endothelin (ET), its receptors, and ultrastructural alterations in the endothelium (35). Several studies have suggested that the endothelin (ET) transduction pathway and its receptor may be involved in diabetic ED. RhoA, a GTP-binding protein, and Rho-kinase, the pathway's effector, make up the pathway (36). The RhoA/Rho-kinase pathway has been found to be connected to ET-1-induced vasoconstriction. The route is activated, which inhibits eNOS and reduces NO generation. Rho-kinase has been found to be increased in diabetic rats and is present in rat, rabbit, and human cavernosal tissue (37). It is proposed that the RhoA/Rho-kinase pathway mediates ED through decreased production of NO in the penis. The RhoA/Rho-kinase pathway is thought to cause ED by reducing NO generation in the penis (38, 39).

3. Glycation

Glycation is a spontaneous non-enzymatically glycosylation reaction between the reactive carbonyl groups or free reducing sugars with nucleophilic free amino groups of proteins, DNA, and lipids that form an Amadori product (40). Glycation products are not stable, and they give rise to numerous and undefined degradation products known as AGEs (41).

3.1. Advanced glycation end products (AGEs)

AGEs are the modifications of proteins or lipids that become non-enzymatically glycated when modified with oxidized aldose sugars. Histopathological studies have shown the accumulation of AGEs in different tissues and dermal layers, including the renal cortex, coronary atheroma, and amyloid plaque in Alzheimer's disease, cardiac muscle, lung, and liver (42). AGEs can be classified into fluorescent AGEs, which include pentosidine and methylglyoxal-lysine dimer (MOLD), and non-fluorescent AGEs, which include carboxymethyl-lysine (CML), carboxyethyl-lysine (CEL), and pyrroline (43).

AGEs have developed different receptors, receptors of advanced glycation end products (RAGE), which mediate the intracellular signaling that disrupts cellular function through the recognition and binding of AGEs represented in Table 1 (44). The therapeutic options include AGE cross-link breaker, AGE inhibitor, RAGE antagonists, clinically approved drugs for antidiabetics, antihypertensive drugs, or statins, as well as dietary and phototherapeutic approaches (45).

Table 1. Classification of AGE receptors

Types of RAGE	AGER1	AGER2	AGER3	sRAGE
Another name	Oligosaccharide transferase-48	Phosphoprotein	Galectin-3	Soluble RAGE
Molecular weight	~48 kDa	~80-90 kDa	~26 kDa	~46-50 kDa
Location	endoplasmic reticulum and cytoplasmic membrane	Endothelial cells	Cytoplasm, nucleus, cell surface	Cell membrane, body fluid
Function	Endocytic uptake and degradation, protective role against the formation of reactive oxygen species, tissue injury	Cell activation	Cell activation and degrade AGEs moieties	Degradation and clearance

The production of AGEs results in the formation of NO as well as a reduction in free radical concentration, resulting in oxidative stress. NO is a vasodilator with an anti-proliferative impact on vascular smooth muscles. As a result of the accumulation of AGEs, hypertension, endothelial dysfunction, and vascular thickening with loss of elasticity occur (42). In patients without diabetes or infection, AGEs and RAGEs are connected with male infertility (46). According to the study, serum, and seminal plasma concentrations of soluble RAGE (sRAGE) show a variation between fertile and infertile men (46). The polyol pathway is activated due to hyperglycemia in the testis, and to investigate the pathway, sorbitol production in the testis and epididymis of mice is measured. This activation of the diacylglycerol pathway is measured by protein kinase C (PKC) in the testis and epididymis of mice. Maresch and his colleagues found that 12 to 24 weeks later, the testicular PKC value increased in diabetic mice compared to non-diabetic mice (14). Identification of CML and immunohistochemical analysis of AGE distribution revealed that the interstitium, Leydig cells, macrophages, and blood vessels are AGE-positive (47).

3.2. Effect of AGEs on ED

In the ED, there were 1.9–4 times more complaints in diabetic men than in non-diabetic men (48). ED has recently been considered the precursor to systemic vascular disease and the

earliest manifestation of atherosclerosis. High amounts of superoxide radicals are present in cavernosal tissue levels, and NO synthase is decreased in men with diabetic-induced erectile dysfunction (DIED) (49).

As shown in Fig. 4, HbA and glucose combine to form glucose and glycosylated hemoglobin, which are freely permeable red blood cells (RBCs). An intermediary, the Amadori product, produces HbA_{1c} formation, and Hb-AGE irreversible accumulation occurs. The formation of AGEs in DM is related to glycaemic control. Fluorescent pentose-mediated protein crosslinks pentosidine, an AGE product that is present in excess amounts in the corpus cavernosum of diabetic patients (50). It states that the preferential location for AGE accumulation is cavernous collagen fibers. AGEs also affect the different channels and receptors present in SM. For relaxation, intracellular calcium is essential, and it is released through potassium channels (11). The early onset of DIED happens due to early damage to potassium channels. The PDE isoform in the cavernosal SM-type 5 is inhibited by drugs, and it is responsible for the degradation of cGMP levels, which leads to an improvement in erection (51).

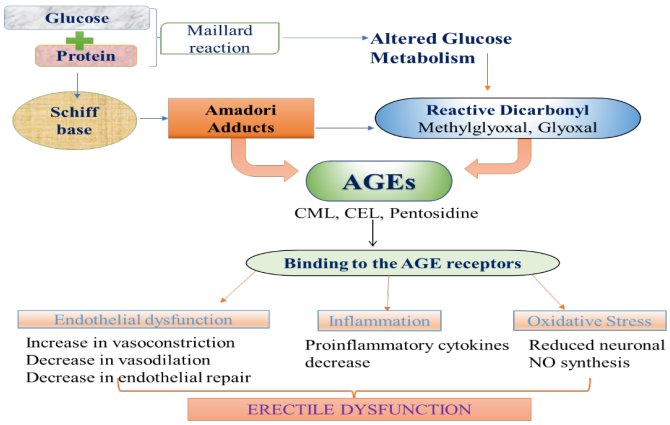

Fig. 4. Consequences of AGE formation on Erectile Dysfunction

AGEs are elevated in diabetic human penile tissues but not in serum and are localized to the collagen of the penile tunica and corpus cavernosum (52). AGEs form a covalent interaction with collagen, which leads to the thickening of the vascular wall and decreases its elasticity, which strongly leads to the dysfunction of cavernous tissue (53). AGEs affect diabetic ED patients by affecting oxygen free radicals, which elevate the oxidative cell damage and quench NO, decrease the cGMP, and impair SM relaxation (54). Some studies reveal that in diabetic animals, reductions in endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) cause SM contraction (54). Studies show that in the human penile cavernosal SM and endothelium, there is a presence of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) (52).

RAGE is attributed to an AGE-binding molecule expressed on cells of monocytes and macrophages, which mediate AGE cell uptake from blood and tissues (55). Macrophages and macrophage derivatives produce AGEs and accumulate these substances in their cytoplasm (56). In diabetic complications, AGEs, and their receptors interact to induce biological effects on the target tissues. Hyperglycemia increased oxidative stress and induced the polyol pathway, both of which are involved in the pathogenesis of diabetic neuropathy. Their effects were nerve tissue damage or vascular dysfunction (56).

AGEs production is one of the important causes of neuropathy (57). AGE receptor 1 (AGER1), or oligosaccharyltransferase-48 or OST-48, is a transmembrane protein present in the cytoplasmic membrane and endoplasmic reticulum (58). It exhibits significant AGE binding affinity, is involved in endocytosis, and contributes to the suppression of AGE-mediated mesangial cell inflammatory injury due to diabetes and aging (55, 59). AGER1 provides protection against AGE-induced ROS, which is generated via NADPH oxidase (60). Periodical oral exposure to methylglyoxal-derived AGEs loses protective effects; it induces the depletion of not only AGER1 but also SIRT1. The vascular protective effect of NAD^+-dependent deacetylase has been identified in the cavernous tissue of rodents and humans. When AGER3 is exposed to AGE, it gets translocated to the cell surface where it promotes endocytosis and degrades AGE moieties. Galectin-3 detection in SM of rat induced-AGE proliferation (11). Excessive deposition of AGEs attracts the monocytes and might bind to the vessel surface, migrate to the vessel wall, and release mediators that contribute to the development of vascular lesions (61). Various activities of the AGER system are modulated by diabetic factors, which include insulin, ROS, and AGEs. Diabetes, dyslipidaemia, and aging highly increased AGE formation, which increases RAGE expressions (55). Sperm nuclear DNA damage implies the presence of RAGE, particularly in diabetic men where the levels are elevated (10).

4. Therapeutic approach and strategies in AGE-induced ED

Several treatment options are available, most of which are associated with high-efficiency rates and safety profiles (62). There is evidence that suggests that for treating sexual dysfunction, pharmacological interventions or combined therapies are more effective than non-pharmacologic ones. Individual patients may consider the risks and benefits of treatment differently (63).

4.1. Oral treatment

PDE-5

Inhibitors of PDE-5 are the most effective oral drugs that help to inhibit the breakdown of intracellular cGMP and help to achieve and maintain an erection (64). Vardenafil (Levitra™), sildenafil (Viagra™), and tadalafil (Cialis™) are drugs in the family of PDE5 inhibitors and are mainly used in the treatment of ED (65). Vardenafil improves erectile function in men with mild to severe ED associated with diabetes mellitus. Oral vardenafil 10-40 mg increased penile rigidity (66). Vardenafil acts on endothelial cells and works as an anti-inflammatory by

reducing RAGE expression by elevating cGMP (67). Sildenafil is well-tolerated and effective -in patients with poor glycaemic control or chronic complications, and it is effective in men who are facing ED with Type II diabetes (68). In the general population, the efficacy of sildenafil is reported to be between 74–97%; it is only 50–56% among diabetic individuals (69). The onset of tadalafil is 2 hours, with a duration of efficacy of 36 hours and no interaction with food (70).

Aminoguanidine

Aminoguanidine (AG) inhibits the formation of highly reactive AGEs and inducible NO synthase (71). Several studies have shown that AG is used in the prevention of AGE formation and AGE-related complications (59). In diabetic conditions, it helps to reverse impairment in neuronal and endothelial NO-mediated penile SM relaxation (15). Usta and his colleagues (2004) found that one-month treatment with AG improved erectile function with no change in AGEs (72). In other words, in the penile vasculature, AG has protective effects through alternative pathways.

4.2. Intracavernosal injection

Intracavernosal injections (ICI) or combined drugs or vacuum constriction devices are included under second-line treatment. ICI is a vasoactive injection with high success rates. This class of drugs includes phentolamine, prostaglandin E1 (PGE-1), vasoactive intestinal peptide (VIP), and papaverine (51). PGE-1 is also known as alprostadil. In this treatment, medication is directly injected into the corpora of the penis at the lateral side (74). The erection lasts for a period of time depending upon the dose injected, and it begins after 5–15 minutes. Complications include penile pain, prolonged erection, priapism, hematoma formation, and penile fibrosis (75). Papaverine is a non-specific inhibitor that increases the level of cyclic adenosine monophosphate (cAMP) or cGMP to inhibit the Ca^{+2} channels and results in SM vasodilation and relaxation (76). Papaverine (30 mg) and phentolamine (1 mg) are collectively marketed as Androskat® and are commonly known as bimix (2 ml). Its efficiency rate is 94% and the incidence of side effects is 0.9-2.6%. Papaverine (30mg), phentolamine (1mg) and PGE1 (40g) combine to form a trimix. Trimix gives a longer-lasting erection than PGE-1 but may also increase the probability of priapism (77).

Table 2. Therapeutic treatment, functions, and side effects of ED

Therapeutic approach	Examples	Function	Side effect	General indications	References
Oral treatment	Phosphodiesterase-5 inhibitors -Vardenafil -Sildenafil -Tadalafil -Avanafil	The increase in cGMP level contributes to the establishment and maintenance of an erection.	Headache, flushing, dyspepsia, nasal congestion, back pain. After continued treatment, side effects may diminish.	First-line treatment	(51, 70, 73]
	Aminoguanidine	Reduced AGE formation in cavernosal tissue	Pancreatic and renal tumor		(11, 61]
Intracavernosal injection	Prostaglandin E-1 Papaverine Phentolamine	cAMP pathway activation Non-selective PDE-5 inhibitor Short-acting alpha-adrenergic receptor antagonist	Common effects include penile pain, bleeding, and bruises. Complicated effects: scar tissue, penile burning	First-line treatment	(51, 78]
Vacuum constriction devices	-	It helps in maintaining an erection.	Decrease in the quality of orgasm and ejaculatory discomfort, pain due to constriction ring	Second-line treatment	(76]
Penile implant	-	It helps to get an erection.	Risk of infection, penile pain, fever	Third-line treatment	(76, 85]

4.3. Vacuum constriction devices

Vacuum erection devices include a suction cylinder and pump, which boost corporal blood flow. A compression band is included at the base of the penis to maintain an erection by lowering corporal venous drainage. About 2–2.5 min is taken to obtain an erection (79). The vacuum constriction device is the safest, least expensive, and most prescribed treatment for men (80). A 100–225 mm Hg vacuum pressure is required to achieve an erection. Since 1982, the United States has approved Vacuum erection devices (81). Most patients quit early because the device may be ineffective, painful, or cumbersome. Moderate ED patients show a higher success rate (82).

