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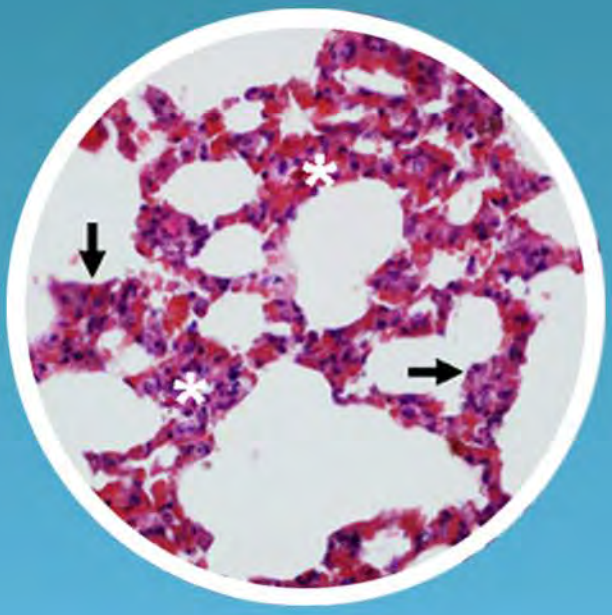

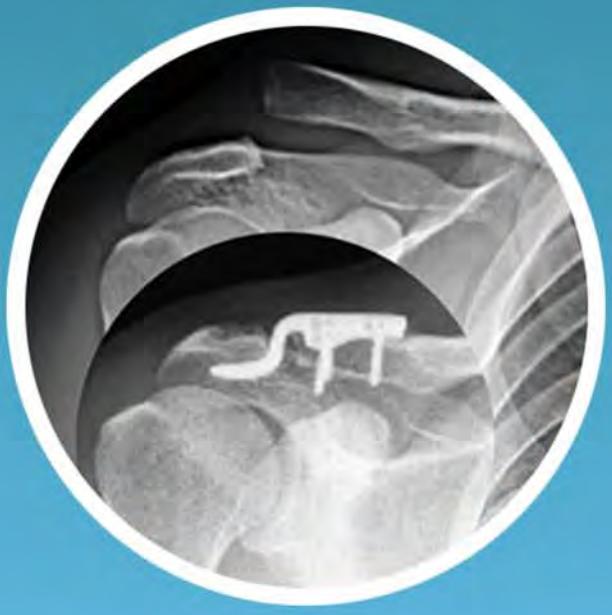

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The European Research Journal

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The European Research Journal 2023;9(5):831-839 Original Article

DOI: 10.18621/eurj.1340926 *Emergency Medicine*

Evaluation of the success of shock index and its derivatives in determining mortality in STEMI cases applied to emergency department

Güner Yurtsever[1], Adem Çakır[2], Ejder Saylav Bora[1]

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ABSTRACT

Objectives: Shock index (SI) and its derivatives play a crucial role in rapid prognosis and risk assessment, particularly in emergent scenarios like ST-segment elevation myocardial infarction (STEMI).

Methods: This study was conducted as single-centered and retrospective. A total of 467 cases who met the study criteria with a confirmed STEMI diagnosis were included. SI, modified SI (MSI), age SI (ASI), and age-modified SI (AMSI) scores of the cases were calculated and compared. In this study, $p < 0.05$ was accepted as the statistical significance level.

Results: Calculated scores were compared among cases meeting STEMI criteria. Mortal cases displayed significantly higher SI, MSI, ASI, and AMSI, as well as elevated heart rates and lowered SBP, DBP, and MAP values. ASI exhibited the highest predictive success for mortality (AUC: 0.802), followed by AMSI (AUC: 0.798). AMSI demonstrated superior significance in estimating major adverse cardiovascular events (MACE) ($p < 0.001$ for each parameter).

Conclusions: ASI proved most effective in gauging mortality risk, while AMSI excelled in predicting MACE risk among SI derivatives. These indices hold promise for guiding patient triage and emergency care in STEMI cases, owing to their simplicity and predictive capacity.

Keywords: Emergency department, mortality, shock index, modified shock index, STEMI

Shock index (SI) was defined as heart rate divided by systolic blood pressure to assess the hemodynamic stabilization of patients and was first described in 1967 [1]. Over time, to evaluate hemodynamic instability, shock index derivatives have been developed by modifying the shock index. Among these modifications, modified SI (MSI), which uses mean arterial pressure instead of systolic blood pressure, and age SI (ASI) are some of the modified indices in the literature [2].

It has been investigated whether it is a useful tool for early risk assessment of underlying diseases in patients, especially in the emergency department [3]. Those critical diseases include traumatic injuries, sepsis, pulmonary embolism, cardiovascular diseases, and ectopic pregnancy [4-7].

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Studies are showing that SI is a successful measure in estimating medium and long-term mortality in ST-segment elevation myocardial infarction (STEMI), which is one of the common cardiovascular emergencies with a high risk of mortality and morbidity, that requires urgent intervention [5, 8, 9]. However, there are few or no studies in the literature investigating whether MSI, ASI, and other SI indices are more successful in determining the risk of mortality in STEMI cases.

In our study, we aimed to investigate the success of SI, MSI, ASI, and age-modified SI (AMSI) in assessing mortality in patients who presented to the emergency department with STEMI.

METHODS

This study was conducted retrospectively between January 1, 2019, and January 1, 2021. A total of 467 STEMI patients admitted to the emergency department of our university hospital were included in the study.

Study Population

This study was carried out in a single center, in the emergency department of a tertiary education and research hospital, retrospectively. Our hospital is the central hospital of the region in terms of PCI and PCI is performed 7 days 24 hours. Patients aged 18 years and older with STEMI who applied to the emergency department between January 1, 2019, and January 1, 2021 were included in the study. Among those, pregnant patients (1), patients not diagnosed with ACS after PCI (23), patients diagnosed with ACS other than STEMI (18), patients presenting tachyarrhythmia (31), patients with primary kidney or blood disease (11), patients with advanced liver (4), kidney (7) or heart failure (13) were excluded from the study. (Fig. 1. Flow Chart). Patients with unknown or undefined medical histories were also excluded from the study. Patients with unstable vital signs at the time of admission were not included in the study either.

Data collection

Electrocardiography (ECG) measurements were taken at the time of application from patients who were diagnosed with STEMI and accepted to participate in the study. Patients with chest pain lasting longer than 30 minutes or equivalent symptoms, patients with ST-segment elevation in at least two adjacent ECG leads (at least 0.2 mV in V2 and V3 in men or at least 0.15 mV in women; at least 0.1 mV) in all leads except V2 and V3) or patients with new-onset left bundle branch block were diagnosed with STEMI regarding current guidelines [10, 11]. The STEMI status of each patient included in the study was evaluated by a cardiologist.

Percutaneous coronary intervention (PCI), the golden standard treatment, was performed in all patients with STEMI. The number of vascular lesions of the patients (lesions of two vessels and above were defined as multi-vessel) was recorded by the cardiologist after the procedure.

Demographic data (age, gender, cardiovascular risk factors, chronic disease history), measured vital signs (systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse rate) and ECG findings of all patients included in the study were recorded. In addition to these, fatal arrhythmia status requiring intervention (ventricular tachycardia (VT) and ventricular fibrillation (VF)), heart failure development according to Killip criteria, mortality. and cardiogenic shock states were named as major adverse cardiovascular event (MACE) and were recorded.

Data Definition and Calculation

Mean blood pressure (MAP) was calculated as [(2x DBP) + SBP]/3.

SI, MSI, ASI, and AMSI were calculated using the following formulas:

SI = Heart rate/ SBP; MSI = Heart rate/ MAP; ASI = SI × age and AMSI = MSI × age.

Statistical Analysis

Statistical analysis was performed using SPSS 23.0 for Windows® statistical program (IBM Inc. Chicago, IL, USA). Number, percentage, mean, standard deviation were used in the presentation of descriptive data. The conformity of the data to the normal distribution was evaluated with the Kolmogorov-Smirnov Test. Pearson chi-square test and Fisher's Exact test were used to compare categorical data. T Test was used to compare two independent nu-

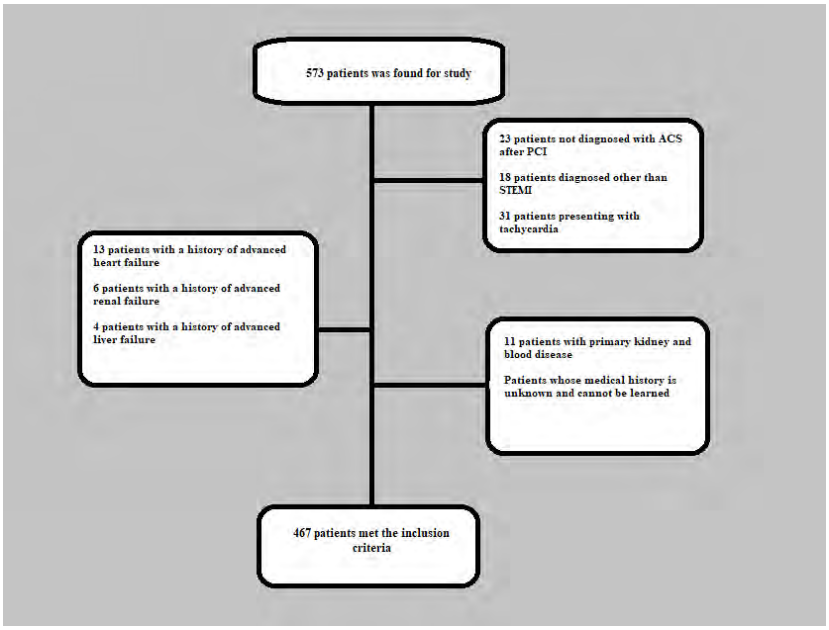

Fig. 1. Flow Chart

meric data, Kruskal Wallis Test and ANOVA test were used to compare triple numeric data. ROC curve analysis was performed to determine the cut-off values, AUC, sensitivity, and specificity of SI and its derivatives. Results were considered significant at $p < 0.05$, with a 95% confidence interval.

RESULTS

Our study was implemented with 467 patients who met the inclusion criteria. 26.3% (n = 123) of the cases were female, 73.7% (n = 344) were male, and the mean age was 61.11 ± 12.33 years in all cases. The mean age was 59.31 ± 11.90 years in men and 66.17 ± 12.17 years in women, which was significantly higher in women ($p < 0.001$).

Demographic and clinical data of the cases were evaluated according to their mortality status. The mean age was significantly higher in cases with mortality ($p < 0.001$). In cases with mortality, SBP, DBP, and MAP were significantly lower ($p < 0.001$ for all) whereas heart rate was significantly higher than the surviving cases ($p < 0.001$). Again, while the history of DM and CAD was significantly higher in cases with mortality, ($p = 0.044$, $p = 0.016$ respectively); HT and smoking were significantly lower ($p = 0.003$, $p = 0.006$; respectively). In cases with mortality, SI, MSI, ASI, and AMSI were significantly higher compared to surviving cases ($p < 0.001$ for all). It was observed that the inferior STEMI type was significantly higher in patients with mortality compared to those who survived. There was no mortality due to posterior and inferolateral STEMI. In cases with mortality, single-vessel occlusion was significantly higher; in addition, RCA occlusion in a single vessel was found to be significantly higher as well. ($p = 0.003$) (Table 1).

ROC analysis was performed to evaluate the success of the SI, MSI, ASI, and AMSI in predicting mortality of the cases (Fig. 2). According to the analysis, the most successful index in predicting mortality was ASI (AUC: 0.802 [95% CI: 0.749-0.855]), followed by AMSI (AUC: 0.798 [95% CI: 0.744-0.851]). AUC and cut-off values of other indices are given in Table 2.

Demographic and clinical data of the cases were analyzed according to the cut-off values obtained from the ROC analysis. It was calculated as 0.603 for SI (SI < 0.603; SI ≥ 0.603); 0.839 for MSI (MSI < 0.839; MSI ≥ 0.839); 34.88 for ASI (ASI < 34.88; ASI ≥ 34.88) and 60.18 for AMSI (AMSI < 60.18; AMSI ≥ 60.18) and the data were compared again according to the cut-off value. AMSI was found to be the most successful index to predict MACE ($p < 0.001$ for each parameter of MACE). The relations of the other demographic and clinical data of the cases according to the cut-off values of the indexes are given in Table 3.

Table 1. Evaluation of the demographic and clinical data of the cases according to their outcomes

Parameters	All cases (n = 467)	Exitus (n = 76)	Surviviors (n = 391)	*p value*
Age (years)	61.11 ± 12.33	66.51 ± 12.65	59.90 ± 12.48	**< 0.001**
Gender, n (%)				
Female	123 (26.3)	21 (17.1)	102 (82.9)	0.780
Male	344 (73.7)	55 (16.0)	289 (84.0)	
Hemodynamics				
SBP (mmHg)	131.67 ± 30.54	111.87 ± 28.05	135.76 ± 29.69	**< 0.001**
DBP (mmHg)	73.14 ± 15.86	63.49 ± 20.18	76.22 ± 16.55	**< 0.001**
MAP (mmHg)	95.92 ± 24.88	79.61 ± 21.88	96.03 ± 19.52	**< 0.001**
Pulse (rate/minutes)	73.98 ± 17.73	89.70 ± 26.20	80.56 ± 18.56	**< 0.001**
Cardiovascular risk factors, n (%)				
Hypertension	217 (46.5)	33 (43.5)	241 (61.8)	**0.003**
Diabetes Mellitus	128 (27.4)	28 (36.8)	100 (25.6)	**0.044**
Dyslipidemia	60 (12.8)	5 (6.6)	55 (14.1)	0.074
Coronary artery disease	159 (34.0)	35 (46.1)	124 (31.7)	**0.016**
Smoking	183 (39.2)	19 (25.0)	164 (41.9)	**0.006**
Indexes				
SI	0.64 ± 0.19	0.83 ± 0.28	0.61 ± 0.15	**< 0.001**
MSI	0.91 ± 0.28	1.18 ± 0.41	0.86 ± 0.22	**< 0.001**
ASI	39.81 ± 16.24	55.06 ± 21.77	36.70 ± 13.16	**< 0.001**
AMSI	56.36 ± 23.40	77.93 ± 30.64	51.88 ± 19.22	**< 0.001**
STEMI type, n (%)				
Anterior	159 (34.0)	26 (34.0)	141 (36.1)	**0.003**
Inferior	240 (51.4)	42 (65.8)	190 (48.6)	
Lateral	30 (6.4)	8 (10.5)	22 (5.6)	
Posterior	25 (5.4)	0 (0.0)	25 (6.4)	
Inferiolateral	13 (2.8)	0 (0.0)	13 (3.3)	
Vessel occlusion type, n (%)				
Single vessel occlusion	298 (63.8)	56 (73.7)	242 (61.9)	**0.003**
LAD	104 (22.3)	13 (17.1)	91 (23.3)	
RCA	159 (34.0)	40 (52.6)	119 (30.4)	
Cx	35 (7.5)	3 (3.9)	32 (8.2)	
Multiple vessel occlusion	169 (36.2)	20 (26.3)	149 (38.1)	

Data are shown mean ± standard deviation or n (%). SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, SI= shock Index, MSI = modified shock index, ASI = age-shock index, AMSI = age-modified SI, LAD = left anterior descending, RCA = right coronary artery, Cx = circumplex

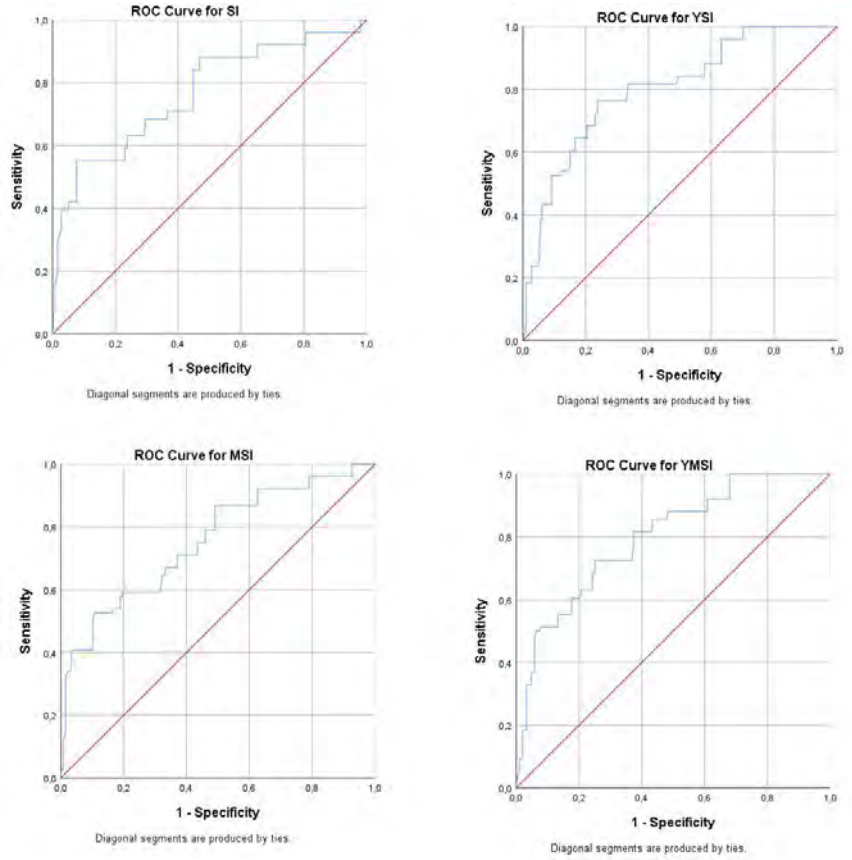

Fig. 2. STEMI ROC analysis results in evaluating the success of SI, MSI, ASI and AMSI in determining mortality.

DISCUSSION

According to the results of our study, for patients with STEMI diagnosis who underwent PCI in the emergency department, AMSI is found to be more accurate than SI, MSI, and ASI in estimating the risk of 30-day in-hospital MACE, whereas ASI was found to be more successful than SI, MSI, and AMSI in determining in-hospital mortality.

The SI was originally introduced to assess hemodynamic stability and then continued to be used as an early shock risk index in cases of trauma, bleeding, sepsis, and cardiogenic shock (pulmonary embolism, etc.) [12]. The use of SI in patients with acute coronary syndrome is not new either. Bilkova *et al*. [13], in 2011, measured the success of SI in the evaluation of in-hospital mortality, and short and long-term MACE in STEMI cases and reported that high SI was a suc-

Table 2. Evaluation of ROC analysis results in evaluating the success of SI, MSI, ASI and AMSI in determining mortality in cases

Parameters	Cut-off Value	Area Under the Curve (AUC)	Sensivity %	Specificity %	*p value*	%95 CI	
						Lower Bound	Upper Bound
SI	0.603	0.766	88	53	**< 0.001**	0.702	0.830
MSI	0.839	0.754	86	51	**< 0.001**	0.691	0.818
ASI	34.88	0.802	84	50	**< 0.001**	0.749	0.855
AMSI	60.18	0.798	72	75	**< 0.001**	0.74	0.851

SI = shock Index, MSI = modified shock index, ASI = age-shock index, AMSI = age-modified SI

Table 3. Comparison of demographic and clinical data of indices according to ROC analysis cut-off values

Parameters	SI			MSI			ASI			AMSI		
	< 0.6 (n = 211)	≥ 0.6 (n = 256)	*p value*	< 0.83 (n = 191)	≥ 0.83 (n = 276)	*p value*	< 34.88 (n = 207)	≥ 34.88 (n = 260)	*p value*	< 60.18 (n = 314)	≥ 60.18 (n = 153)	*p value*
Age (years)	59.82 ± 11.79	62.18 ± 12.69	**0.040**	60.98 ± 12.32	61.21 ± 12.36	0.845	54.32 ± 10.37	66.52 ± 11.05	**< 0.001**	56.34 ± 9.71	70.90 ± 1.35	**< 0.001**
Gender, n (%)												
Female	39 (18.5)	84 (32.8)	**< 0.001**	40 (20.9)	83 (30.1)	**0.028**	33 (15.9)	90 (34.6)	**< 0.001**	73 (23.2)	50 (32.7)	**0.030**
Male	172 (81.5)	172 (67.2)		151 (79.1)	193 (69.9)		174 (84.1)	170 (65.4)		241 (76.8)	103 (67.3)	
MACE (-), n (%)	179 (84.8)	185 (72.3)	**0.001**	164 (85.9)	200 (72.5)	**0.001**	182 (87.9)	182 (70.0)	**< 0.001**	271 (86.3)	93 (60.8)	**< 0.001**
MACE (+), n (%)	32 (15.2)	71 (27.7)		27 (14.1)	76 (27.5)		25 (12.1)	78 (30.0)		43 (13.7)	60 (39.2)	
MACE, n (%)												
VF/VT in ED	12 (5.7)	46 (18.0)	**< 0.001**	11 (5.8)	47 (17.0)	**< 0.001**	11 (5.3)	47 (18.1)	**< 0.001**	17 (5.4)	41 (26.8)	**< 0.001**
CHF-Killip	12 (5.7)	34 (13.3)	**0.006**	14 (7.3)	32 (11.6)	0.128	8 (3.9)	38 (14.6)	**< 0.001**	14 (4.5)	32 (20.9)	**< 0.001**
Cardiogenic shock	22 (10.4)	44 (17.2)	**0.037**	17 (8.9)	49 (17.8)	**0.007**	18 (8.7)	48 (18.5)	**0.003**	21 (6.7)	45 (29.4)	**< 0.001**
Arrest in ED	15 (7.1)	41 (16.0)	**0.003**	14 (7.3)	42 (15.2)	**0.010**	14 (6.8)	42 (16.2)	**0.002**	14 (4.5)	42 (27.5)	**< 0.001**
Mortality, n (%)												
Absent	202 (95.7)	189 (73.8)	**< 0.001**	181(94.8)	210 (76.1)	**< 0.001**	195 (94.2)	196 (75.4)	**< 0.001**	293 (93.3)	98 (64.1)	**<0.001**
Present	9 (4.3)	67 (26.2)		10 (5.2)	66 (23.9)		12 (5.8)	64 (24.6)		21 (6.7)	55 (35.9)	
Cardiovascular risk factors, n (%)												
Hypertension	91 (43.1)	140 (54.8)	0.189	79 (41.4)	138 (50.0)	0.066	56 (27.1)	160 (61.9)	**< 0.001**	114 (36.3)	103 (67.3)	**< 0.001**
Diabetes mellitus	61 (28.9)	67 (26.2)	0.509	62 (32.5)	66 (23.9)	**0.042**	43 (20.8)	85 (32.7)	**0.004**	78 (24.8)	50 (32.7)	0.075
Dyslipidemia	35 (16.6)	25 (9.8)	**0.028**	26 (13.6)	34 (12.3)	0.681	22 (10.6)	38 (14.6)	0.201	42 (13.4)	18 (11.8)	0.625
Coronary artery disease	73 (34.6)	86 (33.6)	0.820	57 (29.8)	102 (37.0)	0.111	51 (24.6)	108 (41.5)	**< 0.001**	102 (32.5)	57 (37.3)	0.307
Smoking	100 (47.4)	83 (32.4)	**0.001**	79 (41.4)	104 (37.7)	0.423	101 (48.8)	82 (31.5)	**< 0.001**	156 (49.7)	27 (17.69)	**< 0.001**
STEMI type, n (%)												
Anterior	72 (34.6)	86 (33.6)	0.651	54 (28.3)	105 (38.0)	**0.004**	66 (31.9)	93 (35.8)	0.106	99 (31.5)	60 (39.2)	0.396
Inferior	108 (51.2)	132 (51.6)		118 (61.8)	122 (44.2)		110 (53.1)	130 (50.0)		166 (52.9)	74 (48.4)	
Lateral	15 (7.1)	15 (5.9)		7 (3.7)	23 (8.3)		19 (9.2)	11 (4.2)		23 (7.3)	7 (4.6)	
Posterior	8 (3.8)	17 (6.6)		8 (4.2)	17 (6.2)		8 (3.9)	17 (6.5)		16 (5.1)	9 (5.9)	
Inferiolateral	7 (3.3)	6 (2.3)		4 (2.1)	9 (3.3)		4 (1.9)	9 (3.5)		10 (3.2)	3 (2.0)	
Vessel occlusion type, n (%)												
Single vessel occlusion	145 (68.7)	153 (59.8)	**0.020**	118 (61.8)	180 (65.2)	0.443	115 (55.7)	115 (44.3)	0.071	195 (62.1)	103 (67.3)	0.081
LAD	58 (27.5)	46 (18.0)		36 (18.8)	68 (24.6)		35 (16.9)	69 (26.5)		59 (18.8)	45 (29.4)	
RCA	67 (31.8)	92 (35.9)		69 (36.1)	90 (32.6)		71 (34.3)	88 (33.8)		111 (35.4)	48 (31.4)	
Cx	20 (9.5)	15 (5.9)		13 (6.8)	22 (8.0)		17 (8.2)	18 (6.9)		25 (8.0)	10 (6.5)	
Multiple vessel occlusion	66 (31.3)	103 (40.2)		73 (38.2)	96 (34.8)		92 (44.3)	145 (55.7)		119 (37.9)	50 (32.7)	

MACE= major adverse cardiovascular events, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAB= mean arterial pressure, SI= shock Index, MSI=modified shock index, ASI= age-shock index, AMSI= age-modified SI, LAD=left anterior descending, RCA=right coronary artery, Cx= circumplex

cessful measure for anticipation of the possible consequences. Later, Reinstadler *et al*. [5], Hemradj *et al*. [14], and Zhou *et al*. [15] reported in their studies that high SI in STEMI cases was significantly associated with determining short- and long-term MACE. Again, Abe *et al*. [8], Kobayashi *et al*. [16] and Yu *et al*. [6] stated that high SI showed significant results in detecting the risk for in-hospital mortality, and short and long-term MACE development in patients with acute coronary syndrome.

In the literature, in addition to SI, modified types of this index have also been used to predict mortality. Abreu *et al*. [9] used MSI in their STEMI study and reported that high MSI was an independent predictor for six-month mortality and fatal arrhythmia. Schmitz *et al*. [17] compared the predictive values of SI and MSI regarding long-term MACE development in both STEMI and non-STEMI cases and reported that MSI was found to be more valuable than SI. Chiang *et al*. [18] found that MSI revealed a better predictive value than SI for mortality acute myocardial infarction (AMI) cases. Shangguan *et al*. [19] reported that MSI was more accurate than SI in predicting all-cause 7-day mortality in 160 cases of STEMI who underwent emergency PCI. When the results of our study were compared with the results of the studies by Schmitz *et al*. [17], Chiang *et al*. [18], and Shangguan *et al*. [19], we observed that high SI had better predictive power than MSI on the mortality of STEMI cases. One possible explanation for this might be since the mean age and admission times of the patients included in the study were not standardized, SBP and MAP measurements differentiated.

Age is one of the independent risk factors in patients with acute coronary syndrome [20, 21]. Therefore, age is integrated into many risk scoring systems, and the effect of age is frequently investigated. For this reason, we expected that ASI and AMSI, which were obtained by integrating age into SI and MSI, would provide better results in predicting mortality and MACE development in STEMI cases. In our results, we found that while ASI was more accurate in predicting mortality; AMSI provided better risk estimation in determining MACE. Yu *et al*. [6] reported that ASI was superior to SI and MSI in estimating all-cause mortality in patients that underwent PCI. In the study of Zhou *et al*. [15]; AMSI was stated to be an independent predictor of MACE development in STEMI cases. Correlatively, we observed that ASI and AMSI, which were designed by the addition of age to SI and MSI, are more significant than SI and MSI in estimating mortality and MACE.

Limitations

Our study has several limitations. One of these limitations is that our study is retrospective. However, both the hospital automation system and patient files were examined in detail to avoid missing data on the patients included in the study, and patient data were tried to be collected completely. Another limitation is that the medical history of the patients was obtained according to the statements of the patients and their relatives. Although we think that there may be errors arising from those statements in this regard, we do not think that this situation will affect our study results.

CONCLUSION

In determining the risk of mortality and MACE development in STEMI cases, ASI demonstrated better predictive power on mortality; whereas AMSI was found to be the most successful index in determining the risk of MACE. It could be concluded that these indexes can be used both in determining the appropriate health center for the patient and in emergency departments due to their easy applicability and their ability to predict mortality and MACE in STEMI cases.

Authors' Contribution

Study Conception: GY, ESB, AÇ; Study Design: GY, ESB, AÇ; Supervision: GY, ESB, AÇ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: GY, ESB, AÇ; Statistical Analysis and/or Data Interpretation: GY, ESB, AÇ; Literature Review: GY, AÇ; Manuscript Preparation: GY, AÇ and Critical Review: ESB.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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Ethical Considerations

Ethics committee approval was obtained from the university hospital ethics committee (Ethics committee dated 21.04.2022 and decision number GOEK-198). Due to the retrospective nature of the study, voluntary consent from patients or their legal heirs to participate in the study was waived. The entire study was performed in accordance with the Declaration of Helsinki.

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The European Research Journal 2023;9(5):840-847 Original Article

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Epicardial adipose and pre-sternal subcutaneous tissues associated with extent of pneumonia and hospitalization in COVID-19

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ABSTRACT

Objectives: The aims of this study were, to analyze epicardial adipose tissue and pre-sternal adipose tissue thicknesses and the relationship of the ratio of these two parameters with radiological progression, age, gender, concomitant diseases, hospitalization, length of hospital stay, need for intensive care and survival status of COVID-19 patients.

Methods: In this retrospective study, a total number of 204 PCR-positive COVID-19 patients, who have initial lung computed tomography (CT) and a second CT within 15 days due to prolonged symptoms or suspected complications were included. According to patterns of lung involvement at the time of diagnosis, patients were divided into 4 groups. In initial CT scans, epicardial adipose tissue and pre-sternal subcutaneous adipose tissue thickness were measured. Progression or regression of the disease is evaluated by comparing the findings in initial and control CTs.

Results: The mean age, epicardial adipose tissue thickness (EAT), pre-sternal adipose tissue thickness (PAT), and the EAT/PAT ratio of patients with involvement in both lungs were found to be higher than those in patients with one lung or without lung involvement and there was a statistically significant positive correlation between them.

Conclusions: This study is thought to be the first in which epicardial adipose tissue and pre-sternal adipose tissue were evaluated together in COVID-19 patients. Epicardial adipose tissue is a metabolically active organ and measurement in initial CT scans may give an easy and quick idea of the evolution of the disease.

Keywords: COVID-19, epicardial adipose tissue, lung inflammation, disease evolution

COVID-19 has emerged as a multisystem complex infectious disease that predominantly affects the lungs and has become a global health problem since it was first identified in December 2019. It is of vital importance to learn about the effects of this pandemic, which continues to threaten human health worldwide. However, it is clear that defining and understanding the different pathogeneses and pathways that explain the severity of clinical findings can facilitate the creation of possible treatment alternatives and the management of patients' clinical findings [1].

Although most patients survive the disease without

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symptoms or with mild symptoms, the disease results in hospitalization with severe symptoms in approximately 20% of cases, with 58% of patients requiring intensive care. Acute respiratory complications may require hospitalization in the intensive care unit for an extended period, and this is one of the major causes of mortality and morbidity in patients [2].

Computed tomography of the thorax is a highly sensitive method in the diagnosis of COVID19 pneumonia and in defining the severity of lung involvement [3]. Epicardial adipose tissue is an active endocrine organ that modulates the metabolic environment of both the coronary arteries and myocardium [4]. Various studies have shown that EAT volume measured by CT is associated with lung function in both healthy individuals and patients with chronic lung disease [5, 6]. Furthermore, the relationship between CT-derived EAT measurement and circulating inflammatory markers, and cardiometabolic risk factors has also been demonstrated [7, 8]. There are assumptions that COVID-19 triggers an immune-mediated systemic inflammatory response and that this inflammation affects the heart via EAT [9, 10]. Besides, EAT volume can potentially accelerate ectopic intrapulmonary fat reserves and increase local lung infiltration [11, 12].

In the light of this information, the present study aims to analyze epicardial adipose tissue and pre-sternal adipose tissue thickness and the relationship of the ratio of these two parameters to each other with radiological progression, age, gender, concomitant diseases, length of hospital stay, need for intensive care unit and survival status of patients.

METHODS

This retrospectively designed study examined initial lung CTs of patients with PCR-positive COVID19 infection. Patients, who had a second CT within 15 days due to prolonged symptoms or suspected complications were included in the study. Patients younger than 18 years of age, with a diagnosis of malignancy, underwent thoracic surgery, and those presented with accompanying pleural effusion or trauma were excluded from the study.

The patients' age, gender, co-morbidities, length of hospitalization, need for intensive care unit, and survival status was researched. A total of 204 patients were included in the study. According to the lung involvement pattern in the initial CT, 4 groups were formed; patients with pneumonia in both lungs (Group 1), patients with pneumonia in one lung but more than one area (Group 2), patients with one focal lung infiltration (Group 3) and patients with normal lung findings (Group 4).

CT imaging of all patients was performed with Philips Ingenuity 128 multi-slice CT device. At the time of diagnosis, epicardial adipose tissue thickness was measured at the level in the front of the right atrium, where it was the widest and pre-sternal subcutaneous adipose tissue thickness was measured from the mid-xiphoid bone level. Progression or regression evaluation was performed by comparing the findings in the second CTs. The relationship between epicardial adipose tissue thickness, pre-sternal fat tissue thickness, and the ratio of these two to radiological progression, the duration of hospitalization, the need for intensive care and length of stay, and survival status were evaluated.

All procedures were performed by institutional ethics committee approval and the Declaration of Helsinki. Approval, numbered 2017-KAEK-120 /2/2021.K-21, was obtained from the Ethics Committee of İstinye University.

Statistical Analysis

The SPSS 25.0 (IBM Corporation, Armonk, New York, United States) program was used to analyze the variables. The conformity of the data to the normal distribution was evaluated with the Shapiro-Wilk Francia test, while the homogeneity of variance was evaluated with the Levene test. The Kruskal-Wallis H test was used according to the Monte Carlo simulation results to compare more than two groups with each other according to the quantitative data, while 's test was used for Post Hoc analysis. The Spearman's rho tests were used to examine the correlations of the variables with each other. In the comparison of categorical variables, the Pearson Chi-Square and Fisher-Freeman-Holton tests were tested with the Monte Carlo Simulation technique, and the column ratios were compared with each other and expressed according to the Benjamini-Hochberg corrected *p* - value results. While quantitative variables were expressed as mean (standard deviation), Median (Minimum / Maximum),

and Median (Percentile 25 / Percentile 75) in the tables, categorical variables were shown as n (%). The variables were analyzed at the 95% confidence level and were considered significant when the *p* - value was less than 0.05.

RESULTS

The mean age of 204 patients was 45.62 ± 16.28 years and 130 (65%) were male. There was no statistically significant difference between the groups in terms of gender ($p = 0.119$). The mean epicardial fat thickness was 18.89 ± 13.76 mm, and the mean pre-sternal fat tissue thickness was 11.75 ± 5.66 mm, and the EAT/PAT ratio was 1.82 ± 1.5 in the whole study group. All the patients were researched for concomitant diseases. The frequency of diabetes mellitus and bronchial asthma was found to be statistically significantly higher in patients with involvement of both lungs (Group 1), compared to other groups ($p = 0.023$ and $p = 0.032$, respectively). There was no significant difference between the groups in terms of the frequencies of hypertension, coronary artery disease, and chronic obstructive pulmonary disease ($p > 0.05$). The comorbidities, CT findings, and clinical features associated with COVID-19 are summarized in Table 1. The mean age, epicardial adipose tissue thickness, pre-sternal adipose tissue thickness, EAT/PAT ratio, length of stay in the department and intensive care unit, and the mortality rates of the patients in Group 1 (both lung involvement) were found to be statistically significantly higher than those in the other groups ($p < 0.05$) (Table 2). The mean age of the patients in Group 1 was significantly higher than that of the patients in the other groups ($p < 0.001$). The EAT thickness of the

Table 1. Distribution analysis of patients

	n	%
Gender (Male)	130	65.0
Control CT results		
Progressed	81	40.5%
Regressed	95	47.5
Stable	24	12.0
Ex patients	9	4.5
Hospitalized patients	86	43,0
Patients with intensive care hospitalization	15	7.7
Hypertension	39	20.3
Coronary artery disease	13	6.8
Diabetes mellitus	20	10.5
Chronic obstructive pulmonary disease	12	6.3
Chronic renal railure	3	1.6
Asthma bronchial	14	7.3
	Mean ± SD	**Median (min-max)**
Age (years)	45.62 ± 16.28	44 (17-87)
Epicardial adipose tissue measurement (mm)	18.89 ± 13.76	15 (1-75)
Pre-sternal adipose tissue measurement (mm)	11.75 ± 5.66	10 (3-36)
EAT/PAT	1.82 ± 1.50	1.33 (0.11-7.92)
Length of stay in the department (days)	5.05 ± 7.64	0 (0-52)
Length of stay in the ICU (days)	1.24 ± 5.98	0 (0-52)

SD = Standard Deviation

Table 2. Comparison of clinical features and demographic data of patients according to their lung involvements

	Group 1 (n = 85)	Group 2 (n = 71)	Group 3 (n = 25)	Group 4 (n = 19)	*p* value
Age, median (q1/q3)	51 (17/87) [bcd]	42 (19/86) [d]	39 (21/83) [d]	26 (21/51)	**< 0.001[k]**
Gender, n (%)					
Female	24 (28.2)	29 (40.8)	7 (28.0)	10 (52.6)	0.119 [c]
Male	61 (71.8)	42 (59.2)	18 (72.0)	9 (47.4)	
Epicardial adipose tissue measurement (mm), median (q1/q3)	28 (3.5/75)[bcd]	12 (3/32)	10 (2/30)	8 (1/22)	**< 0.001[k]**
Presternal adipose tissue measurement (mm)	12 (3.3/30)[bcd]	10 (3/36)	8 (4/22)	10 (7/25)	**< 0.001[k]**
EAT/Presternal ratio	2 (0.23/7.92)[bcd]	1.15 (0.23/7.62)[d]	1.17 (0.33/3)[d]	0.8 (0.11/3.14)	**< 0.001[k]**
Length of stay in the department (days)	8 (0/52) [bc]	1 (0/20)	2 (0/22)	5 (0 / 15)	**< 0.001[k]**
Length of stay in the ICU (days)	0.5 (0/52) [d]	0.5 (0 / 37)	0.5 (0 / 12)	0 (0 / 0)	**0.012[k]**
Mortality, n (%)					
Survived	78 (91.8)	71 (100.0) [ac]	23 (92.0)	19 (100.0)	**0.028[f]**
Ex	7 (8.2) [b]	0 (0.0)	2 (8.0) [b]	0 (0.0)	
Stay in the department, n (%)					
Absent	28 (32.9)	56 (78.9) [a]	20 (80.0) [a]	10 (52.6)	**< 0.001[c]**
Present	57 (67.1) [bc]	15 (21.1)	5 (20.0)	9 (47.4)	
Stay in the ICU, n (%)					
Absent	69 (85.2)	70 (98.6) [a]	23 (92.0)	19 (100.0)	**0.008[f]**
Present	12 (14.8) [b]	1 (1.4)	2 (8.0)	0 (0.0)	
Hypertension, n (%)					
Absent	64 (77.1)	50 (76.9)	21 (84.0)	18 (94.7)	0.319[f]
Present	19 (22.9)	15 (23.1)	4 (16.0)	1 (5.3)	
Coronary artery disease, n (%)					
Absent	76 (91.6)	61 (93.8)	23 (92.0)	19 (100.0)	0.695[f]
Present	7 (8.4)	4 (6.2)	2 (8.0)	0 (0.0)	
Diabetes mellitus, n (%)					
Absent	67 (81.7)	61 (95.3) [a]	23 (92.0)	19 (100.0) [a]	**0.023[f]**
Present	15 (18.3) [bd]	3 (4.7)	2 (8.0)	0 (0.0)	
Chronic obstructive pulmonary disease, n (%)					
Absent	74 (90.2)	61 (93.8)	25 (100.0)	19 (100.0)	0.284[f]
Present	8 (9.8)	4 (6.2)	0 (0.0)	0 (0.0)	
Chronic renal railure, n (%)					
Absent	82 (98.8)	64 (98.5)	24 (96.0)	19 (100.0)	0.657 [f]
Present	1 (1.2)	1 (1.5)	1 (4.0)	0 (0.0)	
Bronchial asthma (n/%)					
Absent	80 (96.4)	57 (87.7)	25 (100.0)	16 (84.2)	**0.032[f]**
Present	3 (3.6)	8 (12.3)	0 (0.0)	3 (15.8)	

[k]Kruskal-Wallis Test (Monte Carlo), Post Hoc Test = Dun's Test, [c]Pearson Chi-Square Test (Monte Carlo), [f]Fisher Freeman Halton Test (Monte Carlo), Post Hoc Test, Benjamini Hochberg Multiple Testing Correction, q1= 1st quartile, q3 = 3rd quartile $p < 0.05$ considered as significant.

patients in Group 1 was found to be significantly higher than in other groups ($p < 0.001$). Also, the pre-sternal fat thickness was found to be significantly higher in Group 1 patients compared to that in Group 2 and Group 3 patients ($p = 0.004$ and $p < 0.001$, respectively). There was no significant difference between Group 1 and Group 4 in this respect ($p > 0.05$). In the comparison of Group 2, 3 and Group 4 patients to each other in terms of epicardial adipose tissue thickness, EAT/PAT ratio, and pre-sternal adipose tissue thickness, no statistically significant difference was found ($p > 0.05$).