4.4. Penile Implant

When pharmacological therapy fails or patients are not able to tolerate vacuum erection devices, then a penile prosthesis is introduced. It's a reliable way to restore an erection with good durability and patient satisfaction (83). There are two types of penile implants: non-inflatable or malleable and inflatable. Patients who can achieve near-normal erection or flaccidity are able to undergo inflatable implants. In secondary diabetes, malleable implants should be avoided due to the risk of erosion (54). After penile prosthesis implantation, the satisfaction rates of partners and patients are higher than those of those who are taking medications or using devices to restore erection (84).

5. Natural products as alternative approaches for the treatment of glycation-induced ED

Natural products have been used since ancient times for the treatment of many diseases and illnesses. About 35% of medicine originates from natural products (86, 87). Some of the plants listed below are used in the treatment of glycation-related ED.

5.1. Curcuma longa

Via upregulation of the heme oxygenase-1-gene and cyclic guanosine monophosphate, curcumin derivative erection is mediated (88). *C. longa* compounds like curcumin and diferuloylmethane show activity on the penile erection response. Oral intake of pure curcumin and water-soluble curcumin by albino male rats for a period result in increased activity of cavernous tissue and heme oxygenase enzyme-1 activity is involved in the penile erectile mechanism (89). A polyphenolic compound of *Curcuma longa,* curcumin may protect carbonyl stress-induced endothelial cells by trapping dicarbonyl compounds (90).

5.2. Epimedium grandiflorum

The genus *Epimedium* includes more than 50 plant species, many of which have been used to treat infertility for over 2000 years (93). Horny goat weed is an active ingredient of icariin, a flavanol glycoside obtained from the aerial part of epimedium (94). Glycoside and its derivatives have been used to increase NO synthesis in the penis, have a positive neurotropic effect on nitrergic nerves, decrease AGEs, and enhance SM proliferation (18). Icariin has enhanced erectile function in its bioactive form, icariside II (95). Icarisid II elevates the intracellular cGMP levels by enhancing nNOS expression and NOS activity in rat corpus cavernosum tissues (96). The upregulation in AGE concentration and downregulation of the NO-cGMP pathway in diabetic ED leads to a decrease in erectile function (95).

5.3. Ginkgo biloba

The release of endothelial NO improved ED which is stimulated by ginkgo (89). An extract of *Ginkgo biloba* (EGb 761) was preserved with nNOS-positive nerve fibers after cavernous nerve injury in rats (97). EGb inhibits AGE production and down-regulates RAGE expression by reducing oxidative stress (98).

5.4. Morus alba

Leaves of mulberry contain antihyperglycemic and antioxidant compounds (99). In diabetic-ED rats cyanidin-3-O-β-d-glucopyranoside can improve and protect erectile function (100). Mulberry anthocyanin, especially C3R by trapping glyoxal and forming several adducts of mulberry anthocyanin-glyoxal, decreases AGE formation (101).

Table 3. Natural plants for the treatment of glycation-induced ED

Plants	Common name	Function	References
Curcuma longa	Turmeric	It inhibits the AGE effect by trapping methylglyoxal. Water-soluble curcumin enhances erectile function. Decrease glycation burden to minimize the Amadori products and prevent corpus cavernosum from accumulation.	(11, 91, 92)
Epimedium grandiflorum	Horny goat weed, or fairy wings	Upregulation in AGE concentration and downregulation of the NO-cGMP pathway, increase NO synthesis in the penis and enhance SM proliferation.	(18, 95)
Ginkgo biloba	Ginkgo	Increases the NO bioavailability, inhibits AGEs production	(18, 98)
Morus alba	Mulberry	Inhibitory effect on the formation of AGEs, cyanidin-3-O-β-D-glucopyranoside from mulberry is used to protect and improve erectile function.	(100)
Nigella sativa	Black cumin, or black seed	Seed extracts directly relax the blood vessels in the corpus cavernosum, enhancing reproductive performance. Inhibit AGEs formation.	(101, 103, 105)
Panax notoginseng	Chinese ginseng, or notoginseng	For protecting endothelial function in the penile corpus cavernosum, restoring erectile responses and anti-hyperglycaemic	(17, 97)
Pausinystalia yohimbe	Yohimbe	Increased NO synthesis in the penis, enhancing the central sexual impulse.	(18)
Punica granatum	Pomegranate	Improved smooth muscle contraction and intracavernosal blood flow. Its extract or compound shows AGE crosslink cleaving activity, preventing AGE formation.	(112, 113)
Tribulus terrestris	Tribulus	Management of erectile dysfunction shows antiglycation activity and improves low sexual desire	(117, 118, 119)

5.5. Nigella sativa

N. sativa seed extracts affect the penile erection response by directly relaxing the blood vessels of the corpus cavernosum (102). *N. sativa* is used as an antiglycation drug in the treatment of diabetes, and other AGE-related diseases and decreases the formation of AGEs (103). *N. sativa* seed extract inhibits AGEs and Amadori activity by up to 80% (104).

5.6. Panax notoginseng saponins

For 600 years, *Panax notoginseng* (PNS) has been used in China, and this traditional herbal medicine has played an important part in the management of diabetes (107). Studies show a wide range of pharmacological applications, which include anti-inflammatory agents, cardiovascular treatment, anti-cancer, prevention of diabetic complications, and neuroprotection (108). The study shows evidence that expression of eNOS increases due to the activity of the peripheral nervous system (PNS) and controls the accumulation of AGEs and inhibits apoptosis of cells (17). It can also be beneficial for restoring endothelial function in the penile corpus cavernosum by adjusting the NO/cGMP pathway (97). PNS is beneficial for restoring erectile responses and protecting endothelial function, which gives a new therapeutic way for the treatment of patients with diabetic-related ED. Further study is essential to improve the mechanism of the peripheral nervous system in erectile function (17). It has an anti-hyperglycaemic and anti-obese effect and can regulate insulin and leptin (107).

5.7. Pausinystalia yohimbe

Yohimbine is a natural indole alkaloid, which is extracted from a variety of plant barks, such as *Pausinystalia yohimbe,* or from the root of *Rauvolfia serpentina* (108). Yohimbine inhibits human alpha-1 & alpha-2 adrenoceptors for the elevation of central sexual impulse as well as enhances the release of NO from cavernosal endothelial cells. The mechanism of enhancement of sexual function is unclear. It is an α-adrenoceptor antagonist that shows superiority in the treatment of ED (18). 5-10 mg of yohimbine is recommended thrice daily. Headache and anxiety, which may be reported as low or mild, blood pressure, nausea, flushing, increased heart rate, and palpitations are some of its side effects. Positive response rates were found in 34-43% of the most effective ED drugs (49).

5.8. Punica granatum

P. granatum juice helps to decrease nNOS and eNOS levels (109). Pomegranate improved intracavernosal blood flow, erectile activity, relaxation of smooth muscle, and reduction of oxidative products (110). *P. granatum* shows anti-erectile dysfunction properties and is measured by intracavernosal blood flow and penile erection in an arteriogenic ED rabbit model (111).

5.9. Tribulus terrestris

T. terrestris extract improves erectile function (114). *T. terrestris* shows an essential role in treating ED and sexual desire problems. Water extracts of *T. terrestris* show antidiabetic activity (115). *T. terrestris* can protect type 2 DM rats' erectile function by inhibiting cavernosal fibrosis and improving penile endothelial function (116).

6. Conclusion

Treatment of ED has progressed from psychosexual therapy and penile prostheses in the 1970s, through revascularization, and vacuum constriction devices in the 1980s, to transurethral and oral drug therapy in the 1990s. Without a firm understanding of genetics, anatomy, physiology, and the complex interplay of the male reproductive system, the evaluation becomes inefficient and fails to define the etiology. Because of our limited understanding of the male contribution, women endure the health risks of treatments for diseases that may not be there. Gene therapy application in ED represents a new field. In humans, more clinical studies are required in gene therapy, which includes cavernous nerve issues, injury, diabetes, and aging. Natural substances, extracts, and various formulations have been found to be effective in the generation of gonadotropic hormones as well as the activation of antioxidative processes such as lipid peroxidation and glutathione formation. Several natural compounds were also found to be effective in regulating hyperglycemia and apoptotic pathways. However, there are still limitations, such as the paucity of clinical trials in infertility research and the indistinguishability of dosage and use techniques of formulations. The mechanisms of pro-fertility effects are expected to be clarified in future studies, as well as the pharmacological effects of natural compounds for clinical application. As a result, they created a novel avenue that should be investigated further in both diabetic and non-diabetic men.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Review Article

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A literature review of waterborne outbreaks in the last decade in Türkiye

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Abstract

The present study aimed to evaluate the studies in the literature on waterborne outbreaks in the last decade. The literature was reviewed using the words "waterborne outbreak, outbreak investigation, Turkey and water" with PubMed, Google Scholar, and TR Index databases for the years 2010-2020. The 14 articles suitable for the outbreak review were reached and evaluated regarding the significant results. The responsible agents were determined as *Norovirus* in six studies, *F. Tularensis* in four, *Shigella spp*. in two, *Rotavirus* in one, and polymicrobial factors in one. In these outbreaks, 51,802 people were affected. Studies were descriptive, case-control, and retrospective cohort. The years with the highest number of outbreaks were 2014 (n=4) and 2010 (n=3). The largest outbreak was a Norovirus outbreak in the Elbistan district of Kahramanmaras, where 34,490 people were affected. In these outbreaks, 641 people received inpatient treatment, while no deaths were reported. In five of the outbreaks, the spots where the water was contaminated were detected, while they were not detected in nine. Failures in the water supply network, the lack of water tanks per the legislation, and the lack of chlorination for different reasons were determined to have caused waterborne outbreaks. Continuous water disinfection, overhauling non-compliance water tanks, timely maintenance and replacement of water networks, and strengthening communication between the institution responsible for water disinfection and health institutions can prevent waterborne outbreaks that could become a significant public health problem.

Keywords: systematic review, waterborne outbreak, Türkiye

1. Introduction

An outbreak is when more individuals are affected by an infectious disease than the usual frequency in a given time and population. For an infection to be qualified as an outbreak, the cases must be epidemiologically related and have at least two cases, whereas, for some infections, even a single case is defined as an outbreak (1, 2).

Poor sewage management, contamination of drinking water as a result of improper sanitation, and exposure to these waters both through consumption and during daily water use can lead to outbreaks that will create a heavy burden on population by bringing some bacterial, viral, and parasitological diseases as well as polluting the environment (3-5). Furthermore, the use of groundwater is common all over the world, especially in rural areas. These waters can be contaminated due to agricultural activities or sewer lines, and using water without treatment, believing it is safe, can lead to outbreaks (4, 6).

Safe drinking water, sanitation, and hygiene (WASH) are vital for human health. Although water-related diseases are very diverse, fecal-oral infections and diseases resulting from exposure to various chemicals and pollutants in drinking water are the leading ones. Other factors, such as climate change, population growth, rapid urbanization, or antimicrobial resistance, may exacerbate these diseases (7).

Waterborne diseases can cause acute gastroenteritis, acute respiratory syndrome, and some neurological disorders, as well as dehydration and electrolyte imbalances due to diarrhea, which can result in death (8). Most of these diseases are of microbiological origin, and some of the agents are *Rotavirus, Norovirus, Salmonella, Shigella, Hepatitis A,* Escherichia coli, *Campylobacter jejuni, Francisella Tularensis, Legionella pneumophila* and *Giardia intestinalis* (9, 10).

According to 2017 data, approximately 1.6 million people worldwide died from diarrheal diseases. In the same year, diarrhea was among the causes of death of one out of every ten children (10). Considering that approximately 88% of diarrhea-related deaths are caused by unsafe water, inadequate sanitation, and inadequate hygiene, it seems that these lives can be saved with simple and inexpensive interventions (11). In our country, waterborne outbreaks have occurred in different regions, and at times, many people have been affected (12-15).

The provisions of the legislation on spring water, drinking

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water, and potable-use water in Türkiye are determined by the "Regulation on Water Intended for Human Consumption." According to this regulation, the residual chlorine level should be 0.2-0.5 mg/L at the extreme point of the distribution network, and monthly residual chlorine measurement should be made in the effluent of the chlorinated tank. Calcium hypochlorite (solid), sodium hypochlorite (liquid), chlorine gas, chlorine dioxide, UV radiation, and ozone are used in water disinfection. The necessary criteria for selecting the disinfection method and the points to be considered are also determined in the legislation. Accordingly, the General Directorates of Water and Sewerage Administration of the Metropolitan Municipalities are responsible for selecting and implementing the appropriate disinfection method in the settlements within the borders of the Metropolitan Municipality. Municipalities are responsible in provinces that do not have metropolitan municipalities. Special Provincial Administrations are responsible for settlements outside the municipality's borders (16, 17).

Drinking water safety plays a significant role in establishing the quality of human life in societies. Waterborne outbreaks are caused by drinking water contamination worldwide. Emerging problems with microbial pathogens in drinking water can have a significant impact on public health. The present study aimed to evaluate the studies in the literature on waterborne outbreaks in the last ten years in Türkiye.

2. Materials and Methods

The data of the systematic review were obtained via the internet between January 15, 2021, and February 28, 2021. The literature was searched via PubMed, Google Scholar, and TR Dizin databases using the words "Waterborne outbreak," "Outbreak investigation," and " Turkey and water" for the years 2010-2020. Some studies were excluded from this study because 41 were same, and 131 were irrelevant. The remaining 84 studies were reassessed in terms of the study's inclusion and exclusion criteria. Case reports, case series, and studies in which the outbreak's source was not investigated were excluded. Among the accessed articles, 14 of which had a content suitable for the outbreak investigation were included in the study (Figure 1). The articles included in the research were evaluated in terms of the place and time of the outbreak, what the pathogens causing the outbreak were, what problems they caused, the leading causes of water pollution, the interventions to the outbreaks, the demographic data of the affected population and the critical results obtained from the studies.

Permission was obtained from the Ondokuz Mayıs University Clinical Research Ethics Committee (OMÜ KAEK 2020/733) for the study. Data were expressed as numbers and percentages.

3. Results

Of the 14 studies included, seven were case-control, six were descriptive, and one was a retrospective cohort study. All of the data in the studies were obtained through face-to-face questionnaires or hospital records.

While four outbreaks occurred in 2014, three in 2010, and two in 2012 and 2013, one was reported in 2015, 2016, and 2017. No studies published in the past three years were found. While most outbreaks occurred in districts and villages, only three occurred in provincial centers. *Norovirus* (Table 1) was held responsible in six of the outbreaks, *Shigella* (Table 2) species in two, *Rotavirus* in one, and polymicrobial agents (*Shigella sonnei, astrovirus, Norovirus, Rotavirus*) in one (Table 3), *F.Tularensis* (Table 4) in four. It was observed that all Norovirus outbreaks occurred in spring and summer, Tularemia in autumn and winter (except for one), *Shigella* in autumn, *Rotavirus* in spring, and polymicrobial outbreaks in winter months.

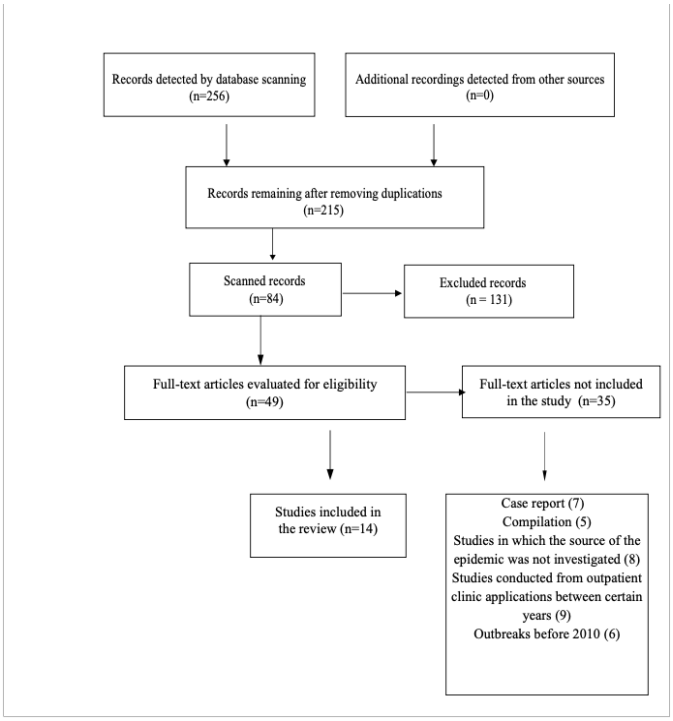

Fig. 1. Selection of studies flow diagram

Table 1. Summary of norovirus-borne outbreaks

Author, Time, Place Type of Study Data source	Characteristics of affected persons/ Complaint Hospital admission and hospitalization status Attack rates	Cause of the Outbreak	Outbreak response studies
Duman et al. (18). May 2014 Akharım, Afyon Case-control 292 cases 292 controls Questionnaire method with a face-to-face	395 suspected cases, 40.7% of probable cases were male, 59.3% female, Abdominal pain (89.8%), diarrhea (89.8%), nausea (80.9%), vomiting	The region has been rainy in recent months, Supplying water to the network system from surface sources, The presence	The waters were started to be chlorinated by an automatic chlorine device, Instead of the surface water source, water was supplied from deep water sources and

interview	(70.3%), fever (67.5%), other (8.1%), 16 people receiving inpatient treatment, the attack rate is 14.3%, the age group with the highest attack rate is 10-14, Norovirus was detected in two of the nine stool examinations, The virological analyzes could not be performed because the water samples taken were extremely dirty.	of cracks and leaks in the pipes, The water tank is not in compliance with the legislation, Water is given to the system without being subjected to chlorination.	given to the network system.
Şahan et al. (12). August 2016 Elbistan, Kahramanmaraş Descriptive Hospital records	34,490 people, 46% male, 54% female, 514 people receiving inpatient treatment, The attack rate is 20%, the age group with the highest attack rate is between 1-4 years old (41.9%), Norovirus was detected in the Ceyhan River, water resources, and irrigation canal, Norovirus was detected in three patients in stool samples taken.	Presence of irrigation water canals and sewerage systems close to water sources, Exposure of the mains water source to environmental pollutants, Insufficient water disinfection The water tank officer does not monitor the chlorine level and does not adjust the device.	A high level of chlorine was given to the network, Clay filling process was done on the riverside, The irrigation channel, which is thought to pollute the water resources, was canceled and covered with earth fill by pouring slaked lime on the ground, The sewerage network located near water sources such as the irrigation canal was also canceled and moved to another region.
Sözen et al. (19). April 2010 Keçiborlu, Isparta Descriptive Hospital records	1482 people, 41.6% male, 58.3% female, Nausea (90%), vomiting (70%), abdominal pain (90%), diarrhea (60%), fever (12%), Norovirus was detected in six of the stool samples taken from nine patients. Norovirus could not be detected in the samples taken in the city mains water, No hospitalization.	Precipitation of the region before the outbreak period, Water mains repairs in the region at that time.	Announcements were made to the public about the use of water after boiling, The training was given on personal hygiene and protection. Outbreak control was achieved in 13 days.

While a total of 51,802 people were affected in these outbreaks, the most affected were in the norovirus-borne outbreaks, with 43,263 people. The epidemic that affected the most people was the Norovirus epidemic that occurred in the Elbistan district of Kahramanmaraş with 34,490 people. In terms of age groups, it was observed that the attack rates were higher in children and young adults.

Table 1. (Cont'd.)

Author, Time, Place Type of Study Data source	**Characteristics of affected persons/ Complaint Hospital admission and hospitalization status Attack rates**	**Cause of the Outbreak**	**Outbreak response studies**
Duman et al. (18). May 2014 Akharım, Afyon Case-control 292 cases 292 controls Questionnaire method with a face-to-face interview	395 suspected cases, 40.7% of probable cases were male, 59.3% female, Abdominal pain (89.8%), diarrhea (89.8%), nausea (80.9%), vomiting (70.3%), fever (67.5%), other (8.1%), 16 people receiving inpatient treatment, the attack rate is 14.3%, the age group with the highest attack rate is 10-14, Norovirus was detected in two of the nine stool examinations, The virological analyzes could not be performed because the water samples taken were extremely dirty.	The region has been rainy in recent months, Supplying water to the network system from surface sources, The presence of cracks and leaks in the pipes, The water tank is not in compliance with the legislation, Water is given to the system without being subjected to chlorination.	The waters were started to be chlorinated by an automatic chlorine device, Instead of the surface water source, water was supplied from deep water sources and given to the network system.
Şahan et al. (12). August 2016 Elbistan, Kahramanmaraş Descriptive Hospital records	34,490 people, 46% male, 54% female, 514 people receiving inpatient treatment, The attack rate	Presence of irrigation water canals and sewerage systems close to water sources, Exposure of	A high level of chlorine was given to the network, Clay filling process was done on the riverside,

	is 20%, the age group with the highest attack rate is between 1-4 years old (41.9%), Norovirus was detected in the Ceyhan River, water resources, and irrigation canal, Norovirus was detected in three patients in stool samples taken.	the mains water source to environmental pollutants, Insufficient water disinfection, The water tank officer does not monitor the chlorine level and does not adjust the device.	The irrigation channel, which is thought to pollute the water resources, was canceled and covered with earth fill by pouring slaked lime on the ground, The sewerage network located near water sources such as the irrigation canal was also canceled and moved to another region.
Sözen et al. (19). April 2010 Keçiborlu, Isparta Descriptive Hospital records	1482 people, 41.6% male, 58.3% female, Nausea (90%), vomiting (70%), abdominal pain (90%), diarrhea (60%), fever (12%), Norovirus was detected in six of the stool samples taken from nine patients. Norovirus could not be detected in the samples taken in the city mains water, No hospitalization.	Precipitation of the region before the outbreak period, Water mains repairs in the region at that time.	Announcements were made to the public about the use of water after boiling, The training was given on personal hygiene and protection. Outbreak control was achieved in 13 days.

While chills-cold, sore throat, lymph node enlargement in the neck or around the ear, fever, muscle and joint pain, and headache were common in tularemia outbreaks; nausea, vomiting, abdominal pain, diarrhea, and fever were more frequent in others. While there were a total of 641 people who were admitted to the hospital for treatment, no fatal cases were reported. It was observed that the points where the water was contaminated in five outbreaks could be determined; in nine of them, it could not be detected. While no causative pathogen could be detected in water in the *Rotavirus, Shigella*, and Tularemia outbreaks, the causative pathogen could be detected in water in only one of the polymicrobial outbreaks and norovirus outbreaks.

Table 2. Summary of Shigella species-borne outbreaks

Author, Time, Place Type of Study Data source	**Characteristics of affected persons Complaint Hospital admission and hospitalization status Attack rates**	**The cause of the outbreak**	**Outbreak response studies**
Topal et al. (14). September 2012 Terme, Samsun Case-control 112 cases 112 controls Hospital records Questionnaire method with a face-to-face interview	4050 people Diarrhea (100%), fever (96.4%), abdominal pain (92.9%), nausea (86.6%), vomiting (80.4%), The attack rate was 9.2%, all age groups were affected by the disease, the 5-9 age group had the highest attack rate (18.7%), A total of 52 patients were hospitalized.	A broken water pipe was found near the water tank. Low chlorine levels (<0.2 ppm), Consuming only tap water increased the risk 2.0 (1.2-3.6) times, Culture results showed S. sonnei in 27 of 33 stool samples, total coliform in 18 of 52 water samples, and Escherichia coli in 4 of them.	The water mains system was examined and repaired.
Özüdoğru et al. (13). October 2014 Bayburt city center Case-control 247 cases, 247 controls Hospital records, Face-to-face survey form	971 people, 55.9% of cases were women, Diarrhea (100.0), abdominal pain (93.9), fever (81.0), nausea (74.5), vomiting (63.6), other (2.8), 23 people were hospitalized for treatment, Cases have been seen in all age groups, 68.0% of cases were under the age of 14, Attack speed 13.0 per thousand, Age groups with the highest attack rates were 0-14 years old (3,0%) and over 90 years old (3,3%)	It was observed that the free chlorine level in all samples was 0 (zero) ppm, In environmental examinations, it was seen that there was no chlorination device in the water tanks and there were rusted pipes, It was determined that there was water and sewerage works in Bayburt city center during the pre-outbreak period and intermittent water outages were made during this period. Before the outbreak period, there	Chlorination has been made in water networks, Public announcements were made to consume water by boiling and not to use tap water, 'Contaminated, undrinkable' statement was written on the fountains by the municipal teams.

		was moderate rainfall in the province.	

Lack of maintenance and control of chlorine tanks, interruption of chlorination as a result of power outages, presence of neighborhood fountain tanks near water channels, contamination of water tanks after heavy rainfall, reactions from the public due to changes in the taste of water with chlorination, and therefore interruption of chlorination, chlorination not being carried out regularly, water tanks not complying with the legislation, breaks and cracks in water pipes, sewer lines passing near water sources, positioning of water sources near environmental contaminants, contact of wild animals with water due to the absence of protection bands around water tanks, the use of unknown underground water sources in some fountains and insufficient chlorine levels in the waters and the miscommunication between the units responsible for water and health units were the main causes of these outbreaks.