Table 3. Correlation findings of EAT, epicardial adipose tissue thickness, and EAT/PAT ratio of the groups

	Age		Length of stay in the department (days)		Length of stay in the ICU (days)		Follow-up period	
	r	*p*	r	*p*	r	*p*	r	*p*
Group 1								
Epicardial adipose tissue measurement (mm)	0.251	**0.021**	0.074	0.500	-0.048	0.673	0.071	0.531
Presternal adipose tissue measurement (mm)	0.045	0.684	0.216	**0.047**	-0.015	0.893	0.190	0.089
EAT/PAT	0.266	**0.014**	-0.085	0.441	-0.001	0.990	-0.054	0.631
Group 2								
Epicardial adipose tissue measurement (mm)	0.460	**< 0.001**	0.053	0.663	0.162	0.185	0.070	0.568
Presternal adipose tissue measurement (mm)	0.214	0.077	0.184	0.131	-0.027	0.823	0.178	0.144
EAT/PAT	0.200	0.099	-0.084	0.490	0.149	0.221	-0.067	0.584
Group 3								
Epicardial adipose tissue measurement (mm)	0.243	0.242	-0.227	0.276	-0.064	0.761	-0.259	0.212
Presternal adipose tissue measurement (mm)	0.180	0.390	-0.161	0.442	-0.016	0.938	-0.137	0.512
EAT/PAT	0.210	0.314	-0.214	0.303	0.025	0.907	-0.211	0.312
Group 4								
Epicardial adipose tissue measurement (mm)	0.128	0.603	0.045	0.854	-	-	0.045	0.854
Presternal adipose tissue measurement (mm)	0.386	0.102	0.123	0.615	-	-	0.123	0.615
EAT/PAT	-0.121	0.622	0.108	0.658	-	-	0.108	0.658
Total								
Epicardial adipose tissue measurement (mm)	0.518	**< 0.001**	0.250	**< 0.001**	0.117	0.103	0.270	**< 0.001**
Presternal adipose tissue measurement (mm)	0.219	**0.002**	0.230	**0.001**	0.024	0.743	0.219	**0.002**
EAT/PAT	0.407	**< 0.001**	0.105	0.142	0.146	0.043	0.138	0.055

Spearman's Rhotest, r: Correlation Coefficient p < 0.05consideredtobesignificant

While a significant positive correlation was found between epicardial adipose tissue thicknesses and age, length of hospital stays, and follow-up periods in all patient groups (r = 0.518, $p < 0.001$; r = 0.250, $p < 0.001$; and r = 0.270 $p < 0.001$, respectively), no correlation was found between the length of intensive care stays (r = 0.117, $p = 0.103$). Again, there was a significant positive correlation between pre-sternal adipose tissue thickness and age, length of hospital stays, and follow-up periods of the patients (r = 0.219, $p = 0.002$; r = 0.230, $p = 0.001$; and r = 0.219 $p = 0.002$, respectively), but no correlation was found between the pre-sternal adipose tissue thickness and the duration of intensive care unit (r = 0.024, $p = 0.743$). All groups showed a significant positive correlation between the EAT/PAT ratio and age (r = 0.407, $p < 0.001$). A significant positive correlation was found between epicardial fat thickness, EAT/PAT ratio, and age in patients with involvement in both lungs (r = 0.251 $p = 0.021$, r = 0.266 $p = 0.014$). There was a significant positive correlation between epicardial adipose tissue thickness and ward length of stay in patients with involvement in both lungs (r = 0.216, $p = 0.047$). There was also a significant positive correlation between epicardial fat measurement and age in patients with involvement in one-lung (r = 0.460. $p < 0.001$) (Table 3).

In the comparison made according to the progression of the control CT findings of the patients in Group 1 and Group 2, it was found that there was no difference between EAT thickness, and the EAT/PAT ratio of the patients with and without progression ($p > 0.05$). However, in the evaluation of all patients, EAT thickness and the EAT/PAT ratio of patients with progressive CT findings were found to be statistically significantly lower ($p < 0.001$ and $p < 0.001$, respectively).

DISCUSSION

The current study determined that there was a statistically significant positive correlation between epicardial adipose tissue thickness, pre-sternal adipose tissue thickness, EAT/PAT ratio, age, and the length of hospital stays of patients diagnosed with COVID-19. The mean age, EAT thickness, pre-sternal adipose tissue thickness, and the EAT/PAT ratio of patients with involvement in both lungs were found to be significantly higher than those in patients with involvement in one lung or without lung involvement.

A high BMI (> 28 kg/m2) and diabetes mellitus were defined as two different independent risk factors that were also associated with the severity of COVID-19 disease. According to the reports of the Center for Disease Control and Prevention of the People's Republic of China, cardiovascular diseases, chronic respiratory diseases, hypertension, diabetes mellitus and cancer increase the risk of death in patients diagnosed with COVID-19 [13]. These diseases mentioned in the published report occur as a result of chronic immune activation in the adipose tissue, liver, pancreas, and vascular system [14]. The contribution of obesity to the increase in inflammatory markers as a result of immune activation is very important. The relationship between hypertension, cardiovascular disease, diabetes mellitus, respiratory disease pathogenesis, and obesity also supports this statement [15, 16]. In this study, the frequency of diabetes mellitus and asthma bronchial was found to be statistically significantly higher in patients with involvement of both lungs compared to that of other groups.

In previous studies, abdominal visceral adipose tissue measured by CT is associated with critical illness in COVID-19 patients [17], but there is not sufficient data on thoracic fat stores. EAT measurements using CT are strongly associated with abdominal visceral adiposity and metabolic risk factors and coronary atherosclerosis [18, 19]. Considering the high spatial resolution of CT and the discrete attenuation values of adipose tissue, EAT volume and density can be measured easily and precisely [20]. Structural and functional properties of visceral and subcutaneous adipose tissue differ from each other. There are molecular, physiological, clinical, and prognostic differences between subcutaneous and visceral adipose tissue in addition to the anatomical region and cellular structure. The visceral adipose tissue found in the mesentery and omentum has more cellular, vascular, and neural innervation structure compared to the subcutaneous adipose tissue and contains more inflammatory and immune cells, less preadipocyte differentiation capacity, and a large number of large adipocytes [21]. It is thought that the imbalance between anti- and pro-inflammatory adipokine secretion from EAT may play a role in the formation of the cytokine storm in criti-

cally ill COVID-19 patients. The relationship between EAT thickness and lung involvement in patients diagnosed with COVID19 disease in the current study may be important.

Different results have been shared in a few publications in the literature evaluating the relationship between EAT thickness and COVID-19 disease findings. In the study by Grodecki *et al*. [21], EAT thickness and EAT attenuation were reported to be associated with clinical worsening and mortality. Deng *et al*. [22], reported that EAT attenuation was lower in patients with severe COVID-19 compared to patients with mild disease, and EAT volume was found to be significantly higher in patients with severe COVID-19 disease. However, in a retrospectively designed study by Iacobellis *et al*. [23], non-gated CT scans of mild and severe COVID-19 patients were evaluated, and EAT thickness was not significantly different among patients with different severities of COVID-19, but EAT attenuation increased significantly with the increase in the severity of COVID-19 [24]. In the current study, epicardial adipose tissue and pre-sternal fat thickness were found to be antly higher in patients with involvement in both lungs.

While the disease primarily affects the respiratory tract, it can lead to multi-organ failure and can be fatal. Acute respiratory complications may require hospitalization in the intensive care unit for a long time and constitute one of the major causes of mortality and morbidity in these patients [25].

Patients admitted to the intensive care unit are generally older and have multiple morbidities, including hypertension and diabetes. Factors associated with increased mortality are advanced age, comorbid diseases (hypertension, diabetes, cardiovascular diseases, chronic lung diseases, and cancer), high disease severity score, high D-dimer, high C-reactive protein levels, and high serum ferritin levels, lymphopenia, and secondary infections [26].

This study is thought to be the first study in which epicardial adipose tissue and pre-sternal adipose tissue were evaluated together in patients diagnosed with COVID-19. A significant positive correlation was found between epicardial fat thickness and EAT/PAT ratio and age in patients with involvement of both lungs. Again, a significant positive correlation was found between epicardial adipose tissue thickness and service length of stay in patients with involvement of both lungs.

Limitations

While the retrospective design of our study, the presence of a relatively low number of patients, and the absence of body mass index data can be counted as shortcomings of the study, the combined evaluation of EAT and subcutaneous adipose tissue in patients with a diagnosis of COVID-19 can be considered strengths.

CONCLUSION

In conclusion, our current knowledge indicates that inflammation plays an important role in the development and progression of COVID-19 disease. The epicardial adipose tissue which is a metabolically active organ is accepted as a new marker of inflammation, it can be easily analyzed on CT scans. Especially in patients with both lung involvement, we can say that morbidity and mortality of COVID-19 patients increase with increasing EAT/PAT ratio.

Authors' Contribution

Study Conception: FST, ŞYT; Study Design: FST, ŞYT; Supervision: FST, ŞYT; Funding: N/A; Materials: N/A; Data Collection and/or Processing: FST, ŞYT GK; Statistical Analysis and/or Data Interpretation: FST; Literature Review: FST, ŞYT; Manuscript Preparation: FST and Critical Review: FST, ŞYT.

Conflict of interest

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The European Research Journal 2023;9(5):848-854 Original Article

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Does chronic venous insufficiency affect cardiac functions? A speckle tracking echocardiography study

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ABSTRACT

Objectives: The aim of this study was to investigate whether there is subtle cardiac dysfunction in patients with chronic venous insufficiency.

Methods: Age and sex matched 56 patients with a score of C3 and above in the Clinical, Etiological, Anatomical, Pathophysiological classification and 56 healthy volunteers were included in the study. All subjects were evaluated by detailed echocardiographic examination, including two-dimensional strain echocardiographic analysis by speckle tracking method.

Results: Mitral E wave deceleration time (EDT), E and A wave velocity, E/e' ratio for left ventricle, tricuspid EDT, E/e' ratio for right ventricle and systolic pulmonary artery pressure were found high as significant statistically in patients groups ($p < 0.05$). But no any statistically significant difference was observed in other parameters between two groups.

Conclusions: There may be an increase in diastolic filling pressures in patients with chronic venous insufficiency due to the increased preload in the supine position. This condition seems to be clinically important in patients at high risk for heart failure due to the presumption of the early treatment of chronic venous insufficiency may reduce the risk of heart failure evolvement.

Keywords: Chronic venous insufficiency, echocardiography, strain, heart failure, speckle tracking

Chronic venous insufficiency (CVI) is a disease characterized by persistent venous hypertension that affects the venous system of the lower extremities, which can lead to pain, edema, skin changes and wounds [1]. The prevalence of patients with varicose veins has been reported up to 60 % in the adult population [2]. The peripheral venous system is a continuation of the arterial system and, thus, of the heart, acting as a reservoir to store blood and as a conduit for its return to the heart. Effective functioning of the peripheral venous system requires patency of the venous system with one-way functioning venous valves and muscle pump efficiency [3, 4]. Venous pathology develops when the return of blood to the heart is impaired due to impaired valvular function of the veins, increased venous pressure due to venous congestion and ineffective muscle pump function. This leads to venous hypertension, increases especially when stand-

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ing or moving [5]. In addition to venous valves, venous return is closely related to the pressure gradient between the heart and systemic capillaries and the right ventricle [6-8].

Several studies show differences in cardiac Doppler findings between patients with CVI and healthy volunteers, especially those with the Clinical, Etiological, Anatomical, Pathophysiological (CEAP) class 3 and above [9-11]. No study has examined subclinical left and right ventricular dysfunctions in patients with dysfunctional peripheral venous system. In this study, we aimed to investigate if any subclinical cardiac dysfunction in patients with CVI by assessing the ventricular function of the heart with two-dimensional (2D) myocardial strain analysis and other cardiac function parameters.

METHODS

The local ethics committee approved the study (University of Health Scinence, Bursa Yuksek Ihtisas Research and Training Hospital, Clinical Research Ethics Committee, REC number: 2011-KAEK-25 2022/04-02). Our study is cross-sectional in design. The CEAP classification system categorizes patients with CVI in ascending order according to the severity of their clinical condition, guides their diagnosis and treatment, and is used in research purposes. The CEAP classification divides patients with CVI into 7 classes (C0-C6) [12].

All patients who applied to cardiovascular surgery outpatient clinic between April 2022 and February 2023 were screened. Applied inclusion criteria were as follows: being over 18 years old and C3 and above according to CEAP classification. Exclusion criteria were as follows (1) Diseases that may have impact on cardiac function such as coronary artery disease, valvular heart disease, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, pericardial constriction; (2) The patient is taking any medication; (3) Anemia and thyroid dysfunction, (4) History of deep venous thrombosis and deep vein insufficiency; (5) Liver diseases; (6) Glomerular filtration rate < 60 ml/min; (7) Previously unknown structural cardiac disease detected during echocardiographic evaluation for the current study; and (8) No giving informed consent for the study. The control group was selected from healthy volunteers who did not have any disease and did not take any medication. 56 patients (min-max age: 23-68 years) and 56 healthy volunteers (min-max age: 25-68 years) with equivalent age, gender and Body Mass Index (BMI) who met the inclusion and exclusion criteria were included in the study. Written informed consent was obtained from all participants.

Ultrasonographic Evaluation

Duplex ultrasound evaluation of lower extremity veins was performed using a 7.5 MHz linear transducer (Toshiba, USDI-790A) while the subjects standing. Evaluation of the iliocaval system was performed in the supine position. The compression/release method was used for venous insufficiency assessment. Venous reflux time was measured after abrupt cessation of manual compression at the saphenofemoral junction and distally along the great saphenous vein tracing. If reflux lasting more than 0.5 seconds was detected, saphenofemoral vein junction/significant saphenous vein insufficiency was diagnosed [6].

Echocardiographic Evaluation

Two-dimensional transthoracic echocardiography was performed in all patients and the control group. Morning fasting blood samples were taken before the procedure. Echocardiography was performed by an experienced echocardiographer blinded to the patient's data. A Vivid E95 platform with a 3.5 MHz transducer was used for the procedure. (GE Vingmed Ultrasound AS, Horten, Norway). Echocardiographic parameters were evaluated according to the recommendations of the American Society of Echocardiography guidelines [13]. Standard 2D, colour, pulse and continuous Doppler data were measured and recorded while the patient was lying supine in the left decubitus position with the patient in end-expiration. Modified Simpson's method was used for volumetric chamber evaluation. Right ventricular end-diastolic and end-systolic area, right ventricular fractional area change (FAC) were measured. Tricuspid valve annular plane systolic excursion (TAPSE), tricuspid valve and mitral valve inflow velocities (E and A waves) and E wave deceleration time (EDT) were measured accordingly. Tricuspid and mitral annular tissue Doppler velocities (s, e' and a' waves) were obtained as recommended by the guidelines [13-15]. Using colour Doppler, systolic pulmonary artery pressure (sPAP) was obtained from

the tricuspid regurgitation jet through different windows using Bernoulli's equation and right atrial pressure was estimated by measuring vena cava inferior diameters and collapsibility index [16, 17]. Left ventricle (LV) and right ventricle (RV) myocardial performance index was calculated as the sum of isovolumetric contraction and relaxation time divided by left ventricular ejection time.

For right ventricular strain analysis (RVS), the Automated Function Imaging (AFI) technique with speckle tracking method was used (Automated Function Imaging, Version 112, GE Healthcare, Horten, Norway) [13]. RV-focused apical four-cavity image was preferred for analysis[18]. Electrocardiography gated loop recording was obtained from the acquired image. Loop recordings' frame rate was > 60-110 frames per second. In particular, "3-click" methods were used to reduce variability. With the 3-click method, a U-shaped region of interest (ROI) was created by automatically determining the endocardial borders by the software after placing a point at the tricuspid annulus lateral, medial and RV apex. ROI was manually corrected if necessary. Once the optimal ROI was obtained and confirmed, right ventricular free wall longitudinal strain (RVFWLS) and RV global longitudinal strain (GLS) were obtained [19, 20].

The same software was used for left ventricular strain analysis (LVS). Similarly, loop recordings were obtained from standard apical four-space, apical two-space and apical three-space windows. At the end-systole of each loop, points were manually placed on both edges of the mitral valve and the apex of the left ventricle using the 3-click method, and the software automatically drew the endocardial borders to create the ROI. Automated endocardial and epicardial borders of the ROI were manually corrected when necessary. Left ventricular global longitudinal strain value (LVGLS) was obtained from the optimal ROI registration of all three loops (Fig. 1a and b) [21].

Doppler and strain measurements were done 3 times consecutively while the subjects were in end-expiration, and the arithmetic mean of the measurements was recorded. Intraclass correlation coefficient test was performed in 10 randomized patients to assess intraobserver reliability in the measurement of each RVGLS and LVGLS. The intra-class correlation coefficient was 0.92 (0.81-0.98) for RVGLS, and was 0.88 (CI: 0.71-0.96) for LVGLS.

Statistical Analysis

All statistical analyses were performed using SPSS version-26. According to the Kolmogorov-Smirnov test, continuous variables showing normal distribution were expressed as mean ± standard variable (SD), continuous variables not showing normal distribution were expressed as median (25th - 75th per-

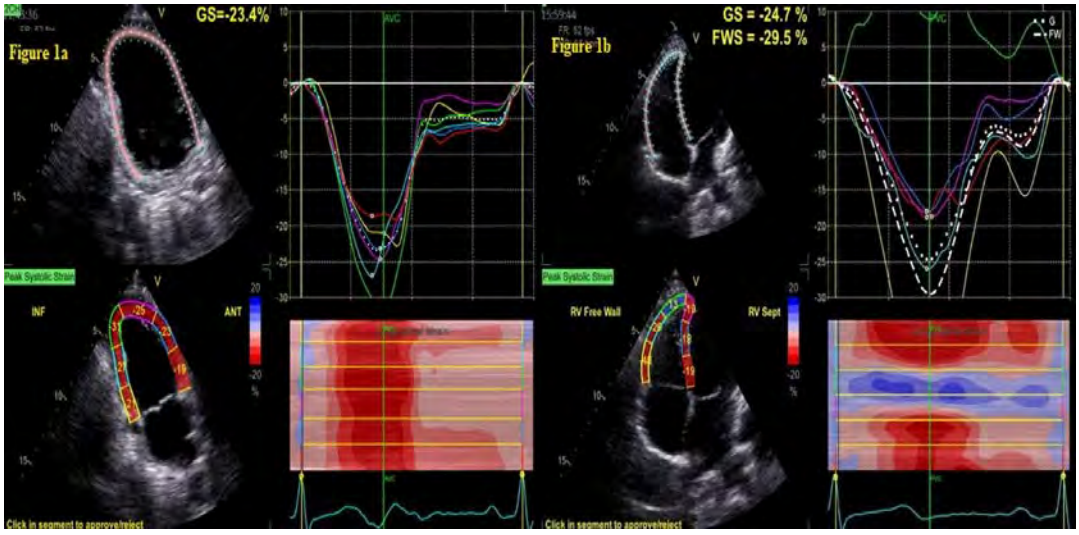

Fig. 1. (a) showing the region of interest of apical two chamber view and left ventricular global longitudinal strain, (b) showing the region of interest of right ventricle focused apical four chamber view and right ventricular free wall and global longitudinal strain

centile), and categorical variables were expressed as a percentage (%). Descriptive statistics of demographic, clinical characteristics and laboratory parameters of the patient and control groups were performed. The patient and control groups' demographic, clinical, laboratory and echocardiographic variables were compared using Independent Samples t-test (for normally distributed variables) or Mann-Whitney U Test (for non-normally distributed variables). A *p* - value less than 0.05 was considered statistically significant.

RESULTS

The mean age was 46.07 ± 12.29 years in the patient group and 46.39 ± 11.92 years in the control group ($p = 0.824$). The gender distribution of both groups was comparable (71.4% male). According to CEAP classification, 25 (44.6%), 24 (42.9%) and 7 (12.5%) patients were categorized in C3, C4 and C5 classes, respectively, while no patient was in C6. In the study group 6 (10.7%) patients had vena saphena parva failure in addition to vena saphena magna and also 2 (3.5 %) patients had perforating vein insufficiency. Demographic, clinical and laboratory parameters of the patients and control group are shown in Table 1.

When left ventricular echocardiographic variables of the patient and control groups were compared, mitral EDT, mitral E and A wave velocities and E/e' ratio were significantly higher in the patient group than in the control group ($p = 0.04$, $p = 0.004$, $p = 0.029$ and $p = 0.002$ respectively). In comparing right ventricular parameters, statistically significant increases in tricuspid EDT, tricuspid E/e' and sPAP values were found in the patient group. Tables 2 and 3 show LV and RV echocardiographic parameters of the patient and control groups.

DISCUSSION

In the present study, patient and control groups were compared in terms of many echocardiographic cardiac

Table 1. Demographic and laboratory findings of the patient and control groups

Parameters	Patients (n = 56)	Controls (n = 56)	*p* value
Age (years)	46.07 ± 12.29	46.39 ± 11.92	0.824
Male, n (%)	40 (71.4)	40 (71.4)	1
Current smoking, n (%)	26 (46.4)	22 (39.3)	0.636
BMI (kg/m^2)	29.65 (26.38-32.68)	28.98 (26.58-32.63)	0.92
GSV diameter (mm)	7.20 (6.35-7.57)	4.05 (3.8-4.30)	**< 0.001**
CrCl* (mL/min)	99.17 (91.93-112.419	106.78 (96.19-11.85)	0.16
Sodium (mEq/L)	139 (137.25-140)	138 (137.25-140.75)	0.63
Potassium (mmol/L)	4.4 (4.1-4.57)	4.4 (4.2-4.5)	0.45
ALT (IU/L)	19.50 (16.00-32.50)	21 (16-32)	0.99
AST (IU/L)	19.32 ± 4.44	18.91 ± 5.58	0.14
Total cholesterol (mg/dL)	204.29 ± 50.15	205.82 ± 39.69	0.07
Triglyceride (mg/dL)	136 (96-256)	155 (118.25-198)	0.99
Hemoglobin (g/dL)	14.95 (13.72-16.22)	14.55 (13.75-15.60)	0.13
White blood cell, (× 10^3/mL)	8.27 ± 1.84	7.74 ± 1.83	0.99
TSH (mIU/L)	1.48 (0.80-2.46)	1.32 (0.75-2.1)	0.43

Data are shown as median ± standard deviation or median (25th-75th) or n (%). ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, BMI = Body mass index, CAD = Coronary artery disease, CrCl = Creatinine Clearance, GSV = Great saphenous vein, TSH = Thyroid-stimulating hormone,

*Calculated according to the Cockcroft-Gault equation

function parameters, including 2D strain analysis. Except for some Doppler parameters, no significant differences were detected between the study groups. To the best of our knowledge, our study is the first to analyze subclinical right and left ventricular functions with 2D strain analysis in patients with CVI.

The present study found a statistically significant elevation in the left ventricular echocardiographic parameters including mitral EDT, E and A wave velocity and E/e' ratio in the patient group. No difference was found in left ventricular strain and other functional parameters between patient and control groups. Among RV echocardiographic parameters, tricuspid EDT, tricuspid E/e' ratio and sPAP were significantly higher in patients in whose sPAP's could be measured. No significant difference were found in RV FWLS, GLS and other functional parameters.

In a study by Rusinovich and Rusinovich [22], mitral and tricuspid E wave velocities, which indicate atrioventricular pressure gradient, were significantly lower, while A wave velocities representing atrial contraction were significantly higher in patients with CVI than the control group. Furthermore, mitral and tricuspid s' velocities representing ventricular systole were significantly increased [13, 22-24]. Mitral and tricuspid e' waves, that indicate myocardial relaxation, were comparable between the study groups [22, 24, 25]. Mitral septal and lateral and tricuspid E/e ratios were significantly lower in patient group compared to control group. These results differ from our study except for a similar increase in mitral A wave and no difference in mitral-tricuspid e' values. However, that study was a retrospective study in which patients from the CEAP 2 class were also included, and the control group was composed of subjects with normal Doppler findings from other studies. Differences in the study population, in particular the fact that all patients in our study were classified as C3 and above, may explain the difference in our results. Rusinovich and Rusinovich [22] explained that the increase in A velocity, a' velocity and s' velocity while no change of tricuspid E and e' velocity in severe venous insufficiency may be attributed to the more increased venous return in the supine position due to the high venous pressure in venous insufficiency.

In another study by Zhang *et al*. [9], a significant increase in tricuspid A-wave velocity, in mitral septal and lateral e' velocities and in septal a', s', lateral s' velocities were observed where as a significant decrease in tricuspid E wave velocity and E/A ratio were recorded in the patients with CVI compared to the control healthy group. No significant differences were found in mitral and tricuspid E/e ratios between the study groups [9]. The mean age of the patient group in this study was 55.2 ± 11.4 years, which is considerably higher than our study. In that study, 51 (40%) of the patients were in C2, 28 (22%) in C3, 50 (38%) in the C4 group and very few in CAEP 5 and 6, consisted mainly C4 patients. 57% of the patients were women. The results of th our study might have differed from the results of this study due to the fact that our study recruited a different study population including lower mean age, higher CEAP class and higher male ratio [11].

The high mitral and tricuspid E/e' ratios, indicators of ventricular filling pressures used to predict diastolic functions, suggest that ventricular filling pressures are high in patients with CVI. E/e' ratio can signify diastolic functions more accurately than other Doppler parameters and has prognostic importance [26, 27]. Mitral E velocity is primarily determined by the early transmitral pressure gradient and may also be elevated in the presence of high left ventricular filling pressures and increased preload [28, 29]. Again, in our study, mitral and tricuspid EDTs, which were not evaluated in previous studies, were found to be significantly higher in the patient group. EDT is a prognostically valid parameter of diastolic dysfunction that rises in stage 1 diastolic dysfunction, reflecting the time required to equalize the pressure difference between the atria and ventricles [30]. In addition, the fact that sPAP was found to be higher if it can be measured, in the patient group also suggests that there may be an increased preload in these patients [31]. These significant differences in diastolic Doppler parameters may suggest a relationship between CVI and diastolic dysfunction or an increased preload in such patients.

In our study, unlike the other studies [9, 22], all echocardiographic examinations were performed by a single echocardiographer between 2 groups whose age, gender, BMI and other clinical features were matched except for CVI. Detailed echocardiographic evaluation of the patients was performed by measuring not only Doppler parameters but also 2D strain analysis, dimensional, volumetric and various other functional parameters. The examination of each subject

took approximately 30 minutes after lying supine for 5 minutes. The decongestion and venous hypertension in the lower extremities during supine position may have caused an increase mitral E-A wave velocity, mitral and tricuspid EDT, E/e ratios and sPAP values due to increased venous return. Detailed preoperative and postoperative echocardiographic evaluation of patients with CVI with a study to be conducted in an adequate patient group may illuminate the underlying mechanism.

Limitations

The study was single-centred and conducted with limited number of subjects.

CONCLUSION

There may be an increase in diastolic filling pressures in patients with CVI due to the increased preload in the supine position. This condition seems to be clinically important in patients at high risk for heart failure due to the presumption of the early treatment of chronic venous insufficiency may reduce the risk of heart failure evolvement.

Authors' Contribution

Study Conception: FK, FL, AKA, ABT; Study Design: FK, FL, FKÖ; Supervision: MD, ET, FV; Funding: FK, FL, FKÖ; Materials: N/A; Data Collection and/or Processing: FK, FL, ABT, AKA, FKÖ; Statistical Analysis and/or Data Interpretation: FL, ET, MD; Literature Review: FK, FL, FV; Manuscript Preparation: ET, FL, AKA, ABT, FK and Critical Review: ET, MD, FV, FK, FL.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The effect of perceived parental attitude score on symptoms of bipolar disorder and schizophrenia

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ABSTRACT

Objectives: We aimed to investigate the effect of perceived parental attitudes on the symptoms of these diseases in patients with schizophrenia and bipolar disorder (BD) and to compare the perceived parental attitudes between these two disorders.

Methods: This cross-sectional study was conducted between August 2020 and August 2021 at a university hospital in Turkey. Fifty-two patients with BD and 50 patients with schizophrenia in remission, aged 16-50 years, who met the respective diagnostic criteria defined by the Diagnostic and Statistical Manual of Mental Disorder-5 were included in the study.

Results: The mean age of patients with BD was 38.90±10.95 years, while it was 39.08 ± 11.51 years for those with schizophrenia. Females comprised 65.38% (n = 34) of the BD group and 78.00% (n = 39) of the schizophrenia group. Our results showed that the severity of various negative schizophrenia symptoms increased with higher levels of perceived parental libertarian attitude. In addition, the severity of delusion, which is one of the positive symptoms of schizophrenia, was found to increase with lower perceived parental interest. We did not find a significant relationship between the severity of mania and depression symptoms and perceived parental attitudes.

Conclusions: In addition to supporting previously reported relationships of various factors and schizophrenia and BD, our results suggest that the increase in the level of liberality of parents has a negative impact on the negative symptoms of schizophrenia. In addition, the decrease in the level of interest of parents towards their children exacerbates delusion symptoms.

Keywords: Bipolar disorder, schizophrenia, parental attitudes, environment

The role of environmental factors in the etiology, course and symptoms of many psychiatric disorders has been and continues to be the subject of research. The most well-known of these diseases are bipolar disorder (BD) and schizophrenia [1, 2]. Schizophrenia and BD are serious psychiatric disorders that are believed to result from the complex interaction of genetic and environmental factors. While there is substantial evidence that these are disorders demonstrate genetic inheritance, the influence of environmental factors is less certain [3].

Family functions may play an important role in the course of symptoms of BD and schizophrenia [4, 5]. It has been shown that children who are maltreated, have oppressive parents, those with separated parents or who have lost a parent or parents,and adults with

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poor socioeconomic status, lower education, and live alone, are more likely to suffer from serious psychiatric disorders such as BD and schizophrenia [2, 6-8]. For instance, in one study, it was reported that family conflicts negatively affect the course of the disease. The same study also found that schizophrenia patients with severe positive symptoms perceived their family environment as being rigid and overly prescriptive [9]. Decreased familial problem-solving ability has been shown to predict the increased persistence of adolescent depressive symptoms independently of pharmacological intervention [4]. Some studies have shown that families of adults with BD and families including children at risk for BD are less organized, less cohesive, and experience more conflict than families of healthy individuals [10, 11]. It was also reported that higher maternal warmth, by both children with BD and their mothers, to be associated with lower rates of post-healing relapse in the 8-year follow-up of adolescents with BD [12]. Existing studies investigating the interaction of these two diseases and the environment have mostly focused on the effect of the environment on the onset of these diseases [3, 13]. In particular, there are very few studies examining the effects of parental attitudes and other environmental factors on the course and symptoms of these diseases.

This study has two aims. The first is to investigate the effect of perceived parental attitudes on the symptoms of these diseases in patients with schizophrenia and bipolar disorder (BD) and the second is to compare the perceived parental attitudes between these two disorders.

METHODS

Design

This comparative cross-sectional study was conducted between August 2020 and August 2021 at Department of Psychiatry Clinic, Hitit University Medical School, Corum Community Mental Health Center, Corum, Turkey.The study received ethical approval from the the Clinical Ethics Committee of Hitit University Faculty of Medicine (Date:14.07.2020, No: 315).

Study Group

Fifty-two BD patients and 50 schizophrenia patients in remission, aged 16-50 years, who met the diagnostic criteria for schizophrenia (for schizophrenia patients) and for BD I or II or another specified bipolar and related disorder (for BD patients), as defined by the Diagnostic and Statistical Manual of Mental Disorder-5. All individuals were given detailed information about the purpose of the study and independently volunteered to participate in the study by signing written informed consent forms. Patients with chronic disease, autoimmune or endocrine disease requiring the use of anti-inflammatory drugs, subjects with other psychiatric or mental diseases, pregnant patients, and non-cooperative patients (for whom the scales could not be applied) were excluded from the study.

Information Form for Data Collection

The information form included questions about the socio-demographic variables of patients, such as age, sex, marital status, cohabiting status (with whom they live), education status, working status, economic status, smoking status, and information about their disease (family history of BD or schizophrenia, age at disease onset, age at first treatment, number of hospitalizations, suicide attempts). The form was prepared by the researchers and the questions were asked to the patients during face-to-face interviews.

Participants in the BD group were assessed using the Young Mania Rating Scale (YMRS) for mania symptoms and the Hamilton Depression Scale (HAMD) for depression symptoms. The severity of symptoms of patients in the schizophrenia group was evaluated by Scale for the Assessment of Positive Symptoms (SAPS) and Scale for the Assessment of Negative Symptoms (SANS) [1]. In addition, the Parental Attitude Scale (PAS) was applied to the patients in both groups to determine their perception of parental behavior towards them [3].

Parental Attitude Scale (PAS)

The PAS was developed by Lamborn *et al*. [14] to evaluate individuals' perceptions of parental attitudes.Three primary factors emerge as a result of the factor analysis applied to the scale scores. These factors are: (1) The acceptance/care factormeasures children's perceptionsregarding their parents' care-giving, love, and participatory attitudes, and is measured with 9 items; (2) The strictness/supervision factor measures children's perceptions regarding their parents' controlling attitude, and is measured with 8 items; and (3) The psychological autonomy factormeasures chil-

dren's perceptionsof their parents democratic attitude and encouragement of individuality, and is measured with 9 items.

All items in each factor receive responses based on use of Likert-type scales. Factors 1 and 2 are evaluated together, often referred to as "acceptance/involvement". Higher scores in both factor 1 and 2 indicate "democratic" attitude, while parents with lower scores in both are defined to have "permissive-neglectful" attitude. Parents with low factor 1 score but high factor 2 score are determined to have "authoritarian" attitude. Parents with high factor 1 score but low factor 2 score are determined to have "permissive-tolerant" attitude. The Turkish validity and reliability of the scale were performed by Yılmaz *et al*. [15].

Scale for the Assessment of Positive Symptoms (SAPS)

The SAPS was developed by Andreasen [16] and is filled on the basis of the information obtained during the interview with the patient and their relatives. Observations during the interview are used to measure the level, distribution and severity of positive symptoms of patients with schizophrenia. The scale measures findings in 4 sub-scales: 7 items under "hallucination", 13 items under "delusion", 5 items under "bizarre behavior", and 7 items under "positive formal thought disorder". The severity of symptoms is evaluated on 6-point Likert-type scales. Total scores are also calculated. As the score obtained from the scale and subscales increases, the severity of the patient's positive symptoms also increases. The Turkish validity and reliability study of the SAPS was carried out by Erkoç *et al*. [17].

Scale for the Assessment of Negative Symptoms (SANS)

The SANS was developed by Andreasen [16] and is filled on the basis of the information obtained during the interview with the patient and their relatives. The observations during the interview are used to measure the level, distribution and severity of negative symptoms in patients with schizophrenia. The SANS used in this study had 5 subscales: 8 items under "affective blunting", 5 items under "alogia", 4 items under "avolition/apathy", 5 items under "anhedonia/asociality", and 3 items under "attention". The severity of symptomsare evaluated on 6-point Likert-type scales, and total scores were also calculated. Higher scores indicate greater severity of negative symptoms. The Turkish validity and reliability study of the scale was carried out by Erkoç *et al*. [18].

Young Mania Rating Scale (YMRS)

TheYMRS is a rating scale used to evaluate manic symptoms at baseline and over time in patients with mania. The scale is usually administered by a clinicianand takes around half an hour to complete. The scale consists of 11 items and is based on the patient's subjective reportsconcerning the previous 48 hours. Additional information is based on clinical observations made during the clinical interview. Four items are rated between 0 and 8 (two-point intervals) (irritability, speech, thought content, and disruptive/aggressive behavior), the remaining seven items are graded between 0 and 4. The strengths of YMRS include its brevity, widely accepted use, and ease of implementation [19, 20].

Hamilton Depression Rating Scale (HAM-D)

This scale was developed by Max Hamilton [21] in 1960 to determine the depression risk among individuals. The scale evaluates the symptoms of depression for the last 7 days. The HAM-D is designed to rate the severity of depression in patients. Although it contains 21 areas, patient scores are calculatedbased on the first 17 answers according to different Likert-type scales. Higher scores indicate greater risk for depression. The Turkish validity and reliability study of the scale was performed by Aydemir *et al*. [22].

Statistical Analysis

The data obtained during the study were assessed via the SPSS v21 software (SPSS Inc., Chicago, IL, USA) with a pre-determined significance threshold of $p < 0.05$. Quantitative variable distributions were checked with the Kolmogorov-Smirnov test. Quantitative data descriptions were given with mean ± standard deviation or median (minimum-maximum) values according to Kolmogorov-Smirnov test results. Categorical descriptive data were given with frequency (number and percentage). Quantitative variables with normal distribution underwent comparisons with the Student's t-test, while those with non-Gaussian distribution underwent Mann-Whitney U test comparisons. Between-group categorical distribution analyses were conducted Pearson chi-square or

Table 1. Patient characteristics and scale scores with regard to diagnosis

	Bipolar AD (n = 52)	Schizophrenia (n = 50)	*p* value
Age (years)	38.90 ± 10.95	39.08 ± 11.51	0.937
Sex			0.233
Female	18 (34.62%)	11 (22.00%)	
Male	34 (65.38%)	39 (78.00%)	
Marital status			**0.018**
Married	27 (51.92%)	12 (24.49%)	
Single	17 (32.69%)	30 (61.22%)	
Widow	4 (7.69%)	2 (4.08%)	
Divorced	4 (7.69%)	5 (10.20%)	
Live with			**0.014**
Alone	3 (5.77%)	10 (20.00%)	
Parents/siblings	22 (42.31%)	28 (56.00%)	
Partner/children	26 (50.00%)	12 (24.00%)	
Parents/partner/children	1 (1.92%)	0 (0.00%)	
Education status			**0.006**
Literate	0 (0.00%)	0 (0.00%)	
Primary school	12 (23.08%)	6 (12.00%)	
Secondary school	12 (23.08%)	11 (22.00%)	
High school	13 (25.00%)	28 (56.00%)	
University	15 (28.85%)	5 (10.00%)	
Working status			0.520
Regularly	18 (34.62%)	13 (26.00%)	
Irregularly	7 (13.46%)	3 (6.00%)	
Doesn't work	22 (42.31%)	28 (56.00%)	
Retired due to disability	3 (5.77%)	4 (8.00%)	
Retired	2 (3.85%)	2 (4.00%)	
Economic condition			0.065
Self-sufficient	46 (88.46%)	36 (72.00%)	
Non-self-sufficient	6 (11.54%)	14 (28.00%)	
Bipolar AD in family	18 (34.62%)	5 (10.00%)	**0.006**
Schizophrenia in family	7 (13.46%)	14 (28.00%)	0.116
Age at onset	23 (14 - 51)	21.5 (14 - 44)	0.720
Age at first treatment	23 (14 - 51)	23 (14 - 52)	0.830
Number of hospitalizations	2 (0 - 14)	2 (0 - 21)	0.232
Suicide attempt	9 (17.31%)	9 (18.00%)	1.000
Smoking			0.167
No	21 (40.38%)	20 (40.00%)	
0-10 cigarettes daily	3 (5.77%)	0 (0.00%)	
1 package daily	20 (38.46%)	16 (32.00%)	
1-2 package daily	8 (15.38%)	14 (28.00%)	
Parental Attitude Scale			
Acceptance/involvement	25 (14 - 32)	24 (11 - 31)	0.286
Psychological autonomy	18.44 ± 4.96	18.26 ± 4.57	0.847

Data are given as mean ± standard deviation or median (minimum - maximum) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables

Fisher's exact tests. Spearman correlation coefficients were calculated to evaluate relationships between scale scores. Multiple linear regression analysis were performed to determine related factors with the disease severity.

RESULTS

The mean age of patients with BD (n = 52 was 38.90 ± 10.95 years, while it was 39.08 ± 11.51 years in patients with schizophrenia (n = 50). There was no significant difference between the two groups in terms of age (p = 0.937). Overall, 65.38% (n = 34) of the BD group and 78% (n = 39) of the schizophrenia group were female, and groups were similar in terms of sex (p = 0.233). The percentage of married individuals was significantly higher among BD patients compared to patients with schizophrenia (p = 0.018). Living alone was significantly more common in patients with schiz-

Table 2. Correlations between Parenteral Attitude Scale and other scale scores

		Acceptance/ Involvement	Psychological autonomy
Young Mania Rating Scale (n = 52)	r	0.188	-0.086
	p value	0.182	0.542
Hamilton Depression Rating Scale (n = 52)	r	-0.178	0.055
	p value	0.206	0.701
SAPS Hallucinations (n=50)	r	-0.159	0.151
	p value	0.270	0.296
SAPS Delusions (n=50)	r	**-0.340**	0.011
	p value	**0.016**	0.938
SAPS Bizarre behavior (n = 50)	r	-0.030	-0.113
	p value	0.834	0.436
SAPS Positive formal thought disorder (n = 50)	r	-0.050	-0.171
	p value	0.731	0.234
SAPS Inappropriate (n = 50)	r	0.008	0.108
	p value	0.954	0.454
SAPS Total (n = 50)	r	-0.271	-0.023
	p value	0.057	0.875
SANS Affective blunting (n = 50)	r	0.178	0.208
	p value	0.216	0.147
SANS Alogia (n = 50)	r	0.254	0.164
	p value	0.076	0.254
SANS Avolition/Apathy (n = 50)	r	0.055	0.349
	p value	0.704	**0.013**
SANS Anhedonia/Asociality (n = 50)	r	0.130	0.302
	p value	0.369	**0.033**
SANS Attention (n = 50)	r	-0.029	0.48**6**
	p value	0.842	**< 0.001**
SANS Total (n = 50)	r	0.149	0.289
	p value	0.301	**0.042**

r = Spearman correlation coefficient

ophrenia, while BD patients more commonly lived with their partners and children (p = 0.014). The majority of schizophrenia patients were high school graduates, while BD patients had a significantly higher rate of university graduation (p = 0.006). In the BD group, family history for BD was present in 34.62% (n = 18); whereas only 10% (n = 5) of patients with schizophrenia reported a family history of the same disease. No difference was observed between the two groups in terms of other parameters, including working status (p = 0.520), economic status (p = 0.065), age at onset (p = 0.720), age at first treatment (p = 0.830), number of hospitalizations (p = 0.232), suicide attempt (p = 1.000), and smoking status (p = 0.167). There was no significant difference between the two groups in terms of the acceptance/involvement and psychological autonomy dimensions of PAS (p = 0.286 and p = 0.847, respectively) (Table 1).