Table 3. Summary of Rotavirus and polymicrobial-borne outbreaks

Author, Time, Place Type of Study Data source	**Number of people affected Complaints Hospital admission and hospitalization status Attack rates**	**The cause of the outbreak**	**Outbreak response studies**
Tozan et al. (20). March 2014 Nigde city center Case-control 88 possible cases 88 controls Hospital records Face-to-face survey form	1288 cases of acute gastroenteritis, Diarrhea (80.7%), abdominal pain (84.1%), nausea (89.8%), vomiting (84.1%), fever (55.7%), Coarse attack speed 10.9 per thousand The age group with the highest attack rate is 0-14 years, Five children were hospitalized. Rotavirus was detected in four of the six-stool samples taken.	Before the increase in the case, there was a water outage for two hours as a result of the power outage, and it was stated that there was light rain in the region on the same day, Insufficient chlorine levels were determined in the mains water samples taken during the outbreak, and the waters were found to be microbiologically unsuitable, It was found that the resulting rotavirus outbreak was caused by contaminated tap water and only tap water consumption was 6.5 (2.1-19.1), p<0.001 times risky, The contamination point of the tap water could not be determined.	Ensuring regular and effective disinfection of the mains water, checking whether the chlorination system works after the interruption, It was suggested that power outages and infrastructure works that may affect the network system should be made by informing the Public Health Directorate in advance, Also, it was suggested that microbiological examinations should be made by detecting the increase in cases and sufficient stool samples should be taken, and the patient's information should be recorded completely, accurately, and completely in Health Institutions.
Sezen et al. (21). December 2012 Erzurum Case-control 95 possible cases 95 controls Hospital records Face-to-face meeting	Number of cases 2096, 49% male, Diarrhea 92%, abdominal pain 86%, vomiting 85%, nausea 84%, fever 59%, other 6.4%, For the city center, the rate of gastroenteritis attack increases 5.6/1000, and the risk between the ages of 2-17 increases OR: 12 (3.3-42) times. Being male increased the risk by 5·6 (2·5-12) times.	It was determined that this outbreak was caused by ancient neighborhood fountains, It was determined that drinking water only from ancient neighborhood fountains increased the risk by 6·4 (3·0–16), The water flowing from these ancient fountains came from unknown underground sources, Of the eight stool samples collected, two were positive for Shigella sonnei, one for astrovirus, the other for astrovirus and Norovirus, and the other for astrovirus and Rotavirus.	A media campaign was organized to warn residents against the use of water from ancient neighborhood fountains, Later, the Provincial Directorate of Public Health initiated a construction project to supply the ancient neighborhood fountains with purified water from the municipal water treatment plant.

Table 4. Summary of tularemia-borne outbreaks

Author, Time, Place Type of Study Data source	**Number of people affected Complaints Hospital admission and**	**The cause of the outbreak**	**Outbreak response studies**

	hospitalization status **Attack rates**		
Boz et al. (22). January 2015 Dinar, Afyonkarahisar Case-control 29 cases 116 controls Face-to-face survey form	The number of cases was 29 people. 72.4% of the cases were women. Chills (89.7%), sore throat (86.2%), lymph node increase around the ear or neck (82.8%), fever (75.9%), muscle and joint pain (69%), headache pain (55.2%), other (10.3%), Although 62.1% of the cases were in the adult age group, cases have been seen in every age group, The oldest case was 72 years old, and the youngest case was five years old, with a mean age of 36.7±17.5 years.	It was found that drinking water from Tank-1 increased the estimated relative risk by 3.6 times in the case group compared to the controls, Although it was found that Tank-1 was the water source in the outbreak, the contamination point of the reservoir could not be found. Tularemia agent could not be detected in the water samples taken, No animal remains were found around the warehouses and in the main water source. Patients were diagnosed late, patients received different treatments for a long time, Since the first exposure period cannot be detected most of the time, it prevents sampling at the appropriate time and from the appropriate places.	The waters of the water tanks in the district were emptied and cleaned and offered for use again, The Public Health Directorate has increased the number of sampling points by tightening the water sampling periods, Physicians in the district were called by phone and informed.
Bozkurt et al. (23). October 21, 2013-January 22, 2014 Kahramanmaraş, Afşin Nadir village, Kargabükü village Descriptive Hospital records	10 patients, 5 (50%) men and 5 (50%) women, the age range of patients 2-68 (mean 25) years, F.tularensis antibody titers were found to be positive between 1/320-1/1280 bmicroagglutination test in serum samples of six out of ten cases, The oropharyngeal form of the disease was detected in all of the cases, and the coexistence of oculoglandular and oropharyngeal forms was observed in one.	Villagers stated that the sewerage system of the neighboring village passes 50 m above the village water tank, that there are leaks in the sewerage system, especially in rainy periods, and that they saw dead rodents in the water tank, None of the patients had a history of contact with rodent animals or their feces, There was no history of insect bites or ticks, contact with game animals, or eating wild animals. F.tularensis positivity was not observed in any of the water samples by PCR.	After the water tank was completely emptied and washed with chlorinated water, it was chlorinated at regular intervals, The villagers and the health center physician were informed about Tularemia.

4. Discussion

Five different factors [Norovirus, F. Tularensis, Shigella spp, Rotavirus, polymicrobial agent (Shigella sonnei, astrovirus, Norovirus, Rotavirus)] observed as the causative pathogens of the outbreaks between the years 2010-2020 were included in the study. Apart from these pathogens, waterborne outbreaks have occurred in Türkiye in the recent past with other factors. An outbreak occurred in a regional boarding school in Kahramanmaraş Central Karacasu town in May 2008, caused by pesticides mixed with the school's drinking water, and 51 children were affected by this outbreak. The contamination of the well water used by the school with pesticides as a result of the spraying of the agricultural lands around the school has been indicated as the cause of the contamination (29). Furthermore, the *Legionella* outbreak occurred in a newly opened hotel in Alanya, and six people were affected. The fact that the water temperature was in the range of 35-45 °C and the amount of iron in the water samples was too high to comply with the regulation paved the way for this outbreak (15).

In our study, the number of people affected by outbreaks ranged from 10 to 34,490 people. A study examining 15 outbreaks from four countries stated that between 47 and 400,000 people were affected by waterborne outbreaks (30). It is observed that there is a change in the number of people affected by these outbreaks according to the type of agent, the settlement where the outbreak occurred, the population of the region, the point where the water networks are contaminated, and when the outbreak prevention works were started. When the causative pathogen was examined, we determined that outbreaks caused by *Norovirus, Rotavirus*, and *Shigella* affect thousands of people in a short time, while Tularemia affects fewer people over a larger period.

Although no fatal cases were reported in the outbreak reviews included in the study, waterborne diseases can cause deaths. In the town of Walkerton, Ontario, Canada, 2300 people fell ill, and seven died after a heavy spring rain in May

2000. This outbreak was caused by *E. Coli 0157:H7* and *C. Jejuni* due to contamination of the water source by animal manure from a nearby farm (30).

From the studies examined, it has been observed that difficult and laborious epidemiological studies are performed to demonstrate that the source of the outbreak is water, to determine the contamination point of the water, to detect the causative pathogen, and to prevent the outbreak early. In nine of the studies examined, the inability to detect the contamination point of the waters delayed the response to the outbreaks. In most waterborne outbreaks, the causative pathogen could not be detected in the water. The cause of the outbreak was tried to be revealed according to the clinics, laboratory analyses, and stool examinations of the patients. These situations cause late intervention to outbreaks and increase the hospital burden in a short time with morbidities and financial losses.

There are various reasons why the agent cannot be obtained from water. Some of these are the late detection of the outbreak, the samples not being taken in the right amounts from the right places, the chlorination made without taking samples when it is understood that there is an outbreak and the absence of laboratories in every province where the samples will be studied. Moreover, the dilute presence of pathogens in water and technical difficulties (e.g., virus loss due to the membrane filtration process in cross-flow microfiltration and RT-PCR technique used in the detection of *Norovirus* and the need to take at least 100 liters of water sample from one point for norovirus detection) in microorganism production are obstacles to the detection of agents from water (31).

Most of the outbreaks we examined occur during periods of heavy rainfall. As a result of excessive precipitation events, fecal contamination of water resources by surface flow and soil movement, excess of the capacities of sewage systems, and discharge of untreated water into drinking water pipelines increase the risk of waterborne diseases in these periods by causing high microorganism density in the waters (32). For this reason, it is even more essential to perform water disinfection effectively and entirely and to take precautions during rainy periods.

It was observed in different studies that the microbiological pollution determined in drinking water in rural areas is higher than in urban areas. In a study in Edirne, 17.8% of the drinking water samples taken from rural areas were microbiologically contaminated, while 5.4% of the samples taken from urban areas were dirty (33,34). In our study, only three (21%) outbreaks occurred in provincial centers. A study investigating the effect of the New Metropolitan Municipality Law No. 6360 on microbiological contamination in water control indicated a positive change in microbiological pollution in drinking water, especially in rural areas in Tekirdağ province. With this law, the responsibility of delivering water to the consumers in a healthy way was transferred to the metropolitan municipality, while it was the special provincial administrations in the villages and the local municipalities in the towns, districts, and provincial centers. It was observed that the improvement in post-law outcomes is greater in rural areas with higher pollution than in urban areas (33). This result suggests that the disruptions that occur in rural areas can be eliminated by centralizing water management institutions, that is, by increasing investments from a single center in the provinces with a holistic approach.

In our country, water that provides less than 10 m3 of water per day or is used by a population of fewer than 50 people is not within the scope of the "Regulation on Water Intended for Human Consumption." In this regulation, it is recommended to inform the people of the region in case of any water contamination, to make recommendations to ensure the protection of public health, and to take the necessary precautions (16). Our study revealed that outbreaks occur mostly in districts and villages. There are neighborhood fountains and water tanks in rural areas in Türkiye, of which many unknown sources are available. Although the population in rural areas is less than in cities, people from the cities obtain drinking water from these fountains, thinking that the water in the village fountains is of higher quality and healthier. Thus, these waters are used by many more people. Continuous analysis and inspection of each source may bring some difficulties and deficiencies in terms of time, personnel, and finances. In these regions, water contamination can develop due to the lack of regular chlorination in the water tanks, the absence of automatic chlorination devices in the tanks, the chlorination being left to one of the village people, and the insufficient knowledge of these people about water disinfection.

A study in which the knowledge and awareness of village headmen in Bitlis province about the chlorination process were determined revealed that the rate of villages with water tanks was 95.3%, the rate of those tanks that comply with the legislation was 52.6%, the rate of those with chlorine devices was 12.2%, and the rate of warehouses where chlorination was performed was only 38.5% (35). This situation suggests a significant lack of legislation and information about water disinfection in rural areas.

Due to the change in the taste of water with chlorination in rural areas, there is a reaction from the public, and therefore chlorination is interrupted. In a study performed in the rural areas of Trabzon, 43.3% of the participants using a domestic water tank stated that the waters do not need to be chlorinated (36). A study on the village headmen of Bitlis indicated that the rate of the headmen who think that the water should be chlorinated is 79.3%. When asked why chlorine should not be added to water, 38.6% of them stated that "it spoils the taste of water," 22.7% said that "chlorine is poisonous," 2.7% said that animals do not drink chlorinated water," and 2.7% said that the chlorination process is challenging and laborious. Among the

headmen (%4.7) there were those who described chlorine as a poison decanted into water (35). Notably, these negative thoughts about water disinfection are at this level, even in a group that is a community leader.

Considering the causes of the outbreaks, general non-compliance with the legislation draws attention. When the outbreaks and the laboratory records of the water samples in that region are examined, it is observed that there were insufficient water chlorine levels and negative microbiological analyzes of the water in previous periods (21). In this respect, efforts should be made to identify and correct deficiencies individually. It should be ensured that devices are installed in water tanks where automatic chlorination devices are not available, additional power measures should be taken against possible power outages, and the necessary controls should be made by sharing the outage situations of electricity suppliers with the local administration and health directorates. Likewise, in the water network works to be conducted in a settlement, health institutions and the public should be informed, and to prevent possible outbreaks, it should be ensured that the water is not used as potable water for a while or it is boiled and consumed in case of contamination of the water after the outages.

This study has some limitations. Our study was carried out only on the data of related articles. For this reason, it cannot reflect the true extent of water-borne epidemics in Türkiye. In addition, insufficient number of related studies can be listed among the limitations.

In conclusion, when the characteristics of the outbreaks included in the study were examined, it was observed that due to the problems related to chlorination, disinfection per the legislation was not provided, there was still the use of neighborhood fountains, there was excessive precipitation, or there was insufficient communication between institutions in case of malfunctions related to the network system. To prevent outbreaks that occur mainly for preventable reasons, education should be provided to all segments of society on issues such as ensuring the water quality standards specified in our legislation, the use of healthy water, and hygiene. Local governments, special provincial administrations and headmen should fulfill their responsibilities more carefully in order to ensure the water quality standards specified in our legislation in order to prevent epidemics that occur mostly due to preventable reasons. Furthermore, effective and solution-oriented communication should be ensured with local governments, health institutions, and other relevant institutions to control the extraordinary situations that may arise and to overcome them with less damage.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: H.N.A., Ö.T., Design: H.N.A., Ö.T., Data Collection or Processing: E.A., Analysis or Interpretation: E.A., Literature Search:E.A., Writing: E.A., H.N.A., Ö.T.