Analysis of correlations revealed that there was a significant negative correlation between delusion severity and acceptance/involvement score (r = -0.340, p = 0.016) (Table 2, Fig. 1). Psychological autonomy subscale score was positively correlated with the avolition/apathy (r = 0.349, p = 0.013) (Fig. 2), anhedonia/asociality (r = 0.302, p = 0.033) (Fig. 3), attention (r = 0.486, p < 0.001) (Fig. 4) subscales of SANS, and also, total SANS score (r = 0.289, p = 0.042) (Fig. 5). There were no other significant correlations between other parameters and scores (Table 2).

Our multiple linear regression analysis results were as follows; We found higher PAS acceptance/involvement score was related with lower SAPS delusions score (p = 0.043) (Table 3). We found

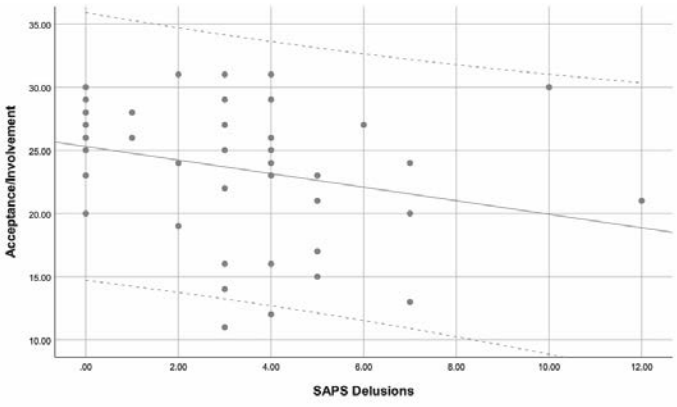

Fig. 2. Scatter plot of the PAS psychological autonomy and SANS avolition/apathy scores.

economically non-self-sufficient patients had higher SAPS inappropriate scores (p = 0.003) (Table 4), SANS affective blunting scores (p < 0.001) (Table 5), SANS alogia score (p = 0.037) (Table 6), SANS avolition/apathy score (p = 0.016) (Table 7), SANS attention score (p < 0.001) (Table 9), SANS total score (p < 0.001) (Table 10). We found male patients had higher SAPS inappropriate scores (p = 0.022) (Table 4) and lower SANS alogia score (p = 0.018) (Table 6). We found higher age was related with lower SANS alogia score (p = 0.002) (Table 6), SANS avolition/apathy score (p < 0.001) (Table 7), SANS total score (p = 0.001) (Table 10). We found higher PAS psychological autonomy score (p = 0.007) was related with higher SANS avolition/apathy score (Table 7). We found patients with schizophrenia history in family (p = 0.003) had higher SANS anhedonia/asociality score (Table 8) and SANS total scores (p = 0.006) (Table

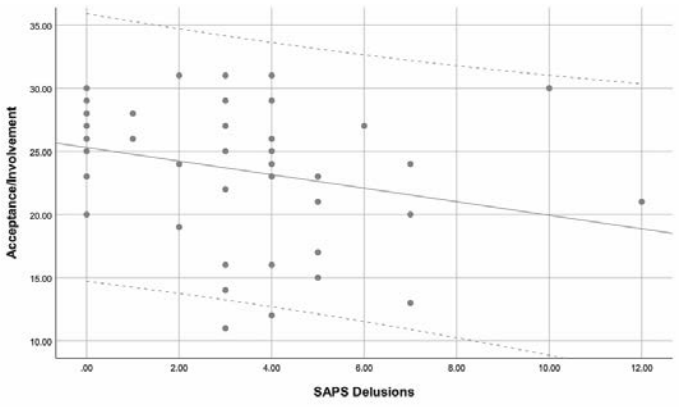

Fig. 1. Scatter plot of the PAS acceptance/involvement and SAPS delusions scores

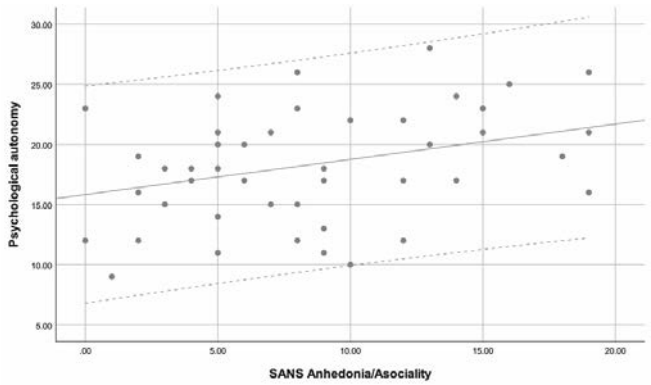

Fig. 3. Scatter plot of the PAS psychological autonomy and SANS anhedonia/asociality scores.

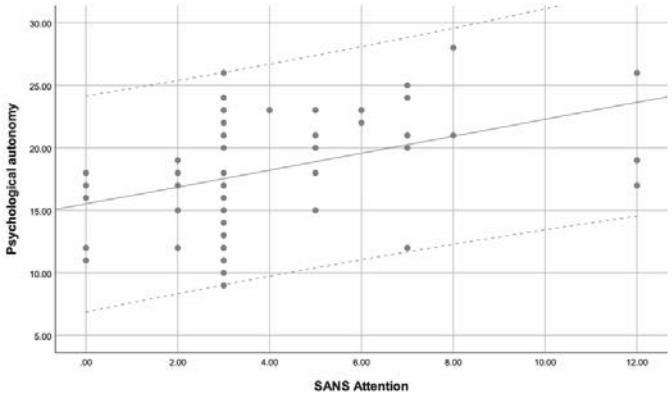

Fig. 4. Scatter plot of the PAS psychological autonomy and SANS attention scores.

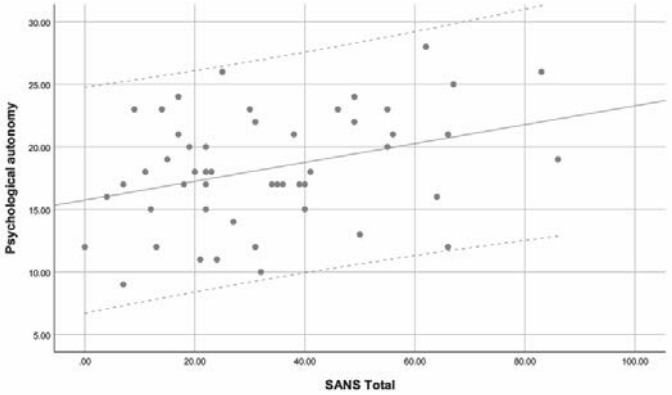

Fig. 5. Scatter plot of the PAS psychological autonomy and SANS total scores.

10). Also, lower age at onset (p = 0.007) was related with higher SANS anhedonia/asociality score (Table 8). Higher PAS psychological autonomy score (p = 0.008) was related with higher SANS attention score (Table 9). Smoking patients had lower SANS total scores than non-smokers (p = 0.039) (Table 10). In the regression analysis, no other significant relationship was detected.

DISCUSSION

Schizophrenia and BD are among the most severe mental disorders that affect patients, their families, and society [23]. While these diseases affect the environment, many environmental factors, especially familial factors, can affect the clinic of these diseases [4]. In this study we aimed to investigate the effects of family attitudes perceived by patients on the symptomatology of BD and schizophrenia and to show the differences in perceived family attitudes between these two diseases. Our results showed that the severity of many of the negative symptoms of schizophrenia including avolition/apathy, anhedonia/asociality, attention and SANS total score increased as the perceived parental libertarian attitude increased. Negative correlation between PAS acceptance/involvement score and SAPS

Table 3. Significant related factors of the SAPS Delusions score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	6.780	1.754		**< 0.001**	3.251	10.309
PAS Acceptance/ Involvement score	-0.152	0.073	-0.290	**0.043**	-0.299	-0.005

Dependent variable: SAPS Delusions score; Adjusted R^2=0.065; F=4.310; p = 0.043. CI = Confidence interval

Table 4. Significant related factors of the SAPS Inappropriate score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	-1.283	0.460		**0.008**	-2.209	-0.358
Gender, Male	0.442	0.187	0.324	**0.022**	0.065	0.818
Economic condition, Non-self-sufficient	0.545	0.173	0.432	**0.003**	0.197	0.892

Dependent variable: SAPS Inappropriate score; Adjusted R^2 = 0.170; F = 6.012; p = 0.005. CI = Confidence interval

Table 5. Significant related factors of the SANS Affective blunting score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	-1.016	2.712		0.710	-6.469	4.437
Economic condition, Non-self-sufficient	8.044	1.999	0.502	**< 0.001**	4.024	12.063

Dependent variable: SANS Affective blunting score; Adjusted $R^2 = 0.237$; F = 16.187; $p < 0.001$. CI = Confidence interval

Table 6. Significant related factors of the SANS Alogia score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	11.285	3.329		**0.001**	4.584	17.986
Age	-0.129	0.040	-0.411	**0.002**	-0.209	-0.048
Gender, Male	-2.792	1.142	-0.324	**0.018**	-5.090	-0.494
Economic condition, Non-self-sufficient	2.212	1.031	0.279	**0.037**	0.136	4.288

Dependent variable: SANS Alogia score; Adjusted $R^2 = 0.260$; F = 6.734; $p = 0.001$. CI = Confidence interval

Table 7. Significant related factors of the SANS Avolition/Apathy score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	4.543	2.910		0.125	-1.314	10.401
Age	-0.181	0.048	-0.446	**< 0.001**	-0.276	-0.085
Economic condition, Non-self-sufficient	3.078	1.234	0.299	**0.016**	0.593	5.562
PAS Psychological autonomy score	0.343	0.121	0.336	**0.007**	0.099	0.587

Dependent variable: SANS Avolition/Apathy score; Adjusted R^2=0.341; F=9.463; $p < 0.001$. CI = Confidence interval

Table 8. Significant related factors of the SANS Anhedonia/Asociality score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	13.428	2.367		< 0.001	8.667	18.190
Schizophrenia in family	4.671	1.476	0.400	0.003	1.702	7.641
Age at onset	-0.272	0.096	-0.357	0.007	-0.466	-0.078

Dependent variable: SANS Anhedonia/Asociality score; Adjusted R^2=0.227; F=8.174; $p = 0.001$. CI = Confidence interval

Table 9. Significant related factors of the SANS Attention score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	-3.803	1.429		**0.011**	-6.678	-0.927
Economic condition, Non-self-sufficient	3.352	0.707	0.528	**< 0.001**	1.930	4.773
PAS Psychological autonomy score	0.196	0.070	0.310	**0.008**	0.055	0.337

Dependent variable: SANS Attention score; Adjusted R^2=0.423; F=18.977; $p < 0.001$. CI = Confidence interval

delusions score, and positive correlation between SANS attention score, SANS avolition/apathy score and PAS psychological autonomy score were also confirmed by regression analysis. However the significant positive correlation between the SANS total score and the psychological autonomy score of PAS could not be confirmed by multivariate analysis. In addition, we saw that the severity of delusion, which is one of the positive symptoms of schizophrenia, increases as the perceived parental interest decreases. We did not find a significant relationship between the severity of positive schizophrenia symptoms other than delusion , all of mania and depression symptoms and perceived parental attitudes.

Positive and negative effects of parents' attitudes and character traits on people's mental health have been shown in many studies [4, 5, 12]. Children exposed to overprotective parent attitudes early in life may become accustomed to see their environment as a dangerous place –a place in which they need help to survive. This perception may negatively affect the child's ability to acquire self-regulation skills, learn to express their negative emotions appropriately, thus reducing the quality of relationships with their family and peers [24]. In a national survey, negative parenting attitudes that included corporal and non-physical punishment were associated with both parent and child mental health problems [25]. Most studies are limited to investigating the role of childhood trauma in the clinical presentation of patients with BD and schizophrenia. However, childhood trauma may also contribute to the development and symptoms of such diseases. Negative attitudes of the parents towards the child and the resulting traumatic effect may affect emotional functioning during disease and, in turn, the general prognosis [26]. In Özütek's study [27] on the subject, a relationship was found between the deterioration in the problem-solving dimension of family functionality of patients with schizophrenia and the positive and negative symptom levels and general psychopathology levels of the patients. In the study of Tüzer *et al*. [9], it was found that patients with schizophrenia with severe positive symptoms perceived their family environment as "strict and overly pre-

Table 10. Significant related factors of the SANS Total score, multiple linear regression analysis

	Unstandardized β	Standard Error	Standardized β	*p* value	95.0%	CI for β
(Constant)	36.925	10.830		0.001	15.113	58.738
Age	-0.760	0.204	-0.422	0.001	-1.171	-0.350
Economic condition, Non-self-sufficient	22.024	5.057	0.482	< 0.001	11.839	32.208
Schizophrenia in family	14.412	5.002	0.315	0.006	4.337	24.486
Smoker	-10.003	4.697	-0.239	0.039	-19.463	-0.543

Dependent variable: SANS Total score; Adjusted R^2=0.420; F=9.859; $p < 0.001$. CI = Confidence interval

scriptive", and greater positive and negative symptoms was associated with increased expression of emotion in the family environment. In a research examining the family functions of patients with schizophrenia showed apparent family dysfunction, and concluded that families should be informed about the crucial nature of family and social support [28]. In another study, it was thought that symptomatic relapse of schizophrenia could be a result of environmental interaction, and that the reactivity of relatives were associated with psychotic symptoms [29]. In our study, we evaluated the symptom severity of schizophrenia patients with SANS and SAPS. In the light of our findings regarding correlations, we concluded that the symptoms of avolition/apathy, anhedonia/asociality, and attention were more severe and the total SANS score was significantly higher in schizophrenia patients whose patients had higher psychological autonomy scores (favoring democratic / libertarian attitude). When we assessed positive symptoms, we observed that the delusion symptom was more severe in schizophrenic patients who defined their parents as having lower acceptance/involvement score. Negative correlation between acceptance/involvement score and delusion severity and positive correlation between the psychological autonomy score and the avolition/apathy and attention severity were also confirmed by regression analysis. However, the r values of the significant correlations found between the perceived PAS score and the positive and negative symptoms of schizophrenia remained below the desired level (0.5). In addition, since the questionnaires measuring both perceived family attitudes and symptoms severity yield subjective results, it would not be correct to say that the results are unbiased. So these results of the study should be supported by other studies with a larger patient population.

Family characteristics such as conflict and adjustment have been associated with the course of mood symptoms in bipolar disorder. Adaptation includes family roles, leadership, discipline, and resilience, while adjustment includes family intimacy (time together, boundaries, and emotional bonds) [30]. Patients with bipolar disorder show levels of family mismatch, like negative family attitudes or interactional behaviors, during and after their illness, although not as high as those seen in patients with schizophrenia. These are suggested to predict poor short-term outcomes related to the disorder [31]. A recent study by Wittenborn *et al*. [32] noted that the poor relationships of people with BD with their parents and spouses were associated with non-remission, relapse, and recurrence of depressive and bipolar symptoms. In the present study, we used YMRS (for mania severity) and HDRS (for depression severity) to assess the possible relationships of the severity of symptoms in BD patients. We could not detect a significant relationship between the severity of neither mania nor depression symptoms in BD patients and their perceived parental attitudes.

It is well known that BD and schizophrenia are highly inherited disorders (some form of family history reported in 59% and 64% of patients, respectively) and significant progress has been made in identifying genetic risk factors. However, 15-40% of the risk from environmental sources is largely unknown [3, 33, 34]. Etain *et al*. [26] assumed that environmental factors were the main causes of the disorder even in diseases such as BD and schizophrenia, where hereditary transmission is known to be the highest, and stated that genes affect the level of sensitivity to these factors. Furthermore, they emphasized that genetic and non-genetic factors are probably interacting in the formation of these diseases and they have a multifactorial origin. In our study, the incidence of BD was found to be significantly higher in patients with a family history of BD compared to schizophrenia. In those with a family history of schizophrenia, the incidence of schizophrenia was higher than the incidence of BD, but it was not statistically significant. Multiple regression analysis results showed that patients with schizophrenia history in family had higher SANS anhedonia/asociality score and SANS total scores. These results support that both the presence and symptoms of BD and schizophrenia may be influenced by familial and perhaps genetic characteristics.

Schizophrenia is associated with lower intelligence and educational performance compared to the general population [35]. It is unclear whether BD patients have lower intellectual and educational performance similar to that observed in schizophrenia. Vreeker *et al*. [35] found that BP patients were more likely to complete higher education despite having lower intelligence quotients (IQs) compared to controls. In contrast, they showed that patients with schizophrenia had both lower IQs and lower levels of education com-

pared to controls, similar to the study by Sletved and colleagues [36]. Vasudeva *et al*. [37] and Pacheco *et al*. [38], on the other hand, did not find any difference between the two diseases in terms of education level. In this study, there was no significant difference between the BD and schizophrenia groups in terms of lower education levels; however, a significant trend for higher education was observed in the BD group compared to the schizophrenia group.

Since schizophrenia and BD affect inter-personal relationships to a great degree depending on symptom severity, they can also cause some problems and restrictions in terms of marriage. Pacheco *et al*. [38] and Sletved *et al*. [36] found that the frequency of being married in schizophrenia patients was lower compared to BD patients. Vasudeva *et al*. [37] did not find any difference between the two diseases in terms of marital status. In our study, while the percentage of married BD patients was higher compared to the schizophrenia group, there was no significant difference between the two groups in terms of being divorced or widowed. In addition, the percentage of participants living alone was higher in the schizophrenia group, similar to the study by Mausbach *et al*. [39], while, consequently the percentage of those living with a partner and child (or children) was higher in the BD group.

Limitations

Our study has several limitations. First, the low number of patients and discrepancies in distribution into categorical subgroups may have negatively affected statistical results. Secondly, the insufficient number of similar studies limited our ability to compare the results of our research. Thirdly, because of its cross-sectional design and reliance on self-report data for some parameters, caution should be exercised when interpreting the findings of this study, particularly when generalizing results. Fourthly, interpretation of results could be improved by considering cross-cultural implications. Finally, as with almost every survey study, the results of this study might be affected from sided and biased thoughts.

CONCLUSION

In conclusion, our results support the idea that some environmental factors, such as marital status, education level, living alone or living with relatives, age, gender and economical and working features may have a role in the development and course of schizophrenia and BD disease. The increase in the level of liberality of parents appears to have a negative impact on the negative symptoms of schizophrenia. In addition, the decrease in the level of interest of parents towards their children appears to exacerbate delusion symptoms. Education of the families of patients with BD and schizophrenia and correction of environmental factors that can be improved can contribute to disease control and remission-free follow-up. Since the present study was a survey study and since the study population consisted of individuals with two major psychiatric illnesses that could affect the thoughts of both patients and their families, the results of the study may have been affected by biased thinking. Therefore, more comprehensive studies with a larger number of patients are needed to support the results of this study.

Authors' Contribution

Study Conception: EY, UA, EO; Study Design: EY, UA, EO; Supervision: EY, UA, EO; Funding: EY, UA; Materials: UA; Data Collection and/or Processing: EY, UA; Statistical Analysis and/or Data Interpretation: E; Literature Review: EY, EC; Manuscript Preparation: EY and Critical Review: EO.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):868-873 Original Article

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Factors predicting transrectal ultrasound-guided systematic prostate biopsy failure

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ABSTRACT

Objectives: To determine the factors that predict the failure of systematic prostate biopsy by examining the clinical, laboratory, and radiological parameters of patients for whom prostate cancer was detected by magnetic resonance imaging (MRI)-targeted biopsy but not by systematic biopsy.

Methods: Patients were included in this study if they had undergone combined targeted and systematic biopsy and had cancer detected in the targeted biopsy. They were biopsy-naive patients and had lesions with a Prostate Imaging Reporting and Data System (PIRADS) score ≥ 3 in the peripheral zone on MRI. The clinical, biochemical, and radiological findings of the groups with and without cancer detected in the systematic biopsy were compared.

Results: A total of 100 patients had an index lesion in the peripheral zone and cancer detected by MRI-targeted biopsy. In 43 (43%) of the patients, no cancer was detected in the systematic biopsy, whereas it was detected in the other 57 (57%). Statistically significant differences were found between the two groups in terms of prostate volume and PSA density ($p < 0.001$ and $p < 0.001$, respectively). Moreover, the findings of univariate and multivariate logistic regression analyses indicated that prostate volume and lesion size are independent predictors of systematic biopsy failure.

Conclusions: The success of systematic biopsy may be lower in patients with high prostate volume and low peripheral zone index lesion size.

Keywords: Prostate biopsy, systematic, failure, targeted biopsy

Currently, the most widely used biopsy technique for diagnosing and grading prostate cancer is the 12-core systematic biopsy procedure performed under transrectal ultrasound (TRUS) guidance [1]. Almost all other cancers are diagnosed by performing a biopsy of the suspected area by radiological or physical examination. However, in systematic prostate biopsy, a total of 12 cores are randomly sampled, six from each lobe of the prostate. Although ultrasound reveals the borders of the prostate gland and adjacent organ structures well, it cannot adequately distinguish malignant from benign lesions [2]. Because approximately 40% of lesions are isoechoic, ultrasound alone is insufficient for targeting specific lesions [3].

With the publication of the Prostate Imaging Reporting and Data System (PIRADS) version 1 in 2012, a standard was set for multiparametric prostate magnetic resonance imaging (MRI) and reporting [4].

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Later, updates were made to the PIRADS system in ver. 2.0 in 2015 and ver. 2.1 in 2019. With the provision of this standardization, MRI-targeted biopsy applications have accelerated. In the 2021 European Association of Urology (EAU) guidelines, multiparametric prostate MRI is recommended for all patients who will undergo prostate biopsy; if PIRADS ≥ 3 lesions are detected through MRI in biopsy-naïve patients, a systematic biopsy together with a targeted biopsy are strongly recommended. In patients with previous negative biopsy, in the case of PIRADS ≥ 3 lesions detected on MRI, a targeted biopsy only from the index lesion is weakly recommended [5].

An MRI-targeted biopsy can be performed in cognitive, software-based MRI-ultrasound fusion as well as in-bore [6]. Many studies have compared all three methods with systematic biopsy.

The present study aimed to determine the factors that predict the failure of systematic biopsy by examining the clinical, laboratory, and radiological parameters of patients whose prostate cancer was detected by MRI-targeted biopsy but could not be detected by systematic biopsy.

METHODS

Our MRI-TRUS fusion-guided prostate biopsy database was reviewed retrospectively. Patients who had undergone combined targeted and systematic biopsy and had cancer detected in the targeted biopsy were included in the study. They were biopsy-naive patients and had PIRADS ≥ 3 lesions in the peripheral zone on MRI. Patients with missing data and additional malignancies were excluded. The clinical, biochemical, and radiological findings of the groups with and without cancer in the systematic biopsy were compared. In addition, logistic regression analysis was performed to determine the parameters that predict systematic biopsy failure. This study was approved by the Clinical Research Ethics Committee (date 02/21/2022, Decision No. 142).

Table 1. Comparison of the variables of the groups with and without cancer in systematic biopsy

	SB Benign (n = 43)	SB Malign (n = 57)	Total (n = 100)	*p* value
Age (year), median (Q1, Q3)	65 (60-69.5)	64 (57-69)	64 (59-69.2)	0.643
tPSA (ng/ml), median (Q1, Q3)	6.2 (5.3-8.6)	7.8 (5.5-11.5)	6.9 (5.4-10.3)	0.057
Prostate Volume (ml), median (Q1, Q3)	58 (39.5-80)	38 (28-50)	43 (32.7-65.2)	**< 0.001**
PSAD (ng/ml/ml), median (Q1, Q3)	0.124 (0.082-0.194)	0.206 (0.138-0.323)	0.170 (0.111-0.280)	**< 0.001**
PIRADS score, n (%)				0.285
3	5 (11.6)	6 (10.5)	11 (11)	
4	25 (58.1)	25 (43.9)	50 (50)	
5	13 (30.2)	26 (45.6)	39 (39)	
Index lesion diameter (mm), median (Q1, Q3)	13 (10-17.7)	14 (11-19.7)	13.5 (10-18.2)	0.223
Number of index lesion, n (%)				0.480
Single	33 (76.7%)	47 (82.5%)	80 (80)	
Multiple	10 (23.3%)	10 (17.5%)	20 (20)	
Type of anesthesia, n (%)				0.116
Local	36 (83.7)	40 (70.2)	76 (76)	
General	7 (16.3)	17 (29.8)	24 (24)	
Core length (mm), median (Q1, Q3)	10.6 (9.2-12.6)	11 (9.1-12.1)	10.8 (9.1-12.3)	0.831

SB = Systematic Biopsy, tPSA = Total Prostate-Specific Antigen, PSAD = PSA Density, PIRADS = Prostate Imaging Reporting and Data System

Statistical Analysis

The normal distribution of continuous variables was evaluated using analytical methods (i.e., Kologorov-Smirnov and Shapiro-Wilk tests). In the descriptive findings, categorical variables were presented as numbers (percentages), and continuous variables were presented with medians (interquartile range) for normal nonscattering data. The cut-off value for prostate volume was determined with a receiver operating characteristic (ROC) curve analysis and Youden's index and then reported using the area under the curve (AUC) with a 95% confidence interval (CI). For the categorical variables, statistical differences among the groups were determined using chi-square tests. For the continuous variables, statistical differences among the groups were determined using Mann–Whitney U tests. Next, univariate and multivariate logistic regression analyses were performed to determine the prognostic factors that predict systematic biopsy failure. Statistical significance was accepted as $p < 0.05$. All statistical analyses were performed with R version 4.0.4 through R Studio version 1.4.1106.

RESULTS

There were a total of 100 patients with an index lesion in the peripheral zone and cancer detected by MRI-targeted biopsy. In 43 (43%) of these patients, no cancer was detected in the systematic biopsy (Group 1), whereas cancer was detected in 57 (57%) of them (Group 2). When the clinical, biochemical, and radiological findings of the two groups were compared, no statistically significant differences were found in terms of age, total prostate-specific antigen (PSA), PIRADS score, lesion size, number of lesions, anesthesia type, and mean core length. However, statistically significant differences were found between the two groups in terms of prostate volume and PSA density (PSAD; $p < 0$ .001 and $p < 0.001$, respectively; Table 1). The findings of the univariate and multivariate logistic re-

Table 2. Univariate and multivariate logistic regression analysis to identify parameters predicting systematic biopsy failure

	Simple Logistic Regression			Multivariate Logistic Regression		
	95% CI	OR	*p* value	95% CI	OR	*p* value
Age	0.949-1.043	0.995	0.839			
tPSA	0.994-1.165	1.062	0.257			
Prostate Volume	0.58-0.852	0.713	**< 0.001**	0.946-0.991	0.969	**0.007**
PSAD	1.024-1.114	1.064	**< 0.004**	0.985-1.068	1.022	0.266
Core length	0.914-1.229	1.055	0.481			
Index lesion diameter	0.995-1.13	1.055	0.091	1.012-1.190	1.089	**0.039**
Number of index lesion						
Single	Reference		0.481			
Multiple	0.26-1.894	0.702				
PIRADS Score						
3	Reference					
4	0.215-3.115	0.833	0.785			
5	0.412-6.587	1.667	0.462			
Type of anesthesia						
Local	Reference					
General	0.839-6.21	2.186	0.121	0.658-6.357	1.978	0.233

CI = Confidence Interval, OR = Odds Ratio, tPSA = Total Prostate-Specific Antigen, PSAD = PSA Density, PIRADS = Prostate Imaging Reporting and Data System

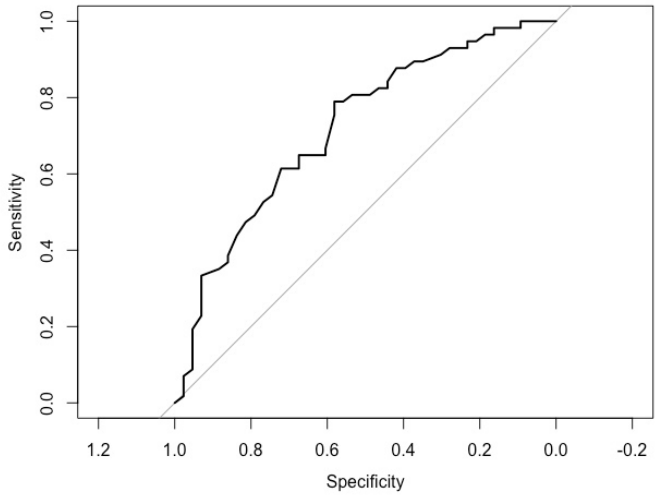

Fig. 1. ROC curve when the cut-off value of the prostate volume was determined as 53 cc.

gression analyses indicated that prostate volume and lesion size are independent predictors of systematic biopsy failure (Table 2). This study determined that a cut-off value of 53 cc on the ROC curve drawn for prostate volume exhibited 78% sensitivity and 58% specificity (Fig. 1).

DISCUSSION

The results of this study revealed that systematic biopsy is more unsuccessful when the patients has small index lesion and large prostate.

The success of targeted and systematic prostate biopsy has been investigated in many studies. A meta-analysis of 29 studies demonstrated that MRI-targeted biopsy has superior diagnostic value to systematic biopsy in terms of detecting clinically important prostate cancer and high-grade cancer in biopsy-naive patients. The same study also demonstrated that not performing a systematic biopsy in this group reduced the rate of clinically insignificant cancer detection without changing the rate of clinically significant and high-grade cancer [7].

Prostate volume is a critical factor that affects the cancer detection rate in systematic biopsy. In a series of 750 patients, Ung *et al*. [8] found the cancer detection rate to be 40% in patients with a prostate volume less than 34 cc, while it was 24% in those whose prostate volume was greater than 64 cc. Similarly, in a series of 1021 patients with sextant biopsy, these rates were 38% and 23%, respectively, when the prostate volume limit was set as 50 cc [9]. Furthermore, in our study, increased prostate volume was determined to be an independent predictive factor for systematic biopsy failure. The cut-off value of 53 cc on the ROC curve exhibited 78% sensitivity and 58% specificity.

In a standard systematic biopsy, a total of 12 cores are sampled, of which six are sampled randomly from both lobes of the prostate. Due to the fact that the number of cores taken is independent of prostate size and as prostate cancer can be multifocal, the probability of taking tissue from the tumor area in a large prostate decreases, and thus, sampling may be insufficient. Therefore, cancer diagnoses can be missed in patients with prostate cancer in addition to benign prostatic hyperplasia, and repeated systematic and saturation biopsies may be required. This result is associated with increased cost, complications, and morbidity [10-12]. On the other hand, one cannot consider prostate volume to be a factor that predicts the detection of cancer under all conditions. In a retrospective study investigating the effect of PSA level on cancer detection, 2079 patients who had undergone 10-core systematic biopsy were evaluated, and prostate volume was found to be a significant parameter only with a PSA level below 10 ng/mL [13].

Another critical parameter in the detection of prostate cancer is PSAD. In a study by Washino *et al*. [14] on 288 patients, PSAD was demonstrated to be an independent predictive factor for clinically signifi-

cant prostate cancer. In addition, a meta-analysis that included 11 studies demonstrated that PSAD is a marker that can be used to predict prostate cancer [15]. In our study, the median PSAD of patients with cancer detected in the systematic biopsy was 0.206, while this value was 0.124 in patients without cancer. A statistically significant difference was found between the two groups ($p < 0.001$), which is consistent with the literature. However, because no significant p value was found in the multivariate logistic regression analysis, we concluded that PSAD cannot be an independent predictive factor for prostate cancer in systematic biopsy. In a study of 5291 patients, Nordström *et al*. [16] demonstrated that by not performing a biopsy in patients with a PSAD < 0.07, 19.7% of them would be saved from unnecessary biopsy; however, 6.9% of clinically important prostate cancer cases would be missed. We believe that the systematic biopsy decision should not be abandoned based only on the PSAD value, since 6.9% is an important rate.

According to the MRI findings in our study, although no statistically significant difference existed in index lesion sizes between the two groups, the multivariate logistic regression analyses revealed that a small lesion size was an independent predictive factor for systematic biopsy failure. Similarly, Park *et al*. [17] performed combined targeted and systematic biopsies on 313 patients. In those with an index lesion smaller and larger than 10 mm, the clinically significant cancer detection rates were 32.5% and 69.5%, respectively, which were also statistically significant ($p < 0.001$). In another study, in which 219 patients underwent combined targeted and systematic biopsy, these rates were 8.6% and 33.1%, respectively, with a 10 mm index lesion size cut-off value; moreover, a statistically significant difference existed between the two groups ($p < 0.001$) [18].

The results of this study revealed that systematic biopsy is more unsuccessful when the patients has small index lesion and large prostate. We believe that 16-core or 20-core systematic biopsy may be preferred instead of 12-core for better sampling for these patients.

In the randomized prospective PRECISION study, it was demonstrated that compared with systematic biopsy, a clinically significant cancer rate was detected when only MRI targeted biopsy was performed (which was statistically significantly higher); however, a lower rate of clinically insignificant cancer was detected [19]. In the present study, we aimed to determine the situations in which systematic biopsy is ineffective, and therefore, clinical significance or non-significance was not distinguished. Therefore, the detection of ISUP grade 1 cancer in the targeted biopsy while the systematic biopsy was negative was accepted as systematic biopsy failure.

Limitations

This study had some limitations. First, it was retrospective; second, the interventions were performed by different clinicians; and third, targeted and systematic biopsies were performed by the same clinician.

CONCLUSION

Systematic biopsy success may be lower in patients with a high prostate volume and low peripheral zone index lesion size. We believe that MRI-targeted biopsy should be performed together with systematic biopsy in these patients. However, we believe that 16-core or 20-core systematic biopsy may be preferred instead of 12-core for better sampling. In future research, if prospective studies with larger patient cohorts are designed, then stronger conclusions can be derived.

Authors' Contribution

Study Conception: TSS, İŞ; Study Design: SÇ, MK; Supervision: TSS, İŞ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: EB, AO; Statistical Analysis and/or Data Interpretation: ECB, MK; Literature Review: EB, AO; Manuscript Preparation: SÇ, MK and Critical Review: SÇ,ECB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Hidden face of chronic obstructive pulmonary disease: effects of patients' psychiatric symptoms on caregivers' burden and quality of life

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ABSTRACT

Objectives: Chronic Obstructive Pulmonary Disease (COPD) faces functional and physical limitations and often needs the help of others at certain times in their lives. Patients and caregivers can affect each other psychologically, physically, and socially. This study aims to examine the relationship between the demographic and clinical characteristics of COPD patients and the quality of life and burden of care of caregivers.

Methods: The study was carried out with 250 COPD patients who applied to the chest diseases hospital and their caregivers. Hospital Anxiety-Depression Scale (HADS), The Zarit Burden Interview (ZBI), and World Health Organization Quality of Life Scale Short Form (WHOQOL-BREF) scales were applied to the patients.

Results: The patients' gender, age, regular drug use, non-invasive mechanical ventilator use, emergency room admissions, number of hospitalizations, number of intensive care admissions, presence of comorbidities were found to be associated with HADS anxiety and depression scores, ZBI, and WHOQOL-BREF. According to the results of multiple linear regression analysis; it is seen that the patient's gender, NIV use, regular device use, presence of comorbidity, HADS anxiety score, and HADS depression score is an independent predictor of the caregiver's burden (ZBI score); and the patient's NIV use, regular device use, HADS anxiety score and HADS depression score is an independent predictor of the caregiver's WHOQOL-BREF score.

Conclusions: It is important to evaluate patients and caregivers in a holistic approach and to realize the factors that may negatively affect them in the early period to take the necessary therapeutic measures.

Keywords: COPD, caregiver, quality of life, caregivers' burden, psychiatry, inpatient

Chronic Obstructive Pulmonary Disease (COPD) is one of the most commonly seen causes of chronic respiratory failure worldwide [1, 2] . COPD, a major cause of mortality and morbidity, has negative effects on quality of life [1, 3]. COPD patients experience functional physical limitations over time and often need the help of others at certain times in their lives [1, 3]. This assistance is undertaken by the care-

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givers of the patients [4]. During the meeting of these needs, the multidimensional response of caregivers to stress caused by the care given to the patients is defined as the burden of care [5]. The burden of care may result in physical, emotional, and financial difficulties and quantitative and qualitative reduction of care provided [6, 7]. It can also lead to decreased quality of life, increased anxiety, and depressive symptoms for the caregiver [8-11]. Additionally, it is known that increasing the burden of care can negatively affect patient care and decreased communication and survival [12].

Timely determination of factors that increase the burden of care and negatively affect the quality of life of the caregiver will ensure that the physical and mental health of both the patient and the caregiver is maintained and thus their quality of life is improved [4, 13]. Studies have shown that the characteristics of caregivers such as age, gender, relationship, coping mechanisms, and social support have negative effects on the mental health of caregivers [14, 15]. Besides, some characteristics of patients such as gender, duration of care, relationship, the functionality of the patient, and tasks undertaken for care are expected to affect the burden of care and quality of life [16-18]. Little is known about the physical-social-psychological effects of patients who are followed up with COPD diagnosis on primary caregivers in Turkey. Furthermore, to the best of our knowledge, there is no study examining the relationship between the characteristics of COPD patients and the burden of care and quality of life of caregivers.

The current study aims to evaluate the relationship between the demographic, clinical, anxiety, and depressive characteristics of COPD patients and the quality of life and burden of care of caregivers.

METHODS

Patients who had hypoxic and/or hypercapnic respiratory failure due to COPD followed up in the inpatient or outpatient clinic between September 2019 and April 2020 in a tertiary chest diseases hospital, who received long-term oxygen therapy (LTOT) and/or non-invasive mechanical ventilation (NIV) at home with the diagnosis of chronic respiratory failure for at least one year, and those who provided care for these patients were included in this study. According to the criteria for inclusion and exclusion in the study, 250 patients and 250 relatives of patients (caregivers) were included. The following criteria were determined as the inclusion criteria in the study: (1) being diagnosed with hypoxic and/or hypercapnic respiratory failure; (2) receiving LTOT and/or NIV at home with a diagnosis of chronic respiratory failure for at least one year; (3) two or more exacerbations leading to hospitalizations per year with a respiratory complaint and/or admissions to emergency at least twice a year due to the same reasons; (4) having a first-degree family member who takes care of the patients included in the study; (5) being over 18 years of age; and (6) voluntary participation of the patient and the caregiver in the study.

The presence of an active psychiatric disorder of both the patient and caregiver or the presence of another chronic disease that may affect the burden of care other than COPD and respiratory failure were considered as criteria for exclusion.

The study was approved by the Ankara Keçiören Training and Research Hospital Clinical Research Ethics Committee with decision no. 1916 dated 28.08.2019. All patients and their relatives were asked to read and complete questionnaires after reading and signing the informed voluntary consent forms.

Evaluation of Patients

1. Anamnesis

Detailed anamnesis of all patients included in the study was taken by clinicians experienced in Pulmonology. Their demographic and clinical variables such as age, gender, smoking status, comorbidities, LTOT and/or NIV receiving durations at home, all hospital admissions, and hospitalizations in the last year were recorded. In the interview with the caregivers of the patients, it was questioned whether they were first-degree relatives and how long they were giving care.

2. Measuring tools

Both the patient and their relatives were allowed to complete the forms under the supervision of the researchers. Patients or their relatives who were not literate were expected to answer the question through an

easy-to-understand reading of the items by the researcher.

Hospital Anxiety and Depression Scale (HADS)

It was developed in 1983 by Zigmond *et al*. [19] to assess the severity of symptoms of anxiety and depression in groups with a medical condition . The validity and reliability study of the scale was done in Turkey by Aydemir *et al*. [20]. 7 out of 14 questions in the 4-point Likert-type scale measure anxiety and 7 measure depression, and are scored between 0-3. The lowest score that patients can get from both subscales is 0 and the highest score is 21.

Turkish Version of the World Health Organization Quality of Life Scale Short Form (WHOQOL-BREF-TR)

The health-related quality of life scale was developed by the WHO, and the validation and reliability study was done by Eser *et al*. [21]. The scale consists of 27 items and evaluates the quality of life in five different dimensions. These are general health, physical health, social relations, environmental health, and psychological health.

Zarit Burden Inventory (ZBI)

This scale was first developed by Zarit [22], Reever, and Bach-Peterson in 1980 for caregivers of dementia patients. It is a 19-item 5-point Likert-type scale used to assess the difficulties and stress experienced by individuals giving care to patients. The validity and reliability study of the scale was carried out by Özlü *et al*. [23] in Turkey.