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Review Article

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The role of stem cell-based extracellular vesicles in male fertility

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Abstract

Fertility tends to decrease over time in physiological or various pathological conditions. Preservation of male and female fertility is of equal importance for successful reproduction. Protection and maintenance of male fertility and infertility treatment are among the medical fields that have attracted attention, especially in recent years. Various therapeutic approaches, especially sperm and testicular tissue cryopreservation, are applied for the preservation and continuity of fertility. In addition to these methods, which are routinely used, there is an increasing interest in stem cell-based therapies, which are not only aimed at preserving fertility but also effective in infertility treatment. Stem cells are therapeutic agents with high differentiation potential found at every stage of life. In the light of the studies, it has been determined that besides all the positive effects of stem cells, they contain nanoparticles known as extracellular vesicles in their structures. This review will discuss the type of extracellular vesicles, their biological functions, the use of stem cell-based extracellular vesicles in men, and current studies.

Keywords: fertility; stem cells; extracellular vesicles; exosome

1. Introduction

In living things, fertility tends to decrease over time under physiological conditions. In addition, it is known that various pathologies (such as spermatogenic insufficiency, severe oligozoospermia), immunological diseases, and various medical procedures applied to patients (such as vasectomy, testicular surgery, chemotherapy) cause fertility to decline or infertility. Therefore, it is possible to say that fertility may decrease due to natural causes, diseases, and treatments applied against diseases. For all these reasons, fertility preservation; has become an increasingly important issue in all living things (1). Preservation of male and female fertility is of equal importance for reproductive success. Preservation of male fertility; is among the essential medical fields that have attracted attention in recent years, and various therapeutic approaches are used for this purpose. Among the main methods available are sperm and testicular tissue cryopreservation. The routinely applied method is sperm cryopreservation (2). However, with these methods, it is impossible to produce new and fertile spermatozoa or increase the fertility of existing spermatozoa; only existing spermatozoa are preserved for future storage. In other words, semen cryopreservation; is not infertility treatment in men; It is only a fertility preservation method. This situation has led researchers to search for alternative approaches for maintaining fertility and treating infertility.

Stem cell therapy; is a relatively new treatment method used in many areas such as healing of degenerated tissues and organs, treatment of immunosuppressive diseases, treatment of congenital anomaly, and cancer treatment. Stem cell therapy today; has also started to take place in the reproductive field, and various studies are being carried out on this subject. Reproductive stem cell studies generally use mesenchymal (MSCs) and spermatogonial stem cells (SSCs) (3). Recent studies are; showed that stem cells can also produce nano-sized cell membrane particles known as extracellular vesicles (EVs) (4). It is known that EVs are produced in the male and female genital tract, as in many paracrine tissues, and there are various studies on the effects of EVs on the reproductive systems. Studies carried out in recent years; EVs derived from stem cells seem to have a regenerative and therapeutic effect like the stem cells themselves (5). In light of all this information, stem cells and EVs obtained from stem cells have become the focus of attention in reproductive fields such as fertility preservation and infertility. This review will discuss the type of extracellular vesicles, their biological functions, the use of stem cell-based extracellular vesicles in men, and current studies.

2. Extracellular Vesicles

Along with releasing secretory vesicles by specialized cells such as hormones or neurotransmitters, various membrane vesicles, known as extracellular vesicles, are also released from all cells. The release of membrane vesicles from the cell surface has been evolutionarily conserved from bacteria to fungi, from parasites to humans (6). Extracellular vesicles (EVs) are nanoparticles released from all cell types with membrane-bound bi-lipid structures containing proteins, lipids, and nucleic acids (microRNAs and mRNAs) involved in cellular communication (7,8). EVs carry a large number of molecules. These molecules include various receptors,

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adhesion molecules, proteins involved in cell trafficking and intracellular signal transduction, cytoskeletal proteins, cytoplasmic enzymes, cytokines, chemokines, and cell-specific antigens (Ags). Moreover, mRNA is enriched with several nucleic acids, including long non-coding RNAs, microRNAs (miRNA), and even extra-chromosomal DNA (9). Lipids enable cells to exchange information because they contain a complex load of signalling proteins, small non-coding RNAs (sncRNAs), and regulatory RNAs (10) (Fig.1).

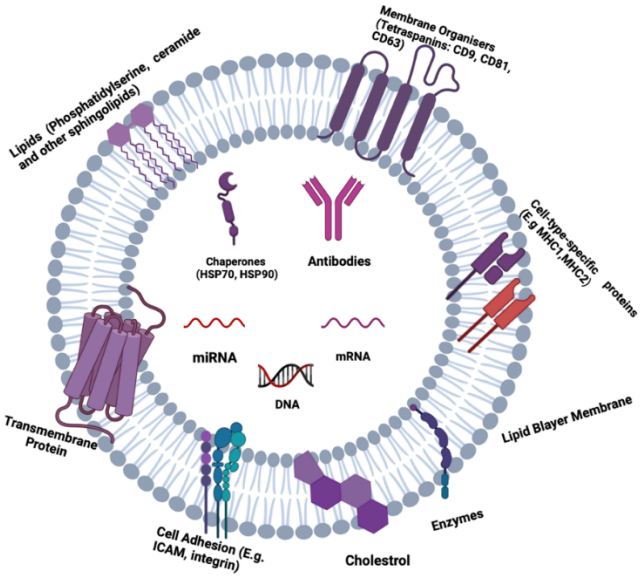

Fig. 1. Cargo contents carried by extracellular vesicles.

EVs; are membrane-packed vesicles secreted by various cell types, including T cells, B cells, dendritic cells, platelets, mast cells, epithelial cells, endothelial cells, neuronal cells, cancerous cells, oligodendrocytes, Schwann cells, embryonic cells, and MSCs (11). It has also been isolated from many biological fluids, including blood, milk, saliva, amniotic fluid, and urine (12, 13). EVs are known to play an important role in cell-to-cell communication. They are involved in physiological and pathological important processes such as immune responses, maintenance of homeostasis, coagulation, inflammation, cancer progression, angiogenesis, and antigen presentation (11, 14). It has been observed that EVs participate in different processes effectively in the physiology of the organism, such as tissue repair (15), preservation of the stem cell status of progenitor cells (4), immunosurveillance (16). They are essential information carriers between cells through the transmission of various proteins, bioactive lipids, and genetic information to alter the phenotype and function of recipient cells. Therefore, extracellular vesicles have been associated with numerous biological and pathological processes (17).

The transported content may vary depending on the cell type and activation state. Once released, EVs can interact with neighboring cells or disperse in the bloodstream and other organic fluids such as semen, saliva, and urine. Their ubiquitination makes EVs critical effectors of cell-to-cell communication, which can occur autocrine, paracrine, exocrine, or endocrine (9). They also trigger cell proliferation, migration, angiogenesis, and apoptosis. It affects altering gene expression, suppresses the immune system, and induces implantation of EVs produced by embryonic tissues (18-20).

Extracellular vesicles can be isolated using differential centrifugation, density gradient centrifugation, ultrafiltration, chromatography, polymeric precipitation, and microfluidic devices. However, there is no single standard applied procedure in isolation methods, and all existing methods have advantages and disadvantages. The chosen method also affects the quality and purity of the vesicles obtained. The most common isolation method used today is the ultracentrifuge method. (21,22). Many isolation methods, including ultracentrifugation, damage the structure of EVs due to the forces involved in isolating these tiny particles. Ultracentrifugal EV isolation methods, ultrafiltration, and immunoaffinity columns can isolate highly pure EVs at the expense of long isolation times that eventually result in few EVs. Polymeric precipitation of EVs, which allows their isolation at average centrifugation speeds, contaminates proteins and other extracellular vesicles (23).

2.1. Biogenesis of Extracellular Vesicles

EVs consist of 3 different groups according to their biogenetic pathways and sizes; exosomes, microvesicles (MVs), and apoptotic bodies. MVs are formed by budding directly from the cell membrane and are reported to have sizes between 100 nm and 1000 nm. They carry a cargo of proteins, lipids, mRNAs, and microRNAs and interact with recipient cells with specific receptor-ligand complexes (11). They are rich in selectins, integrins, CD-40, phosphatidylserine and metalloproteinase. Apoptotic bodies are fragments of dying cells formed and secreted in the extracellular space by the budding of the plasma membrane during the apoptotic process. They are irregular in shape and range in size from 500 to 4000 nm. They are rich in DNA and histones (24). Exosomes are 30-100 nm vesicles in size of endosomal origin and are found in multidimensional bodies that fuse with the cell membrane and are then released into the extracellular space (9) (Fig. 2).

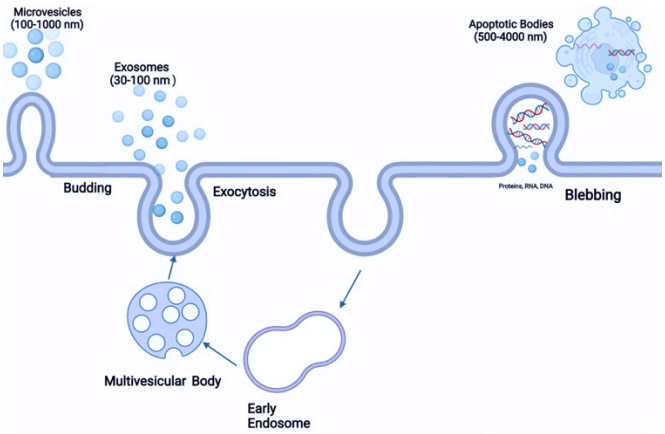

Fig. 2. Schematic representation of EVs biogenesis.

Exosomes are enclosed in a bilayer membrane that protects their contents and allows them to travel long distances in tissues. While this bilayer membrane contains a small amount of phosphatidylserine, it contains more cholesterol, ceramide, and sphingolipid (12,14). Exosomes carry membrane proteins, cytosolic proteins, transcription factors, DNA, mRNA, rRNA, miRNA, and various signal transduction molecules. They are rich in heat shock proteins (HSPs), annexins, cytoskeletal proteins, signal transduction proteins, and multidimensional body synthesis proteins (9). The most important evolutionarily conserved proteins in exosomes; are heat shock proteins (HSP), CD63, and tetraspanins (25). Exosomes carry free fatty acids and bioactive lipids derived from arachidonic acid. Signalling mediators such as prostaglandins, arachidonic acid, phospholipase A2, phospholipase C, and phospholipase D are also components of the exosome lipid pool (26). Exosomes are small particles with major functions, and they stand out thanks to their big role in intercellular and intracellular communication (27). It is well known that cholesterol and phospholipids are essential components of mammalian cell membranes, including exosomes, and cellular membrane integrity can be maintained by balancing the physiological cholesterol/phospholipid ratio. Growth factors associated with exosomes have also been found to play an essential role in the repair and healing of damaged tissue (28).

3. The Role of Extracellular Vesicles in the Male Reproductive System

Studies have shown that EVs and their cargoes have influential roles in male reproduction. From puberty to adulthood, the spermatogonial stem cells in the testis differentiate in the seminiferous tubules and mature into spermatozoa. Spermatozoa undergo many changes as they leave the testicles and pass into the epididymis (29). Especially, EVs coming from the epididymis and prostate gland interact with spermatozoa during these transitions, giving them many changes and functions. EVs; are secreted in different parts of the reproductive tract (male and female), epididymosomes, prostasomes, uterosomes, and oviductosomes (30). Although isolated from all tissues found in the male reproductive tract, they are best characterized by the epididymis (epididymosomes) and seminal fluid (prostasomes). Although first documented in hamsters, they have been identified in all mammals studied, including rats, bulls, rams, and humans. In general, EVs support the development and function of spermatozoa and support reproductive success by affecting the physiology of female reproductive system cells. Through cargoes carried by EVs to spermatozoa, spermatozoa gain motility, attachment of the zona pellucida, and the ability to fertilize the oocyte (31,32). Membrane vesicles derived from seminal plasma were first described in humans and named prostasomes because they are secreted by the acinar epithelial cells of the prostate gland (33). Prostasomes are the most abundant extracellular vesicles in seminal plasma. These vesicles have also been isolated in animal seminal plasma after human. The seminal fluid inhibits sperm capacitation mainly due to its high cholesterol content. Prostasomes are also the primary sources of cholesterol found in seminal fluid. It is known that they have many protective properties and can transfer proteins and lipids to spermatozoa. It is accepted that the primary function of prostasomes is to interact with sperm and prepare them to encounter oocyte after ejaculation while maintaining and maintaining their fertilization capacity (34). Human prostasomes, including exosomes, have played essential roles in increasing sperm motility, influencing tyrosine phosphorylation of sperm proteins, delaying the acrosomal reaction, and influencing sperm-oocyte interaction by transferring related proteins to spermatozoa. It has been determined that prostasomes play a role in sperm maturation, capacitation, and acrosome reaction and have antioxidant and antibacterial properties (35-37). Prostasomes are classified as exosomes because of their size. At the same time, epididymosomes are larger and are more commonly referred to as microvesicles because they are shed from the plasma membrane of epididymis epithelial cells. Prostasomes have many important functions, including stimulation of immune activity, sperm motility, and capacitation in the female reproductive tract. Epididymosomes transfer proteins to spermatozoa that pass through the epididymis epithelium (38). These are surface proteins required for male gamete function (39). The epididymosomes of mice, humans, and bulls contain abundant antioxidant enzymes. Thus, they have a protective effect on spermatozoa that are sensitive to oxidative stress and play a role in eliminating damaged spermatozoa. In other words, thanks to the sperm quality control mechanism, protection against quality spermatozoa is formed (40). miRNAs in proximal epididymosomes are known to alter the gene expression of epididymal cells. Thanks to these properties, it has been seen that it can change the essential functions that ensure the maturation of spermatozoa and regulate fertilization ability and can be used as a biomarker (41). Seminal EVs transfer different molecules involved in $Ca2+$ signalling, including receptors and enzymes that maintain sperm motility. One such molecule, CRISP1, was also more abundant in seminal extracellular vesicles from normozoospermic men than in asthenozoospermic men. EVs derived from normozoospermic males have been shown to improve sperm motility and induce capacitation (42).