Statistical Analysis

The SPSS 22 package program was used in the

Table 1. Representation of sociodemographic and clinical characteristics of the participants included in the study

Patient's age (years) (mean ± SD)		69.84 ± 14.02
The Number of cigarettes smoked by the patient (pcs/day) (Mean ± SD)		28.23 ± 26.29
Number of emergency department admissions of the patient, (Mean ± SD)		5.52 ± 3.79
Number of hospitalizations of the patient (Mean ± SD)		2.90 ± 1.83
Number of intensive care hospitalizations of the patient (Mean ± SD)		1.70 ± 1.09
Patient's gender, n (%)	Male	164 (5.6)
	Female	86 (34.4)
Patient's use of LTOT, n (%)	Yes	250 (100)
	No	0 (0)
Patient's use of NIV, n (%)	Yes	85 (34)
	No	165 (66)
Patient's regular use of devices, n (%)	Yes	190 (76)
	No	60 (24)
The patient's presence of comorbidity, n (%)	Yes	214 (85.1)
	No	36 (14.4)
Psychiatric treatment status of the patient, n (%)	Yes	38 (15.2)
	No	212 (84.8)
The age of the caregiver (year) (Mean ± SD)		51.19 ± 11.64
Caretaking time (years) (Mean ± SD)		7.77 ± 6.39
The gender of the caregiver, n (%)	Male	71 (28.4)
	Female	179(71.6)

LTOT = Long-term oxygen therapy, NIV = Non-invasive mechanical ventilator, SD = standard deviation

statistical analysis of the data. Descriptive analysis methods were applied after the sociodemographic and clinical characteristics of the participating patients and caregivers were duly recorded in the appropriate data set in order. Continuous variables were expressed as mean ± standard deviation and categorical variables as frequency (percentage). In the comparison of continuous variables, it was examined primarily whether they met the assumptions required for parametric tests. Student-T Test was used for those who met the assumptions for parametric tests. The Pearson correlation analysis was applied if they met the assumptions for parametric tests to examine the relationship between the two numerical variables. The Linear Regression analysis was applied to determine the predictors of the caregiving burden. In all statistical analyses, a p - value of ≤ 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the participants are presented in Table 1. The mean age of COPD patients included in the study was 69.84 ± 14.02 years and 34.4% (n = 86) were female. The mean age of the caregivers was 51.19 ± 11.64 years, 71.6% (n = 179) were female and the mean caregiving period was 7.77 ± 6.39 years.

Comparison of WHOQOL-BREF and ABI scores of caregivers by gender and clinical variables of COPD patients is presented in Table 2. There was a statistical difference between General Health (p = 0.001), Psychological Health (p = 0.024), Social Relations (p = 0.018), WHOQOL-BREF Total (p = 0.005), and ZBI (p = 0.037) scores according to the comparison by the patient's gender. A statistically significant difference was found between General Health ($p < 0.001$), Physical Health (p = 0.045), Social relations ($p < 0.001$), Environmental Health ($p < 0.001$), WHOQOL-BREF Total ($p < 0.001$), and ZBI ($p < 0.001$) scores according to the comparison by regular LTOT and/or NIV device usage of the patient. Physical Health ($p < 0.001$), Environmental Health (p = 0.001), WHOQOL-BREF Total (p = 0.001), and ZBI (p = 0.001) scores were found to have a statistically significant difference according to the comparison by the NIV usage of the patient. There was a statistical difference in Psychological Health (p = 0.041) score according to the comparison by the presence of comorbid disease.

The relationship between the age and clinical variables of COPD patients and the WHOAOL-BREF and ZBI scores of the caregivers are presented in Table 3. There was a negative statistically significant relationship (r = -0.134) between the ZBI score and the age of the patient, and the HADS Anxiety subscale (r = -0.413), and a positive statistically significant relationship between the number of emergency admissions (r = 0.180), the number of hospitalizations (r = 0.214), the number of intensive care hospitalizations (r = 0.181) and the HADS (r = 0.176) Depression subscale. In addition, there was a positive statistically significant relationship between the WHOQOL-BREF score of the caregiver and the patient's age (r = 0.197), and the HADS Anxiety subscale (r = 0.325); and a negative statistically significant relationship between the number of hospitalizations (r = -0.179) and the number of intensive care hospitalizations of the patient (r = -0.131).

A significant relationship was found between the patient's age, gender, number of hospitalizations, NIV usage, regular device usage, presence of comorbidity, antidepressant use, which are thought to affect the caregiver's total scores of ZBI and WHOQOL-BREF, the variables as a result of multiple linear regression analyses to reveal the predictiveness of HADS anxiety and depression scores, and the ZBI score (R = 0.607, R2 = 0.369, F (9-240) = 15.594, $p < 0.001$); and a significant relationship with the WHOQOL-BREF total score (R = 0.480, R2= 0.230, F (9-240) = 7.980, $p < 0.001$). These variables explain 36% of the change in the ZBI scores. According to the model; patient's gender ($p < 0.001$), NIV use ($p < 0.001$), regular device usage ($p < 0.001$), presence of comorbidity (p = 0.014), HADS anxiety score ($p < 0.001$), and HADS depression score ($p < 0.001$) are significant predictors of the caregiver's ZBI score. The variables included in the model explain 23% of the change in the WHOQOL-BREF total scores. According to the model; NIV usage ($p < 0.001$), regular device usage ($p < 0.001$), HADS anxiety score (p = 0.012), and HADS depression score ($p < 0.001$) are seen to be significant predictors of the caregiver's WHOQOL-BREF total score.

Table 2. Comparison of gender and clinical variables of COPD patients with caregivers' WHOQOL-BREF and Zarit Burden Inventory scores

	Caregiver's WHOQOL-BREF						Caregiver's Zarit Burden Inventory
	General Health	Physical Health	Psychological Health	Social Relations	Environmental Health	Total	
Patient's gender							
Male	52.99 ± 14.40	40.39 ± 12.67	49.00 ± 9.46	43.07 ± 14.43	58.31 ± 11.36	52.14 ± 9.77	59.37 ± 11.21
Female	39.03 ± 22.78	38.40 ± 12.01	45.39 ± 12.00	37.70 ± 16.69	56.52 ± 9.50	48.37 ± 9.42	62.85 ± 12.10
	t = 5.785	t = 1.160	t = 2.270	t = 2.378	t = 1.174	t = 2.819	t = -2.093
	***p* = 0.001**	*p* = 0.247	***p* = 0.024**	***p* = 0.018**	*p* = 0.243	***p* = 0.005**	***p* = 0.037**
Patient's status of regular device use							
Yes	46.57 ± 20.40	39.73 ± 12.78	47.19 ± 10.92	42.23 ± 15.96	58.60 ± 10.18	51.00 ± 9.72	59.82 ± 11.30
No	31.76 ± 21.76	36.54 ± 9.87	43.95 ± 12.70	29.72 ± 13.23	52.08 ± 7.94	44.52 ± 7.61	68.33 ± 11.67
	t = -4.855	t = -2.023	t = -1.920	t = -5.501	t = -5.156	t = -5.359	t = 5.047
	***p* < 0.001**	***p* = 0.045**	*p* = 0.056	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**
Patient's status of NIV use							
Yes	40.88 ± 19.03	34.95 ± 12.29	45.10 ± 10.74	36.96 ± 16.03	54.08 ± 9.33	46.65 ± 8.52	65.36 ± 10.63
No	44.09 ± 22.87	41.03 ± 11.67	47.04 ± 11.75	40.40 ± 16.27	58.56 ± 10.13	50.88 ± 9.91	60.05 ± 12.20
	t = 1.177	t = 3.832	t = 1.212	t = 1.593	t = 3.400	t = 3.354	t = -3.400
	p = 0.241	***p* < 0.001**	*p* = 0.227	*p* = 0.112	***p* = 0.001**	***p* = 0.001**	***p* = 0.001**
Patient's comorbidity status							
Yes	43.69 ± 21.31	39.15 ± 12.15	47.02 ± 11.38	39.25 ± 15.65	56.57 ± 10.21	49.55 ± 9.58	61.26 ± 11.41
No	38.88 ± 23.38	37.89 ± 12.67	42.82 ± 11.25	39.12 ± 19.60	59.80 ± 8.87	48.83 ± 10.21	65.42 ± 14.38
	t = -1.232	t = -0.570	t = -2.050	t = -0.038	t = 1.792	t = 0.409	t = 1.942
	p = 0.219	*p* = 0.569	***p* = 0.041**	*p* = 0.970	*p* = 0.074	*p* = 0.683	*p* = 0.053
Patient's status of antidepressant use							
Yes	43.09 ± 20.89	37.96 ± 12.68	49.45 ± 10.07	40.57 ± 13.16	58.30 ± 7.34	50.47 ± 7.86	62.50 ± 11.99
No	42.98 ± 21.84	39.15 ± 12.14	45.87 ± 11.60	38.99 ± 16.74	56.81 ± 10.48	49.26 ± 9.95	61.75 ± 11.95
	t = 0.028	t = 0.548	t = 1.784	t = 0.650	t = 1.074	t = 0.839	t = 0.358
	p = 0.977	*p* = 0.584	*p* = 0.076	*p* = 0.518	*p* = 0.287	*p* = 0.405	*p* = 0.721

COPD = Chronic Obstructive Pulmonary Disease, WHOQOL-BREF =World Health Organization Quality of Life Scale Short Form, NIV = Non-invasive mechanical ventilator

Table 3. Relationship between the age and clinical variables of COPD patients and WHOQOL-BREF and Zarit Burden Inventory scores

		Age	Number of Emergency Department Admissions	Number of Hospitalizations	Number of Intensive Care Hospitalizations	HADS Depression Subscale	HADS Anxiety Subscale
Caregiver's WHOQOL-BREF score							
General health	r	0.314**	-0.180**	-.254**	-0.003	-0.181**	0.476**
	p value	**< 0.001**	**0.004**	**< 0.001**	0.959	**0.004**	**< 0.001**
Physical health	r	0.122	-0.072**	-0.098**	-0.159	0.091	0.205**
	p value	0.054	0.257	0.122	**0.012**	0.154	**0.001**
Psychological health	r	0.154*	-0.140	-0.071	-0.018	-0.036	0.171
	p value	**0.015**	**0.027**	0.261	0.779	0.575	**0.007**
Social relations	r	0.192	-0.168	-0.232	-0.196	-0.223	0.260*
	p value	**0.002**	**0.008**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**
Environmental health	r	0.046	0.060	-0.087**	-0.088**	-0.040	0.193**
	p value	0.465	0.342	0.169	0.163	0.528	**0.002**
Total	r	0.197**	-0.112**	-0.179	-0.131**	-0.069	0.325**
	p value	**0.002**	0.077	**0.005**	**0.038**	0.276	**< 0.001**
Caregiver's Zarit Burden Inventory Score	r	-0.134*	0.180**	0.214**	0.181	0.176	-0.413**
	p value	**0.034**	**0.004**	**0.001**	**0.004**	**0.005**	**< 0.001**

COPD = Chronic Obstructive Pulmonary Disease, WHOQOL-BREF = World Health Organization Quality of Life Scale Short Form, HADS = Hospital Anxiety and Depression Scale.
*< 0.05. **< 0.001.

DISCUSSION

This study evaluated the relationship between the demographic, clinical, anxiety, and depressive characteristics of COPD patients and the quality of life and the burden of caregivers. The most important finding of the current study is that the patients' gender, NIV usage, regular LTOT and/or NIV device usage, presence of comorbid diseases, anxiety, and depressive symptoms are independent predictors of the burden of caregivers; and that the patients' NIV use, regular LTOT and/or NIV usage, anxiety, and depressive symptoms are independent predictors of quality of life of caregivers. The results of our study reveal that the patients' factors affecting the caregiving burden and quality of life of caregivers and that it has the potential to be a roadmap for the measures to be taken.

It is natural for some characteristics of patients with COPD, which is one of the most important causes of chronic respiratory failure, to affect caregivers. Our study has shown that patients' regular usage of LTOT and/or NIV devices improves the quality of life of caregivers and reduces the burden of care. To the best of our knowledge, although there has been no previous study addressing this issue, it is a normal result that patients' regular use of their devices positively affects caregivers. The most likely reason for this result may be that regular device usage reduces the workload of caregivers as it reduces hospital admissions and hospitalizations of patients [2, 3]. On the other hand, the

Table 4. The examination of the predictiveness of some demographic and clinical characteristics of COPD patients for the Zarit Burden Inventory WHOQOL-BREF scores of caregivers with the multiple linear regression analysis models

	Zarit Burden Inventory				WHOQOL-BREF			
	Coefficient of regression (95%CI)	SE	t value	*p value*	Coefficient of regression (95%CI)	SE	t value	*p* value
Age (year)	-0.075 (-0.169-0.020)	0.048	-1.553	0.122	0.061 (-0.022-0.145)	7.1940	1.4349	0.153
Gender (Female)	5.186 (2.582-7.789)	1.322	3.924	**< 0.001**	-2.317 (-4.642-0.008)	0.0429	-1.9629	0.051
Number of Hospitalizations (pcs)	-0.640 (-1.424-0.143)	0.398	-1.609	0.109	0.429 (-0.270-1.129)	1.1804	1.2083	0.228
Noninvasive mechanical ventilation (yes)	5.817 (3.160-8.473)	1.349	4.313	**< 0.001**	-4.472 (-6.845- -2.099)	0.3553	-3.7131	**< 0.001**
Regular device usage (yes)	-5.989 (-8.984- -2.995)	1.520	-3.940	**< 0.001**	4.702 (2.028-7.377)	1.2046	3.4640	**< 0.001**
Comorbidity (yes)	-4.568 (-8.187- -0.948)	1.837	-2.486	**0.014**	0.144 (-3.088-3.376)	1.3576	0.0878	0.930
Antidepressant use (yes)	-0.291 (-3.940-3.357)	1.852	-.157	0.875	2.290 (-0967-5.549)	1.6411	1.3849	0.167
HADS depression sub-scale score	1.787 (1.141-2.434)	0.328	5.444	**< 0.001**	-0.739 (-1.316- -0.161)	1.6542	-2.5200	**0.012**
HADS anxiety sub-scale score	-1.833 (-2.434- -1232)	0.305	-6.009	**< 0.001**	1.1587 (0.622-1.695)	0.2932	4.2534	**< 0.001**

COPD = Chronic Obstructive Pulmonary Disease, WHOQOL-BREF = World Health Organization Quality of Life Scale Short Form, HADS = Hospital Anxiety and Depression Scale

fact that the devices are being used as recommended by the clinician, and that they reduce the risk of sudden mortality/morbidity of patients may have a positive effect on caregivers [17]. We believe that this result is important because it objectively demonstrates the need for patients to follow the advice given by their physician.

The patients and caregivers spend a significant part of their time together, it is expected that the mental state of the patients will affect their caregivers. In our study, we found that patients' severity of the depression symptoms negatively affects the burden of care and quality of life of the caregivers. In a study of patients with COPD, it was reported that the caregivers' burden of providing care to patients who were depressed with a comorbid disease, which supports our results [9, 15, 16, 24, 25]. Contrary to the literature, there is a linear relationship between the increased severity of anxiety symptoms of patients and the burden of caregivers [7]. This is most likely because patients are struggling to recover due to anxiety, thereby reducing the burden on the caregiver [26]. This situation needs to be investigated through in-depth studies in the future. We think that social-psychological support should be provided in the early period to prevent the negative effects of the burden of care on the mental state of the caregivers.

In our study, similar to studies in the literature, it was found that the patient's female gender increased the burden of care and led to a decrease in the quality of life of the caregiver [7]. This may be associated with the increased workload of caregivers due to higher emotional expressions of female patients [7, 27]. Female patients' greater focus on the physical symptoms thus increasing the number of hospital and emergency department admissions may also be another reason [28]. Another reason may be that it is harder to care for female patients than male patients.

A similar study about caregivers burden was published by Baha *et al*. in 2022. The patients with COPD which were symptomatic and had comorbidities were related to increased burden of caregivers [29]. COPD already itself is associated with comorbidities. The patients with advanced COPD likely had chronic hypoxemia and this is related with pulmonary vascular remodeling, leading to an increase in pulmonary artery pressure [30]. The clinical severity of COPD progresses, hypoxia increases, pulmonary hypertension appears and pathological changes occur in the right heart, which leads to atrial fibrillation as one of the main comorbidity [31]. Our results were supported by the previous data.

Another finding of our work is that patient's use of NIV negatively affects the burden of care and the quality of life of the caregiver. As far as we know, this is the first time this result has been presented in the literature. This is most probably because that the patient's use of NIV leads caregivers to consider that the patients' disease is in an advanced stage. On the other hand, the use of NIV may cause the presence of secondary negative consequences such as more hospitalization and comorbidity [26].

Limitations

We should evaluate the results of our study within some limitations. First of all, the sample of the study may not reflect all COPD patients as our study population was provided from a tertiary chest diseases hospital. Secondly, the fact that the data was collected through the scales applied to patients may provide a clear case for the manipulation of patients. Finally, there was no control group in our study. Future studies with a control group will ensure that possible errors are excluded.

CONCLUSION

The results of our study, which evaluated the patient characteristics affecting the burden of care and quality of life of caregivers, may support sustainable psychosocial support policies for patients and caregivers. A positive impact on the quality of life and burden of care of caregivers will indirectly positively affect patient care and reduce mortality/morbidity. We think that patients and caregivers should be evaluated with a holistic approach, risk factors that may affect them should be identified in the early period and measures should be taken to eliminate the risks. Both the patient and caregivers should be provided with psychological and social support and culture-based communication.

Authors' Contribution

Study Conception: ZK, DÇ, MSA, İG, YTŞ; Study Design: ZK, DÇ, MSA, İG, YTŞ; Supervision: ZK, DÇ, MSA, İG, YTŞ; Funding: ZK, DÇ, MSA, İG,

YTŞ; Materials: ZK, DÇ, MSA, İG, YTŞ; Data Collection and/or Processing: ZK, DÇ, MSA, İG, YTŞ; Statistical Analysis and/or Data Interpretation: ZK, DÇ, MSA, İG, YTŞ; Literature Review: ZK, DÇ, MSA, İG, YTŞ; Manuscript Preparation: ZK, DÇ, MSA, İG, YTŞ and Critical Review: ZK, DÇ, MSA, İG, YTŞ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Characterization of clinical features of monosensitized and polysensitized allergic rhinitis patients with pollen allergy

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ABSTRACT

Objectives: The present study evaluates the prevalence of monosensitization and polysensitization in patients with pollen-hypersensitive moderate-to-severe persistent allergic rhinitis (AR), and determines the clinical characteristics of the two phenotypes.

Methods: This retrospective cohort study included 160 patients with moderate-to-severe persistent AR among the 3,699 patients who presented to allergy outpatient clinics who were found to have hypersensitivity to pollen based on a skin prick test and/or allergen-specific IgE positivity. The patients were divided into two groups: monosensitized (hypersensitivity to pollen alone), and polysensitized (hypersensitivity to pollen and other allergens). Both groups were evaluated for allergen hypersensitivity, symptoms of AR, symptom frequency and comorbidities related to AR.

Results: Of the 160 patients, 83 (51.9%) were monosensitized and 77 (48.1%) were polysensitized. The mean age was 29.5 ± 10.7 yeasrs and 28.3 ± 8.3 years, respectively and the female-to-male ratio was 42/41 and 47/30 in the two groups. Nasal congestion was remarkably more common in the polysensitized patients than in the monosensitized patients ($p = 0.01$). Hypersensitivity to weed mix and Cupressus arizonica pollen identified with a skin prick test was significantly more common in the polysensitized patients than in the monosensitized patients ($p = 0.03$ and $p = 0.01$, respectively). The two groups were similar in terms of the prevalence of asthma and other comorbidities related to rhinitis ($p = 0.78$).

Conclusions: In this single-center study, the rates of monosensitization and polysensitization were found to be similar in patients with pollen-hypersensitive moderate-to-severe AR, and the clinical characteristics of the polysensitized phenotype were different from those of the monosensitized phenotype.

Keywords: Pollen allergy, allergic rhinitis, monosensitization, polysensitization, skin prick test

Allergic rhinitis (AR) is a non-infectious form of rhinitis affecting 10-30% of all adults, and is characterized by a runny nose, congestion, itching and sneezing. Epidemiological studies have reported an increasing global prevalence of AR. Severe AR significantly affects quality of life, sleep and work performance. It is often associated with asthma, and is the main risk factor for the development of the condition [1].

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AR is characterized by nasal inflammation, occurring as a result of IgE-mediated hypersensitivity reactions triggered by the inhalation of respiratory allergens. Nasal symptoms are generally accompanied by eye symptoms. The inhaled allergens associated with AR are airborne protein-based antigens such as pollens, the fecal particles of house dust mites, cockroach residues and animal hair [2].

Hypersensitivity to more than one structurally different allergen (polysensitization) is the most common phenotype in patients with AR. Polysensitization is of considerable clinical and epidemiological significance and has been reported to be responsible for more than 50% of respiratory allergies among patients. Polysensitized patients have been reported to have quite different clinical characteristics from monosensitized patients, with more severe symptoms and greater detriment to quality of life [3, 4]. Patients may remain monosensitized for years and polysensitization may never develop. It should be noted that nasal allergic inflammations resulting from exposure to structurally different allergens can lead to the development of different symptoms and clinical characteristics [5].

Airborne pollens are the main triggers of respiratory allergies. The prevalence of pollen hypersensitivity is increasing worldwide under the effects of global climate change [6]. Hypersensitivity to other inhaled allergens may be detected alongside pollen hypersensitivity in patients with AR. A review of literature revealed no study comparing the prevalence and clinical characteristics of pollen-hypersensitive monosensitized and polysensitized patients with AR. It is not known whether the clinical characteristics of pollen-hypersensitive polysensitized patients with AR differ from those of monosensitized patients.

We present here a retrospective analysis of the prevalence of monosensitization and polysensitization among the patients with pollen-hypersensitive moderate-to-severe persistent AR who presented to our outpatient clinic, and evaluate the clinical characteristics of the two phenotypes.

METHODS

This retrospective cohort study included 160 adult patients with moderate-to-severe persistent AR who presented to the Ankara City Hospital Allergic Diseases outpatient clinics between April 1, 2022 and December 31, 2022, and who were found to have hypersensitivity to pollen based on a skin prick testing and/or allergen-specific IgE positivity. The study inclusion criteria were aged between 18-80 years, diagnosed with moderate-to-severe persistent AR, and identified with pollen hypersensitivity based on a skin prick test (grass and/or tree and/or weed and/or rye) and/or serum allergen-specific IgE positivity. Patients younger than 18 years, those with mild persistent AR and those without pollen hypersensitivity based on a skin prick test and/or serum allergen-specific IgE positivity were excluded from the study. Patients with moderate-to-severe persistent AR were identified using the ARIA (Allergic Rhinitis and its Impact on Asthma) criteria (symptoms of allergic rhinitis occurring at least 4 days a week over a period of at least 4 weeks, and symptoms resulting in sleep disorders, impairment in daily, entertainment and/or sport activities and/or impairment in school or work performance) [1].

Ethical approval was obtained from the Ankara City Hospital Ethics Committee (Date:18.01.2023, Decision no: E2-23-3263), and written informed consent was obtained from all study participants.

Skin Prick Tests

The skin prick tests made use of a standard panel consisting of grass mix *(Timothy, Orchard, june, Redtop, Meadow fescue, Perennial rye, Sweet vernal), Rye,* trees *(White birch, Olive tree, Salix nigra, Populus alba, Pinus strobus)*, weed mix *(Cocklebur, Rough pigweed, English plantain, Chenopodium album)* and *Mugwort, English plantain, Lamb's quarters, Short ragweed,* house-dust mites *(Dermatophagoides pteronyssinus and Dermatophagoides farinae),* cat and dog dander, cockroach *(Blatella germanica)* and molds *(Aspergillus fumigatus, Penicillium notatum, Alternaria alternata, Cladosporium herbarum)* (ALK®, Hørsholm, Denmark). A weal (edema with erythema) measuring at least 3 mm or greater in diameter than the negative control after 20 minutes was considered a positive reaction. Histamine dihydrochloride (10 mg/mL) was used for the positive control and physiological saline was used for the negative control.

Determination of Specific IgE and Total IgE Levels

The levels of allergen-specific IgE [Mite mix *(Dermatophagoides pteronyssinus, Dermatophagoides*

farinae, Dermatophagoides microceras, Lepidoglyphus destructor, Tyrophagus putrescentiae, Glycyphagus domesticus, Euroglyphus maynei, Blomia tropicalis) grass mix *(Viscum album, Festuca, Lolium temulentum, Phleum pratense, Poa pratensis),* weed mix *(Senecio vulgaris, Artemisia vulgaris, Plantago lanceolata, Chenopodium album, Silybum Marianum),* trees mix *(Quercus petraea, Ulmaceae, Platanus orientalis, Salix, Populus),* animal dander mix *(Cat, dog, horse, cow)* and mold mix *(Penicillium notatum, Cladosporium herbarum, Aspergillus, fumigatus, Candida albicans, Alternaria tenius)*] were quantified using the solid-phase, two-step chemiluminescent immunoassay system according to the manufacturer's instructions (Siemens, Immulite 2000 XP, USA). For allergen-specific IgE, the reference value was taken as > 0.35 kUA/L. Serum allergen-specific IgE levels were classified as follows: Class 0: 0-0.35 kUA/L; Class 1: 0.35-0.69 kUA/L; Class 2: 0.70-3.49 kUA/L; Class 3: 3.50-17.49 kUA/L; Class 4: 17.5-49.9 kUA/L; Class 5: 50–100 kUA/L; and Class 6: > 100 kUA/L. Total IgE levels were measured using a two-site sandwich immunoassay technology and direct chemiluminescence (Siemens, Atellica, IM 1600, Ireland).

The blood eosinophil count was determined from leucocyte measurements (Siemens Advia 2120i, Ireland).

The patients were divided into two groups as monosensitized and polysensitized, based on the results of the skin prick test and/or serum allergen-specific IgE levels. Patients with pollen hypersensitivity alone were defined as monosensitized (grass pollen and/or tree pollen and/or weed pollen and/or rye pollen) and those with hypersensitivity to both pollens and structurally different antigens (hypersensitivity to house dust mites and/or fungi and/or cat/dog and/or cockroach) were defined as polysensitized.

Statistical Analysis

IBM SPSS Statistics (Version 26.0. Armonk, NY: IBM Corp.) was used for the statistical analysis. The fitness of the variables to normal distribution was tested visually (histogram and probability graphs) and using analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Among the descriptive statistics, normally distributed variables were expressed as mean and standard deviation, variables without normal distribution were expressed as median and interquartile ranges, and ordinal variables were expressed as frequencies. An independent samples t-test was used to compare normally distributed variables and a Mann-Whitney U test was used to compare variables without normal distribution between the pollen-hypersensitive monosensitized and polysensitized patients with AR. The differences between variables were tested with a Chi-square test or Fischer's exact test (the latter was used when the cell value did not meet the assumptions of the Chi-square test). A p - value of less than 0.05 was considered statistically significant.

RESULTS

The medical charts of 407 patients with moderate-to-severe persistent allergic rhinitis, randomly selected from among 3,699 patients examined in a single allergy outpatient clinic between April 1, 2022 and December 31, 2022, were reviewed. No hypersensitivity to any allergen was determined in 48% (n = 195) of the patients based on a skin prick test and/or allergen-specific IgE measurement, while 12.7% (n = 52) had no pollen hypersensitivity. The study thus continued with 160 patients (39.3%) who met the study inclusion criteria and who had pollen hypersensitivity based on a skin prick test and/or serum allergen-specific IgE positivity. The mean age of the patients was 28.9 ± 9.6 years and the female-to-male ratio was 89:71. The mean duration of AR symptoms was 59.7 ± 45.4 months.

Of the 160 patients, 83 (51.9%) were monosensitized and 77 (48.1%) were polysensitized. The mean age was 29.5 ± 10.7 and 28.3 ± 8.3, respectively and the female-to-male ratio was 42/41 and 47/30 in the two groups (p = 0.96 and p = 0.18, respectively). The duration of AR symptoms did not differ between the two groups (p = 0.72). The analysis of symptom frequency revealed that although the rate of patients with symptoms during the pollen season was higher among the monosensitized patients, the rate of those with seasonal (pollen season) and perennial (throughout the year) symptoms and perennial symptoms with seasonal exacerbations did not differ significantly between the two groups (p = 0.29). Non-smokers constituted the majority in both groups (p = 0.73). Although the prevalence of asthma was higher among

Table 1. Characteristics of the study population

	Monosensitized n = 83	Polisensitized n = 77	*p value**
Sex (F/M)	42/41	47/30	0.18
Age (years), (Mean±SD)	29.5 ± 10.7	28.3 ± 8.3	0.96
Duration of rhinitis (months), median (min-max)	48 (3-240)	48 (3-180)	0.72
Frequency of the symptoms, n (%)			
Perennial	23 (31.1)	23 (33.8)	0.29
Seasonal	40 (54.1)	29 (42.6)	
Perennial but seasonally exacerbated	11 (14.9)	16 (23.5)	
Smoking history, n (%)			
Non-smoker	21 (61.8)	23 (65.7)	0.73
Smoker	13 (38.2)	12 (34.3)	
Rhinitis-related comorbitidies, n (%)			
None	76 (91.6)	67 (87)	0.78
Asthma	4 (4.8)	7 (9.1)	
CRS with NP	2 (2.4)	2 (2.6)	
CRS without NP	1 (1.2)	1 (1.3)	
Symptoms, n (%)			
Runny nose	68 (81.9)	64 (84.2)	0.7
Sneezing	66 (79.5)	59 (78.7)	0.89
Itchy nose	33 (40.2)	24 (31.6)	0.25
Postnasal drip	34 (41)	30 (40)	0.9
Nasal obstruction	32 (38.6)	44 (57.9)	**0.01**
Conjuctivitis	46 (55.4)	36 (47.4)	0.31
Cough	11 (13.3)	7 (9.1)	0.4
Prick test positivity, n (%)	80 (96.4)	75 (97.4)	0.51
Pollen allergen spesific IgE positivity, n (%)	15 (18.1)	13 (16.9)	0.91
Total IgE (IU/ml), median (min-max)	111 (3.9-275.5)	234 (28.6-1494.4)	0.06
Eosinophil (cell/mcL), median (min-max)	170 (0-840)	170 (10-740)	0.73

CRS = Chronic sinusitis, NP = nasal polyp.
*$p < .05$ was considered statistically significant

polysensitized patients (n = 7 vs n = 4), the prevalence rates of asthma and other comorbidities related to AR [Chronic sinusitis (CRS) with nasal polyp (NP) and CRS without NP] were similar between the two groups ($p = 0.78$). The most common symptoms were runny nose (81.9%), sneezing (79.5%) and eye symptoms (conjunctivitis) (55.4%) among the monosensitized patients, and runny nose (84.2%), sneezing (78.7%) and nasal congestion (57.9%) among the polysensitized patients. Although itchy nose, sneezing and eye symptoms were more common in the monosensitized patients than in the polysensitized patients, the difference between the two groups was not statistically significant ($p = 0.25$, $p = 0.89$, and $p = 0.31$, respectively). In contrast, nasal congestion was a remarkable symptom that was more common in the polysensitized patients than in the monosensitized patients ($p = 0.01$) (Table 1).

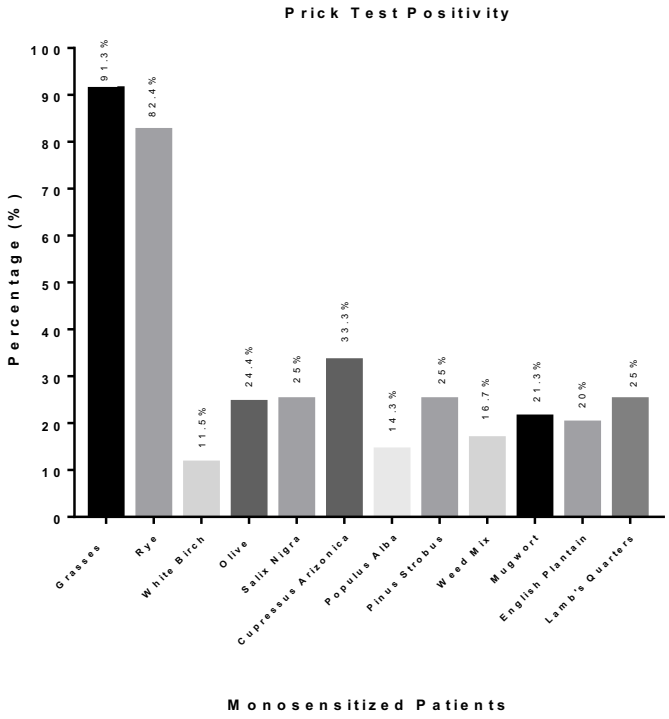

Fig. 1. Prevalence of pollen allergen positivity based on a skin prick test in monosensitized patients.

A positive skin prick test to pollen was found in 96.4% of the monosensitized patients and 97.4% of the polysensitized patients, while the rate of pollen-specific IgE positivity was 18.1% and 16.9%, respectively. Although the total IgE was higher in the polysensitized patients, there was no statistically significant difference between the two groups ($p = 0.06$), and the serum eosinophil count of the groups was also similar ($p = 0.73$) (Table 1).

The distribution of pollen hypersensitivity in the skin prick test in the monosensitized patients was as follows: grass pollen (91.3%), rye pollen (82.4%),

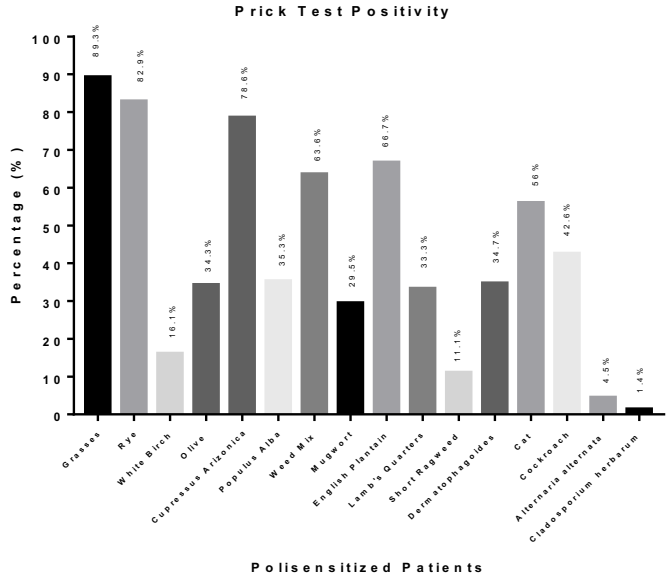

Fig. 2. The prevalence of pollen allergen positivity based on a skin prick test of polysensitized patients.

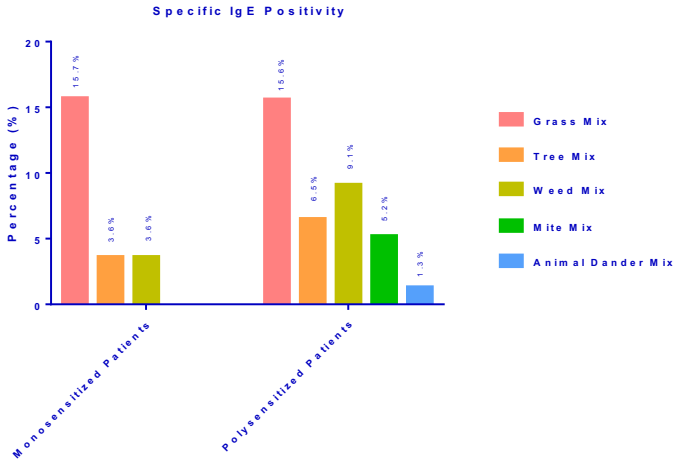

Fig. 3. Distribution of pollen-specific IgE positivity in monosensitized and polysensitized patients.

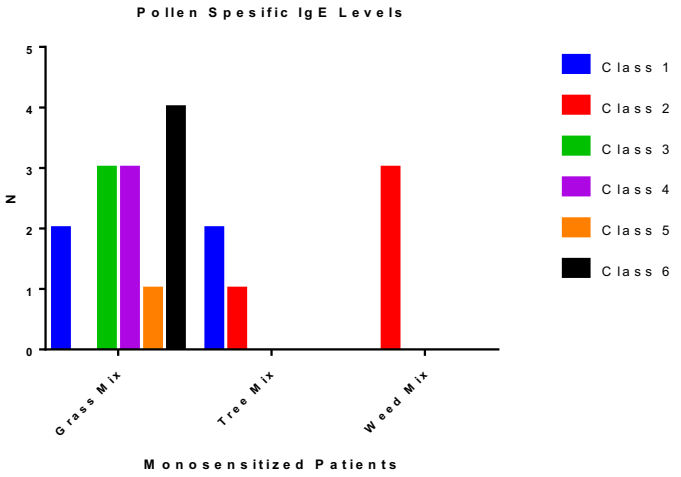

Fig. 4. Distribution of pollen-specific IgE levels in monosensitized patients.

White birch pollen (11.5%), olive pollen (24.4%), Salix nigra pollen (25%), Cupressus arizonica pollen (33.3%), Populus alba pollen (14.3%), Pinus strobus pollen (25%), Weed mix pollen (16.7%), Mugwort pollen (21.3%), English plantain pollen (20%) and Lamb's quarters pollen (25%) (Fig. 1).

The distribution of allergen hypersensitivity in the polysensitized patients was as follows: grass pollen (89.3%), rye pollen (82.9%), White birch pollen (16.1%), olive pollen (34.3%), Cupressus arizonica pollen (78.6%), Populus alba pollen (35.3%), Weed mix pollen (63.6%), Mugwort pollen (29.5%), English plantain pollen (66.7%), Lamb's quarters pollen (33.3%), Short ragweed pollen (11.1%), house dust mites (34.7%), cat (56%), cockroach (42.6%), Alternaria (4.5%) and Cladosporium (1.4%) (Fig. 2). A comparison of pollen hypersensitivity in the monosensitized and polysensitized patients based on a skin prick test showed hypersensitivity to weed mix and Cupressus arizonica pollen to be significantly more common among the polysensitized patients than the monosensitized patients ($p = 0.03$ and $p = 0.01$, respectively).

One striking finding was that hypersensitivity to weed mix identified through the skin prick test was more common among the polysensitized patients with seasonal AR symptoms, while hypersensitivity to Cupressus Arizonica and Lamb's Quarters was significantly more common than the monosensitized patients with perennial symptoms ($p = 0.008$, $p = 0.03$, and $p = 0.01$, respectively).

Among the serum pollen-specific IgE values, grass mix pollen positivity was the most common (15.7% vs 15.6%) in both the monosensitized and polysensitized groups ($p = 0.6$). Furthermore, tree mix and weed mix specific-IgE positivity were more common among the polysensitized patients than the monosensitized patients but not statistically different ($p = 0.23$, $p = 0.05$, respectively). Furthermore, 5.2% and 1.3% of polysensitized patients were identified with house dust mite and animal dander mix specific IgE positivity, respectively (Fig. 3). Figs. 4 and 5 presents the serum specific IgE levels of the monosensitized and polysensitized patients. No significant difference

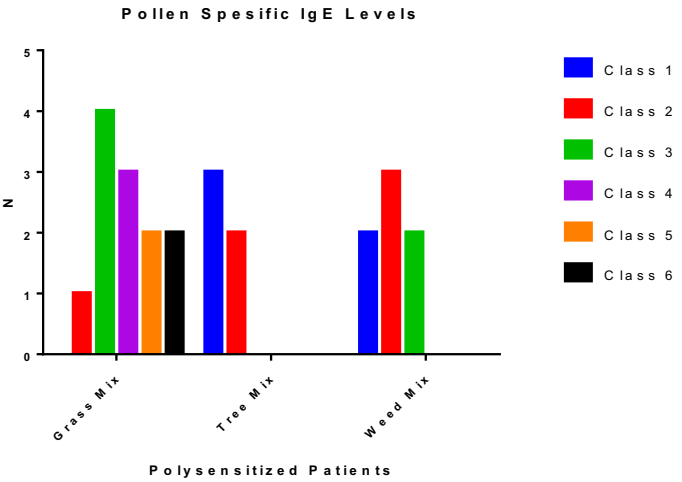

Fig. 5. Distribution of pollen- and non-pollen allergen specific IgE levels in polysensitized patients.

was found in the grass mix, tree mix and weed mix-specific IgE levels of the two groups ($p = 0.7$, $p = 1$, and $p = 0.3$, respectively).

DISCUSSION

IgE-mediated immune response can vary among those exposed to environmental aeroallergens and hypersensitivity to a single allergen (monosensitization), or multiple allergens (polysensitization) may occur. Monosensitized and polysensitized patients also differ in terms of their immune responses. It has also been reported that polysensitized patients exhibit different clinical characteristics to monosensitized patients [7]. This has necessitated the characterization of monosensitized and polysensitized patients in epidemiological studies. There has, however, been no study to date identified comparing the prevalence and clinical characteristics of monosensitized and polysensitized patients who present to allergy outpatient clinics with pollen-hypersensitive AR. The present study can thus be considered the first retrospective cohort study conducted on this subject.

According to general population data, the rate of polysensitized patients ranges from 20% to 90%. In a cohort study by Arbes *et al*. analyzing the results of the skin prick test screening of 10,863 people, no hypersensitivity to any allergen was reported in 45.7% while 15.5% were classified as monosensitized and 38.8% as polysensitized [8]. In our review of literature, the reported rate of polysensitization among patients who presented with respiratory allergies was 27.5%, 73.5%, 62%, 31% and 74.3%, respectively [5, 9-12].

Although the data in literature on allergic patients varies depending on the studied population and the study region, all report polysensitization to be more common in this patient population. It has been speculated that monosensitized patients develop hypersensitivity to other allergens over time. In a retrospective analysis of 165 monosensitized children, Silvestri *et al*. reported that 43.6% became polysensitized during follow-up, and also that hypersensitivity to house dust mites, and to a lesser extent, pollen, acts as a triggering factor for the development of polysensitization [13]. It does not seem feasible, however, to directly compare the data derived from different studies. In a review of epidemiological and clinical studies, Calderon *et al*. highlighted that the rate of polysensitization is in the range of 51-81% [14]. In the present study involving a cohort of 3,699 patients, moderate-to-severe persistent allergic rhinitis was identified in 11% of the sample, and pollen hypersensitivity was found in 39.3% of these patients based on a skin prick test and/or allergen-specific IgE measurement, with 51.9% being monosensitized and 48.1% polysensitized. Monosensitization and polysensitization rates that are close to each other appear as a different finding to those reported in literature.

Allergen immunotherapy is the sole treatment method with the ability to change the natural course of allergic disorders. That said, the presence of hypersensitivity to multiple structurally different allergens (polysensitization) in patients presenting to the allergy outpatient clinic with moderate-to-severe persistent AR can make decisions of whether or not to deliver immunotherapy challenging. The symptoms of AR may not be seasonal in patients with pollen hypersensitivity and may occur throughout the year. Knowing patient-specific clinical characteristics can guide the therapy, and is of particular importance in the selection of the allergen as the specific target of immunotherapy in patients with hypersensitivity to pollens and structurally different allergens (i.e. house dust mite, animal dander) associated with perennial symptoms. For the above reasons, real-life, multicenter and observational studies named POLISMAIL (Polysensitization Impact on Allergen Immunotherapy) have been conducted to characterize polysensitized patients in clinical practice [3]. The first study in this series investigated the clinical characteristics of 418 polysensitized patients, and identified 220 patients with AR and 198 patients with AR accompanying asthma, with a median allergen hypersensitivity score of 3.65. Nasal symptoms were found to be more severe in the polysensitized patients than in the monosensitized patients, and the identified allergen hypersensitivities were grass pollen (76.4%), Parietaria pollen (38.9%), birch pollen (38.3%), olive pollen (26.7%), cypress pollen (9.6%), ragweed pollen (23.3%), house dust mites (47.4%), cat dander (22.5%), dog dander (13.2%), and Alternaria (10.4%) (15). In the present study, grass pollen, rye pollen, Cupressus arizonica and weed mix in the pollen group,

and cat dander and cockroach were the most commonly observed allergens in the polysensitized patients.

Studies in literature have reported that polysensitized patients exhibit different clinical characteristics to monosensitized patients, with a particular negative impact on quality of life [16]. One study reported that polysensitized patients develop more severe symptoms than monosensitized patients, and polysensitization has been found to be associated with a higher frequency of concurrent asthma than monosensitization [16]. The present study identified no difference between monosensitized and polysensitized patients in terms of the presence of asthma and other comorbidities related to AR.

In their study, Ciprandi *et al*. [17] reported polysensitized patients to have higher rhinitis symptom scores than monosensitized patients, but no difference in the symptom durations of monosensitized and polysensitized patients. The higher symptom scores, indicating symptom severity, among the polysensitized patients were attributed to the contribution of perennial allergens to the development of chronic inflammation. It was observed that irritative symptoms (runny nose, sneezing and itchy nose) and conjunctivitis were more common among monosensitized patients than polysensitized patients, although the rate of nasal congestion was similar in the two groups [17]. The symptom severity of the two groups could not be compared in the present study due to retrospective nature of the study and the lack of accessible data related to the symptom severity. The most common symptoms, however, were runny nose, sneezing and conjunctivitis in the monosensitized patients, and itchy nose, sneezing and nasal congestion in the polysensitized patients. In contrast to the above-mentioned study, the rate of nasal congestion in the present study was significantly higher in the polysensitized patients than in the monosensitized patients.

Regarding the issue of lifelong monosensitization, studies have reported functional T regulatory cell defects in polysensitized patients and higher IL-10 and IFN-γ levels in monosensitized children than in polysensitized children [18], which supports the notion that monosensitization and polysensitization are two different phenotypes. There is thus a need for large cohort studies providing a comparative evaluation of the immunological and clinical characteristics of monosensitized and polysensitized patients. There has been no large cohort study in literature to date comparing monosensitized and polysensitized patients and their experience with pollen-related complaints. Our present preliminary data as a pilot, retrospective cohort study.

The type of allergens involved also seems to be related to the clinical characteristics of AR. It has been demonstrated that each structurally different allergen is associated with different immunological, inflammatory, functional and clinical consequences [3]. For this reason, the presence of hypersensitivity alongside perennial allergens in polysensitized patients with pollen hypersensitivity suggests that the symptoms in polysensitized patients may differ from those observed in monosensitized patients.

Studies in literature have reported that approximately 50% of patients with allergic rhinitis are hypersensitive to any pollen allergen, and that the prevalence of pollen allergies has doubled in recent years [19]. One study determined pollen hypersensitivity in 72% patients with moderate-to-severe persistent AR. In European countries in particular, grass pollen is reported to be responsible for 40% of cases of pollen allergy [20]. A study conducted in Mexico, consistent with previous studies, reported higher rates of polysensitization and house dust mite hypersensitivity, accompanied by at least one pollen hypersensitivity (one of Lamiales, Fagales, and Cupressales). In contrast, in European countries, the rate of hypersensitivity to tree pollen is four times the hypersensitivity associated with grass pollen [21]. In the present study, hypersensitivity to grass pollen took first place in both groups, while the positive reaction to weed mix and Cupressus arizonica pollens was remarkably higher among the polysensitized patients than the monosensitized patients.

AR is traditionally divided into two groups, being seasonal and perennial, depending on the frequency of symptoms throughout the year. Pollens are the leading allergen causing to symptoms of seasonal allergic rhinitis. That said, some patients who are hypersensitive to pollens may exhibit perennial symptoms due to the fact that some plants have prolonged pollination periods, that pollen seasons vary from one country to another, and based on such factors as the presence of

polysensitization. In support of this observation, studies in literature have reported perennial symptoms (throughout the year) in 44.6% of patients with AR and pollen allergies [18]. In the present study, the majority of patients exhibited seasonal AR symptoms, while only 28.7% had perennial AR symptoms, although monosensitized and polysensitized patients were similar in terms of symptom frequency. Interestingly, the present study observed that the Cupressus arizonica and Lamb's quarters pollen positivity identified through a skin prick test increased perennial symptoms and weed mix pollen positivity, and increased seasonal symptoms in polysensitized patients.

Limitations

The main limitations of the present study are its retrospective study design and the small number of patients in the study cohort. Due to retrospective nature of the study, the absence of symptom scores and quality of life parameters in the patient charts is another limitation.

CONCLUSION

Polysensitized patients with pollen hypersensitivity exhibit different clinical characteristics to those identified in monosensitized patients. Weed mix and Cupressus arizonica pollen positivity would appear to be associated with the development of different clinical characteristics. The authors consider the present study to be an important pilot study that contributes to literature in the sense that there has, as yet, been no prospective cohort study in literature comparing the clinical characteristics of monosensitized and polysensitized patients with pollen hypersensitivity. The authors stress that there is a need for large cohort studies of patients with pollen allergy to investigate this issue further.

Authors' Contribution

Study Conception: ŞS; Study Design: ŞS; Supervision: ŞS; Funding: ŞS; Materials: DÖÇ; Data Collection and/or Processing: DÖÇ; Statistical Analysis and/or Data Interpretation: ŞS; Literature Review: DÖÇ; Manuscript Preparation: ŞS and Critical Review: ŞS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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Investigation of the relationship between modified Glasgow prognostic score and no-reflow phenomenon in patients undergoing primary percutaneous coronary intervention for ST-elevation myocardial infarction

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ABSTRACT

Objectives: No-reflow phenomenon (NRP) is a complication associated with poor clinical outcome in patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (pPCI). The modified Glasgow prognostic score (mGPS) is a novel immune-inflammatory index, derived from C-reactive protein (CRP) and serum albumin levels and has been shown to be associated with prognosis in heart disease. In this study we aimed to investigated the relationship between mGPS and NRP in patients undergoing pPCI for STEMI.

Methods: A total of 379 patients (aged 59 ± 9.9 years; 54.9% male) were enrollled. The patients were divided into 2 groups:no-reflow (n = 72) and reflow (n = 307). No-reflow was defined as thrombolysis in myocardial infarction (TIMI) ≤ 2 flow. The mGPS of all patients was calculated from blood samples at admission. Logistic regression analysis was performed to determine the independent predictive factors for NRP.

Results: Mean age, pain to balloon duration, troponin T, white blood cell (WBC), Syntax score, neutrophil to lymphocyte ratio (NLR), glucose level, C-reactive protein level (CRP), diabetic and female patient ratio were higher, while left ventricular ejection fraction, ST segment resolution ratio at 60 min, and serum albumin level were lower in the NRP group.Logistic regression analysis showed that WBC count [Hazard ratio (HR): 0.816, 95% confidence interval (CI): 0.728-0.914, $p < 0.001$], NLR (HR: 0.482, CI: 0.355-0.654, $p < 0.001$), pain-to-balloon time (HR: 0.976, CI:0.960-0.991, $p = 0.002$) and mGPS (HR: 3.213, CI: 1.643- 6.283, $p = 0.001$) were independent predictive factors for NRP.

Conclusions: Modified GPS is an independent predictive factor for NRP in patients undergoing pPCI for STEMI.

Keywords: ST-segment elevation myocardial infarction, modified Glasgow prognostic score, no-reflow phenomenon, primary percutaneous coronary intervention

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The aim of treatment of ST-segment elevation myocardial infarction (STEMI) is to achieve rapid and permanent reperfusion as soon as possible. Currently, the most effective and widely used reperfusion therapy is primary percutaneous coronary intervention (pPCI) [1]. No-reflow phenomenon (NRP) is defined as a tissue-level perfusion defect despite restoration of epicardial coronary arteries during percutaneous coronary intervention (PCI). NRP is frequently observed in patients undergoing PCI for acute myocardial infarction [2]. Hypertension, smoking, dyslipidemia, diabetes, renal failure and inflammatory parameters have been shown to be risk factors for NRP in many studies. In addition, a recent study has also demonstrated an association between increased inflammatory status and NRP in elderly individuals [3].

The Modified Glasgow Prognostic Score (mGPS) is a scoring system based on elevated C-reactive protein (CRP) and low serum albumin (SA) levels among inflammatory parameters and has been shown to be effective in prognosis mainly in patients with malignancy [4]. In addition, recent studies have shown an association of mGPS with prognosis in patients with heart failure and acute coronary syndrome [5, 6]. There are also substantial studies demonstrating the association of CRP and albumin, components of mGPS, with NRP [7, 8]. However, according to our literature, there is no study investigating the relationship between NRP and mGPS in patients undergoing pPCI for STEMI and this study was performed to demonstrate this relationship.

METHODS

Patient Population

A total of 379 patients aged 18 to 80 years who underwent pPCI for STEMI between November 2022 and March 2023 were included in this retrospective cross-sectional study. STEMI was diagnosed with symptoms of myocardial ischemia and ST-segment elevation ≥ 1 mm in the two adjacent inferior leads or ≥ 2 mm in the precordial leads, or the presence of newly developed left bundle branch block and elevated cardiac markers. Patients with previous history of coronary artery disease, dysrhythmia, cardiogenic shock, pain for more than 12 hours, fibrinolytic therapy, active infection or chronic autoimmune disease, hematological disease, end-stage renal or hepatic failure, and known malignancy were excluded from the study. The study protocol was approved by the Ethics Committee of Bilecik Şeyh Edebali University Faculty of Medicine in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants.

Coronary Angiography

All patients diagnosed with STEMI received 300 mg acetylsalicylic acid with 600 mg clopidogrel or 180 mg ticagrelor. Intravenous bolus unfractionated heparin at a dose of 50-70 units/kg was administered to patients who were decided to undergo pPCI. Angiographic evaluations were performed by two experienced invasive cardiologists blinded to the study design using the Artis Zee Floor (Siemens Medical Solution, Erlingen, GERMANY). NRP was visually assessed according to Thrombolysis In Myocardial Infarction (TIMI) flow grade after pPCI.Accordingly, TIMI 0: no flow after the responsible lesion, TIMI 1: opaque is present after occlusion but fails to fill the entire vessel, TIMI 2: opaque fills the entire vessel but with a slower than normal flow, and TIMI 3: normal coronary flow is present. No-reflow was defined as flow in the responsible artery (IRA) ≤ TIMI 2 in the absence of dissection, thrombus or spasm. Patients with residual stenosis below 20% and TIMI 3 flow on IRA were defined as the reflow group.

Laboratory Measurements

Routine biochemical and hematologic parameters of the patients were obtained from the results of analysis of venous blood samples obtained from the antecubital region before coronary angiography. Creatinine kinase MB (CK-MB) and cardiac troponin T (Tn-T), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, platelet count, neutrophil count, lymphocyte count, serum creatinine, blood glucose, CRP, SA level and other biochemical and hemogram parameters were analyzed. Neutrophil to lymphocyte ratio (NLR) was calculated as the ratio of neutrophil count to lymphocyte count. Glomerular filtration rate was calculated using the Cockcroft-Gault equation.

Definition of Cardiovascular Risk Factors

Hypertension was diagnosed if systolic blood

pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or both; or if the individual was taking antihypertensive medication. Patients were defined as having diabetes mellitus (DM) if they were taking anti-diabetic medication or had a fasting glucose level ≥ 126 mg/dL or HbA1c value ≥ 6.5% in at least two measurements. Patients who smoked at least 1 cigarette per day for at least 1 year were defined as smokers. Familial coronary artery disease was defined as the presence of coronary artery disease in a first-degree female relative aged < 65 years or a first-degree male relative aged < 55 years. Hyperlipidemia was diagnosed if total cholesterol >200 mg/dL, LDL-cholesterol > 130 mg/dL, triglycerides > 150 mg/dL or if the patient was on lipid-lowering medication.

Modified Glasgow Prognostic Score (mGPS):

The modified GPS score was calculated as score 0: SA level ≥ 3.5 g/dL and CRP ≤ 1 mg/dL, score 1: SA level ≥ 3.5 g/dL and CRP > 1 mg/dL, score 2: SA level < 3.5 g/dL and CRP > 1 mg/dL.

Electrocardiographic Analysis

A 12-lead standard electrocardiogram (ECG) was performed in all patients at admission and 60 minutes after pPCI. ECG evaluation was performed by two expert cardiologists blinded to the other data of the patients. ST segment elevation was measured in millivolts 20 ms after the J point. Total ST segment elevation in leads DI, aVL, V1-V6 was calculated for noninferior infarction and total ST segment elevation in leads D2, D3, aVF, V5, V6 was calculated for inferior infarction. ST resolution (STR) was calculated as a percentage by calculating the total depression in ST elevation at the specified localizations 60 min after pPCI and proportioning it to the total ST elevation at baseline. Accordingly, patients were classified according to STR as complete STR (≥ 50%) and incomplete (STR < 50%) [9].

Statistical Analysis

SPSS 24.0 version software package (Chicago, IL, USA) was used to analyze the data obtained. A p value < 0.05 was accepted for statistical significance. Visual histogram and Kolmogorow-Smirnow test variables were used for normal distribution assessment. Levene's test was used for homogeneity of variances. Mean ± standard deviation and median and interquartile ranges (25^{th}-75^{th} percentiles) were used for normally distributed continuous variables and abnormally distributed continuous variables, respectively. Categorical variables were expressed as percentages and Chi-square test was used for comparison. Student's t-test was used for the comparison of normally distributed continuous variables and Whitney U test was used for variables that did not fit normal distribution. Univariate regression analysis was performed to determine the variables associated with no-flow and the factors that were significant were included in multivariate regression analysis to determine the independent predictors of no-reflow.

RESULTS

A total of 379 patients, 72 in the NRF group and 307 in the reflow group, were included in the study. The mean age of the patients was 59.9 ± 9.9 years and the male proportion of the population (n = 208) was 54.9%. Demographic characteristics and baseline clinical data of the patients are shown in Table 1. Patients in the NRF group had a higher mean age [62 (58-67) vs. 59 (51-67), $p = 0.002$], diabetes (54.2 % vs. 38.4 %, $p = 0.015$) and female (55.6 % vs. 42.7 %, $p = 0.048$) ratio. The history of drug use was similar in both groups. Pain-to-balloon time was observed to be longer in the NRP group [80 (60-90) vs. 60 (45-80), $p < 0.001$]. When both groups were compared in terms of laboratory and admission physical examination findings, peak TnT [4567 (3031-6536) vs. 3654 (1745-5231), $p < 0.001$], WBC count [13.8 (11.4-15.4) vs. 11.2 (10.2-13), $p < 0.001$], Syntax score [20 (16-24) vs. 18 (12-21), $p < 0.001$], proportion of patients with < 50% STR at 60 min (86.1% vs. 18.9%, $p < 0.001$), NLR [3.2 (2.5-4.2) vs. 2.1 (1.5-2.8), $p < 0.002$], glucose level [187 (146-219) vs.155 (127-200), $p = 0.001$], CRP level [4 (0.8-6) vs. 1.8 (0.7-4.3), $p < 0.001$] and Killip class ≥ 2 (9.7 % vs. 2.6 %, $p = 0.005$) were higher, while LVEF [43 (40-45) vs. 45 (40-50), $p = 0.008$] and SA level [4.0 (3.8-4.1) vs. 4.2 (4.1-4.3), $p < 0.001$] were lower in the NRP group. Modified GPS 1 (50 % vs. 20.8%) and mGPS 2 (9.7% vs. 2.3%) rates were higher in the NRP group (Table 1).

Patients were further categorized as mGPS =0 (group 1) and mGPS ≥ 1 (group 2). Group 2 patients had higher age [62 (55-68) vs. 60 (52-66), $p = 0.037$],

Table 1. Baseline characteristics of the study population

Variable	No-reflow (n = 72)	Reflow (n = 307)	*p* value
Age (years)	62 (58-67)	59(51-67)	0.002
Gender (female), n (%)	40(55.6 %)	130(42.7%)	0.048
BMI (kg/m2)	26.9(24.4-29.4)	27.4(24.8-30.1)	0.420
Diabetes mellitus, n (%)	39(54.2%)	118(38.4%)	0.015
Hypertension, n (%)	28(38.9%)	95(30.9%)	0.195
Current smoking, n (%)	19(26.4 %)	86(28 %)	0.453
ASA/P2Y12-inh, n (%)	18(25%)	80(26.1%)	0.492
ACEI/ARB, n (%)	23(31.9%)	92(30.0%)	0.743
BB, n (%)	18(25%)	76(24.8%)	0.966
CCB, n (%)	6(8.0%)	22(7.1%)	0.756
Statin, n (%)	15(20.8%)	62(20.2%)	0.904
SKB (mmHg)	132(110-140)	130(110-140)	0.089
DKB (mmHg)	85(76-91)	80(70-90)	0.153
Heart rate, (beat/min)	80(70-86)	78(68-86)	0.706
Pain-to-balon time (sec)	80(60-90)	60(45-80)	**< 0.001**
LVEF (%)	43(40-45)	45(40-50)	**0.008**
SYNTAX score	20(16-24)	18(12-21)	**< 0.001**
Peak troponın T (ng/L)	4567(3031-6536)	3654(1745-5231)	**<0.003**
WBC Count (10^3/μL)	13.8(11.4-15.4)	11.2(10.2-13)	**< 0.001**
NLR	3.2(2.5-4.2)	2.1(1.5-2.8)	**< 0.001**
Hemoglobin (g/dL)	14(13-15)	14.1(13-15)	0.474
Platelet count ($\times10^9$/L)	261(157-361)	257(165-401)	0.244
Creatinine (mg/dL)	0.93(0.8-1.1)	0.9(0.7-1.0)	0.850
Glucose (mg/dL)	187(146-219)	155(127-200)	**0.001**
Total cholesterol (mg/dL)	200(186-212)	199(181-213)	0.721
Triglycerides (mg/dL)	179(158-198)	178(158-199)	0.893
HDL-C (mg/dL)	37(33-39)	36(33-40)	0.820
LDL-C (mg/dL)	149(133-160)	142(127-157)	0.107
CRP (mg/L)	4.0(0.8-6)	1.8(0.75-5.2)	**< 0.001**
Albumin (mg/dL)	4.0(3.8-4.1)	4.2(4.1-4.3)	**< 0.001**
mGPS, n (%)			
0	29(40.3%)	236(76.9%)	**< 0.001**
1	36(50.0%)	64(20.8%)	**< 0.001**
2	7(9.7%)	7(2.3%)	**< 0.001**
STR (%)			
≥ 50	10(13.9%)	249(81.1%)	**< 0.001**
< 50	62(86.1%)	58(18.9%)	**< 0.001**
Killip class, n(%)			
Class 1	65(90.3%)	299(97.4%)	**0.005**
Class ≥2	7(9.7%)	8(2.6%)	**0.005**

CCB = Calcium channel blocker, ACEI = Angiotensin-converting enzyme inhibitor, ARB = Angiotensin receptor blockers, ASA = acetylsalicylic acid, BB = Beta-blocker, BMI = Body Mass Index, CRP = C-reactive protein, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, LVEF = Left ventricle ejection fraction, WBC = white blood cell, STR = ST-segment resolution, SBP = systolic blood pressure, DBP = diastolic blood pressure, SYNTAX = Synergy between PCI with Taxus and Cardiac Surgery, NLR = Neutrophıl lymphocyte ratıo, mGPS = modified Glasgow prognostic score

Table 2. Baseline characteristics according to the mGPS groups

Variable	mGPS ≥ 1 (n = 82)	mGPS = 0 (n = 297)	*p* value
Age (years)	62 (55-68)	60 (52-66)	**0.037**
Gender (female), n (%)	45 (54.9%)	126 (42.4%)	**0.045**
BMI (kg/m^2)	27.2 (24.2-28.7)	27.3 (24.9-30.1)	0.177
Diabetes mellitus, n (%)	40 (48.8%)	117 (39.4%)	0.127
Hypertension, n (%)	28 (34.1%)	95 (32.0%)	0.712
Current smoking, n (%)	26 (31.7 %)	79 (26.6 %)	0.218
ASA/P2Y12-inh, n (%)	19 (23.2%)	79 (26.6%)	0.317
ACEI/ARB, n (%)	24 (29.3%9	91 (30.6%)	0.811
BB, n (%)	21 (25.6%)	73 (24.6%)	0.848
CCB, n (%)			
Statin, n (%)	18 (22%)	59 (19.9%)	0.678
SKB (mmHg)	130 (110-140)	131 (110-142)	0.820
DKB (mmHg)	81 (70-90)	80 (72-90)	0.676
Heart rate (beat/min)	79 (66-88)	78 (68-86)	0.484
Pain-to-balon time (sec)	70 (50-90)	60 (50-80)	**0.045**
LVEF (%)	44.5 (40-48)	45 (40-48)	0.281
SYNTAX score	18 (14.7-23)	19.8 (12-21.5)	**0.036**
Peak troponın T (ng/L)	4696 (2504-6605)	3654 (1980-5231)	**0.002**
WBC Count (10^3/μL)	12.6 (11-15)	11.3 (10.1-13.4)	**< 0.001**
NLR	2.8 (1.7-3.9)	2.1 (1.6-2.9)	**0.002**
Hemoglobin (g/dL)	14 (13-15)	14.1 (13-15)	0.706
Platelet count (×10^9/L)	265 (168-401)	268 (165-420)	0.765
Creatinine (mg/dL)	0.97 (0.8-1.12)	0.9 (0.8-1.04)	0.130
Glucose (mg/dL)	180.5 (143-209)	156 (126-200)	**0.002**
Total cholesterol (mg/dL)	199.5 (187-213)	200 (181-213)	0.818
Triglycerides (mg/dL)	178.5 (160-197)	178 (158-199)	0.795
HDL-C (mg/dL)	36.9 (33-41)	37 (33-40)	0.641
LDL-C (mg/dL)	142 (125-156)	144 (127-158)	0.582
CRP (mg/L)	5 (4-6)	1.78 (0.8-4.3)	**<0.001**
Albumin (mg/dL)	4.0 (3.8-4.2)	4.1 (4-4.3)	**0.001**
No-reflow (%)	35 (42.7%)	37 (12.5%)	<0.001
STR (%)			
≥ 50	33 (40.2%)	226 (76.1%)	**< 0.001**
< 50	49 (59.8%)	71 (23.9 %)	
Killip class, n (%)			
Class 1	78 (95.1%)	286 (96.3%)	0.629
Class ≥2	4 (4.9%)	11 (3.7%)	

CCB = Calcium channel blocker, ACEI = Angiotensin-converting enzyme inhibitor, ARB = Angiotensin receptor blockers, ASA = acetylsalicylic acid, BB = Beta-blocker, BMI = Body Mass Index, CRP = C-reactive protein, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, LVEF = Left ventricle ejection fraction, WBC = white blood cell, STR = ST-segment resolution, SBP = systolic blood pressure, DBP = diastolic blood pressure, SYNTAX = Synergy between PCI with Taxus and Cardiac Surgery, NLR = Neutrophıl lymphocyte ratio, mGPS = modified Glasgow prognostic score

Table 3. Univariate and multivariate logistic regression analysis of the association between the no-reflow phenomenon and multiple parameters

	Univariate analysis		Multivariate analysis	
Variables	**OR (95% CI)**	***p value***	**OR (95% CI)**	***p value***
Gender (female)	1.670 (0.993-2.808)	0.053		
Age	0.961 (0.935-0.987)	**0.004**	1.020 (0.981-1.060)	0.323
Diabetes mellitus	0.528 (0.315-0.886)	**0.016**	0.814 (0.409-1.620)	0.557
ASA/P2Y12-inh	1.051 (0.588-1.879)	0.867		
WBC Count	0.732 (0.652-0.821)	**< 0.001**	0.816 (0.728-0.914)	**< 0.001**
Glucose	0.991 (0.985-0.996)	**0.001**	0.997 (0.990-1.005)	0.491
NLR	0.424 (0.332-0.543)	**0.013**	0.482 (0.355-0.654)	**< 0.001**
Pain-to-balloon time	0.963 (0.950- 0.976)	**< 0.001**	0.976 (0.960-0.991)	**0.002**
SYNTAX score	0.926 (0.887-0.968)	**< 0.001**	0.975 (0.920-1.034)	0.396
CRP	0.729 (0.653-0.813)	**< 0.001**	0.892 (0.772-1.031)	0.123[a]
Albumin	16.416 (5.613-48.016)	**< 0.001**	2.353 (0.557-9.942)	0.244[a]
mGPS	5.233 (2.998-9.132)	**< 0.001**	3.213 (1.643-6.283)	**0.001**[b]

WBC = White blood cell, NLR = Neutrophil lymphocyte ratio, CRP = C-reactive protein, SYNTAX = Synergy between PCI with Taxus and Cardiac Surgery, mGPS = modified Glasgow prognostic score.

[a]The variables (Age, Diabetes mellitus, Glucose, Pain-to-balloon time, SYNTAX score, CRP and albumin) were tested in a multivariable analysis.

[b]The variables (Age, Diabetes mellitus, WBC Count, Glucose, NLR, Pain-to-balloon timeSYNTAX score and mGPS) were tested in a multivariable analysis

female ratio [54.9% vs. 42.4%, $p = 0.045$], Syntax score [18 (14.7-23) vs. 19.8 (12-21.5), $p = 0.036$], pain to balloon time [70 (50-90) vs. 60 (50-80), $p = 0.045$], peak TnT level [4696 (2504-6605) vs. 3654 (1980-5231), $p = 0.002$], WBC count [12.6 (11-15) vs. 11.3 (10.1-13.4), $p < 0.001$], NLR [2.8 (1.7-3.9) vs. 2.1 (1.6-2.9), $p = 0.002$], glucose level [180.5 (143-209) vs. 156 (126-200), $p = 0.002$], no-reflow rate (42.7% vs. 12.5%, $p < 0.001$) and CRP level [5 (4-6) vs. 1.78 (0.8-4.3), $p < 0.001$], while SA level [4.0 (3.8-4.2) vs. 4.1 (4-4.3), $p < 0.001$], and the proportion of patients with ≥ 50% STR at 60 min (40.2% vs. 76.1%, $p < 0.001$) were lower (Table 2).

The results of the multivariate analysis performed to determine the predictive factors for NRP based on demographic, clinical and procedural parameters that were found to be significantly associated with NRP according to univariate analysis are shown in Table 3. According to the results of this analysis, WBC count [Hazard ratio (HR): 0.816, 95% confidence interval (CI): 0.728-0.914, $p < 0.001$], NLR (HR: 0.482, CI: 0.355-0.654, $p < 0.001$), pain to balloon time (HR: 0.976, CI: 0.960-0.991, $p = 0.002$) and mGPS (HR: 3.213, CI: 1.643-6.283, $p = 0.001$) were found to be significant and independent predictive factors for NRP.

DISCUSSION

NRP is a serious complication associated with mortality and morbidity frequently seen during pPCI [10]. Although it has been an area of interest for invasive cardiologists in recent years, its pathophysiology is still unclear. However, vasoconstriction, platelet and leukocyte activation, oxygen radicals released after reperfusion, endothelial damage, and dysfunction are the parameters blamed in pathophysiology [11]. The presence of an invisible microthrombus during PCI may also contribute to the development of NRP, the pathophysiology of which is unknown. Therefore, tests to identify patients who may be at risk for NRP before the procedure becomes even more important. In this study, we investigated the relationship between

mGPS, an inexpensive and easy-to-calculate scoring system, and NRP and demonstrated that mGPS is an independent and important predictor factor for NRP.

Diabetes, hypertension, smoking, dyslipidemia, renal insufficiency, age, and inflammatory parameters are accepted cardiovascular risk factors for NRP [3]. In our study, in parallel with these findings, it was observed that NRP group patients were older, female, and had a higher proportion of diabetic patients.

It is thought that endothelial damage, leukocyte and platelet accumulation at the microvascular level, oxygen radicals released after reperfusion and the inflammatory process play an important role in the pathophysiology of NRP [12]. There are also studies demonstrating the association of CRP and SA, components of modified GPS, with NRP [7, 8]. Recent data clearly demonstrate the relationship between inflammation and atherosclerosis. Therefore, inflammatory biomarkers are frequently used in both the diagnosis and prognosis of coronary artery disease. Among these, CRP is the most commonly used parameter. Increased CRP is associated with the risk of myocardial infarction and stroke in asymptomatic individuals and with recurrent coronary events, morbidity, and mortality in patients with stable coronary artery disease and acute coronary syndrome [13]. It is thought that increased CRP level decreases vasodilation at the microvascular level directly and by affecting endothelium-dependent mediators [14]. CRP is also thought to contribute to vasoconstriction at the microvascular level in which NRP is also thought to be involved. CRP is also thought to play a role in endothelial damage and activation of lymphocytes and platelets accumulated in this region [15]. In this study, SA level, the other component of mGPS, was found to be lower in the NRP group. Kurtul *et al*. [7] also demonstrated that low SA was a risk for NRP in patients undergoing pPCI for STEMI and this finding is in parallel with the results of our study. The pro-inflammatory state caused by plaque rupture may contribute to the development of NRP after PCI by increasing platelet aggregation. In addition, albumin inhibits platelet aggregation by increasing PGD2 production, which has direct and indirect antiaggregant effects [16, 17]. On the other hand, hypoalbuminemia is thought to cause endothelial damage by increasing both blood viscosity and free lysophosphatidylcholine concentration [18]. The results of this study support the mechanisms mentioned above and are thought to be involved in the pathophysiology of NRP.

In this study, pain-balloon duration was also shown to be an independent factor for NRP. Early after AMI, the thrombus at the site of the responsible lesion contains platelet-rich, erythrocyte-rich, and red fibrin. Thrombus of this character is usually resolvable with antiaggregant therapy such as tirofiban and abciximab. However, as time progresses, the thrombus becomes more robust and it is thought that fragmented fragments after ballooning and stenting performed during pPCI disrupt microvascular perfusion in the distal region and cause NRP [19].

In this study, another finding supporting the relationship between inflammation and NRP was that increased WBC count and NLR were shown to be predictor factors for NRP. Leukocytes accumulate in small vessel beds in the infarct zone, which plays a key role in the pathophysiology of NRP. The increase in neutrophil adhesion molecules in this region leads to the activation of leukocytes and monocytes. Aggregation of activated leukocytes in the capillary region may directly disrupt blood flow [20]. In addition, leukocyte aggregation is thought to cause additional vascular injury by leading to edema in the endothelial bed, increased permeability and oxygen radicals [21]. Consistent with these mechanisms, WBC count is usually high in patients with acute myocardial infarction. Increased WBC count is associated with poor prognosis and risk of developing NRP, as shown in previous studies [22, 23].

Limitations

Single centers and a small number of patients are the main limitations of the study.

CONCLUSION

The aim of STEMI treatment is to achieve revascularization as effectively and as early as possible. However, NRP, defined as impaired myocardial perfusion despite epicardial coronary revascularization, is frequently seen during pPCI and is also associated with mortality. In this study, mGPS based on CRP and SA levels, which are routine laboratory parameters, was

shown to be an independent predictor factor for NRP. For patients undergoing pPCI for STEMI, this score may help identify individuals at risk for NRP before the procedure and determine adjuvant treatment. However, larger studies are needed before mGPS can be routinely used to predict NRP.

Authors' Contribution

Study Conception: MK, KT; Study Design: MK, KT; Supervision: MK, KT; Funding: N/A; Materials: N/A; Data Collection and/or Processing: MK, RA, CA; Statistical Analysis and/or Data Interpretation: MK, KT; Literature Review: MK, RA, CA; Manuscript Preparation: MK and Critical Review: MK, RA, CA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):903-909 Original Article

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Evaluation of intraductal papillary mucinous neoplasms detected incidentally with magnetic resonance cholangiopancreatography

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ABSTRACT

Objectives: The aim of this study was to estimate the prevalence of coincidentally found intraductal papillary mucinous neoplasms (IPMNs) and assess their features with magnetic resonance cholangiopancreatography (MRCP) imaging.

Methods: The prevalence of incidentally detected IPMN was evaluated in 951 patients who underwent MRCP examination for various indications. MRCP images were assessed to analyze the number, size, location, and internal structure of lesions in patients with IPMN. Furthermore, the association between IPMN prevalence and age and gender was evaluated.

Results: IPMN was detected in 102 (10.7%) of 951 patients. Solitary IPMNs were located in different parts of the pancreas: in the uncinate process in 8 (7.8%), in the head and neck in 19 (18.6%), in the corpus in 10 (9.8%), and in the tail in 7 (6.9%) patients. IPMN was multiple in 58 (56.9%) patients. IPMN was identified in 41 (6.18%) patients under 65 years and 61 (21.18%) patients over 65 years, and the variance was statistically substantial ($p < 0.001$). IPMN diameter was 7.22 ± 4.3 mm in patients under 65 years and 9.21 ± 4.74 mm in those over 65 years, which was statistically significant ($p = 0.048$). Patients who were older were more likely to have multiple IPMNs ($p = 0.010$).

Conclusions: IPMNs increase in frequency, quantity, and size with age. MRCP is the most essential sequence for determining main pancreatic duct (MPD) involvement or communication, a critical finding for diagnosis. Since MRCP is capable of screening patients at very short intervals, it may be utilized for follow-up imaging in IPMN patients.

Keywords: Intraductal papillary mucinous neoplasm, pancreatic duct, pancreatic cystic lesion, magnetic resonance cholangiopancreatography

Cystic neoplasms of the pancreas are rare, comprising approximately 10% of pancreatic cysts and 1% of pancreatic carcinomas [1, 2]. As a consequence of developments in imaging technology, including computed tomography (CT), ultrasonography (USG), and magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP), they are one of the most commonly observed

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pathologies [3-8]. Pancreatic cystic neoplasms encompass an extensive spectrum of genetic, inflammatory, and malignant etiological factors [9].

Given the possibility of malignancy in these cystic neoplasms, accurate diagnosis and treatment are critical. Nonetheless, little data is available regarding its occurrence and clinical relevance in the general populace. Intraductal papillary mucinous neoplasm (IPMN) is one of the cystic neoplasms that is a precancerous mass of the pancreas [10-12]. IPMN has been divided into branch duct (BD-IPMN) and main duct (MD-IPMN) types depending on the site of the affected pancreatic duct [13, 14]. Consequently, individuals with a branch duct IPMN are frequently directed to monitoring programs, and surgery is suggested when follow-up findings imply the development of high-grade dysplasia or malignancy. After a 5-year observation period, it is suggested that the surveillance of asymptomatic patients with IPMNs that have not changed or have changed only moderately should be terminated [15-17].

Increasing data indicate that carcinoma progression in individuals with IPMNs occurs by two primary routes: de novo pancreatic ductal adenocarcinoma (PDAC) or arising from IPMN [18, 19]. On the basis of imaging features and/or pathological analyses, these carcinomas are distinguished clinically.

Few studies have been conducted to determine the prevalence of IPMNs to date. The objective of our study was to determine the prevalence of incidentally detected IPMN and their evaluation based on gender, age, size, location, and internal structure.

METHODS

Patient Data

Our institution's Ethics Committee approved this retrospective research (approval number: 2023/36). The patient files were examined for those who underwent MRCP examinations with different clinical indications between August 2020 and November 2022. The following were the inclusion criteria for the research: Patients with (a) MRCP imaging; (b) pancreatic cystic lesions and imaging results consistent with IPMN; (c) no known pancreatic cyst; and (d) adequate image quality for optimal evaluation.

The search turned up a sum of 1011 patients. 10 patients under 18 years of age; 5 patients with a connection between the cyst and the main pancreatic duct that could not be clearly established; 24 patients known to have a pancreatic cyst; 18 patients for whom the quality of the image was inadequate for assessment; and 3 patients with main duct IPMN (Fig. 1) were not included in the research. The investigation included 951 patients, 569 females, and 382 males with a mean age of 56.43 ± 15.07 years and a range of 31-85 years.

MRI Examination

A 3.0-T MR unit (Verio; Siemens Medical Solutions, Erlangen, Germany) was used to perform MRI. Thin-section turbo spin-echo T2-weighted (TSE) images were acquired in the axial, coronal, and sagittal planes (20 slices; thickness: 4 mm; TR/TE: 7800/150 ms; the amount of signals obtained: 2; resolution: 0.6 mm × 0.8 mm). Prior to the MRI scan, patients had to

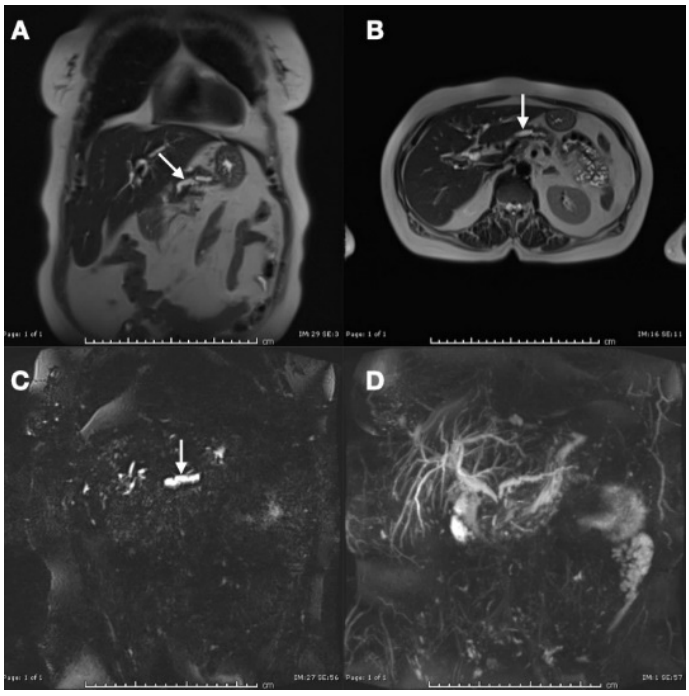

Fig. 1. Main duct intraductal papillary mucinous neoplasm in a 57-year-old male patient. (a) Coronal T2W, (b) axial T2W, and (c) MRCP images show dilatation of the main pancreatic duct without any stone or mass (white arrows). Accompanying dilatation is observed in the common bile duct in MRCP maximum intensity projection (MIP) image (d). The histopathology of the lesion was compatible with the main duct intraductal papillary mucinous neoplasm after surgery

fast for a minimum of six hours. At least six T2-weighted MRCP sequences, comprising coronal and sagittal planes, were taken during breath-holding using the quick SE technique. For 3D MRCP, a 3D fast-recovery turbo SE sequence was performed. Data was transmitted to a personal computer, where maximum intensity projection was used to recreate 3D MRCP images.

Image Analysis

All scans were assessed by a radiologist with seven years of hepatopancreatobiliary system MRI interpreting expertise, who was unaware of whether there was a cystic lesion in the pancreas. In patients with IPMN, the number, size, location, and internal structure of the lesions were analyzed using MRCP images. In addition, the association between IPMN frequency and age and gender was investigated.

Statistical Analysis

With the aid of the SPSS 25.0 software, statistical analyses were conducted. Using histograms and the Kolmogorov-Smirnov test, it was determined whether the variables followed a normal distribution. Descriptive statistics use mean, standard deviation, median, and IQR values. The Pearson Chi-Square Test was utilized to evaluate independent parameters. Between the two groups, nonparametric variables were analyzed using the Mann-Whitney U test. Statistical significance was accepted when the p value was below 0.05.