After combining with target cells, these functional molecules cause different phenotypic and functional modifications and ultimately affect adhesion, regeneration, resistance to external factors, and viability. Spermatozoa interact with these vesicles during their passage through the reproductive system. This spermatozoa-vesicle interaction is crucial for the proper functioning of spermatozoa (critical biological events such as maturation and capacitation). These vesicles contribute molecules required for different physiological events such as maturation, motility, activation, protection, capacitation, acrosomal reaction, and fertilization

(30). In human and bull studies on EVs, it has been shown to prevent early acrosome reaction and premature capacitation (34,43) and is important for capacitation, acrosome reaction, and fertilization in mice and pigs (44) has been reported to inhibit polyspermia in mice (45). Due to these features, limited studies have been conducted on whether it has a curative effect during freezing and storage in semen cryopreservation. As a result of the studies, it was observed that sperm motility, viability, membrane integrity, and percentage of acrosome integrity were improved in spermatozoa after thawing (46-49). Vesicles, including exosomes, are thought to prevent premature acrosome reaction by early attachment to or fusion with the sperm membrane during capacitation (50) and it has been found that exosomes have important bioactive functions such as maturation, capacitation, acrosome reaction and fertilization of spermatozoa. It has been observed that spermatozoa improve the integrity of the plasma membrane and protect their function by binding to the membrane of spermatozoa and transferring proteins (such as AWN and PSP1) to spermatozoa. EVs can transfer Spermadezines to the sperm membrane, which can help maintain sperm function by inhibiting premature capacitation during long-term storage. It has been reported that there is a positive correlation between increasing exosomal concentration and improving both motility and vitality (51). Many studies have been conducted on their use in physiological events and as disease biomarkers. Especially in the diagnosis of prostate cancer, PCA-3 and TMPRSS2: ERG, which is prostate cancer biomarkers, have been detected in exosomes obtained from urine (52).

E EVs are vital structures for basic biological processes in reproduction. Assisted reproductive methods are a promising therapeutic tool in many fields, such as diagnosis and treatment of reproductive diseases and fertility preservation.

4. Stem Cell Based Extracellular Vesicles

Stem cells (SC) are reserve cells that can self-renew and differentiate into various cell types. Stem cells can divide indefinitely, regenerate damaged cells and produce differentiated progeny, and they also have vital roles in maintaining cellular homeostasis and repairing tissue damage. Studies have shown that stem cells also secrete small vesicles known as extracellular vesicles into the extracellular environment (4,21). During the regeneration of tissues, stem cells have to reach target cells at a distance or near. They communicate with target cells via long thin tubular processes such as soluble factors, cytonema, cilia, or extracellular vesicles (53). EVs are thought to play a role in morphogen, nucleic acid release and distribution, establishment and maintenance of cell-tissue polarity during embryonic development, and adult tissue regeneration. Stem cells are controlled by specific microenvironments known as niches. Niche-emitted Wnt signals are self-renewal factors for stem cells in many mammalian tissues. In other words, extracellular developmental signalling proteins known as Wnt signals provide lifelong renewal and protection of tissues by feeding stem cell activity. These Wnt signalling ligands are located across the plasma membrane, between cells, and on the surface of EVs. Thus, it also contributes to the tasks of EVs in cellular homeostasis and tissue repair after damage (54,55). At the same time, it has been found that stem cell-derived EVs stimulate tissue regeneration in studies conducted on terms of injuries in the kidney, heart, liver, and nerve tissue (56).

Stem cells with differentiation and self-renewal gain importance in many areas, such as male fertility preservation and male infertility treatment. Stem cell-based extracellular vesicles have extraordinary potential for regenerative and therapeutic medicine.

4.1. Use of Stem Cell-Based Extracellular Vesicles in Male Fertility

Today, the most commonly used stem cell types to be used in the protection of male fertility; mainly mesenchymal stem cells and spermatogonial stem cells (57). Mesenchymal stem cells (MSCs) are a type of cell commonly used in clinical studies. Clinical and animal studies have shown that mesenchymal stem cells exert their therapeutic effects, not through their differentiation potential but through extracellular vesicles and paracrine factors (58). EVs obtained from MSCs can reprogram by stimulating damaged areas in target cells (59). Mesenchymal stem cells can be isolated from almost all body tissues. However, bone marrow and adipose tissue (adipose tissue) are the most common and essential sources due to easy stem cell healing and minimal donor site morbidity (60). Adipose tissue offers some advantages over other stem cell sources under investigation. Because the tissue is easy to collect, the number of cells obtained from the isolation is very high, and a small piece of fat is sufficient for isolation. In addition, adipose tissue has high initial cell yields, robust in vitro proliferative capacities, and higher immunomodulatory properties (61). Adipose tissue mesenchymal stem cells have specific therapeutic mechanisms of action such as anti-inflammatory, antibacterial, antiviral, tissue regeneration, and extracellular vesicle production (62). Microvesicles derived from bone marrow mesenchymal stem cells improved the quality of mouse spermatozoa after freezing and thawing. It has also been suggested to enhance the properties of surface adhesion molecules (CD29, CD44, ICAM-I, and VCAM-I), which are involved in spermatozoa's fusogenic and signalling properties. Decreased levels of necrosis and apoptosis were detected in spermatozoa. This study also shows that supplementation with microvesicles derived from mesenchymal stem cells improves semen quality (46). The presence of exosomes obtained from adipose tissue mesenchymal stem cells in dogs during cryopreservation has been reported to improve post-thaw motility, acrosome integrity, and plasma membrane integrity. It has been reported that exosome-treated spermatozoa show an increased lateral head displacement (ALH) amplitude compared to untreated spermatozoa (47). Conversely, no effect was found on parameters related to semen quality after thawing in a study

with mesenchymal stem cells derived from the amniotic membrane. However, it has been said that the reason for this may be the low concentration of the added vesicles, and different concentrations should be tried (63). Four miRNAs were detected in a study with EVs derived from adipose tissue mesenchymal stem cells in pigs. Compared to stem cells, EVs were found to have richer miRNAs. Among them, genes involved in cellular pathways such as angiogenesis, cellular transport, apoptosis, and proteolysis have been identified. Thus, tissue repair potentials have been identified (64). Mesenchymal stem cells are also promising cells for the treatment of diseases. In a study on diabetes mellitus-associated erectile dysfunction in mice, dysfunction treatment was tried using exosomes derived from adipose tissue-derived stem cells. As a result of the study, it was observed that fibrosis decreased, the number of endothelial cells increased, and erectile function was restored when treated with exosomes obtained from adipose tissue-derived stem cells. At the same time, it has been determined that miRNAs (miR-126, miR-130a, miR-132, miR-let7b, miR-let7c) play a crucial role in healing and repair and are transported to target cells by exosomes (65). A previous study observed that exosomes obtained from mesenchymal stem cells decreased corpus cavernosum apoptosis and increased the amount of both smooth muscle and endothelium in mice with erectile dysfunction (66).

The stem cells found at the origin of spermatogenesis in mammalian testicles are called spermatogonial stem cells (SSCs). Spermatogonial stem cells are found in the basal part of the seminiferous epithelium. These cells are the only stem cells in the body that transmit genetic information and self-renewal to offspring throughout life (67). There needs to be a balance between self-renewal and differentiation of SSCs. Excessive self-renewal or differentiation can negatively affect spermatogenesis and cause infertility by causing impaired fertility. Some niches provide endocrine and paracrine signals to regulate these mechanisms. In mammalian testicles and factors released from these cells, Sertoli and Leydig cells are primary contributors to SSC niches (68). Spermatogonial stem cell development and maturation of spermatozoa occur in waves in the highly regulated environment of the seminiferous tubules and spermatogonial stem cell niche (29). However, the molecular mechanisms in the differentiation of SSCs are still not fully known. In a study, it was observed that Sertoli cell-derived exosomes favored differentiation more than Leydig cell-derived exosomes. In order to better understand how it works, miRNA analysis was also performed, and it was found that miR-486-rich exosomes were transferred from Sertoli cells to SSCs. miR-486 is important in the regulation of male germ cell differentiation. Thus, the effect of exosomes on differentiation has been contributed, and it has been thought that it can be used as a biomarker in diagnosing male infertility (69). Although many studies have been conducted on spermatogonial stem cells and their importance in male fertility, there are not many studies in terms of extracellular vesicles. In a recent study, extracellular vesicles were detected in the basal part of the seminiferous epithelium in mice, rats, rabbits, and humans at different stages of spermatogenesis (70).

The number of studies on bioactive vesicles has increased significantly in recent years. EVs are emerging as critical players in both normal physiology and pathophysiology. Stem cell EVs exert their therapeutic and regenerative effects by transferring biologically active molecules in their vesicular cargo containing proteins, lipids, mRNA, and microRNA. EVs and their secretions act as regulators of both reproductive physiology and reproductive pathology processes. Spermatozoa interact with extracellular vesicles passing through the male and female reproductive tracts. It affects male gamete quality by acting as a regulator of reproductive success in males. The importance of the intercellular communication provided by EVs in the male reproductive system is becoming more and more a matter of curiosity. With many new dark spots, EVs and their tasks are increasingly being understood and discovered more and more.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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Diagnosing hyper-IgE syndrome in adulthood: A case based discussion in resource-limited setting

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Abstract

Hyper-IgE syndrome (HIES) in adult patients is a very rare occurrence with heterogeneous clinical manifestations. Most of the previously described cases were from developed countries, capable of performing comprehensive testing to pinpoint the underlying genetic defect. Such a facility is often unavailable in resource-limited countries, creating a great diagnostic challenge. Nevertheless, several important clinical clues can significantly aid in hypothesis formulation, such as the presence of primary immunodeficiency, eosinophilia, and a history of atopy. We describe the process of diagnosing HIES on an 18-year-old Asian male through systematic symptom analysis and strategic use of a simple laboratory examination.

Keywords: hyper-IgE syndrome; DOCK8; adulthood; clinical diagnosis; primary immunodeficiency

1. Introduction

Hyper-IgE syndrome (HIES) in adult patients is a very rare occurrence with heterogeneous clinical manifestations. There is very limited existing literature on the subject, most of which describes cases from developed countries with facilities to perform comprehensive genetic testing (1-4). The scarcity of such technology, combined with the absence of strong clinical diagnostic criteria, often causes misdiagnosis and sub-optimal treatment, especially in resource-limited countries (5,6). Nevertheless, several important clinical clues can significantly aid in hypothesis formulation, such as the presence of primary immunodeficiency, eosinophilia, and a history of atopy. We describe a case of 18-year-old Asian male suffering from primary immunodeficiency with eosinophilia, clinically diagnosed as HIES.

2. Case Presentation

An 18-year-old Asian male suffered from progressively declining health over a four-month period. He was a previously healthy individual with no history of severe infection, allergic reaction, as well as hospitalization. He had normal growth and development as a child. He was an only child with no significant history of familial disease. He initially suffered recurrent abdominal pain, which was diagnosed as appendicitis. He underwent an appendectomy and experienced repeated post-operative wound dehiscence. One month after surgery, he experienced a pruritic rash on his back which spread to most of his body and facial edema after consuming a multivitamin product consisting of vitamin B1, B12 and vitamin C. Most of the rash and edema resolved after cessation of the aforementioned multivitamin. However, the skin around his right thigh worsened and started to show signs of ischemia. He also started to develop persistent diarrhoea, roughly 5-7 times daily with feces of a consistency of type 7 on Bristol stool scale. The wound on his right thigh eventually developed a secondary infection and he fell into septic shock, causing a grade 3 acute kidney injury requiring dialysis. He had lost roughly 15 kg in 3 months and looked cachexic. His physical examination was unremarkable except for the wound on his right thigh (Fig. 1).

Fig. 1. Non-healing wound on patient's right thigh taken before (A, B) and after surgical debridement (C).

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No lymphadenopathy was detected on physical and ultrasound examination. His bloodwork showed an absolute eosinophil count of 7000 cells/µL with negative tests for HIV, TBC, and ANA-IF. His IgE level was >2500 kIU/L with a specific IgE level elevated only towards shellfish (24.5 kIU/L). His stool analysis showed fungal infestation, and his wound culture isolated *Pseudomonas stutzeri* and *Candida tropicalis*. Genetic testing wasn't available for our patient. However, he had a DOCK8 score of 49.08, which predicted DOCK8 mutation (Table 1).