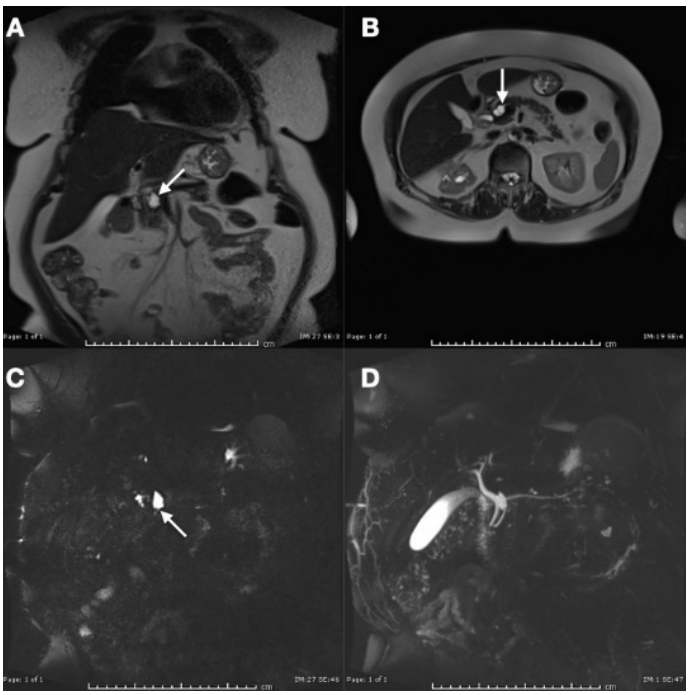

Fig. 2. Branch duct intraductal papillary mucinous neoplasm in a 60-year-old female patient. Coronal T2W (a) and axial T2W (b) images show a hyperintense lesion in the head of the pancreas (white arrows). The MRCP (c) image reveals the connection between the lesion and the main pancreatic duct (white arrow). The MRCP-MIP image displays the pure content of the cyst (d)

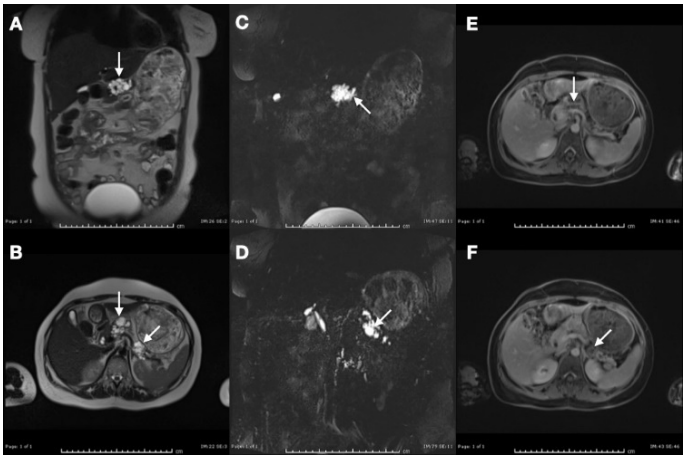

Fig. 3. Branch duct intraductal papillary mucinous neoplasms in a 51-year-old female patient. Coronal T2W (a) and axial T2W (b) images show hyperintense lesions containing multiple septa in the corpus and tail of the pancreas (white arrows). The MRCP images (c, d) demonstrate the connection between the lesions and the main pancreatic duct (white arrows). In the contrast-enhanced sequences, contrast enhancement is observed in the septa of the cysts

RESULTS

The research included 951 patients with a mean age of 56.43 ± 15.07 years, consisting of 382 males and 569 females. There were 663 patients under the age of 65 and 288 patients older than 65. There was no statistically significant difference in the incidence of IPMN between young and elderly patients based on gender ($p = 0.306$).

In our study, IPMN was detected in 102 (10.7%) of 951 patients. MRCP indications were choledocholithiasis in 506 (53.2%) patients, pancreatitis in 93 (9.8%) patients, malignancy in 348 (36.6%) patients, and biliary duct injury in 4 (0.4%) patients.

The mean tumor diameter was 8.51 ± 4.63 mm for

IPMNs. While IPMN was solitary in 44 (43.1%) patients, it was multiple in 58 (56.9%) patients. Eight (7.8%) patients had solitary IPMNs in the uncinate process, 19 (18.6%) patients in the head and neck of the pancreas, 10 (9.8%) patients in the corpus of the pancreas, and 7 (6.9%) patients in the tail of the pancreas. In 58 (56.9%) patients, IPMN was multiple and localized in different parts of the pancreas. In 98 (96.1%) patients, the internal structure of IPMN was pure (Fig. 2), whereas 4 (3.9%) patients exhibited a complicated appearance with septations (Fig. 3) (Table 1).

Table 1. Findings detected in the MRCP examination

Characteristics	Data
Age (years) (mean ± SD) (range)	56.43 ± 15.07 (31-85)
Sex	
Male	382 (40.2)
Female	569 (59.8)
Quantity of patients with IPMN, n (%)	102 (10.7)
MRCP indications, n (%)	
Choledocholithiasis	506 (53.2)
Pancreatitis	93 (9.8)
Malignancy	348 (36.6)
Biliary duct injury	4 (0.4)
Quantity of IPMN, n (%)	
Solitary	44 (43.1)
Multiple	58 (56.9)
Location of IPMN, n (%)	
Uncinate process of pancreas	8 (7.8)
Head and neck of the pancreas	19 (18.6)
Corpus of the pancreas	10 (9.8)
Tail of the pancreas	7 (6.9)
Multiple	58 (56.9)
Internal structure of the IPMN, n (%)	
Pure	98 (96.1)
Complicated	4 (3.9)
Tumor diameter (mm) (mean ± SD) (range)	8.51 ± 4.63 (3-32)

MRCP = magnetic resonance cholangiopancreatography, IPMN = intraductal papillary mucinous neoplasm, SD = standard deviation

IPMN was detected in 41 patients under the age of 65 (6.18%) and in 61 patients over the age of 65 (21.18%) and there was a statistically significant difference ($p < 0.001$). In the group of patients under 65 years of age, the mean diameter of the IPMN was 7.22 ± 4.3 mm, whereas it was 9.21 ± 4.74 mm in the group of patients over 65. This variance was statistically substantial ($p = 0.048$). In the older patient group, the number of IPMNs was higher than in the younger cohort, and they tended to be multiple in the older patient group ($p = 0.010$) (Table 2).

DISCUSSION

The frequency of coincidental pancreatic IPMNs has grown recently as a result of technological advancements in imaging. However, their incidence has not been thoroughly investigated. In our research, we collected data from patients who underwent MRCP, the most effective method for investigating incidental IPMNs.

Several investigations have examined the pancreatic cystic neoplasm prevalence to date, with reported rates ranging from 0.2% to 36.0% [6-9, 20]. According to our research, this rate was 10.7%. IPMN is the most prevalent cystic pancreatic neoplasia (70%) and may be malignant and multiple. IPMN can exhibit a whole range of histologic alterations, with a variable incidence between BD-IPMN and MD-IPMN [21, 22]. Based on its localization, there are three morphological forms of IPMN: the MD-IPMN, the BD-IPMN, and the mixed type. Malignancy rates are considerably greater for MD-IPMN and mixed types and lower for BD-IPMN [23].

IPMN is typically seen in asymptomatic individuals, has a median age of diagnosis of 60 years old, and disproportionately affects males compared to females. However, in this research, no significant difference was found in terms of gender in the incidence of IPMN. On imaging, mucin secretion causes cystic ductal segment dilatation in IPMN. MRI is the most effective tool for defining IPMN, and MRCP is the most essential sequence for evaluating MPD communication or involvement, which are key points for IPMN identification [21].

Without obstruction, MD-IPMN can cause diffuse or segmental MPD dilatation (> 5 mm). In diffuse

Table 2. Comparison of IPMN findings for patients under and over 65 years of age

	< 65 years	≥ 65 years	*p* value
Quantity of patients with IPMN, n (%)	41/663 (6.18)	61/288 (21.18)	**< 0.001[b]**
Sex, n (%)			0.306[b]
Male	14 (34.1)	27 (44.3)	
Female	27 (65.9)	34 (55.7)	
IPMN diameter (mm), (mean±SD)	7.22 ± 4.3	9.21 ± 4.74	**0.048[a]**
Quantity of IPMN, n (%)			**0.010[b]**
Solitary	24 (58.5)	20 (32.8)	
Multiple	17 (41.5)	41 (67.2)	

IPMN = intraductal papillary mucinous neoplasm, SD = standard deviation
[a]Mann Whitney U Test; [b]Chi-squared test.

MD-IPMN, MPD dilatation is more homogenous with regular margins, helping to differentiate from chronic pancreatitis. In diffuse MD-IPMN, the MPD enlargement is more symmetrical and has uniform outlines, distinguishing it from chronic pancreatitis [24]. Parenchymal atrophy is typically observed in MD-IPMN. Segmental MD-IPMN can spread through MPD if left untreated [25, 26]. Diffuse or segmental dilatation of branch ducts and MPD is a hallmark of mixed-type IPMN. Conversely, BD-IPMN may cause MPD dilatation due to mucin overproduction, thereby imitating mixed-type IPMN [27].

BD-IPMN manifests as a multifocal or unifocal cystic lesion that communicates with the main pancreatic duct. Cysts may be multi- or unilocular, with diameters varying from a few millimeters to a few centimeters; they are frequently grouped in clusters like clusters of grapes; and they are typically separated by small septa, which enhance after contrast injection [28]. The demonstration of communication with the MPD is essential for BD-IPMN diagnosis; hence, a high-quality MRCP is the most crucial step in the entire imaging procedure [29].

IPMN has a varied malignant potential; hence, the Fukuoka consensus was published with two-tiered malignancy prediction categories. The first tier consists of "worrisome features," a set of diagnostic observations indicating that the mass may progress to malignancy. EUS is needed to risk-strategize the lesion, and follow-up is required. The second tier is "high-risk stigmata," which signal the lesion may be cancerous and require surgical excision if the patient is eligible [14]. To summarize the 2017 Fukuoka revised consensus on the management of IPMN, patients with high-risk stigmata must have excision if physically possible; patients with worrisome features require additional workup; and individuals without either need follow-up at varied periods based on the dimensions of the biggest cyst [14].

In patients with IPMN, terminating or extending monitoring may be risky due to the persistent risk of concurrent PDAC [30]. In the research of 197 patients with IPMN and other cystic pancreatic lesions, Tada *et al*. found that the IPMN group is "at high risk" of advancing to pancreatic cancer, with a frequency that is 22.5 times greater than the estimated population mortality [10]. In a research study of 130 cases on surveillance following pancreatic resection for IPMN, He *et al*. [31] found that after 1, 5, and 10 years, the probability of PDAC or a new IPMN needing surgery was 0%, 7%, and 38%, respectively.

Owing to the increased number of CPLs, especially in elderly cases, the follow-up strategy overwhelms radiological facilities with a huge number of asymptomatic subjects [32-36]. As MRI is the best method for monitoring these patients, and as it is "time-consuming," pancreas-specific MRI protocols ought to be examined. In the identification of significant cystic lesion alterations and mural nodules, some publications have already demonstrated that a brief MR technique provides the same information as a more time-consuming and expensive complete ap-

proach. We think that MRCP examination is a fast and optimal modality for screening patients with IPMN at short intervals.

Limitations

Our study included a number of limitations. Due to the retrospective nature of our study, selection bias was inevitable despite our use of tight inclusion criteria. We did not assess the progression on follow-up imaging in IPMN patients since we were primarily interested in determining the incidence of IPMN observed incidentally on MRCP in this study. Additional investigation is essential for verification.

CONCLUSION

IPMN is the most frequent cystic pancreatic neoplasia that can be multifocal and cancerous, which is typically detected incidentally with cross-sectional imaging. It occurs more frequently, in greater numbers, and at larger sizes with age. MRCP is the most crucial imaging method for identifying MPD communication or involvement, a crucial finding for IPMN diagnosis. Follow-up imaging in IPMN patients is of great importance, so MRCP can be used for screening patients at short intervals.

Ethics Committee Approval

This retrospective study was performed at the University of Health Sciences, Bakirkoy Dr. Sadi Konuk Training and Research Hospital. The study protocol (2023/36) was approved by the Institutional Review Board on January 23, 2023. Written informed consent was obtained from all patients.

Authors' Contribution

Study Conception: MON; Study Design: MON; Supervision: MON; Funding: N/A; Materials: MON; Data Collection and/or Processing: MON; Statistical Analysis and/or Data Interpretation: MON; Literature Review MON; Manuscript Preparation: MON, and Critical Review: MON.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The effect of orally administered metoprolol on the frequency and severity of rocuronium injection pain

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ABSTRACT

Objectives: This study aimed to examine the effects of orally administered metoprolol on the frequency and severity of pain caused by rocuronium injection in patients who started to use and were currently using oral metoprolol for any reason such as ischemic heart disease, hypertension, and arrhythmias.

Methods: Patients were evaluated in four groups. Group M: patients currently using metoprolol and who did not receive lidocaine before the application of rocuronium. Group ML: patients currently using metoprolol and who received lidocaine before rocuronium application. Group L: patients currently not using metoprolol and received lidocaine before rocuronium application. Group C: patients currently not using metoprolol and who did not receive lidocaine before rocuronium application. Following the induction of general anesthesia with thiopental sodium, a researcher blind to the groups observed the pain during rocuronium injection based on the following scale: (1) no reaction, (2) movement only in the ankle, (3) movement or withdrawal only in the arm (shoulder and ankle), and (4) diffuse reaction (movement or withdrawal in more than one extremity, coughing and holding breath).

Results: Two hundred patients with 50 in each of four groups were included. The incidence of pain was statistically significantly lower in Group ML compared to Groups M and C ($p = 0.001$). The correlations between pain caused by rocuronium injection and duration of metoprolol usage and the time since the last dose were not statistically significant (for all, $p > 0.05$).

Conclusions: Oral metoprolol combined with lidocaine reduced pain and withdrawal reflex caused by rocuronium injection. No significant difference was observed between the last dose and the duration of metoprolol usage.

Keywords: Rocuronium, metoprolol, lidocaine, general anesthesia, pain, reflex

Rocuronium is a nondepolarizing neuromuscular blocker that produces muscle relaxation to help facilitate surgery and lung ventilation during elective and emergency procedures [1]. It is preferred for rapid onset of action and reversibility [2]. However, the injection of rocuronium is associated with severe burn-

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ing pain and spontaneous movement in the arm observed after induction of anesthesia, known as rocuronium withdrawal reflex [3]. Rocuronium administered in awake patients often leads to complaints of burning pain at the injection site [4]. If rocuronium is used following the loss of consciousness, some patients show withdrawal reflex and recall injection pain postoperatively. Rocuronium-induced withdrawal reflex has been reported between 50and 84% [5]. This reflex's exact mechanism has not been clearly understood, but it can cause severe complications such as aspiration pneumonia [6]. It has been proposed that this reflex occurs due to the release of local mediators or stimulation of C nociceptors [7].

Non-pharmacological and pharmacological methods have decreased the frequency of withdrawal reflex following rocuronium injection. Non-pharmacological techniques include cooling the reagent, topical warming, and decelerating the injection rate [8]. Numerous pharmacological agents such as opioids, ketamine, tramadol, magnesium sulfate, and lidocaine have been studied to decrease the severity and frequency of pain and reflexes following rocuronium injection [3]. Also, animal models using new rocuronium formulations not causing vascular pain in flexor reflex anesthetized rats are ongoing [9].

Metoprolol is a selective β1 receptor blocker. β blockers, as a class of drugs, are primarily used to treat cardiovascular diseases and other conditions. The possibilities for their use in treating different conditions continue to evolve. β adrenoreceptors are localized in the areas directly associated with pain pathways and have proinflammatory properties with the production of IL1β and IL6. Pain control of β adrenoreceptors depends on the type of nociceptive stimulus. Whereas both β1 and β2 are effective in physical stimuli, β1 adrenoceptors are more effective in chemical stimuli [10]. Some β blockers have been proven to show local anesthetic effects and cause activation of GTPase in vitro. Other studies have demonstrated that esmolol, a β1 blocker, was used to prevent rocuronium injection pain and effectively reduce this pain [119. Asik *et al.* [12] found that iv metoprolol was as effective as lidocaine in preventing propofol-induced pain, like rocuronium.

There are studies investigating of the effects of metoprolol administered intravenously. However, no study was found to investigate its effects via oral way. This study aimed to investigate the effect of metoprolol on the frequency and severity of pain after rocuronium injection in patients who were using oral metoprolol for any reason.

METHODS

The local ethics committee approved the study protocol of our hospital with the 07/05/2014 dated and 2014-4/94 numbered decision. The study was registered with the Clinical Registration Number: NCT05457751. All patients were informed about the study and gave informed written consent. The study was conducted per the ethical principles of the Declaration of Helsinki and was planned as a prospective, placebo-controlled cohort study.

Inclusion criteria included patients aged between 18 - 75 years, ASA 1-III, patients undergoing elective surgery under generala anesthesia, patients using thiopental sodium for induction of general anesthesia, and patients who used oral metoprolol for any medical reason in the preoperative period.

Patients under 18 and above 75 years of age, with ASA IV class, those with known allergy to rocuronium and lidocaine, patients with chronic pain, pregnant women, those who had received analgesics or sedatives, and patients who were receiving calcium channel blocker that could affect pain were excluded from the study.

After being taken to the operating table, patients were routinely monitored with 3-channel ECG, non-invasive blood pressure, and oxygen saturation (SpO_2). We provided IV access, with a 22 G branule from the most prominent vein, the dorsum of the hand. Time since the last use of the metoprolol and metoprolol usage duration were recorded. Before the rocuronium injection, anesthetic agents administered for induction were recorded, and only patients who received thiopental sodium were included in the analysis to provide standardization.

Following the induction of general anesthesia with thiopental sodium, a researcher blind to the groups observed the pain during rocuronium injection based on the following scale: (1) no reaction, (2) movement only in the ankle, (3) movement or withdrawal only in

the arm (shoulder and ankle), and (4) diffuse reaction (movement or withdrawal in more than one extremity, coughing and holding breath).

Patients were evaluated in four groups. Group M: patients currently using metoprolol and who did not receive lidocaine before the application of rocuronium. Group ML: patients currently using metoprolol and who received lidocaine before rocuronium application. Group L: patients currently not using metoprolol and received lidocaine before rocuronium application. Group C: patients currently not using metoprolol and who did not receive lidocaine before rocuronium application.

Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), and SpO_2 values were recorded before rocuronium administration, 1 and 3 minutes after rocuronium induction, and after intubation. Anesthesia maintenance was performed according to the discretion of the anesthetist. We checkedthe injection site regarding edema, rash, and thrombophlebitis after the first 24 hours of the operation.

Statistical Analysis

Considering withdrawal of 70% as described in the previous studies [13], the power analysis determined that each group should have 50 patients at 95% confidence interval ($\alpha = 0.05$) and 92% power. Sampling analysis was performed using PASS 13 statistical software. Data obtained in the study were statistically analyzedutilizing SPSS version 20.0 (SPSS, Statistical Package for Social Sciences, IBM Inc. Armonk, NU, USA) program. All data are presented as mean ± standard deviation or number (percentage) of patients. The normal distribution of variables was studied using the Kolmogorov-Smirnov test. One-way analysis of variance and χ^2 test were used in the analysis of the demographic data of the patients. χ^2 test was employed to evaluate the incidence of the pain caused by rocuronium bromide injection. In the variables showing normal distribution, such as HR, SBP, DBP, and MBP, statistically, significant differencesamong the groups wereanalyzed with the Repeated Measures ANOVA test. Bonferroni adjusted test was utilized to reveal the measurement times that led to the significant difference between values. $P < 0.05$ values were considered statistically significant.

RESULTS

A total of 200 patients, with 50 being in each group, were included in the study. The mean overall age was found as 55.95 ± 12.33 years. The mean age was statistically significantly higher in Groups M and ML compared to Groups L and C ($p < 0.001$). No statistically significant difference was found between the groups in terms of gender, height, weight, BMI, time

Table 1. Demographic data of the patients

	Group M (n = 50)	Group ML (n = 50)	Group L (n = 50)	Group C (n = 50)	*p* value
Gender (F/M)	22/28	17/33	15/35	10/40	0.079
Age	61.1 ± 11.6	63.4 ± 11.5	48.7 ± 11.5	50.6 ± 14.7	**0.001***
Height	164.4 ± 16.1	165.4 ± 8.0	165.2 ± 7.7	164.7 ± 5.0	0.956
Weight	78.2 ± 12.3	76.4 ± 15.2	77.5 ± 13.3	76.3 ± 12.3	0.880
BMI	30.9 ± 17.6	28.0 ± 5.6	28.5 ± 5.0	28.2 ± 4.6	0.419
Last time (hr)	6.3 ± 5.8	8.7 ± 11.5	--	--	0.183
Metoprolol usage time (day)	42.1 ± 53.8	35.6 ± 49.7	--	--	0.534

All values are presented as mean ± standard deviation or number of patients. BMI = body mass index, F = female, M = male, Group M = patients currently using metoprolol and who did not receive lidocaine before the application of rocuronium, Group ML = patients currently using metoprolol and who received lidocaine before rocuronium application, Group L = patients currently not using metoprolol and received lidocaine before rocuronium application, Group C = patients currently not using metoprolol and who did not receive lidocaine before rocuronium application. *Group M and ML compared with group L and group C.

Table 2. The intensity and incidence of rocuronium-induced injection pain between the groups

Pain Score	Group M (n = 50)	Group ML (n = 50)	Group L (n = 50)	Group C (n = 50)	*p* value
1	31 (62%)	44 (88%)	36 (72%)	26 (52%)	
2	10 (20%)	3 (6%)	5 (10%)	4 (8%)	
3	9 (18%)	3 (6%)	8 (16%)	17 (34%)	
4	0 (0%)	0 (0%)	1 (2%)	3 (6%)	
Overall incidence (2+3+4)	19(38%)*	6(12%)†	14(28%)‡	24(48%)§	**0.001**

All values are presented as number of patients (percentages). Group M = patients currently using metoprolol and who did not receive lidocaine before the application of rocuronium, Group ML = patients currently using metoprolol and who received lidocaine before rocuronium application, Group L = patients currently not using metoprolol and received lidocaine before rocuronium application, Group C = patients currently not using metoprolol and who did not receive lidocaine before rocuronium application.

*p = 0.003, Group M compared with Group ML

†p = 0.001, Group ML compared with Group C

‡p = 0.047, Group L compared with Group ML

§p = 0.040, Group C compared Group L

since last use of the drug, and metoprolol usage duration (for all, $p > 0.05$) (Table 1).

The data relating toincidence and intensity of the pain during rocuronium injection were statistically different among all study groups ($p = 0.001$). In group ML, 88% of patients did not have rocuronium-related injection pain. The incidence of pain induced by rocuronium injection in metoprolol plus lidocaine group was 12%, compared with 28%, 38% and 48% in lidocaine, metoprolol, and control groups; respectively ($p = 0.001$) (Table 2). The incidence of the pain was statistically significantly lower in Group ML compared to Groups L, M, and C ($p = 0.047$, $p = 0.003$, and $p = 0.001$; respectively). On the other hand, the pain score was statistically significantly lower in Group L compared to Groups M and C ($p = 0.001$).There were no statistically significant differences between Group M and Group C ($p = 0.315$) and Groups M and L ($p = 0.290$) in terms of overall incidence.

The pain induced by rocuroniuminjection was significantly higher in Group C than in Groups L ML ($p = 0.02$ and $p = 0.001$). There were no statistically significant differences between Groups L M ($p = 0.416$)

Table 3. Comparison of heart rate values between the groups

Heart Rate	Group M (n = 50)	Group ML (n = 50)	Group L (n = 50)	Group C (n = 50)	*p* value	Difference
Pre-induction	77.9 ± 12.3	80.3 ± 13.5	83.1 ± 11.8	81.8 ±13.5	0.206	--
Post-induction	85.5 ± 13.0	84.8 ± 13.9	93.7 ± 11.7	89.7 ± 13.2	**0.002***	C and M-ML
Metoprolol 1st min	82.6 ± 14.5	85.7 ± 13.9	88.2 ± 11.0	88.3 ± 15.2	0.127	--
Metoprolol 3rd min	78.6 ± 13.7	80.0 ± 14.1	103.1 ± 117.1	85.0 ± 15.0	0.151	--
Post-intubation 1st min	85.9 ± 13.2	86.8 ± 14.5	97.7 ± 15.1	97.0 ± 15.6	**< 0.001**	M-ML and L-C
Post-intubation 5th min	77.7 ± 14.0	79.1 ± 13.3	87.6 ± 14.8	86.8 ± 14.2	**< 0.001**	M-ML and L-C

All values are presented as mean ± standard deviation. Group M = patients currently using metoprolol and who did not receive lidocaine before the application of rocuronium, Group ML = patients currently using metoprolol and who received lidocaine before rocuronium application, Group L = patients currently not using metoprolol and received lidocaine before rocuronium application, Group C = patients currently not using metoprolol and who did not receive lidocaine before rocuronium application. *Group M and ML compared with group L and group C.

Tablo 4. Correlation between pain and metoprolol usage duration- Last dose (hour)

Pain		Usage Duration	Last dose/hour
Group M	r	-0.80	0.153
	p value	0.579	0.289
Group ML	r	-0.172	-0.132
	p value	0.233	0.360
Overall	r	-0.091	-0.070
	p value	0.366	0.491

Group M = patients currently using metoprolol and who did not receive lidocaine before the application of rocuronium, Group ML = patients currently using metoprolol and who received lidocaine before rocuronium application

and Group M and C ($p = 0.08$).

ASA classes were statistically significantly lower in Group L and Group C compared to Groups M and ML ($p < 0.001$).

When the groups were compared in terms of comorbidities, Groups M and ML were found to have more comorbidities compared to Groups L and C ($p < 0.001$).

No statistically significant differences were detected between the groups in HR measured pre-induction and 1st and 3rd minutes of metoprolol administration (for all $p > 0.05$). At the same time, statistically significant differences were observed between the groups regarding the HR measured post-induction and 1st and 5th minutes of intubation. Accordingly, HR was significantly higher post-induction in Group L patients than in Groups M and ML. In addition, HR was significantly higher in Groups L and C than in M and ML at the 1st and 5th minutes of the intubation (Table 3).

SBP, DBP, and MBP values were compared between the groups at pre- and post-induction, 1st and 3rd minutes after metoprolol administration, and 1st and 5th minutes of the intubation. The mean SBP value was significantly higher in Group C compared to Groups M and L at the 1st minute of metoprolol administration. We found no statistically significant difference between the groups in DBP values at all times (for all, $p > 0.05$).

Similarly, we found no statistically significant difference between the groups in MBP values at all times (for all, $p > 0.05$).

We examined the correlations between pain and duration of metoprolol usage and the time since the last dose, and no statistically significant difference was found (for all, $p > 0.05$). In addition, the pain was not correlated with other demographic and clinical features of the patients (for all, $p > 0.05$) (Table 4).

A comparison of pain scores according to the demographics was given in Table 5.No statistically sig-

Tablo 5. Comparison of demographics according to pain scores

	Pain 1 (n = 137)	Pain 2 (n = 22)	Pain 3 (n = 37)	Pain 4 (n = 4)	*p* value*	Difference
Age	58.3 ± 12.9	57.6 ± 13.8	48.1 ± 13.3	38.0 ± 17.3	**< 0.001**	4 and 1-2
Height	165.1 ± 11.5	164.5 ± 4.7	164.6 ± 6.2	164.0 ± 7.9	0.987	--
Weight	77.7 ± 13.5	77.6 ± 13.1	76.0 ± 11.9	62.3 ± 13.8	0.131	--
BMI	29.3 ± 11.4	28.7 ± 5.1	28.2 ± 4.9	23.2 ± 5.2	0.632	--
Last hour	7.9 ± 9.9	5.2 ± 5.9	7.6 ± 6.2	--	0.620	--
Usage duration	41.4 ± 54.5	35.1 ± 36.1	27.5 ± 48.8	--	0.666	--

All data were presented as mean ± standard deviation. BMI = body mass index. *One Way Anova

nificant difference was found among the pain scores in terms of height, weight, BMI, the time elapsed after the last use of metoprolol, and duration of metoprolol usage ($p > 0.05$). A statistically significant difference was found in age values ($p < 0.05$). A multiple comparison test (post-hoc) wasused to determine which group/groups caused the difference. There was a statistically significant difference between the patients with a pain score of 4 and those with a score of 1-2. The patients with a pain score of 4 were younger.There was a moderate negative correlation between pain score and age (r= -0.321, $p = 0.001$).

DISCUSSION

Rocuronium bromide is a nondepolarizing neuromuscular blocking agent characterized by a rapid onset and intermediate time of action. Rocuronium is often used to induce and maintain general anesthesia with its superior properties. However, during induction of anesthesia, the amino steroid neuromuscular blocking drug rocuronium usually causes pain and withdrawal reactions in the arm [14]. It is generally accepted that short duration burning severe pain is the cause of these spontaneous movements. In a similar study, Jimbo *et al*. [9] claimed that the primary cause of pain associated with rocuronium is not the active ingredient, but a high acetate buffer used as a solvent. So, the authors developed a new formulation of rocuronium using a low-acid concentration in a rat model, which caused no pain [9].

Although this is considered well-tolerated during injection, recent reports indicate severe pain and withdrawal reflexes after iv injection of rocuronium. Pain has been attributed to the effect of the acidic pH of rocuronium because it is supplied as an isotonic solution with a pH of 4. Blunk *et al*. postulated that the allogenic effect of amino steroid neuromuscular blocking drugs could be attributed to the direct activation of C-nociceptors, which causes pain [14].

Clinical studies are ongoing on using various agents with rocuronium as a nerve blockade agent and different variations and formulations of rocuronium [15]). In this observational study, we aimed to examine the effects of oral metoprolol or oral metoprolol plus lidocaine on rocuronium pain and reflex. Our findings showed that, although metoprolol was effective in pain reduction, its effect significantly increased when combined with lidocaine. We used the following scores were included to evaluate pain severity: (1) No reaction, (2) Movement only in the ankle, (3) Movement or withdrawal only in the arm, and (4) Diffuse reactions.

In our study, 62% of patients in Group M had no pain, while this rate was 88% in Group M+L. It means that, when added to lidocaine, the effect of metoprolol on the pain increased. On the other hand, none of the patients in Groups M and ML exhibited diffuse reactions, while three patients in the control group and one in the lidocaine groupshowed diffuse responses. In addition, the duration of using metoprolol and the time since the last dose did not affect the impact of metoprolol on reducing rocuronium pain. Lee *et al*. showed that simply through fast injection, the withdrawal response of rocuronium could be significantly reduced without using lidocaine as pretreatment [16].

Yavascaoglu *et al*. aimed to determine the effect of esmolol on the frequency and severity of pain and withdrawal reflex after injection of rocuronium with lidocaine and placebo. The authors reported that esmolol, like lidocaine, reduces the frequency of pain and withdrawal reactions associated with rocuronium injection [11].

Various pharmacological alternative agents have been attempted to reduce withdrawal reflex and pain induced by rocuronium injection. A study by Jeon *et al*. aimed to reduce withdrawal movements associated with rocuronium injection by pretreatment with acetaminophen; the pain was significantly reduced and lidocaine [17].

Davidson *et al*. [18] studied esmolol formalin's antinociceptive and cardiovascular properties in rats and found that esmolol leads to analgesia and reduces cardiovascular responses to pain.

Cheong *et al*. [19] investigated the effect of two doses of pretreatment lidocaine on the incidence of pain caused by rocuronium injection. They found that prior administration of lidocaine 10 mg or 30 mg iv decreased the incidence and severity of pain.

Mahajan *et al*. [5] investigated the effect of ketamine on rocuronium pain. Ketamine acts on a multitude of receptors. It is a non-competitive N-methyl-D-aspartic acid receptor antagonist and opioid μ receptor agonist in the central nervous system and vascular endothelium. The authors concluded that

these actions of ketamine might have attenuated the pain caused by rocuronium [59.

Asik *et al*. [12] compared the effects of lidocaine and metoprolol on pain with propofol injection. The pain mechanism of propofol is still unclear, but several factors have been accused, such as the speed of injection. It was suggested in the same study that metoprolol has a vasodilator effect, decreasing the contact of propofol with the endothelium of the vein used for the injection. This study showed that pretreatment with metoprolol was as effective as lidocaine in reducing pain associated with propofol injection [12]. As mentioned above, several studies have shown the effects of different agents on rocuronium pain and reflex. Some studies have used new formulations of rocuronium to reduce pain and reflex. However, there is still no standard method for this purpose, and reflections on this topic are still underworking.

There were some statistically significant differences between the groups regarding the other study parameters such as age, ASA, comorbidities, and heart rate. We found that the pain related to rocuronium injection was higher in younger patients. The mean age of patients using metoprolol was higher. Pain-related rocuronium injection was less among these patients. In the metoprolol group, a lower incidence of pain may not be related to metoprolol. It may be associated with their older age.

Limitations

The main limitation of the present study was its observational nature. Furthermore, participants in the groups are not enough to draw a definitive conclusion from the study. However, there is no study in the literature investigating the direct effects of orally administered metoprolol on rocuronium pain and reflex. We believe that our findings will be guiding for future studies.

CONCLUSION

This study shows that oral metoprolol reduces pain and withdrawal reflex of the arm into which the drug is injected. On the other hand, this effect further increased when metoprolol plus lidocaine were combined. There was no significant difference in the time since the last dose and duration of metoprolol usage. Further comprehensive prospective and multicenter studies are needed to clarify these effects.

Authors' Contribution

Study Conception: ÖŞ, DK; Study Design: ÖŞ, DK, TK, MK, SY; Supervision: ÖŞ; Funding: N/A; Materials: ÖŞ, DK, TK, MK; Data Collection and/or Processing: ÖŞ, SY; Statistical Analysis and/or Data Interpretation: ÖŞ, MK, SY; Literature Review: ÖŞ, MK, ŞY; Manuscript Preparation: ÖŞ, TK and Critical Review: ÖŞ, TK, MK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):918-925 Original Article

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Determining the traditional methods used by newborn women by giving birth: a descriptive study

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ABSTRACT

Objectives: This study was carried out to determine the traditional practices that women who have just given birth apply to their babies.

Methods: The research is descriptive. The participants were recruited from women who had just given birth to 198 women in a maternity hospital. The data was collected through the personal information form. The chi-square test was used to plan the categorical data in detail as the mean of the research data, as numbers, and as percentages. $p < 0.05$ was accepted as meaning.

Results: It was determined that traditional practices frequently used by women; The use of the yellow blanket against jaundice, swaddling so that the waist does not sink in, staying warm, being hard as steel, and having straight legs and the use of salt. It was determined that these practices were applied more by mothers who graduated from primary school. Although the frequency of use of applications such as burying the belly in the garden and throwing it in the water was found to be significant by age, it was determined to be used more in the group aged 31 and over. It has been determined that wearing evil eye beads is more common among mothers under the age of 25.

Conclusions: In our study, it was observed that traditional practices were widely applied in the neonatal period. Newborn health should be supported by providing continuous midwifery care to women, education, and health checks.

Keywords: Newborn, newborn care, traditional practices, culture

Birth has been accepted as a pleasing situation in every age and everywhere, and being a parent has increased the dignity of the individual in society. The relationship between health, culture, and religious belief is very important in the realization of women's health behaviors [1]. Healthcare professionals need to focus on traditional practices that can have an impact on maternal and child health practices. Families often resort to traditional methods if they cannot access or provide health care or maintenance management with modern methods. Especially in many cultures, traditional practices for maternal and child health are known [2, 3].

Tradition is a term that encompasses cultural values, habits, and behaviors transmitted from generation to generation. Traditions affect people's lifestyles. Ac-

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cordingly, the health status of people can be affected by traditions [4].

In underdeveloped, developing countries and traditional societies, they try to solve their health problems themselves and resort to traditional practices more frequently when the health service is inadequate and access to health services is difficult. Pressure from family elders and spouses, accepting the problems seen in the postpartum period as a normal situation, women's place in society, economic deficiencies, inability to reach health centers immediately, religious beliefs, lack of trust in health personnel, lack of health insurance, etc. It is not possible to go to any health institution unless it is necessary for reasons. For such reasons, individuals try to solve their health problems with traditional methods that they have seen from their families and relatives [5].

The issue of public health has covered the importance of newborn health. Parents who assume the first responsibility for the care of babies in newborn health, should be closely related to the knowledge, attitudes, and practices related to newborn care [6]. The services applied according to the modern health understanding have been examined and defined as preventive, restorative, and rehabilitation services in general health [7]. According to the developments in health services; the beliefs and practices of the traditional period continue among the people.

Turkish culture has also been under the influence of different beliefs and cultures due to the traces of the geography, cultural richness, and the influence of religions. While these practices arising from beliefs sometimes do not affect human health, its sometimes affect public health positively or negatively. However, it is undoubtedly the newborns who are most vulnerable and unprotected from the applications. Newborns are under the influence of the practices existing in the culture of the society they live in, and in time, newborns adapt to this culture and reflect their behaviors [8, 9].

The neonatal period includes the first four weeks or the first 28 days following the birth of the baby. It is the neonatal period with the highest mortality rate in childhood. It is important to provide routine health checks and home care during the newborn period and to inform family members about newborn care [4].
Traditional practices for nutrition, hygiene, jaundice, and general care are carried out after the birth of the newborn in Turkey [9]. Although these practices differ according to culture, region, and religious beliefs, it is known that they are frequently applied in Anatolian Asian regions. An example of these applications, giving sugary water, not giving colostrum [10], not breastfeeding until a few hours after the birth of the newborn or until the three azan times have passed [11], and giving water to the newborn after each feeding [12], for care; applying honey, giving lemon juice in case of hiccups, praying for mothers to protect newborns from the evil eye, wearing blue beads, wiping the mouth of their babies with carbonated water or soda, powder, soap, salting and applying margarine or olive oil when the newborn has a rash [13], not to take a bath until the navel drops, to remove the host and to prevent it, to wait for a few hours for the crusts to soften by applying olive oil, baby oil Vaseline to the baby's head [11], o put a höllük (covering with parched earth) under the newborn, to apply cologne or olive oil so that the navel of the newborn falls off as soon as possible. two women in their forties do not visit each other to prevent and protect them from flushing [14], if newborn flushing occurs, red gauze covers the face of the newborn, they have the teacher read it, they use amulets, bathing the newborn 7 days, 20 days and 40 days after birth [15], The nails are not cut until the newborn is forty days old, and if they are cut, it is thought to be a thief. When the baby is forty days old, the tradition of clipping is to first wash the newborn and then the newborn's laundry with forty-stone water, and finally to wash the mother, to use flour for bleaching or to take a bath with 40 eggshell water [10]. To prevent the newborn from developing jaundice, bathing with egg yolk, drinking mineral water and covering himself with a yellow cloth [15], bathing with gold water, giving herbal drinks and making the newborn drink his own urine, wearing gold to eliminate neonatal jaundice, wearing yellow clothes, wrists and tying a yellow thread around the neck, draining blood from the heel with a razor blade, cutting behind the ear or under the tongue, making an incision in the middle of the two eyebrows of the newborn, washing with poppy juice and feeding chicken liver [12], keeping the fluorescent lamp on to prevent neonatal jaundice [16], allowing the face of the newborn to cover the writing, to wrap a newspaper with gas oil on the back of the newborn when coughing [17]. Other applications made are; applying indigo on the fontanelle,

wrapping the soil under the newborn, putting the mother's hair in the mouth of the newborn with thrush, tying a shrine cloth in the crib to prevent the newborn from getting evil eye, adding basil, blueberry, chamomile, rose and some salt to the bath water, collecting his laundry before the evening prayer time, such as wearing a lock [16, 18], applying bat blood to prevent hair in the perineum and armpits when the newborn grows up, not taking the newborn out for forty days and not showing it to anyone [19, 20] is in the form.

While some of the methods and applications applied to the newborn in the postpartum period do not cause any negative effects on the health, some affect the newborn health negatively. Give the newborn mineral water, sugar water, honey, etc. such as feeding, swaddling, salting, placing foreign materials such as foreign currency in the belly, prolonging breastfeeding, not giving the first milk, etc. Practices such as these can have a negative effect on newborn health. However, practices such as keeping the newborn away from unknown people, not being left alone, being with his mother, singing lullabies can have positive effects on newborn health [4].

As stated in the literature; women trying to find solutions to some of their problems in the postpartum period by doing the traditional practices they have seen in their own families, especially trying this on newborns may bring some negative results. With this study, it is aimed to determine the practices made, to replace the behaviors that may cause negative results with beneficial behaviors, and to contribute to the literature for midwives and gynecology nurses who are service providers.

METHODS

Participants

The population of the research consists of women who gave birth. Women who were hospitalized in the delivery room and gynecology service of a maternity and children's hospital of a hospital affiliated to the provincial health directorate in the Mediterranean region and who met the criteria for inclusion in the study were included. There were 7413 births registered for 2021 in the hospital where the research was conducted. The minimum sample size was calculated as 191 when the 95% confidence interval, 5% margin of error, and the traditional application frequency to the newborn were 15% using the population-specific sampling method [21]. The study was carried out cross-sectionally between 20.07.2021 and 20.10.2021 with a total of 198 women who gave live birth and volunteered to participate in the study. This study is inclusion criteria; 18 years and over, able to speak and understand Turkish, had at least 1 live birth, to be voluntary, without a mental illness and who did not meet this criteria was not included this study.

Data collection

The data were collected using the "Personal Information Form (4 questions) prepared by the researchers by scanning the literature, and the questionnaire form in which traditional practices were questioned (19 questions). The pre-trial of the questionnaire used to collect data was applied to 20 women who gave birth in a private hospital and necessary corrections were made. The data of the women who filled out the questionnaire completely and volunteered to participate in the research were evaluated.

Ethical statement

Ethical approval for the study was obtained from the Clinical Research Ethics Committee (Date/No:2021/02). In addition, informed consent was obtained from women to participate in the study within the scope of the Declaration of Helsinki. In addition, the women participating in the study were informed that they could withdraw from the study at any time.

Statistical Analysis

Data analysis was performed using SPSS (Stastical Package for Social Science) 22.0 package program. Study data did not show a normal distribution. The chi-square test was used to compare numbers, percentage distributions and data. The results were evaluated within the 95% confidence interval, and $p < 0.05$ was accepted as statistically significant.

RESULTS

The mean age of the women participating in the study was 30.69 ± 8.53 years (min: 17, max: 63). 32.8% of the mothers reported that they were primary school

Table 1. Some traditional practice according to purposes

Traditional Practices for Nutrition	n	%
Waiting for three adhans to feed the newborn	3	1.5
Not giving the first milk (colostrum) of the newborn	29	14.9
Giving the newborn sugar water as the first food	33	16.9
Putting dates in the mouth of the newborn	21	10.8
Giving the newborn a teaspoon of zamzam first	36	18.5
Applications for Maintenance		
Remove forty	161	**82.6**
Not rubbing anything into the belly	55	28.2
Belly powder smearing	10	5.1
Burning cloth and smearing its ash	4	2.1
Rubbing oily dough	1	0.5
Saltwater-oil ploughing	11	5.6
Applying olive oil to the belly	191	**97.9**
Burying the belly in the courtyard of the mosque	84	43.1
Burying the belly in the schoolyard	52	26.7
Burying the belly in the garden	38	19.5
Storing the belly at home	45	23.1
Throwing the belly into the water	7	3.6
Burying in a place where there is no foot	29	14.9
Throwing the girl's home and the boy's outside	11	5.6
Cloth tie	94	48.2
Laying a hölluk under it	10	5.1
Salt	118	**60.5**
Arson	110	56.4
Giving herbal tea for gas pains	20	10.3
Cutting your nail after forty	20	10.3
Using a yellow dressing to remove jaundice	141	**72.3**
Applying clay for diaper rash	150	**76.9**
Squeezing the nose of the newborn	59	30.3

graduates and 7.7% of them were university graduates. 5.1% of those who put a hole 1.5% so that it does not get nappy rash, so that it gets its strength from the soil, 4.1% without gas pains, 3.1% for easy cleaning, 5.6% of those who take the placenta and throw it into the water, 60% of those who salt the newborn reported that they salt it so that it does not smell bad. For those who swaddle 53.8% to have straight legs, 31.8% to be hard as steel, 48.7% to keep their body temperature warm, 53.4% to sleep comfortably, and 25.6% to keep their backs from sinking; It has been reported that anise (21.5), fennel (9.2) linden (2.6), cumin (12.3) water is drunk to relieve gas pain. Wearing gold (21.5%) for jaundice, washing with gold-filled water (5.1%). Reading prayers to protect from the evil eye 75.4%, wearing evil eye beads 24.6%, wearing eggshells 2.6%, 36.4% having the teacher read it, 8.2% making amulets, 4.6% rubbing the hair of a woman with two pregnancies, rubbing soda in the baby's mouth 19.5%, applying sugar 7.7%, applying olive oil to remove diaper rash 50.3%; It has been reported that 49.7% of those who apply powder are applied. Applications such as applying eyeliner to the eyes 6.7% and applying almond oil 5.6% have been reported to make the newborn beautiful (Table 1).

While applications such as burying the belly in the garden and throwing it into the water were found to be significant according to age, it was determined that they were used more in the 31 and over group. It has been determined that wearing evil eye beads is more common in mothers under the age of 25 (Table 2).
It was determined that mothers who graduated from primary school practiced using a yellow cover to get rid of jaundice, swaddling to keep the waist from sinking, swaddling to keep warm, swaddling and salting so that they are hard as steel and their legs are straight (Table 3).

DISCUSSION

In this study, 32.8% of the mothers reported that they were primary school graduates and 7.7% of them were university graduates. The educational status statistics of our research were similar to the similar studies in the literature and the TNSA 2018 data [21, 22].

It was determined that 14.9% of the women did not give the newborn's first milk (colostrum), and it

Table 2. Comparision of age groups and some traditional applications

Traditional applications	Age groups		
	< 25 years old n (%)	25-30 n (%)	31 and up n (%)
Burying the belly in the garden			
Yes	4 (10.5)	12 (31.6)	22 (52.9)
No	50 (31.8)	45 (28.7)	62 (39.5)
X^2/ *p* value*	7.517/**0.017**		
Throwing the belly into the water			
Yes	1 (14.3)	0 (0.0)	6 (85.7)
No	53 (28.2)	57 (30.3)	78 (41.5)
X^2/ *p* value*	5.658/**0.048**		
Tie a cloth under it			
Yes	19 (20.2)	24 (25.5)	51 (54.3)
No	35 (34.7)	33 (32.7)	33 (32.7)
X^2/ *p* value*	9.780/**0.007**		
Wearing an evil eye bead			
Yes	20 (42.6)	9 (19.1)	18 (38.3)
No	34 (23.0)	48 (32.4)	66 (44.6)
X^2/ *p* value*	7.420/**0.028**		

*Chi-square test, $p < 0.05$

was also observed that 46.2% gave the baby foods other than breast milk such as zamzam, date and sugar water. Most common traditional practices in our study, it was determined that 60.5% salted their baby, 72.3% covered the newborn with a yellow cover to remove jaundice, 82.6% of them brought their fortieth off, 97.9% applied olive oil to their navel. Uysal *et al*. In their study with women who applied to a family health center in a different city to determine the traditional practices of parents with 0-12 month old babies, it was stated that 62.3% of women milked and emptied colostrum and did not give it to the newborn. 80% salt their baby; 12.1% covered the yellow cover for neonatal jaundice, and 60.9% washed with egg yolk; It was stated that 56.7% of them applied cologne to the navel of nature, 30.7% of them applied olive oil [15]. The research conducted is similar to our study, it has been observed that women use traditional practices at high rates. According to the basic newborn care guide of the Ministry of Health; It is recommended to initiate breastfeeding within the first 30-60 minutes after birth and to give colostrum to the newborn [23]. According to the WHO guidelines for maternal and newborn care recommendations for a positive postpartum experience; For neonatal jaundice, which affects approximately 60-80% of newborns, it has been recommended to check the total serum bilirubin level before discharge from the health institution [24].

In our study, 56.4% of the women (110 women) swaddled the newborn; When asked why; 53.8% (59 women) have straight legs, 31.8% (35 women) have hard as steel, 48.7% (54 women) keep their body temperature warm, 53.4% (59 women) sleep comfortably, 25.6% (28 women) keep their waist down They stated that they were arson. In the research conducted by Arabacı *et al*. in a family health center to determine the traditional methods applied to babies; 48% of women (47 women) swaddle the newborn; They reported the reasons for swaddling their babies as having straight legs (22 women), not getting a cold (11 women), sleeping well (9 women), not hitting their hands on their face (2 women), and "I learned that

Table 3. Comparision of education status and some traditional applications

Traditional Applications	Education Status			
	Primary school n (%)	Secondary school n (%)	High school n (%)	University n (%)
Using a yellow dressing to pass jaundice				
Yes	44 (52.4)	22 (26.2)	15 (17.9)	3 (3.6)
No	31 (27.9)	31 (27.9	37 (33.3)	12 (10.8)
X^2/ *p* value*	15.039/**0.002**			
Arson so that the waist does not sink				
Yes	28 (56.0)	11 (22.0)	9 (18.0)	2 (4.0)
No	47 (32.4)	42 (29.0)	43 (29.7)	13 (9.0)
X^2/ *p* value*	9.127/**0.003**			
Arson so that it stays warm				
Yes	46 (48.4)	24 (25.3)	17 (17.9)	8 (8.4)
No	29 (29.0)	29 (29.09	35 (35.0)	7 (7.0)
X^2/ *p* value*	10.501/**0.014**			
Arson so that it is hard as steel				
Yes	33 (53.2)	19 (30.6)	8 (12.9)	2 (3.2)
No	42 (31.6)	34 (25.6)	44 (33.1)	13 (9.8)
X^2/ *p* value*	14.369/**0.002**			
Arson so that the legs are smooth				
Yes	49 (46.7)	33 (31.4)	20 (19.0)	3 (2.9)
No	26 (28.9)	20 (22.2)	32 (35.6)	12 (13.3)
X^2/ *p* value*	17.360/**0.001**			
Salt				
Yes	43 (49.4)	22 (25.3)	17 (19.5)	5 (5.7)
No	32 (29.6)	31 (28.7)	35 (32.4)	10 (9.3)
X^2/ *p* value*	8.881/**0.031**			

*Chi-square test, $p < 0.05$

way" (2 women) [21]. The rates of the research conducted with our research are similar in this aspect.

In this stuy, 50.3% of those who apply olive oil to remove diaper rash; powder application was determined as 49.7%. In the research of Arabacı *et al*. [21], those who apply olive oil to remove diaper rash were 5.1%; those who use powder are 1%, those who use diaper rash cream are 74.5%; In the study of Arısoy *et al*. [25], those who apply powder to protect against diaper rash are 40.3%, those who apply olive oil 19.4%, those who apply powder when they have rashes, 10.1%, those who use olive oil, and 68% of those who use the diaper cream given by the doctor.

Pathak *et al*. [26] in their research, 49.5% of women applied anything on the cord after delivery; Of these, 85.8% applied oil, 14.2% powder, 52.5% applied a liner/pencil to the eyes of the newborn after birth, 8% did not give colostrum to the newborn, 16% gave honey except for breast milk, 4% she reported that gave water. In a systematic review of quantitative and qualitative data compiled by Bee *et al*. to related newborn care practices in sub-Saharan Africa (Ethiopia, Ghana, Malawi, Tanzania and Uganda) were evaluated; It has been determined that agents are

applied to the cord, most commonly to prevent infections or to aid wound healing. this has been reported to occur due to lack of knowledge about breastfeeding and not giving colostrum with the belief that it is unclean or harmful [27].

According to the WHO guidelines It has been recommended to keep the umbilical cord clean and dry for the prevention of neonatal infection, and not to routinely apply creams, ointments, lotions, oils, gels, sprays and emulsions specified as topical emollients to the skin of term and healthy newborns [24]. The health benefit of newborns, where traditional practices are frequently applied in many countries and societies, should be considered. For this purpose, according to the WHO guideline for maternal and newborn care recommendations for a positive postpartum experience; it was stated that providing information, educational interventions and counseling are necessary to prepare women and parents for postpartum discharge from the health facility in order to improve maternal and newborn health outcomes and facilitate home care. It is suggested that there should be written/digital educational booklets and illustrated educational materials for the semi-literate population.

The necessity of ensuring continuity of care under the leadership of the midwife was emphasized. The application of midwife-led continuity of care models, in which a midwife or a group of midwives support the woman throughout the prenatal, intrapartum and postpartum period, is recommended for women. It has also been proposed to share tasks with a wide range of staff, including nurses, midwives and doctors, to promote health-related good behavior towards maternal and newborn health. Again, for all mothers and newborns, on day 3 (48-72 hours), 7-14 days after birth. recommended at least three additional postpartum visits on days and six weeks postpartum. This visits are important opportunities for observing traditional practices [24].

CONCLUSION

As a result; in our study, it is seen that traditional practices are widely applied in the neonatal period. Societies that ignore traditional practices should be effectively use health care services. For this reason, midwives and nurses have important duties. Informing the society about traditional practices that have drawbacks should be among their main duties.

Authors' Contribution

Study Conception: AB, MS, MO, AG; Study Design: AB, MS; Supervision: AB; Funding: N/A; Materials: AB, MO, AG; Data Collection and/or Processing: AB, MS, MO; Statistical Analysis and/or Data Interpretation: AB, MS; Literature Review: AB, MS, MO, AG; Manuscript Preparation: AB, MS, MO, AG and Critical Review: AB, MS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The relationship of laboratory values with prognosis in acute stroke recanalization treatment applied patients

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ABSTRACT

Objectives: Many factors affect the results of acute recanalization treatment and prognosis of ischemic stroke patients. Some markers which has a role in inflammation process cause atherosclerosis that leads to ischemic stroke. We aimed to evaluate the relationship between admission laboratory findings and prognosis in patients to whom acute recanalization therapy were applied.

Methods: In our study, we evaluated 139 acute stroke patients to whom acute recanalization therapies had been applied. Demographic data, glomerular filtration rate, uric acid, albumin, lipid profile, C-reactive protein, fibrinogen values were evaluated. Admission and discharge National Institutes of Health Stroke Scale and modified Rankin Scale scores were recorded. The effect of laboratory parameters on prognosis was examined. $P < 0.05$ was considered significant.

Results: Tissue plasminogen activator (tPA) therapy was applied to 53 (38.1%) patients, thrombectomy to 62 (44.6%) patients, tPA bolus+thrombectomy to 3 (2.2%) patients, tPA full dose+thrombectomy to 19 (13.7%), and thrombectomy+stent to 2 (1.4%) patients. None of the laboratory were statistically related to prognosis except for lymphocytes count ($p = 0.012$) and albumin ($p = 0.01$). There was no relationship between laboratory findings with hemorrhagic transformation and acute recanalization treatment outcome.

Conclusions: In the etiology of ischemic stroke, there are many inflammatory processes that cause atherosclerosis such as hypertension, hyperlipidemia, diabetes mellitus. The effect of admission laboratory values on prognosis has not been clarified. In patients with acute recanalization therapies, admission laboratory findings has no effect on patient management. Consequently, laboratory parameters provide limited information about the prognosis of patients who underwent acute recanalization therapies.

Keywords: Acute stroke, thrombectomy, thrombolysis in cerebral infarction (TICI), laboratory findings

In the etiology of ischemic stroke, hypertension, hyperlipidemia, diabetes mellitus and smoking are major risk factors [1]. The role of C-reactive protein (CRP), fibrinogen, white blood cell (WBC), complement fragments, lipoprotein (a) and acute phase reactants (AFR) in the etiology of ischemic stroke is not clear [2]. There are multiple factors affecting the prognosis of stroke patients who underwent acute recanalization therapies such as perfusion grade of vessels after thrombolysis and hemorrhagic transformation of ischemic parenchyma. The perfusion grade of vessels after thrombolysis was stated as grade 0 is 'no perfu-

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sion'.Grade 1 means minimal perfusion and grade 2 means partial perfusion. Partial perfusion is divided into grade 2A and 2B. 2A means that only a portion of the vascular region (less than two-thirds) is visible. Grade 2B is indicated when the estimated vascular territory is completely filled, but the filling is slower than usual. Complete perfusion implies grade 3 [3]. There are limited satisfactory data on whether admission laboratory findings affect Thrombolysis in Cerebral Infarction (TICI) and the European Cooperative Acute Stroke Study II (ECASS II), which evaluated hemorrhagic transformation. ECASS II defines HI as petechial hyperdensities in the infarct region. If it is punctat petechiae, it is referred to as HI1, and if it is confluent petechiae, it is referred to as HI2. PH means for homogenously hyperdense hematoma, and it is referred to as PH1 if it covers less than 30% of the infarct region, and PH2 if it covers more than 30% of the infarct area with mass effect [4].

CRP, an inflammatory marker, reveals vascular inflammation caused by a cytokine-dependent inflammatory process in the vascular system, as well as arterial atherosclerosis caused by the inflammation. Decreased albumin levels are also linked to peripheral vascular disease. According to certain research, the CRP/albumin ratio (CAR) may be a factor influencing stroke mortality [5].

We aimed to evaluate the relationship between admission laboratory findings and prognosis of stroke patients who underwent acute recanalization treatments.

METHODS

Study Design and Patients

Total 139 patients who underwent acute recanalization treatment between 2019-2022 were evaluated retrospectively. All of the patients and their families signed written informed consent forms. Before receiving acute recanalization treatment, each patient was examined by a physician. Demographic data of the patients, CRP, albumin, uric asid, fibrinogen values, lipid profile, National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS) scores, results of recanalization treatments according to TICI, consideration of 24th CT according to ECASS II were recorded. In the assessment of laboratory findings, patients with elevated CRP values secondary to infection were excluded from the study.

Study Outcomes

The relationship between the admission laboratory findings and the acute recanalization treatment results (TICI) or the 24th hour CT results (ECAS II) were evaluated. The effect of laboratory findings on prognosis was examined by comparing the NIHSS and mRS scores at admission and discharge. Among the laboratory measures, inflammation-related indicators were explicitly chosen. Simultaneously, the lipid profile was chosen since hyperlipidemia plays a role in the etiology of stroke.

Ethical Approval

Ethics committee approval was obtained with protocol number 2022/83 (Decision no.:2022-05, Date: 07.03.2022) from Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethical Committee.

Statistical Analysis

Statistical analyzes were performed using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Shapiro-Wilk test was used to detect normal distribution of the data. ANOVA test (post hoc: Bonferroni test) or Kruskal Wallis H tests (post hoc: Dunn's test) were used to compare numerical variables in groups of three or more. Chi-square test, Yates correction and Fisher exact test were used for comparison of categorical data. The relationship between numerical variables was examined by Pearson or Spearman correlation analysis. $P < 0.05$ was considered statistically significant.

RESULTS

The mean age of 139 acute stroke patients who underwent recanalization treatment was 70.6 ± 14 years, 64 of the patients were female and 75 were male. Sixty (43.2%) patients had hypertension, 40 (28.9%) patients had diabetes mellitus, 42 (30.2%) patients had coroner artery disease, 22 (15.8%) of them had history of ischemic cerebrovascular disease, 9 (6.5%) patients had hyperlipidemia, 2 (1.4%) patients had malignancy, and 47 (33.8%) had other comorbidities. Thrombec-

Table 1. Comparison of TICI and laboratory findings

Variables	TICI (1) n = 13	(2A) n = 11	(2B) n = 15	(3) n = 45	*p value*
GFR (mL/min)	77.6 ± 21.8	87.6 ± 15.9	83.9 ± 19.9	83 ± 24.6	0.747
Uric acid (mg/dL)	5.9 ± 1.2	5.3 ± 0.9	5.4 ± 1.0	5.7 ± 1.5	0.701
CRP (mg/L)	10.9 (0-148.3)	7 (1-85)	3.4 (0-24)	6 (0-161)	0.244
Albumin (g/L)	36.4 ± 4.1	37.2 ± 3.7	38.8 ± 2.2	37.8 ± 3.9	0.383
HDL (mg/dL)	44.7 ± 12	35.4 ± 9.3	42.5 ± 12.5	41.8 ± 10.6	0.280
LDL (mg/dL)	137.1 ± 43.6	90.3 ± 30.5	127.1 ± 32.8	125.5 ± 62.8	0.227
TG (mg/dL)	99 (37-178)	95 (37-145)	92.5 (50-284)	92.5 (28-329)	0.949
Fibrinogen (mg/dL)	338 (338-338)	338 (338-338)	447 (447-447)	386 (317-457)	0.706
CAR	0.3 (0-5.2)	0.2 (0-2.1)	0.1 (0-0.6)	0.2 (0-5.4)	0.207

TICI = Thrombolysis in Cerebral Infarction, GFR = glomeruler filtration rate, CRP = C-reactive protein, HDL = high density lipoprotein, LDL = low density lipoprotein, TG = triglyceride, CAR = CRP-albumin ratio

TICI 0: no perfusion, **1**: antegrade reperfusion past the initial occlusion, but limited distal branch filling with little or slow distal reperfusion, **2a**: antegrade reperfusion of less than half of the occluded target artery previously ischemic territory, **2b:** antegrade reperfusion of more than half of the previously occluded target artery ischemic territory, **3:** complete antegrade reperfusion of the previously occluded target artery ischemic territory, with absence of visualized occlusion in all distal branches

Table 2. Comparison of ECASS II and laboratory findings

Variables	ECASS II HI1 n =2 5	HI2 n = 15	PH1 n = 21	PH2 n = 12	Hemorrhage (-) n = 61	*p value*
GFR (mL/min)	79.6 ± 22.9	82.8 ± 31	75.3 ± 20.9	79.3 ± 20.4	86.3 ± 19.7	0.319
Uric acid (mg/dL)	5.6 ± 1.1	5.9 ± 1.6	5.2 ± 1.6	5.9 ± 2.8	5.7 ± 1.5	0.639
CRP (mg/L)	8 (0-85)	3.1 (0-17.5)	5.1 (0.7-148.3)	5.1 (1-161)	6 (0.6-100.1)	0.244
Albumin (g/L)	38.2 ± 2.7	38.1 ± 3.5	37.9 ± 4.2	37 ± 3.2	37.6 ± 4.9	0.932
HDL (mg/dL)	41.9 ± 10.6	44 ± 14.4	57.6 ± 56.3	41.2 ± 9.7	41.7 ± 10.5	0.142
LDL (mg/dL)	134 ± 70.1	119.7 ± 53.2	125.6 ± 44.9	117.5 ± 44.3	128.8 ± 41.6	0.863
TG (mg/dL)	100 (37-329)	91.5 (45-275)	96 (37-284)	113 (28-161)	105 (50-436)	0.828
Fibrinogen (mg/dL)	338 (337-386)	317 (317-317)	-	372.5 (260-428)	384 (0.7-615)	0.711
CAR	0.2 (0-1.9)	0.1 (0-0.5)	0.1 (0-5.2)	0.1 (0-5.4)	0.2 (0-3.1)	0.268

ECASS II = European Cooperative Acute Stroke Study II, GFR = glomeruler filtration rate, CRP = C-reactive protein, HDL = high density lipoprotein, LDL = low density lipoprotein, TG = triglyceride, CAR = CRP-albumin ratio

Hemorrhagic infarction type 1 (HI1) indicates petechial hemorrhages at the infarct margins. Hemorrhagic infarction type 2 (HI2) shows petechial hemorrhages throughout the infarct and there is no mass-effect attributable to the hemorrhages. Parenchymal hematoma type 1 (PH1) defines less than 30% of the infarcted area has hemorrage and there's minor mass effect attributable to the hematoma. Parenchymal hematoma type 2 (PH2) if it covers more than 30% of the infarct area with mass effect

tomy was applied to 62 (44.6%) patients, 53 (38.1%) patients treated by full dose tissue plazminogen activator (tPA), 19 (13.7%) patients by full dose tPA with thrombectomy, 3 (2.2%) patients by bolus tPA with thrombectomy, and 2 (1.4%) patients underwent to thrombectomy with stent procedure. In the assessment of laboratory values, the mean glomeruler filtration rate (GFR) was 82.5 ± 21.7 mL/min, uric acid: 5.6 ± 1.6 mg/dL, CRP: 6 mg/L, albumin: 37.8 ± 4.1 g/L, high density lipoprotein (HDL): 44.3 ± 23.6 mg/dL, low density lipoprotein (LDL): 127 ± 9.2 mg/dL, triglyceride (TG): 101 mg/dL, and the CAR: 0.2. The laboratory findings were compared according to TICI grades and there was no statistical significance (Table 1). There was no significant difference between the laboratory findings according to the groups of hemorrhagic transformation (Table 2). Admission and discharge NIHSS and mRS scores were compared to laboratory findings but there was no significant difference (Table 3). There was a direct relationship between albumin level and admission mRS ($p = 0.001$). The same relationship between discharge mRS and albumin level was not discovered. At the same time, there was no significant difference in NIHSS and albumin levels.

DISCUSSION

Comorbid diseases, hemodynamic, metabolic or infectious status of patients at admission usually affect the success of the acute recanalization therapy process and the follow-up period. Prognosis of the patients may be affected by these multiple conditions. Systemic infections which caused endothelial damage, impaired hemostasis and clot formation have been shown to be associated with stroke [6]. It was considered that the CRP and fibrinogen values at admission may be ineffective regarding the outcome of the acute recanalization process. In a meta-analysis, it was stated that the logarithmic increase in CRP and fibrinogen values is associated with increased risk of recurrent major vascular events in stroke patients [7]. We don't know whether CRP and fibrinogen levels are effective in the acute, chronic, or both processes. In our study, CRP and fibrinogen values of the patients were compared according to the TICI results, there was no significant difference between the groups. It was considered that the CRP and fibrinogen values of admission may be ineffective for the outcome of the acute recanalization process. The limited influence of our study results on the acute process does not allow us to assess their ef-

Table 3. Comparison of laboratory findings with admission and discharge NIHSS and mRS scores

	Admission				Discharge			
Variables	**NIHHS**		**mRS**		**NIHHS**		**mRS**	
	r	*p*	r	*p*	r	*p*	r	*p*
GFR	-0.090	0.295	-0.115	0.188	-0.109	0.282	-0.139	0.109
Uric acid	0.022	0.805	0.012	0.890	-0.088	0.394	0.012	0.891
CRP	0.020	0.819	0.072	0.413	0.036	0.722	-0.007	0.940
Albumin	-0.055	0.524	-0.305	**0.001**	-0.124	0.220	-0.030	0.726
HDL	0.076	0.398	0.049	0.588	-0.074	0.468	-0.040	0.657
LDL	-0.055	0.546	-0.100	0.264	-0.027	0.796	0.025	0.781
TG	-0.039	0.671	-0.048	0.600	-0.077	0.453	0.104	0.249
Fibrinogen	0.040	0.854	0.041	0.851	0.022	0.927	0.078	0.716
CAR	0.013	0.878	0.094	0.279	0.034	0.735	-0.006	0.948

CAR: CRP albumin ratio.

NIHHS = National Institutes of Health Stroke Scale, mRS =modified Rankin Scale scores, GFR = glomeruler filtration rate, CRP = C-reactive protein, HDL = high density lipoprotein, LDL = low density lipoprotein, TG = triglyceride, CAR = CRP-albumin ratio

fect on the chronic process. Similarly, NIHSS and mRS scores (admission and discharge) and hemorrhagic transformation according to ECASS II were not affected by admission CRP and fibrinogen values. We suggest that, admission CRP and fibrinogen values do not affect the short-term prognosis.

Albumin values, a negative acute phase reactant was reported to be lower in patients with high level carotid artery stenosis compared to patients without stenosis [8]. On the contrary, in our study, the albumin, CRP and CRP albumin ratio did not affect the admission-discharge NIHSS, mRS scores, the results of the procedure (TICI) and the degree of hemorrhagic transformation (ECASS II). There was only a slight correlation between the admission albumin level and the admission mRS score. In general, the admission albumin level could be ignored in patients who will undergo acute recanalization treatment. Many hemodynamic alterations and systemic illnesses might affect albumin value. Although if it is difficult to assign meaning solely, it can be thought of as a factor when combined with other laboratory variables.

Serum uric acid level is strongly correlated with GFR and triglyceride level in diabetic patients and therefore there is a high risk for stroke in these hyperuricemic patients [9, 10]. Because of the common ethiology atherosclerosis, it is reasonable to assume that stroke patients may have high uric acid levels. However, in our study, we found that uric acid and GFR did not affect the outcome of the procedure, hemorrhagic transformation and short-term prognosis in patients treated with acute recanalization therapies. Since this presence largely correlates with chronic processes, it is difficult to take into account increased uric acid for stroke patients in the acute stage.

Dislipidemia is also a risk factor for acute stroke and TG < 100.2 mg/dL was found as an independent risk factor in the acute-term stroke mortality in a study [11]. A high LDL cholesterol concentration in acute stroke patients with large artery occlusion is independently associated with a favorable prognosis at 3 months [12]. On the other hand, there is a perplex effect of dyslipidemia on reperfusion therapies. According to our results the admission lipid profile did not effect the TICI score and short-term ECASS II results. Similarly admission and discharge NIHSS, mRS scores were not affected by the lipid profile. Although a positive correlation between ischemic stroke and LDL cholesterol and triglyceride levels and a negative correlation with HDL cholesterol were reported, we did not find any relationship in the acute period. Dyslipidemia clearly has an impact on stroke risk. Lack of long-term follow-up may be the cause of our study's inability to detect a significant difference. These findings also raise questions about how crucial it is to take into account the lipid profile when choosing a course of treatment for the acute phase of the disease.

Limitations

Lack of consideration of lipid subgroups in our study is a shortcoming. At the same time, there is insufficient data to provide information about the long-term prognosis.

CONCLUSION

Our study implies that the laboratory findings such as lipid profile, infectious markers at admission do not affect the outcome of the procedure and do not have an effect on the short term prognosis in the patients who underwent to acute stroke recanalization treatments.

Authors' Contribution

Study Conception: İA; Study Design: İA; Supervision: VAY; Funding: N/A; Materials: MS; Data Collection and/or Processing: MS, HOY; Statistical Analysis and/or Data Interpretation: İA, HOY; Literature Review: HAE; Manuscript Preparation: İA, HAE and Critical Review: HAE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Long-term outcomes of COVID-19 infection in patients with solid tumors

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ABSTRACT

Objectives: We analyzed the impact of some clinical and disease-specific factors on the long-term outcomes of SARS-CoV-2 infection in patients with solid tumors.

Methods: Total of 739 patients with known solid malignancy and infected by SARS-CoV-2 before the beginning of vaccination were examined.

Results: Seventy-six cancer patients died from COVID-19 infection-related effects such as mostly pulmonary and cardiovascular system disorders after a median 16-month follow-up (67.1% and 14.5%; respectively). Compared with survivors (n = 468), non-survivors due to COVID-19 infection related effects (n = 76) were more likely to be aged ≥ 65 years and diagnosis with lung cancer ($p = 0.01$). Also, female patients were at decreased risk of mortality [OR: 0.34 (95% CI: 0.18-0.65)]. Furthermore, patients with tumor stage IV, active/stable/progressive disease and patients receiving active anticancer therapy were at increased risk of mortality ($p = 0.01$).

Conclusions: The patients with aged ≥ 65 years, diagnosed with lung cancer, receiving active anticancer therapy, with active/stable/progressive and advanced cancer stage were at increased risk of mortality from COVID-19 infection in long-term follow-up.

Keywords: Cancer patients, COVID-19, mortality, long-term outcomes

The pandemic of SARS-CoV-2 infection have led to considerably increased mortality and morbidity [1, 2]. Furthermore, it is known that COVID-19 survivors could survive many multisystem sequelae ranging from pulmonary, cardiovascular system changes to neurocognitive disorders [3, 4]. As shown in the studies, patients with a diagnosis of cancer have increased risk for worse SARS-CoV-2 infection-related outcomes compared to the healthy population [5-8]. Approximately 60%-80% of the patients with cancer infected with SARS-CoV-2 survive infection critically [9-11].

SARS-CoV-2 infection-related case fatality rates in patients with solid cancer have been reported to range from 13% to 41% in the literature [9, 12, 13]. While ElGohary *et al*. [14] showed that a mortality rate of up to 21% and an intensive care unit hospitalization rate of 14% in patients with cancer, Barlesi *et al*. [15] did not show any difference in mortality compared to the normal population. The large population-

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based data on outcomes of 20.133 patients with COVID-19 demonstrated that mortality was significantly increased in patients with neoplasm [16]. Also, the study revealed that when needed to stay in the intensive care unit, patients with immunosuppressed systems showed increased mortality [17].

In the literature, increasing age, comorbidities and immunosuppression by the disease and/or its treatment are shown to be risk factors for poor outcomes after COVID-19 in patients with cancer [18, 19]. Also, smoking, poor performance status, oncological characteristics such as specific cancer types, tumor stage, presence of active cancer and recent systemic anticancer therapy may contribute to worse prognosis in patients with cancer during COVID-19 infection [13, 20-22].

Increased SARS-CoV-2 testing capacity and discovering of SARS-CoV-2 biology and clinical management of the infection and complications by several new therapies, vaccinal campaigns have improved the outcomes of COVID-19 infection in this vulnerable population [23-26]. In this report, we intended to analyze the impact of some clinical and disease specific factors on the outcomes of COVID-19 infection in patients with solid tumors before the begining of vaccination period.

METHODS

Patient Population

A total of 739 patients, followed up with the diagnosis of solid malignancy in our department and infected by SARS-CoV-2 (positive nasopharyngeal swab) between May 2020 and December 2020 (pre-vaccine period in our country) were examined retrospectively. The patients with aged ≥ 18 years, diagnosis of SARS-CoV-2 infection confirmed by nasopharyngeal swab, diagnosis of active or in remission solid cancer were included in the study. Active cancer therapy was defined as patients who received anti-cancer treatment (Chemotherapy, hormonal therapy, immunotherapy, targeted therapy) within 4 weeks period before the diagnosis of COVID-19 infection.

Data Collection

Data about demographic characteristics, cancer diagnosis, SARS-CoV-2 infection, mortality, morbidity and long-term outcomes of the patients were retrospectively collected from the hospital database. The cancer status of the patients was collected at the time of SARS-CoV-2 infection and at follow-up period.
The study was conducted by the Declaration of Helsinki and was approved by the Gazi University local ethics committee. Ethics Committee approval has been obtained (Date: 05.07.2022 , Decision no.: 13).

Statistical Analysis

The Statistical Package for the Social Sciences software version 23 (SPSS) was used for the statistical analysis. The presence of a normal distribution was investigated using visual and analytical methods. Categorical measurements were presented as number (n) and percentage (%). As normally distributed quantitative variables were described as mean values ± standard deviation, non-normally distributed variables were expressed as median values (range). While, non-parametric tests were used to compare the parameters and the ordinal variables, the chisquare test was used the compare the proportions in different groups. For multivariate analysis, logistic regression analysis was performed to determine independent predictors of patient outcome. A value of $p < 0.05$ was considered statistically significant.

RESULTS

A total of 739 patients (378 males, 361 females) median aged 61 years (18-94) were included in the study. The baseline characteristics of the patients during COVID-19 infection are demonstrated in Table 1. The most common diagnosed malignancies were lung cancer, gastrointestinal cancer and breast cancer (25.7%, 24.4%, and 19.4%; respectively). Thirteen point nine percent of the patients had at least two comorbidities, such as hypertension, diabetes mellitus, chronic obstructive lung disease. Most (42.4%) of the patients had stage IV disease. While 312 (42.2%) patients were in remission from cancer, 427 (57.8%) patients were with active cancer. Also, 362 (49%) patients were on active anticancer therapy in the last 4 weeks before COVID-19 infection.

The clinical characteristics of the patients during COVID-19 infection are demonstrated in Table 2.

Table 1. Baseline characteristics of the patients (n = 739)

Characteristics		Data
Age, n (%)	< 65 years	462 (62.5)
	≥ 65 years	277 (37.5)
Sex, n (%)	Male	378 (51.2)
	Female	361 (48.8)
Comorbidities, n (%)	0-1	636 (86.1)
	≥ 2	103 (13.9)
Cancer diagnosis, n (%)	Lung	190 (25.7)
	Gastrointestinal tract	180 (24.4)
	Breast	143 (19.4)
	Urogenital	53 (7.2)
	Gynecologic	37 (5)
	Head and neck	20 (2.7)
	Others	89 (12)
	Two primary	27 (3.6)
Tumor stage at diagnosis, n (%)	I	91 (12.3)
	II	198 (26.8)
	III	137 (18.5)
	IV	313 (42.4)
Cancer status during COVID-19 infection, n (%)	Active/stable/progressive	427 (57.8)
	Remission/no evidence of disease	312 (42.2)
Active anticancer therapy during COVID-19 infection, n (%)	Yes	362 (49)
	No	342 (46.3)
	Missing	35 (4.7)

Most of the patients were not hospitalized (n = 646, 87.4%), however 35 (4.8%) patients were hospitalized in the intensive care unit. While 22 (3%) patients needed the use of a mechanical ventilator, 42 (5.7%)patients needed to use supplemental oxygen.

The median follow-up period of the patients from the diagnosis of cancer to COVID-19 infection was 16 (1-24) months. During the follow-up, 36.7% (n = 271) of the patients died (Table 3). Most of the patients died from tumor related and other reasons (n = 195, 72%). Seventy-six cancer patients died from COVID-19 infection-related effects such as mostly pulmonary and cardiovascular system complications (67.1% and 14.5%; respectively). The mortality rate of COVID-19 infection was calculated as 14% (76/544). When the active cancer status of the survivors was examined, it was seen that most patients were in remission (n = 336, 71.8%) (Table 3).

Compared with survivors (n = 468), non-survivors due to COVID-19 infection-related effects were more likely to be aged ≥ 65 years and diagnosis with lung cancer ($p = 0.01$) (Table 4). Also, female patients were at decreased risk of mortality ($p = 0.01$). Patients with tumor stage IV, active/stable/progressive disease and patients receiving active anticancer therapy were at increased risk of mortality ($p = 0.01$). Non-survivors were more likely to be not hospitalizated for COVID-19 or COVID-19 complications in the intensive care unit ($p = 0.01$) (Table 4). Also, survivors were more likely to use supplemental oxygen ($p = 0.01$).

Table 2. Characteristics of the patients with COVID-19 infection (n = 739)

Characteristics		Data
Hospitalization for COVID-19 or COVID-19 complications, n (%)	Yes, but no intensive care	58 (7.8)
	Yes, and intensive care	35 (4.8)
	No	646 (87.4)
Use of mechanical ventilator, n, (%)	Yes	22 (3)
	No	717 (97)
Use of supplemental oxygen, n (%)	Yes	42 (5.7)
	No	697 (94.3)

When the predictors of mortality were analyzed using a multivariable ordinal logistic regression model, being aged ≥ 65 years has increased risk of mortality related to COVID-19 infection [OR: 4.07 (95% CI: 2.14-7.75)]. Also, being a female has decreased the risk of mortality [OR: 0.34 (95% CI: 0.18-0.65)].

DISCUSSION

This study underscores some clinical and disease-spesific factors on outcomes of COVID-19 infection. In this report, we highlighted that gender, age, cancer diagnosis, tumor stage, cancer status and whether or not receiving active cancer therapy are important factors for mortality related to COVID-19 infection. Although a decrease in mortality has been achieved after effective vaccination programs, prevention strategies such as organization of healthcare visits and timing of imaging for cancer patients with risk factors will significantly contribute to the decrease in mortality and morbidity.

It is known that COVID-19 has affected more than 220 million individuals worldwide [27]. Cancer patients are susceptible to infection with COVID-19 due to immunocompromised status secondary to malignancy, predisposition for malnutrition, and immunosuppressive treatment strategies and these patient population suffer worse disease outcomes [28, 29]. In the studies, it was seen that COVID-19 infection in cancer patients leads to poor illness, more need to stay in the intensive care unit and use of ventilator. In the current study, 4.8% of the patients were hospitalized in the intensive care unit. While 3% of the patients needed to use of ventilator, 5.7% of the patients needed to use supplemental oxygen.

The mortality rate of COVID-19 infection was

Table 3. Demographic and clinical characteristics of the patients at the post COVID-19 period

Characteristics			Data
Non-survivor, n (%)			271 (36.7)
Reasons for mortality, n (%)	COVID-19 infection-related effects	Cardiovascular system	11 (14.5)
		Pulmonary system	51 (67.1)
		Renal system	4 (5.3)
		Neurological system	2 (2.6)
		Others	8 (10.5)
	Tumor-related and others		195 (72)

Table 4. Clinical characteristics of the non-survivors due to COVID-19 infection (n = 76) compared with survivors (n = 468)

Parameter		Data	*p* value
Age, n (%)	< 65 years	24 (31.6)	**0.01**
	≥ 65 years	52 (68.4)	
Gender, n (%)	Female	27 (35.5)	**0.01**
	Male	49 (64.5)	
Comorbidities, n (%)	0-1	63 (82.9)	0.65
	≥ 2	13 (17.1)	
Cancer diagnosis, n (%)	Lung cancer	39 (51.3)	**0.01**
	*Non-lung cancer	*37	
Cancer status during COVID-19 infection, n (%)	Active/stable/progressive	64 (84.2)	**0.01**
	Remission/no evidence of disease	12 (15.8)	
Active anticancer therapy, n (%)	Yes	56 (73.7)	**0.01**
	No	18 (23.7)	
	Missing	2 (2.6)	
Hospitalization for COVID-19 or COVID19 complications, n (%)	No	46 (60.5)	**0.01**
	Yes, but no intensive care	4 (5.3)	
	Yes, and intensive care	26 (34.3)	
Use of supplemental oxygen, n (%)	Yes	27 (35.5)	**0.01**
	No	49 (64.5)	

*Cancer types other than lung cancer

ranges from 13% to 41% in the literature [9, 12, 13, 30-32]. In our study, mortality rate of COVID-19 infection was calculated as 14% (76/544) in accordance with the other studies.

Patients with cancer diagnosis are also at an increased risk of developing post-COVID complications including the heart, lung, kidney, skin and brain [33-35]. In our study, 76 cancer patients died from COVID-19 infection-related effects such as mostly pulmonary and cardiovascular system complications (respectively; 67.1%, 14.5%) with the median follow-up period of 16 (1-24) months.

Many risk factors for mortality related to COVID-19 infection are defined in the guidelines [36]. Increasing age, comorbidities and immunosuppression by the disease and/or its treatment have shown as the risk factors for poor outcomes after COVID-19 infection in patients with cancer in the literature [18, 19]. Immune responses to viral infections are weaker in males than in females and compatible with this information, female patients were at decreased risk of mortality in our study ($p = 0.01$) [37]. Furthermore, ≥ 65 years was associated with dysregulated immune system, more severe disease and with a high mortality rate in the studies [38]. In our study, compared with survivors (n = 468), non-survivors due to COVID-19 infection-related effects were more likely to be aged ≥ 65 years.

The cancer patients infected with SARS-CoV-2 mostly had lung cancer, gastrointestinal cancer and breast cancer in our study which are in fact the common types of cancers. Therefore, this finding may be a related to the frequency of these tumors. In regression analysis, it was seen that non-survivors due to COVID-19 infection-related effects were more likely to be diagnosed with lung cancer ($p = 0.01$). In the studies, lung cancer during COVID-19 infection was

demonstrated to be associated with pulmonary complications and mortality [39]. Defective pulmonary architecture related to thoracic surgery or radiotherapy, smoking associated lung injury, the alterations of alveolar epithelium and pulmonary vessels lead to the development of pulmonary complications in patients with lung cancer during the SARS-CoV-2 infection [7, 39, 40]. In the literature, patients with comorbidities such as hypertension, diabetes, cardiovascular disease or cerebrovascular disease are found to have an increased risk of COVID19 related mortality due to increased the complexity and difficulty of treatment of comorbidities after COVID-19 infection. In our study, we did not demonstrate the same association. This can be due to rigorous management of the patients with comorbitidies. Furthermore, the preventive measures for COVID-19 infection especially in patients with comorbitidies may be led to decreased mortality in this special population.

Cancer patients on active anticancer treatment are at increased risk of a severe form of COVID19 infection due to their immunosuppression [41]. A meta-analysis showed that cancer patients who received active anticancer treatment within 2-4 weeks of developing COVID-19 were associated with a fourfold increased rate of mortality [42]. Our study also demonstrated that patients receiving active anticancer therapy were at increased risk of mortality ($p = 0.01$). Furthermore, in the studies, it was shown that patients with cancer stage IV and active cancer were more likely to experience severe events [43]. In our study, we also demonstrated that the patients with tumor stage IV and active/stable/progressive disease were at increased risk of mortality ($p = 0.01$).