Table 1. The DOCK8 score. A total number of scaled points > 30 predicts a DOCK8 mutation (7)

			Feature	Points x Scale		Scaled points
1	**Mandatory: IgE> 10x normal range**					
2	A	Parenchymal lung abnormalities	No structural lung damage	0	-5.00	0.00
			Bronchiectasis	6	-5.00	-30.00
			Pneumatoceles	8	-5.00	-40.00
	B	Highest eosinophiles/µl	< 700	0	8.18	0.00
			701-800	3	8.18	24.54
			>800	6	8.18	49.08
	C	Sinusitis, otitis (# episodes in worst year)	1-2	0	15.50	0.00
			3	1	15.50	15.50
			4-6	2	15.50	31.00
			>6	4	15.50	62.00
	D	Retained primary teeth	None	0	-4.54	0.00
			1	1	-4.54	-4.54
			2	2	-4.54	-9.08
			3	4	-4.54	-18.16
			>3	8	-4.54	-36.32
	E	Fractures with minor trauma	0	0	-9.09	0.00
			1-2	4	-9.09	-36.36
			>2	8	-9.09	-72.72
			Total (Sum A-E) Scaled points			49.08

*IgE: Immunoglobulin E

He was treated empirically with meropenem 1 gram TID and was adjusted according to the culture result to ampicillin-sulbactam 375 mg BID with fluconazole 150 mg QD, as well as fluid and nutritional support. He was prescribed methyl prednisolone 31.25 mg QD during septic shock and was tapered as his condition stabilized to 8 mg QD. His septic shock and kidney injury were resolved after 3 weeks. Surgical wound debridement and biopsy were performed, followed by vacuum-assisted wound closure. The wound biopsy result showed non-specific suppurative inflammation with no sign of vasculitis and eosinophilic infiltration (Fig. 2). On discharge, his condition was stable with signs of improved wound healing. His IgE level and absolute eosinophil count improved to 370 kIU/L and 500 cells/µL, respectively.

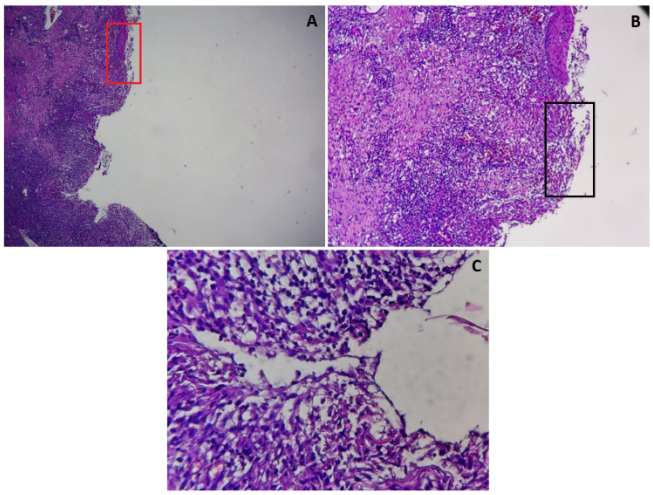

Fig. 2. Hematoxylin and eosin-stained skin biopsy taken during surgical debridement showing lymphocyte and neutrophil infiltration, suggesting non-specific suppurative inflammation. There were no signs of vasculitis or eosinophilic infiltration. Magnifications: A: 4x; B: 10x of the red rectangle, and C: 40x of the black rectangle.

3. Discussion

To date, more than 400 types of PIDs have been described with overlapping clinical manifestation, requiring comprehensive genetic testing to reach a definitive diagnosis, which was not available in our region. Due to fund and resource limitations, we had to strategically chose which diagnostic modality to use and exclude most of the differential diagnoses (DDx) clinically. Firstly, we reduced the DDx substantially by including only PIDs with eosinophilia in adulthood, which includes, but not limited to: HIES, Wiskott Aldrich syndrome (WAS), immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, and Netherton's syndrome, autoimmune lymphoproliferative syndrome (ALPS), selective IgA deficiency, and adenosine deaminase (ADA) deficiency (8). Secondly, we performed an IgE level examination due to the possible allergic reaction preceding the patient's skin lesion, which showed an extremely high level of >2500 kIU/L with a specific IgE level elevated only towards shellfish (24.5 kIU/L). We excluded WAS due to normal platelet level, IPEX syndrome due to absence of endocrinopathy, Netherton's syndrome due to absence of typical ichthyotic skin and bamboo hair shaft defects, ALPS and ADA deficiency due to normal lymphocyte count and morphology, while extremely elevated IgE level that had never been described in selective IgA deficiency, leaving HIES as the best fitting diagnosis (8,9).

Table 2. The HIES Score. A total score of >40: likely , 20-40: uncertain of HIES (10)

CLINICAL FINDINGS	Points									
	0	1	2	3	4	5	6	7	8	10
Highest serum-IgE level (IU/ml)	< 200	200-500			501-1000				1001-2000	>2000
Skin abscesses	None		1-2		2-3				>4	
Pneumonia (episodes over lifetime)	None		1		2		3		>3	
Parenchymal lung anomalies	Absent						Bronchiectasis		Pneumatocele	
Retained primary teeth	None	1	2		3				>3	
Scoliosis, maximum curvature	$< 10^0$		$10\text{-}14^0$		15-20				> 20	
Fractures with minor trauma	None				1-2				>2	
Highest eosinophil count/µl	< 700			700-800			> 800			
Characteristic face	Absent		Mildly present			Present				
Midline anomaly	Absent					Present				
Newborn rash	Absent				Present					
Eczema (worst stage)	Absent	Mild	Moderate		Severe					
Upper respiratory infections/year	1-2	3	4-6		> 6					
Candidiasis	None	Oral	Fingernails		Systemic					
Other serious infections	None				Severe					
Fatal infection	Absent				Present					
Hyperextensibility	Absent				Present					
Lymphoma	Absent				Present					
Increased nasal width	< 1 SD	1-2 SD		> 2 SD						
High palate	Absent		Present							
Young-age correction	>5 years			2-5 years		1-2 years		≤ 1 year		
Total	20									

*IgE: Immunoglobulin E

According to its inheritance pattern, HIES can be broadly categorized as AD, AR, or sporadic form (1,10,11). Our patient didn't fit the somatic description of classical AD-HIES due to STAT3 deficiency, which usually presents in childhood (Table 2) (10,12). On the other hand, our patient had the immunological characteristics of HIES, such as: IgE level > 2000 kIU/L, eosinophilia, a history of atopy, mucocutaneous candidiasis, severe skin infection, and GI malabsorption, which matched the characteristics of AR-HIES, especially DOCK8 immune deficiency syndrome (DIDS) (12-14). Furthermore, the result of DOCK8 scoring in our patient clinically supports the diagnosis of DIDS (Table 1). Similar to other forms of HIES, clinical manifestation of DIDS tend to occur in childhood (7). While significantly less frequent, DIDS in adulthood have previously been reported with cutaneous manifestations and atopy being the most dominant complaints (2,7). Chronic diarrhoea is frequently observed in DIDS and is caused by malabsorption due to intestinal infection and allergic or autoimmune enteropathy, which might lead to malnutrition and failure to thrive (14,15). Poor wound healing in DIDS can be caused by IL-22 deficiency, causing apoptosis and proliferation inhibition of intestinal cells (16). Although, other factors such as infection and malnutrition might also contribute to poor wound healing in this patient. Hematopoietic cell transplantation is the only curative treatment for AR-HIES and DIDS, whereas no curative treatment is currently available for AD-HIES (17,18). Antibiotic and antifungal agents are routinely given regardless of the type of HIES (19). In the absence of facility to perform comprehensive genetic examination, diagnosis of HIES can still be achieved through systematic symptom analysis and strategic use of simple laboratory examination. Antibiotic and antifungal agents are the mainstay of HIES management and can be given regardless of the type of underlying genetic mutation.

Conflict of interest

The authors declared no conflict of interest.

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Written informed consent was obtained from the patient for the publication of this case report and the accompanying data.

Authors' contributions

Concept: D.P.M., B.L., D.P.W., N.R., M.J., Design: D.P.M., B.L., N.R., Data Collection or Processing: D.P.M., B.L., Analysis or Interpretation: D.P.M., B.L., D.P.W., N.R., M.J., Literature Search: D.P.M., B.L., Writing: D.P.M., B.L., D.P.W., N.R., M.J.

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Case Report

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Giant intrapulmonary bronchgenic cyst

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Abstract

Bronchogenic cysts are common congenital bronchopulmonary malformations that are thought to develop as a result of abnormal budding of the tracheal diverticulum during the embryonic period. A 39-year-old female patient underwent a right lower lobectomy with a right thoracotomy due to a giant intraparenchymal cyst in the lower lobe of the right lung. At the end of the 1-year follow-up period, no clinical and radiological problem was detected in the case. It can be cured by complete resection of the cystic structure without leaving any residue of the cyst wall epithelium.

Keywords: bronchogenic cyst, surgery, intrapulmonary cyst, bronchopulmonary malformations

1. Introduction

Bronchogenic cysts are common congenital bronchopulmonary malformations that are thought to develop as a result of abnormal budding of the tracheal diverticulum during the embryonic period (1). Bronchogenic cysts, usually located in the mediastinum around the trachea and main bronchi, may show intraparenchymal localization, especially in the lower lobes (2).

2. Case Report

A 39-year-old female patient presented to our clinic with complaints of cough, sputum, and back pain. Anamnesis revealed that the patient had frequent lung infections in the last eight years as well as exertional dyspnea that had worsened in the last one month. On physical examination, auscultation revealed a decrease in respiratory sounds in the lower zones of the right lung.

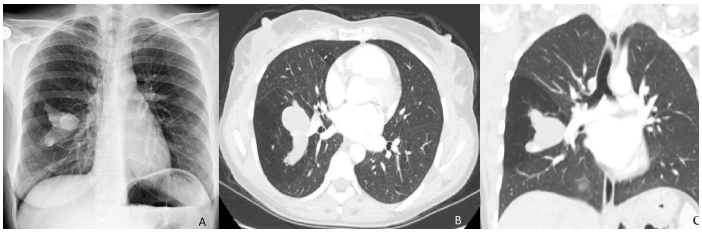

Fig 1.A) A well-circumscribed opaque lesion near the hilar region in the lower lobe of the right lung is visible on posteroanterior chest roentgenogram. **B)** and **C)** Axial and coronal section of thorax tomography shows a lesion in the lower lobe of the right lung

Posteroanterior chest X-ray revealed a well-circumscribed opaque lesion in the lower lobe of the right lung, close to the hilar region (Fig. 1A). Thoracic tomography revealed a 43-mm, ovaloid-shaped, well-contoured mass lesion centrally located in the lower lobe of the right lung containing hypodense material thought to be dense mucoid accumulation and accompanied by sporadic calcifications, and areas of entrapped air lateral to the lesion were detected (Fig. 1B and 1C). When the airways were evaluated using flexible bronchoscopy, no association with the bronchial system was detected, but the right lower lobe bronchus was rotated medially due to external compression.

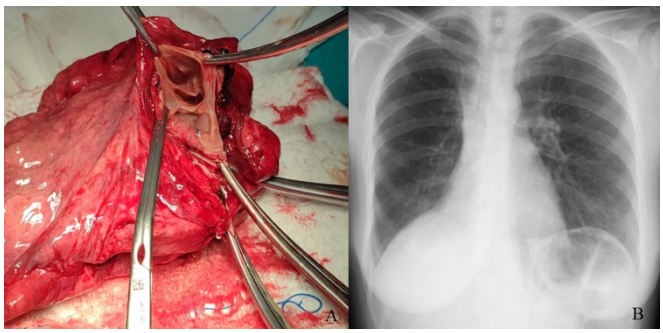

Fig. 2. A) Pathology specimen opened during the operation **B)** Posteroanterior chest roentgenogram seen at 1 year postoperatively

The subject underwent surgery under general anaesthesia with the preliminary diagnosis of an infected air cyst or an intraparenchymal bronchogenic cyst, and a right posterolateral muscle-sparing thoracotomy incision was made. During exploration, a centrally located lesion in the lower lobe of the right lung was palpated, and it was observed that the right lower lobe was mostly emphysematous. Therefore, a right lower lobectomy was performed. During the operation, the pathology specimen was opened, and it was observed that the cavity was filled with copious amounts of mucoid secretion (Fig. 2A). Histopathological examination showed a cystic

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formation with a wall structure, including mucus glands and fibrovascular and cartilage tissues lined with ciliated columnar epithelium, and the diagnosis of an intrapulmonary bronchogenic cyst was made. The patient had no problems during the postoperative period and was discharged on the 5th day. The patient was followed up for one year, and no clinical or radiological issues were detected (Fig. 2B).

3. Discussion

Bronchogenic cysts are usually located in the mediastinum around the trachea and main bronchi. However, 15% of cases demonstrate intraparenchymal localization, especially in the lower lobes. While most cases are asymptomatic, they may present with symptoms such as chest pain, cough, and dyspnea as a result of infection and rupture or compression of the adjacent structures by the bronchogenic cyst (2). Posteroanterior chest X-ray and thoracic tomography are sufficient for radiological diagnosis (3). In these examinations, bronchogenic cysts are often seen as oval or circular homogeneous solitary opacities with smooth borders, thin walls, and no calcifications. The cyst content may be of fluid or soft tissue density. Due to the high protein and calcium oxalate content in mucoid cysts, the radiodensity measurement value may be more than 100 HU. Air is rarely observed in the cyst (3). The differential diagnosis should consider a lung abscess, hydatid cyst, infected air cyst, and tuberculosis. Surgery is the main treatment modality for bronchogenic cysts, even in asymptomatic cases, because of future complications, the risk of malignant transformation, and the successful results of surgical treatment (1). It can be cured by complete resection of the cystic structure without leaving any residue of the cyst wall epithelium. Although total excision is sufficient in mediastinally located bronchogenic cysts, wedge resection, segmentectomy, or lobectomy may be needed in intraparenchymal bronchogenic cysts (4). Among the surgical techniques that can be applied for this purpose are thoracotomy, VATS and mediastinoscopy. Recently, VATS has become a frequently used method with the help of developing technology. A study showed that thoracoscopic excision used in the treatment of mediastinal bronchogenic cysts has similar results to the approach with thoracotomy (5). Jung et al. showed in their series of 113 cases that the VATS approach is reliable and has encouraging results. In addition, they recommend VATS excision as a primary treatment option (6).

Although rare, adult-onset intrapulmonary bronchogenic cysts should be considered in the differential diagnosis of pulmonary space-occupying lesions. In intraparenchymal bronchogenic cysts, the extent of resection is determined by the size of the cyst, its localization, and complications such as emphysema or destruction that may develop in the lobe in which the cyst is located.

Conflict of interest

The authors declared no conflict of interest.

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Acknowledgments

None to declare.

Authors' contributions

Concept: O.T., A.B., E.S.T., Design: O.T., S.K., Data Collection or Processing: O.T., E.S.T., Analysis or Interpretation: O.T., S.K., E.S.T., Literature Search: O.T., A.B., E.S.T., Writing: O.T., S.K.

Ethical Statement

This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki as revised in 2000. Written informed consent was obtained from the patient or the next of kin for publication.

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Case Report

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Nontraumatic pneumocephalus and subarachnoid hemorrhage case: Brain abscess related to complicated otitis media

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Abstract

We wanted to present this case to emphasize that the diagnosis of complicated otitis media should be kept in mind in patients presenting to the emergency department with headaches, nausea, vomiting, and confusion. Our case was a 30-year-old male patient. Although he was on oral treatment for otitis media, his symptoms persisted, and a headache started. A brain CT scan performed in the emergency room revealed no pathology. Five days later, pneumocephalus and surrounding subarachnoid hemorrhage were detected on brain CT imaging due to nausea, vomiting, headache, and confusion. The patient was discharged 70 days after receiving medical care. As seen in our case, it should be kept in mind that pneumocephalus in an unconscious patient may occur due to non-traumatic causes and may be a consequence of complicated otitis media, and this situation should be thoroughly investigated.

Keywords: intracranial abscess, complications, lateral sinus thrombosis, suppurative otitis media, temporal abscess

1. Introduction

The middle ear is a thin temporal bone gap, with inner and outer surfaces adjacent. The majority of the outer wall of the middle ear is composed of the tympanic membrane (TM), while the medial wall of the middle ear is the outer wall of the inner ear. It is connected to the cellulae mastoideae via the antrum mastoideum in its posterior part and to the pharynx via the eustachian tube in its anterior part (1).

Acute Otitis Media (AOM) is characterized by acute, suppurative inflammation of the middle ear fluid and mucosa. Purulent otorrhea may be observed if the tympanic membrane (TM) is perforated (2, 3). Malignancies, sinusoidal illnesses, reflux, eustachian dysfunction, smoking, intensive care follow-up, sarcoidosis, and HIV infection are all known to be risk factors for acute otitis media (AOM) (4, 5). AOM that is not treated appropriately and for an adequate amount of time can lead to complications such as TM perforation, mastoiditis, labyrinthitis, petrositis, meningitis, brain abscess, hearing loss, and lateral and cavernous sinus thrombosis (3).

We wanted to present this case to bring attention to the fact that the diagnosis of complicated otitis media should be kept in mind in patients brought into the emergency department with headaches, nausea, vomiting, and confusion. The remarkable aspects of our case are the detection of a brain abscess after the worsening of clinical features while receiving antibiotic and antifungal treatment under supervision, the occurrence of this condition in a short period of time when brain CT imaging performed a few days earlier was normal, and the diagnosis of pneumocephalus when the differential diagnosis is more suggestive of traumatic events.

2. Case Report

A 30-year-old male patient was given oral treatment and ear drops (antibiotics and NSAIDs) in a primary health care facility due to otitis media. As the patient's complaints persisted, he sought therapy from an otorhinolaryngology specialist; IM treatment was planned due to purulent discharge in the left ear, the antibiotic spectrum was expanded, and an antifungal was commenced. Six days after receiving this treatment, the patient presented to the emergency department complaining of a headache. Brain CT imaging was conducted and reported normal (Fig. 1).

After five days, the patient was brought to the emergency room with nausea, vomiting, a headache, and blurred consciousness. Blood tests revealed WBC: 7.3 10^9/L, LYM: 0.61 10^9/L, AST: 42.6 U/L, ALT: 57.8 U/L, and CRP: 94 mg/L. After an initial diagnosis of encephalitis, the patient was referred to a higher-level facility, where the brain CT scans revealed pneumocephalus and the presence of subarachnoid hemorrhage (SAH) in the area (Fig. 2).

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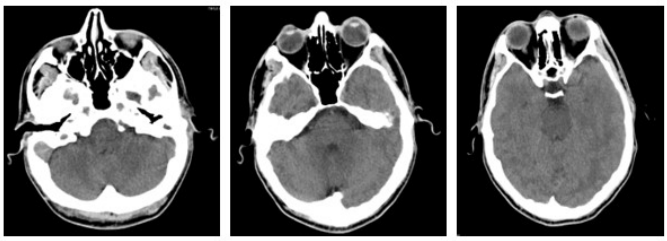

Fig. 1. Brain CT imaging 5 days prior to diagnosis of pneumocephalus and brain abscess

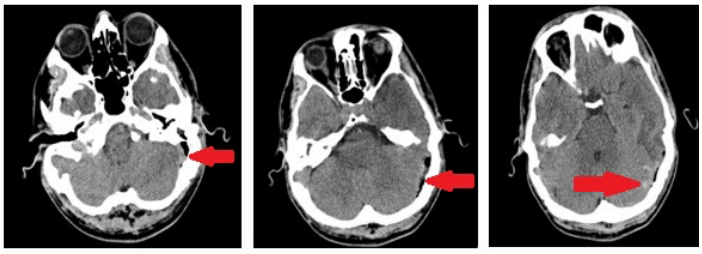

Fig. 2. Brain CT scan when pneumocephalus is detected

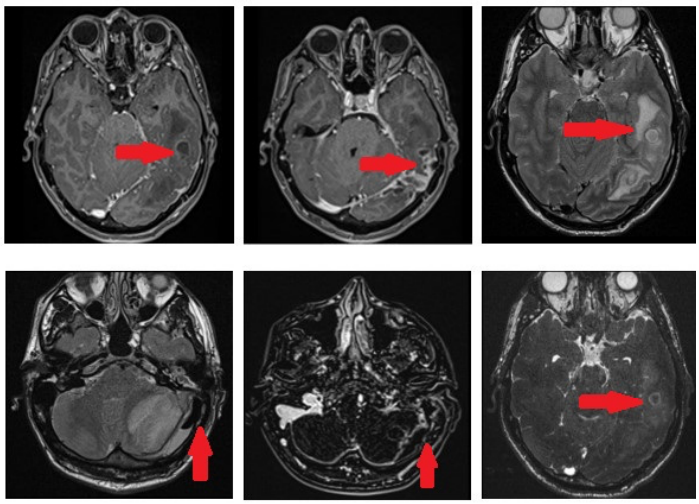

Fig. 3. Brain MRI image performed with pre-diagnosis of brain abscess

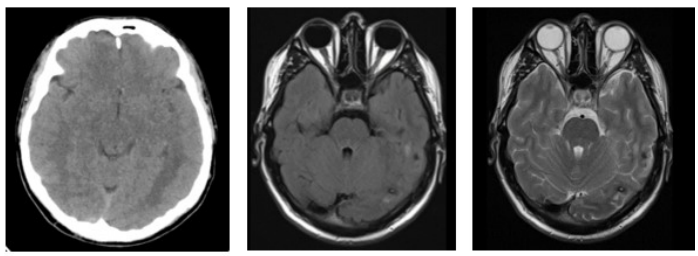

Fig. 4. Post-treatment brain CT and MRI images

After MRI scans revealed abnormalities suggestive of an abscess in a patient with no prior history of trauma, the patient was admitted to the Surgical Intensive Care Unit for further evaluation and treatment under neurosurgery care (Fig. 3). The patient, for whom a medical treatment decision had been made, was transported to the service the following day.

The patient, who had a history of antibiotic use, had a significantly reduced amount of purulent ear discharge, and no microorganism growth was detected in his repeated cultures. The Brucella test was negative as well.

The patient's abscesses were determined to shrank after a prolonged course of intensive and broad-spectrum antibiotic treatment (piperacillin/tazobactam, meropenem, and vancomycin). During this period, the patient developed left sinus vein thrombosis and was discharged with anticoagulant therapy 70 days after hospitalization (Fig. 4). The patient, who had been symptom-free for 15 to 20 days before discharge, had a normal neurological assessment, and complete well-being was achieved with no motor deficits.

3. Discussion

It is common knowledge that the incidence of AOM decreases with age, and it is recognized as one of the most significant diseases requiring antibiotic treatment. Because of this, the majority of AOM investigations in the scientific literature are conducted on children, focusing mostly on pediatric treatment and complication processes. Consistent with our case, it has been reported in the literature that AOM in adults is more prevalent in men and those in the 3rd decade age group (2, 6, 7).

Pneumocephalus is known for the presence of air in the cranial cavity. Some publications report that it mostly occurs due to traumatic events, surgical procedures that require opening of the skull, and very infrequently as a result of intracranial vascular and infectious events. Some publications in the literature report that pneumocephalus and SAH develop following meningitis, which is extremely uncommon. Similar to our case, the chief complaint is a headache, and meningitis secondary to middle ear infection is among the differential diagnoses (8, 9).

A brain abscess following AOM is one of the most dreaded complications of otitis media. Mortality rates rise to 10%. It has been determined that abscesses occur most often in the temporal lobe, less commonly in the cerebellum, and rarely in both regions. The malodorous purulent ear discharge and subsequent symptoms of an abscess, such as fever and malaise, followed by symptoms suggestive of an intracranial space-occupying formation such as headache, nausea, and vomiting, as well as meningitis symptoms such as nuchal rigidity and confusion of consciousness due to the spread of the abscess, are also compatible with the clinical process of our case (10, 11).

When diagnosing patients, symptoms such as high fever and headache should be carefully checked after symptoms such as ear pain, purulent ear discharge, hearing difficulties, and headache, which typically accompany or follow an upper respiratory infection. On physical examination, as in our patient, otoscope findings of purulent discharge are supportive. In addition, the asinus vein thrombosis seen in our case is one of the conditions of complicated otitis media (2, 12).

Brain abscess treatment includes surgical and medicinal approaches, aiming to remove the infection's primary focus.

Age, underlying etiology, and comorbid conditions are just a few factors that have an impact on the pathogens that cause this condition. Evacuation of abscesses of 2.5 cm or greater for surgical treatment is described in the literature, but evaluating the treatment approach by observing whether there is a reduction in the abscess size with antibiotic treatment is one of the strategies that can be followed. In our case, the abscess lesions were found to shrink with IV antibiotherapy treatment, and the patient was discharged with full recovery without the need for surgical intervention (12–15).

Diagnosing individuals who present to the emergency department with confused consciousness is a highly challenging and complex process. The patient's medical history and known conditions will be the most helpful in shedding light on this process. In order to establish the source of this confused state of consciousness in managing this critical situation, it is crucial to swiftly select the appropriate investigations and analyses from among a large number of options. When considering a conscious patient who has been diagnosed with pneumocephalus, the first possible cause that comes to mind is trauma. As seen in our case, it is a condition that should be kept in mind that this may be the result of complicated otitis media and that the patient's medical history needs to be meticulously investigated.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: F.C.T., M.S., Design: M.S., Data Collection or Processing: F.C.T., M.S., F.S.Ö., Analysis or Interpretation: F.C.T., M.S., F.S.Ö., Literature Search: F.C.T., M.S., Writing: F.C.T.

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