Limitations

There were some limitations to our study. Firstly, this study had a retrospective design. Therefore, post-COVID complications and post-COVID symptoms and sequelae could not be interpreted in detailed. Also, we could not examine smoking status, Eastern Cooperative Oncology Group (ECOG) performance of the patients due to the retrospective nature of the study. Secondly, the median follow-up period was short to recognize and interpret COVID-19 sequelae. Further efforts with larger population are needed to compare the long-term outcomes between inpatients and outpatients.

CONCLUSION

The current study showed statistically significant effects of older age, male gender, diagnosis of lung cancer, active/stable/progressive and advanced cancer stage and active anticancer therapy on mortality related to COVID-19 infection. Preventive measures should be taken for these vulnerable groups to reduce the risk of mortality in COVID-19 infection.

Authors' Contribution

Study Conception: OY, NG, AÖ; Study Design: OÜ; Supervision: AÜ, AÖ; Funding: OÜ, ÖFÖ; Materials: OÜ, ÖFÖ; Data Collection and/or Processing: OÜ, OY, ÖFÖ, GŞ, NÖ; Statistical Analysis and/or Data Interpretation: OÜ, GŞ; Literature Review: OY, GŞ, NÖ, AÖ; Manuscript Preparation: OÜ, ÖFÖ, AÜ and Critical Review: OÜ, OY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Musculoskeletal pain and quality of life in patients undergoing hemodialysis: a single-center study

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ABSTRACT

Objectives: In this study, we aimed to research the frequency of musculoskeletal manifestations of patients receiving hemodialysis treatment, and evaluate the relationship between quality of life and musculoskeletal manifestations of these patients.

Methods: Patients undergoing hemodialysis for at least 6 months were included in our study. Patients were asked to complete a questionnaire that formed by the investigators including demographic, clinical features, and musculoskeletal symptoms that frequently seen in patients who receive hemodialysis. Kidney Disease Quality of Life Short Form Version 1.3 (KDQOL-SF 1.3) and Short Form Health Survey (SF 36) questionnaires were completed by the patients and the control group. Blood urea nitrogen, serum creatinine, serum albumin, C-reactive protein (CRP), parathormone (PTH), ferritin, calcium, phosphate, hemoglobin, and Kt/V were measured. Also, the patients were divided into two subgroups according to musculoskeletal symptoms and these subgroups were compared in terms of dialysis adequacy, quality of life scoring and laboratory findings.

Results: Seventy-four patients (42 males, 32 females) were enrolled in our study. The mean age of the patients was 60.85 ± 12.29 years. Six-five (87.83%) patients had musculoskeletal symptoms. There was statistically major difference in terms of smoking between subgroups ($p = 0.046$). We did not detect any correlation between two groups in terms of Kt/V ($p = 0.411$). Pain in shoulder/neck (41.9%), pain in limbs (58.1%) and pain in back (56.8%) were the most detected musculoskeletal symptoms of the patients. There was statistically significant difference between genders in terms of joint swelling, muscle cramps, pain in limbs, back and neck /shoulder ($p = 0.015$, $p = 0.001$, $p = 0.008$, $p = 0.001$, and $p = 0.004$, respectively). We detected that all subunits of KDQOL scores were higher in control group than patients who were included in our study. There was statistically significant relation between emotional role and energy subunits of KDQOL scores and CRP ($p = 0.031$ and $p = 0.025$, respectively).

Conclusion: The results of our study were not as significant as the results of similar studies, however they are valuable because they show demographic, clinical characteristics, and quality of life of patients receiving hemodialysis in our region.

Keywords: Musculoskeletal pain, quality of life, hemodialysis

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The findings of end stage renal disease (ESRD) are very various. Musculoskeletal pain is a frequent complaint in patients undergoing dialysis [1]. Musculoskeletal symptoms have significant impact on patients' health and quality of life [1]. Laboratory parameters (hemoglobin, albumin, c reactive protein), clinical features like duration of dialysis, demographic factors like age, gender, education, and marital status are associated with quality of life in patients undergoing hemodialysis [2].

The treatment of these patients usually focused on mainly medical conditions. Psychological, emotional, nutritional, and social conditions that affect the general condition of the patients are overlooked.

In this study, we aimed to focus on the relationship between musculoskeletal symptoms and quality of life of patients undergoing hemodialysis. In addition, we tried to evaluate the association between patients' demographic, clinical and laboratory findings, and their life quality.

METHODS

Patients undergoing hemodialysis treatment due to end stage kidney disease applied to Umraniye Training and Research Hospital Nephrology outpatient clinics for follow-up from June 2022 to November 2022 were included in the study. 35 healthy controls were enrolled to the study as well.

Criteria for inclusion are following: (1) Age between 18-75 years, (2) Patients undergoing hemodialysis treatment for at least 6 months, and (3) Patients speak and understand Turkish. However, criteria for exclusion are; (1) Age < 18 and > 75 years; (2) Patients undergoing hemodialysis treatment less than 6 months; (3) Patients with cognitive impairment; (4) Patients with psychiatric disorders; and (5) Patients don't speak or understand Turkish.

Informed consent was received. The research was carried out under the original Declaration of Helsinki, and it is approved by the ethical committee of Umraniye Training and Research Hospital on 21 April 2022 (No: B.10.1.TKH.4.34.H.GP.0.01/142).
Patients were asked to complete a questionnaire under the supervision of a nurse during their hemodialysis session, created by the investigators of the study including demographic and clinical questions, as well as common musculoskeletal symptoms of patients undergoing hemodialysis.

The questionnaire included age, gender, education, marital status, smoking, duration of hemodialysis (months), number of sessions / week, etiology (primary cause of renal disease), musculoskeletal symptoms (myalgia, morning stiffness, pain in shoulder/neck, pain in limbs, pain in back/low back, and muscle cramps).

Clinical data were extracted from the hospital database. Blood samples were taken for laboratory analysis before the mid-week session. Blood urea nitrogen, serum creatinine, serum albumin, C-reactive protein (CRP), parathormone (PTH), ferritin, calcium, phosphate, and hemoglobin were measured. Kt/V (K: dialyzer clearance, t: dialysis duration, V: volume of bodily water) for dialysis adequacy, primary cause of renal disease, and duration of dialysis (months) were recorded.

To measure hemodialysis treatment sufficiency, Kt/V was used as an indicator (where: K-D dialyzer clearance of urea, t-dialysis time, V-volume of distribution of urea, approximately equal to patient's total body water). Kt/V was calculated by the online calculator: http://www.davita.com/ktvcalculator/. The US National Kidney Foundation. Insufficient dialysis quality was defined when Kt/v was below 1.2 as recommended by National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines [3]. We divided the patients into two groups according to dialysis efficiency, utilizing a Kt/V value of 1.2 as a separator. We compared two groups in terms of quality-of-life scoring.

In addition, the patients were divided into two groups according to musculoskeletal symptoms and these groups were compared in terms of dialysis adequacy, quality of life scoring and laboratory findings. Kidney Disease Quality of Life Short Form Version 1.3 (KDQOL-SF 1.3) questionnaires were completed by the patients undergoing hemodialysis. Short Form Health Survey (SF-36) quality of life measurements scale was used for the healthy controls.

Kidney Disease Quality of Life Short Form Version 1.3 (KDQOL-SF 1.3)

KDQOL-SF 1.3, a self-report measure developed for individuals suffering from kidney disease used to assess health related quality of life [4]. It includes a

general part (SF 36) and a disease-specific part. SF36 has 36 questions measuring eight measures (physical functioning, physical role, pain, general health, emotional well-being, emotional role, social functioning, and energy/fatigue). The disease particular section includes symptom/ problem list, effects of kidney disease, the burden of kidney disease, work status, cognitive function, quality of social interaction, sexual function, sleep, social support, dialysis staff encouragement and patient satisfaction. Overall health assessment part is evaluated apart. All measure points range between 0 and 100, where higher scores indicate a better quality of life. Yildirim *et al*. [5] conducted the currency and credibility of the Turkish language version of KDQOL-SF 1.3 questionnaire16. Cronbach's alpha coefficient of the Turkish KDQOL-SF questionnaire was 0.84 to 0.916.

SF-36

The SF-36 form is one of the questionnaires which is not prepared for a certain disease that measure the quality of life. Quality of life is self-evaluated in eight different parts in the questionnaire which includes 36 questions. Comorbidity was self-reported and reported as impaired functioning in the following domains as: physical functions (PF), limitation of role functions (RP) – physical, bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role functioning- limitations due to emotional problems (RE) and mental health (MH). The score ranges from 0 (lowest) to 100 (highest). Scores of the eight domains are collected to two total scores, the physical (PF) and mental component (MH) [6].

Statistical Analysis

All analyses were made by the IBM SPSS Statistics Version 15.0 statistical software package. Categorical factors were defined as numbers and percentages, whereas continuous variables were defined as mean and standard deviation and as median and minimum-maximum where convenient. For the comparison between two groups, the student's t-test was used for normally distributed variables, and the Mann-Whitney U test was used for the abnormally distributed variables. The Chi-square test or Fisher exact test was used to compare the categorical factors between the groups. The statistical level of significance for all tests was accepted as 0.05. The data were evaluated in IBM SPSS Statistics 15.0 (IBM Corp. Armong, New York, AB) program.

RESULTS

Seventy-four patients (42 males, 32 females) were enrolled in our study. The mean age of the patients was 60,85 ± 12,29 years. Mean time in dialysis was 70.73 ± 72.24 months. The sociodemographic, clinical, and laboratory features of patients are listed in Table 1. The most frequent causes of end stage renal disease were hypertension (46.7%) and diabetes mellitus (25%). Most patients (92%) were receiving hemodialysis treatment 3 days a week.

Sixty-five (87.83%) patients were suffering from musculoskeletal symptoms. There was no significant difference in terms of etiology, educational status, marital status, duration of dialysis, and age between the subgroups which were separated according to musculoskeletal symptoms (p = 0.698, p = 0.718, p = 0.504, p = 0.690 and p = 0.608, respectively) There was statistically significant difference in terms of smoking between subgroups (p = 0.046). We did not detect any difference between two subgroups in terms of Kt/V (p = 0.411).

Pain in shoulder/neck (41.9%), limbs (58.1%), and back (56.8%) were the most detected musculoskeletal symptoms of the patients. Muscle cramps were also seen frequently in patients undergoing hemodialysis (33%). All musculoskeletal symptoms were detected more frequently in female patients than male patients. There was statistically major difference between genders in terms of joint swelling, muscle cramps, pain in limbs, back and neck /shoulder (p = 0.015, p = 0.001, p = 0.008, p = 0.001 and p = 0.004, respectively). Table 2 demonstrates musculoskeletal symptoms of patients.

According to KDQOL questionnaire, patients suffering from musculoskeletal complaints had higher scores than patients without complaints except pain and emotional role subunits. There was no statistically major difference between two groups in terms of physical functioning, physical role, emotional role, emotional well-being, energy, pain, general health, and social functioning (p = 0.685, p =0.860, p =0.290, p = 0.646, p = 0.095, p = 0.851, p = 0.419 and p = 0.111, respectively).

Table1. Comparison of demographic, clinical and biochemical features of hemodialysis patients

	Total (n = 74)	Patients with musculoskeletal symptoms (n = 65)	Patients without musculoskeletal symptoms (n = 9)	*p* value
Age (years), mean ± SD	60.85 ± 12.29	61.08 ± 12.33	59.22 ± 12.60	0.608
Male/Female, n (%)	42/32 (56/42.7)	34/31 (52.3/47.7)	8/1 (88.9/11.1)	**0.038**
Smoking (+/-), n (%)	15/59 (20.27/78.7)	13/52 (20/80)	2/7 (22.2/77.8)	**0.046**
Education, n (%)				0.718
Illiterate	5 (6.7)	5 (7.7)	0 (0)	
Primary school	37 (49.3)	34 (52.3)	3 (33.3)	
Middle school	10 (13.3)	8 (12,3)	2 (22.2)	
High school	9 (12)	7 (10.8)	2 (22.2)	
University	12 (16)	10 (15.4)	2 (22.2)	
Marital status, n (%)				0.504
Single	14 (18.9)	12 (18.5)	2 (11.1)	
Married	60 (81)	53 (81.5)	7 (88.9)	
Duration of dialysis (month)	70.73 ± 72.24	71.42 ± 73.95	65.78 ± 61.96	0.690
Number of sessions/weeks, n (%)				0.389
2 sessions/w	5 (6.7)	5 (7.7)	0 (0)	
3 sessions/w	69 (92)	60 (9.23)	9 (100)	
Etiology, n (%)				0.698
Diabetes mellitus	19 (25)	7 (26.2)	2 (22.2)	
Hypertension	35 (46.7)	29 (44.6)	6 (66.7)	
Glomerulonephritis	8 (10.7)	8 (12.3)	0 (0)	
Polycystic kidney disease	1 (1.3)	1 (1.5)	0 (0)	
Others	11 (14.7)	10 (15.4)	1 (11.1)	
Serum creatinine (mg/dL)	7.39 ± 2.07	7.42 ± 2.10	7.13 ± 1.86	0.725
Blood urea nitrogen (mg/dL)	121.17 ± 31.82	122.30 ± 30.36	112.83 ±.41.89	0.535
Albumin (g/L)	40.66 ± 3.29	40.58 ± 3.41	41.22 ± 2.30	0.540
Ferritin (ng/mL)	697.32 ± 373.52	671.91 ±364.74	894.23 ± 407.79	0.155
CRP (mg/dL)	12.37 ± 15.22	12.23 ± 14.15	13.37 ± 21.54	0.946
Hemoglobin (g/dL)	10.83 ± 1.24	10.81 ± 1.27	10.96 ± 1.02	0.953
PTH (pg/mL)	367.57 ± 405.05	348.74 ± 358.25	515.86 ± 698.69	0.649
Calcium (mg/dL)	8.76 ± 0.58	8.74 ± 0.61	8.86 ± 0.30	0.597
Inorganic phosphate(mg/dL)	4.80 ±1.10	4.80 ± 1.13	4.83 ± 0.93	0.860
Venous bicarbonate (mEq/L)	19.70 ± 1.61	19.69 ± 1.64	19.73 ± 1.40	0.713
Kt/V	1.51 ± 0.29	1.52 ± 0.29	1.44 ± 0.29	0.411

Data are shown as mean ± standard deviation or n (%). Kt/V = K: dialyzer clearence, t: dialysis duration, V: volume of bodily water, PTH = Parathormone, CRP = C- reactive protein

Table 2. Distribution of musculoskeletal symptoms in hemodialysis patients

	Total (n = 74)	Male (n = 42)	Female (n = 32)	*p* value
Pain in shoulder/neck, n (%)	31 (41.9)	11 (26.2)	19 (59.4)	**0.004**
Pain in back/low back, n (%)	42 (56.8)	16 (38.1)	25 (78.2)	**0.001**
Pain in limbs, n (%)	43 (58.1)	20 (47.6)	25 (78.1)	**0.008**
Myalgia, n (%)	8 (10.8)	2 (4.8)	8 (25)	0.120
Morning stiffness, n (%)	12 (16.2)	4 (9.5)	8 (25)	0.074
Joint swelling, n (%)	12 (16.2)	3 (4.8)	9 (28.1)	**0.015**
Muscle cramps, n (%)	33 (44.6)	10 (23.8)	22 (68.8)	**0.001**

We detected that all subunits of KDQOL scores were higher in control group than patients in our study. There was statistically significant difference between patients and the control group in terms of KDQOL subunits; physical function, physical role, general health, social functioning, pain, and emotional well-being ($p = 0.001$, $p = 0.001$, $p = 0.001$, $p = 0.006$, $p = 0.004$ and $p = 0.012$, respectively). KDQOL subunits' scores and correlation between subgroups are described in Table 3.

There was statistically significant relation between emotional role and energy subunits of KDQOL scores and CRP ($p = 0.031$ and $p = 0.025$, respectively). According to this result, the present study showed that patients whose CRP values were lower than 4 mg/dl had higher emotional role and energy scores.

DISCUSSION

In our study, we found that hypertension and diabetes mellitus were the leading causes of end stage renal disease. There was no statistically significant difference between the subgroups separated according to musculoskeletal symptoms in terms of etiology, educational status, marital status, duration of dialysis and age.

Table 3. Comparison of KDQOL-SF 1.3 scores and Sf- 36 scores of hemodialysis patients and control group

	Patients with musculoskeletal symptoms (n = 65)	Patients without musculoskeletal symptoms (n = 9)	*p* value	All patients (n = 74)	Control group (n = 33)	*p* value
Physical functioning	37.56 ± 35.25	23.33 ± 32.14	0.685	36.59 ± 34.88	87.73 ± 14.25	**0.001**
Physical role	36.58 ± 44.75	16.66 ± 14.43	0.860	35.22 ± 43.57	80.30 ± 30.46	**0.001**
Pain	51.83 ± 27.84	55.83 ± 32.53	0.851	52.10 ± 69.31	69.31 ±.21.05	**0.004**
General health	47.68 ± 13.28	41.66 ± 10.40	0.419	47.27 ± 13.09	64.70 ± 15.90	**0.001**
Emotional well being	54.53 ± 12.45	49.33 ± 8.33	0.646	54.18 ± 12.21	60.61 ± 15.84	**0.012**
Emotional role	45.71 ± 50.54	100.00	0.290	47.22 ± 14.24	62.60 ± 37.04	0.142
Social functioning	36.58 ± 44.75	16.66 ± 14.43	0.111	53.13 ± 27.11	61.56 ± 20.19	**0.006**
Energy	45.73 ± 14.12	33.33 ± 12.58	0.095	44.88 ± 14.24	51.18 ± 19.09	0.123

Data are shown as mean ± standard deviation. KDQOL-SF1.3 = Kidney Disease Quality of Life Short Form Version 1.3, SF-36 = Short Form Health Survey

Nevertheless, all musculoskeletal symptoms were detected more frequently in female patients than male patients. There was statistically major difference between genders in terms of joint swelling, muscle cramps, pain in limbs, back and neck /shoulder. According to KDQOL questionnaire, patients with musculoskeletal symptoms had higher scores than patients without musculoskeletal symptoms except subunits pain and emotional role. In addition, all subunits of KDQOL scores were markedly higher in control group than patients who were included in our study. We did not find any relationship between serum albumin, hemoglobin, ferritin, Kt/V, gender, age, duration of hemodialysis, and KDQOL scores. According to our study we only found that patients with lower CRP values had higher scores of emotional role and energy.

Musculoskeletal manifestations are often detected in patients who have been on hemodialysis treatment for 5 to 10 years [7]. Nevertheless, according to another study which included younger participants who had duration of dialysis longer than 10 years had fewer musculoskeletal symptoms [8]. Hage *et al*. [7] demonstrated that pain was the most detected musculoskeletal manifestation in patients undergoing hemodialysis like other similar studies [9]. In our study we found increased rate of musculoskeletal symptoms in patients undergoing hemodialysis, while pain in limbs, back and shoulder/neck were the most detected musculoskeletal symptoms.

In a study by Kesikburun *et al*. [10], low back pain with nonspecific etiology was detected more than 1/3 of patients. They also found relationship between advanced age, smoking, increased body mass index (BMI) and low back pain. We also found significant relation between smoking and patients' musculoskeletal manifestations. Nevertheless, we could not find any relationship between musculoskeletal pain and other demographic features of patients in our study except gender. This may be due to limited number of participants included in the study.

As in general population [11], Caravaca *et al*. [12] found higher prevalence of musculoskeletal pain among women than men. They claimed that the reason for musculoskeletal pain being more likely to occur in females may be due to a hypothesis that females had greater sensitivity to pain [12]. We found that female patients had more musculoskeletal manifestations than male patients like this study.

According to Molsted *et al*. [13] musculoskeletal pain was associated with low quality of life scores in patients with chronic kidney disease. Additionally, Hsu *et al*. [14] declared that body pain was the leading qualitative parameter for the evolution of the quality of life in patients undergoing hemodialysis. Kesikburun *et al*. [10] investigated the impact of low back pain on quality of life in patients undergoing hemodialysis. According to that study patients receiving hemodialysis with low back pain had significantly worse scores in the energy, pain, and physical activity subunits of quality of life. In our study we could not find any significant difference between subgroups in terms of musculoskeletal symptoms according to KDQOL scores. This result may be due to disproportionate increased number of patients with musculoskeletal symptoms included in the study.

In general population, chronic musculoskeletal pain was related with increased levels of inflammatory indicators [15], and this may be a clue that make us to understand the mechanism of pain in chronic kidney disease [16]. Caravaco *et al*. [17] found high levels of inflammatory markers in patients with pain as general population. Nevertheless, according to Kesikburun *et al* no significant difference detected between subgroups in terms of low back pain according to laboratory findings such as hemoglobin, albumin, PTH, and calcium phosphate [10]. In the present study we did not detect relationship between inflammatory laboratory findings and musculoskeletal symptoms.

Decreased quality of life scores in patients receiving dialysis compared to general population [18]. The present study showed increased quality of life scores in control group compared to patients undergoing hemodialysis like other studies.

In patients undergoing hemodialysis low values of hemoglobin, albumin and increased CRP and ferritin levels may be predictors of chronic inflammation and this may cause inadequate hemodialysis and low quality of life. According to Turk *et al*. [19], negative correlation was found between KDQOL scores and hemoglobin levels. In this study also ferritin was found negatively correlated with five subunits of KDQOL-SF (work status, cognitive functions, dialysis staff encouragement, patient satisfaction, and energy) in patients undergoing dialysis. According to Ma *et al*.

[20], prealbumin and hemoglobin were significantly associated with poorer mental health status of patients undergoing hemodialysis, and patients with poorer psychological states were more significantly associated with decreased quality of life [20]. In the present study we only found that lower CRP values ended up higher emotional role and energy scores. This result may be useful in demonstrating the effect of elevated inflammatory markers on quality of life.

Kt/v and URR shows the adequacy of dialysis that may affect quality of life of patients. Kt/v, determined by single pool urea kinetic modeling, is the most preferred method for the numerical expression of dialysis dose [21]. Some studies showed little effect on kt/v on quality-of-life scores [19]. We did not find any correlation between kt/v and KDQOL scores like other studies mentioned above. Based on these findings we can suggest that there is not significant correlation between kt/v and quality of life in patients receive dialysis.

Limitations

Inclusion of small number of participants is one of the limitations of our study. In addition, it is a single center study. Patients receive peritoneal dialysis and patients in pre-dialysis stage were not included in the study and we could not use imaging methods to diagnose patients' musculoskeletal symptoms.

CONCLUSION

Our study showed decreased scores of KDQOL subunits compared to control group. We did not find any significant relationship between serum albumin, hemoglobin, ferritin, Kt/v, gender, age, duration of hemodialysis, and KDQOL scores. We found that lower CRP values result in higher scores of emotional role and energy. In addition, while no statistically significant difference was found between the subgroups in terms of etiology, educational status, marital status, duration of dialysis and age, significant difference was detected in terms of musculoskeletal symptoms between male and female patients. Although the results are not as significant as the results of similar studies, our study is valuable because it demonstrates the demographic, clinical characteristics, and quality of life of patients undergoing dialysis in our region.

Authors' Contribution

Study Conception: ÖP; Study Design: EEY; Supervision: DŞ; Funding: EEY, ZT; Materials: EEY, ZT; Data Collection and/or Processing: EEY, ÖP; Statistical Analysis and/or Data Interpretation: EEY; Literature Review: HK, DŞ; Manuscript Preparation: EEY and Critical Review: HK, DŞ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Turkish speech-language therapists' perceptions and experiences of augmentative and alternative communication

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ABSTRACT

Objectives: In this study, it is aimed to reveal what extent speech and language therapists (SLT) working in Turkey used augmentative and alternative communication (AAC) systems in their working area. For this purpose, it was investigated how they perceive AAC in terms of its scope and role; AAC applications within the scope of interventions for communication, language and speech disorders; best practice insights on AAC; what factors are seen as facilitating or limiting the implementation of AAC within the scope of intervention and suggestions for providing the best practice for AAC.

Methods: Phenomenology, one of the qualitative research methods, was used in the study. The subject of the study is the opinions of SLTs working in Turkey on their clinical practices and thoughts on the use of AAC. The study group consists of 15 SLTs from Turkey and determined by using maximum diversity sampling method. The semi-structured interview forms were used in which SLTs' views, suggestions and expectations about AAC applications in the service delivery as a data collection tool. The obstacles and difficulties in these applications were discussed. Content analysis was used and also carried out using the qualitative data analysis program MAXQDA 2018. In order to ensure the consistency of the data analysis, the data were analyzed by another field expert and the 92% consensus was tried to be reached by using the consistency formula.

Results: Participant opinions consist of benefiting status from AAC, opinions on the importance of AAC, preferred case groups and reasons for AAC implementation, opinions on current best practice understanding on communication and language intervention/use of AAC, opinions on current working conditions on AAC practices, opinions on the limitations of the use of AAC in communication and language intervention and recommendations for ensuring effective use of AAC themes.

Conclusions: The results of the study show that supporting individuals who can benefit from AAC in the context of intervention services for communication disorders requires great effort. In addition, SLTs stated that they strongly believed in AAC and its potential value for individuals with communication disorders, but did not have sufficient self-confidence about their current or developing skills in this area. It is also seen that clinicians need training and support from employers, professional or government agencies that set policies and standards to achieve their AAC related goals.

Keywords: Augmentative and alternative communication systems, speech and language therapy services, qualitative research, speech, language

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Augmentative and alternative communication (AAC) systems are defined as a clinical practice field addressing the requirements of individuals who have significant and complex communication disabilities characterized by impairments in language-speech production and/or understanding, including oral and written communication modes [1].

AAC refers to an area of study as well as clinical and educational practices. AAC includes attempts to examine and, when necessary, compensate for the temporary or permanent impairments, activity limitations, and participation restrictions of individuals who have severe language-speech production and/or comprehension impairments, including oral and written communication modes [1]. AAC response services and technologies are part of habilitation and rehabilitation services. Rehabilitation refers to intervention strategies and technologies intended to help people who have acquired disabilities regain competence, but habilitation refers to intervention strategies and technologies intended to help people who have a developmental disability develop competence for the first time. In this context, there is a wide variety of AAC systems designed to meet the requirements of individuals who have complex communication requirements. These systems include both unassisted and assisted options. Although unassisted AAC does not require any external equipment or technology, assisted AAC requires some type of equipment or technology. Examples of unassisted AAC include vocalizations and approximate speech, gestures, cues, and blink codes (e.g., raising eyes to indicate "No" or closing eyes to indicate "Yes") [2].

In the national literature, a limited number of studies on AAC can be found [3]. One of them deals with the AAC use of health personnel working in the intensive care unit [3]. In the the study, it was found that healthcare personnel and patients mostly preferred to use non-technology AAC; It is stated that healthcare personnel are more aware of low-tech AAC than high-tech AAC and they are more knowledgeable about their use. At the same time, it is observed that very few of the health personnel have received training on AAC. In another similar study [4], the necessity of using a high-tech application to provide alternative communication for individuals treated in the intensive care unit is emphasized to the current literature and the lack of clinical practice in the field of speech and language therapy.

It is emphasized in the international literature that SLTs must show expertise in some fields by exploring the concepts of best practice and professional expectation [5] and AAC is accepted as one of these application areas. However, previous studies reported that limited specialist experience can be achieved among SLTs because of the specific nature of the job [6] and the inadequacies in undergraduate education [7]. One aspect of clinical skill is the extent to which professional practice is based on study evidence. The ability to implement such approaches depends on the quality of the approach and the clinician's familiarity with the underlying evidence base.

It is not known to what extent the study literature influences the practice of SLT in intervention settings for communication disorders; however, basic knowledge and skills that are listed by the American Speech-Language-Hearing Association (ASHA) include understanding of developmental disabilities, family-centered approaches, different service delivery models and teams, AAC, evidence-based interventions and ability to conduct evidence-based reviews [8]. These areas follow a set of guiding principles according to best practices for early evaluation and intervention [9]. Services in this field must be (a) family-centered and culturally and linguistically sensitive; (b) support the child's development and participation in his or her natural environment; (c) coordinated and team-based, but comprehensive and (d) based on "the highest quality evidence in other words available". The evidence mentioned in the last principle may be internal or external. Clinical opinion, which is supported by professional training experiences, theory, internal evidence, values, and beliefs comes from a variety of sources, including the application of evidence. The values and beliefs of other professionals and clients, professional consensus, and government policy also contribute to internal evidence. The scope of external evidence is more limited and is based on empirical published studies and for this reason relevant to contemporary concepts of evidence-based practice [10, 11].

Key study areas for clinicians providing speech and language therapy services include family-centered practice, early language intervention strategies, and AAC. It is reported that the levels of evidence in these

areas can vary considerably. It is already known that the evidence base for the use of AAC generally consists of clinical studies with some single-subject experimental studies that support the use of AAC to increase functional communication skills [12]. In this context, accepted practices in the AAC field, the selection of such simultaneous communication and graphic symbol teaching strategies are supported by only very limited evidence under the criteria of evidence-based practice. For this reason, the purpose of the present study was to explore these issues for SLTs and to explore the extent to which clinicians include AAC in their studies. The obvious point of this purpose was to address their perceptions of what constitutes AAC and its use. The study questions were as follows.

1. How do SLTs describe AAC in terms of its scope and role?
2. What are the AAC practices of SLTs in the scope of interventions for communication, language, and speech disorders?
3. What is SLTs' understanding of best practices regarding AAC in general?
4. What factors facilitate or limit the implementation of AAC in the context of best practice presentation and intervention for communication, language, and speech disorders?
5. What are the SLTs' recommendations for ensuring best practices for AAC?

METHODS

Participants and Ethics Committee Approval

The study group consisted of 15 SLTs, which were determined by using Maximum Diversity Sampling, one of the purposeful sampling methods, from SLTs working in Turkey. Every effort was made to accommodate as many SLTs as possible by adapting to their programs. Participants worked in speech and language therapy services for less than one year to 5 years. One of the participants worked in Ankara, one in Kocaeli, one in Eskişehir, and the others in Istanbul. Although one participant worked in a private hospital, another participant worked in a disability-free living center, the other participants worked in private counseling centers (n = 5), special education and rehabilitation centers (n = 3), a private counseling center, and a special education and rehabilitation center (n = 5). Since very few male therapists work in the field of speech and language therapy in Turkey, only 3 male SLT participants were included in the study, and all the other participants were female (n = 12). To ensure anonymity, the names of all participants were referred to by coding as "AA, BB" without any causal relationship.

The study was approved by the Bahçeşehir University Research and Publication Ethics Committee (Date: 25.01.2023, Decision no: 2023/01). Also, care was taken to act in line with ethics in all study processes. Participants filled out a written consent form declaring that they voluntarily participated in the study. Before starting the interviews, the participants were given preliminary information on the study, and they were reminded that their personal information would be protected by the researchers and that they had the right to stop participating in the study at any time.

Study Pattern

In the present study, phenomenology, which is one of the qualitative study methods, was used. The case of this study was the opinions of SLTs working in Turkey on their clinical practices and thoughts on the use of AAC. The concept of "phenomenon" is at the center of the phenomenological pattern. The concept of phenomenon, in another common usage, refers to everything experienced in life. Each of the subjects such as perception, consciousness, thought, emotion, memory, language is within the concept of the phenomenon. According to Vagle (2014) [13], the phenomenon is about how we associate ourselves with the world in our daily lives. Phenomenological studies try to understand and explain the experiences and the experiences of the individuals in these experiences in the process. The main purpose is to try to understand and interpret experiences in the deepest and richest way possible. For this reason, in such studies, it is ensured that the cases are handled with a descriptive approach. The phenomenological design was revealed in the study by describing the current situation regarding the use of AAC for the intervention of language and speech disorders in Turkey, and to determine the factors that were considered to limit the use of AAC in

clinical practice and to present recommendations for its effective use. The data obtained with the phenomenology design in the study is an important source for the detailed analysis of qualitative study questions.

Interviews

Semi-structured interview forms were used as the data collection tool in the study in which SLTs' views, suggestions, and expectations about AAC practices in service delivery and the obstacles and difficulties in these applications were discussed. To prepare semi-structured interview questions, the literature was reviewed [5] and a draft interview form was prepared. Draft questions were presented to the field experts. Opinions were received to check the correctness and comprehensibility of the questions in terms of grammar. Semi-structured interview questions were prepared in line with the feedback of the experts. In this context, 9 open-ended questions were included in the interview form (Appendix 1).

The data were collected in the study with video recordings of individual interviews with speech and language therapists online with "Zoom Video Communications". In this context, the participants were interviewed online between 25.01.2023 and 15.03.2023 at a convenient time. The interviews were video-recorded to be transcribed later, with the permission of the participants.

Qualitative Analysis

The Content Analysis Method was used in the analysis of the data obtained in the study by using the qualitative data analysis program MAXQDA 2018, along with the analysis stages used by Thomas and Hardene (2008) [14]. These stages are described below.

Coding the Findings

At this step, the findings in the form of direct quotations or basic concepts that were extracted from the primary study were coded by reading them line by line. After all the findings were coded, the second step was started.

Developing Descriptive Themes

The codes obtained at this step were compared according to similarities and differences and grouped to form a hierarchical tree structure. Each group created was called a theme. Each theme was created to cover the definitions and meanings of the grouped codes.

Generation of Analytical Themes

Although this step is close to the findings of primary studies in the development of themes, new interpretative structures, and explanations are generated by going beyond primary studies to produce analytical themes. Going beyond primary studies requires the use of descriptive themes that are derived from the inductive analysis to answer study questions that were suspended for a while. For this purpose, more abstract analytical themes are generated as a result of comparing descriptive themes and discussing them with other researchers.

Credibility and Consistency Step

The most important factor in accepting a qualitative study as a scientific study is credibility and consistency. In this context, the credibility and consistency of the study must be ensured.

The data were analyzed by another field expert besides the researcher and a consensus was tried to be reached to ensure the consistency of the data analysis in the study. The consistency formula of Miles and Huberman (1994) [15] was used to calculate the consensus.

The formula is as follows.

Reliability = [Consensus / (Consensus + Disagreement)] × 100 = 189/ (189+15)] × 100 = [189/204] × 100 = 0.926

As a result of the analyses, the consensus between the researcher and the field expert was found to be 92%. Miles and Huberman (1994) [15] reported in the literature that the consistency of study results of 70% and above is high.

The credibility of the study was supported by an expert review. In order to strengthen the validity of the research findings, direct quotations were made from the statements of the participants while the result was compiled into a report. In the study, different views were tried to be given in all aspects in the findings in line with the diversification strategy. Instead of revealing a common view from the expressions of the participants, the differences were emphasized. In the "triangulation" strategy, a wide variety of sources, people and opinions are discussed in order to allow the truth to be expressed from different perspectives [16].

One of the applications to ensure transferability in

qualitative research is "detailed description". Detailed description is defined as “transferring the raw data in a rearranged manner according to the emerging concepts and themes, without adding comments to the reader” [17]. It is a common method to quote directly from the statements of the participants in order to make detailed descriptions. In this study, the transferability aspect of the research was tried to be strengthened by giving place to the direct statements of the participants.

For ensuring internal reliability, the data was transferred as it is without adding any comments in the findings section so that the reader can have the opportunity to read the data without including the researcher’s comments. Secondly, in order to increase the rate of acceptance of the research by others, the principal researcher and the assistant researcher agreed on all processes including data collection, analysis process and conclusion stage.

The researcher also tried to provide the opportunity to obtain comparable results for researchers who will undertake similar studies by sharing information expressing his/her own position. Participants, who are also data sources, are described in detail. This is one of the features that can be taken into account in determining the sample group in terms of reproducibility of the research and revealing the differences in similar studies to be conducted in the future. In addition to all these, it was given importance to express the data collection and data analysis processes of the research in detail for those who will do similar research.

In order to ensure the confirmability of the research, the raw data (audio recordings) of the study are protected and stored by the researchers, open to review by other researchers at any time.

A total of 15 participants were interviewed and the dataset obtained as a result of translating the interviews was 52 pages long.

RESULTS

The findings obtained from the individual interviews conducted with the speech-language therapists, who constituted the study group, were analyzed under categories in this part of the study. These categories can be seen in Table 1. In this section, the information obtained as a result of the qualitative analysis of the data set obtained by translating the semi-structured interviews conducted with the 15 SLTs included in the research is explained. In order to reveal the opinions, practices and suggestions of SLTs towards AAC, 7 main themes were determined. These are Benefiting Status from AAC, Opinions on the Importance of AAC, Preferred Case Groups and Reasons for AAC Implementation, Opinions on Current Best Practice Understanding on Communication and Language Intervention/Use of AAC, Opinions on Current Working Conditions on AAC Practices, Opinions on the Limitations of the Use of AAC in Communication and Language Intervention and Recommendations for Ensuring Effective Use of AAC. Then, the expressions of the participants within the framework of these themes were examined and categories belonging to each theme were created.

Status of Benefiting from AAC

The status of the participants who made up the

Table 1. Categories of participant opinions

Categories
Status of injury from AAC
Opinions on the importance of AAC
Preferred case groups and reasons for AAC practices
Opinions on current best practice approach on communication and language intervention/use of AAC
Opinions on current working conditions regarding AAC practices
Opinions on limitations on the use of AAC in communication and language interventions
Recommendations for effective use of AAC

AAC = augmentative and alternative communication

Table 2. Benefit status of participants from AAC

Category	Themes	Frequency (f)
Status of benefiting from AAC	Those who do not benefit from AAC	9
	Low-tech systems	5
	High-tech systems	1

AAC = augmentative and alternative communication

study group is given in Table 2. AA, who is among the participants who said that s/he never benefited from AAC, said, *"Unfortunately, no. I have never been able to benefit from AAC because I did not have such a case portfolio, therefore, I did not benefit from it"*. The opinion of a participant on the use of low-tech AAC is as follows. *"Well, usually, if we talk about these applications, I can use a little more picture matching, sometimes a little more PECS-like things, or being able to continue the written statements, written instructions, or I can benefit from the research in the part we call communication board, I can say that, well, or rather half-cost, there are 3 different types of AAC, which is paid, as far as I know, I can say that I have benefited from AAC with those that are a little more unassisted and assisted, a little more assisted, or do not have more technological tools"*. GG, who expressed his opinion within the scope of the high-tech systems theme, said, *"Once, I tried in a dysarthric case, who was in an old, very old group, but we could not do it very well because he was too old. His wife was also very old so they did not want to use it, so we did not use it either. Well, I wanted him to write in the form of write-voice, in other words, from a place like Google Translation, because writing, reading everything was fine, just because he was dysarthric, he had severe articulation problems, well… he had to use writing and write-voice practice"*.

Opinions on the Importance of AAC

The opinions of the participants, who constituted the study group, regarding the importance of AAC are given in Table 3. Some examples of participant responses to the themes of this category are as follows. *"I find AAC important if I am not aiming for a verbal* output" (Supporting Conversational Non-Linguistic Communication Skills) (EE). *"I find AAC very important. Because, well… if verbal production is limited, I think the case must be used as a supplement"*. (Supporting Speaking Skills) (FF).

Preferred Case Groups and Reasons for AAC Application

Some examples of participant responses to the themes in this category are as follows. *"I mean, I think that I might need AAC, especially in individuals who have aphasia. Therefore, this is as a comment this way"* (Acquired Language and Speech Disorders) (AA). *"Especially motor disorders, advanced dysarthria. I see that children with an advanced course of dysarthric conditions have a sense of humor in this context. I think that AAC is especially valuable in such children. In other words, as the group, I work most specifically with. I need it very much in dysarthria and Broca's Aphasia"* (Motor Speech Disorder and Acquired Language and Speech Disorders) (CC). *"Dysarthria. Or It could be Apraxia. Then, maybe those with mental retardation. We either looked*

Table 3. Opinions of participants on the importance of AAC

Category	Themes	Frequency (f)
Opinions on the importance of AAC	Supporting speaking skills	5
	Supporting conversational non-linguistic communication skills	10

AAC = augmentative and alternative communication

at severely mentally retarded people, it could be autism with limited verbal output, or it could be Down Syndrome. In other words, there may be any problem because of genetic diseases. It can be used in language disorders, in severe language disorders" (Motor Speech Disorder Cases, Cases with Communication Disorder Because of Intellectual Disability, Cases of Developmental Language Disorders) (EE). *"Firstly, Autism, then, Aphasia. In this way, the language features expected here cannot be framed by certain standards. In other words, it can be seen in many different varieties. For example, of course, we do not recommend AAC to every autistic person, but we can come across children to whom we can recommend it. In other words, I can say that language skills differ greatly from each other. In other words, because of other physical or physiological limitations accompanying Aphasia"* (Autism Spectrum Disorder Cases, Aphasia Cases) (JJ). The case groups are given in Table 4.

Opinions on Current Best Practice Approach on Communication and Language Intervention/Use of AAC

Some examples of the participant responses to the themes in this category are as follows. *"It must be planned individually because it is not possible to say anything average. In line with the individuals' capacity, it can be planned following their strengths, by considering their competence and their physical movement for a case with Cerebral Palsy, for example, supporting the areas where they are not strong. For example, some AACs even occur with some eye movements, if it is necessary to develop a system that can be applied to the case by the family completely suitable for the case"* (Individual Approach Selection) (DD). *"When the expectation is completely focused on AAC in terms of parents and other educator groups, because when we say AAC directly, the reaction we face when they work on the most basic is from the parents, there are reactions such as 'Sir, will we not study speaking? Isn't it our purpose to talk?' Rather, our first goal is to explain to the family that this is a system that will support language skills to support speaking, and to convey that the priority of the family is the development of this system rather than speaking"*. (Family Being a Model) (BB). *".Well, then, it must be an application where the child is studied on a certain material and the family also participates in it, and if the child goes to school, it must be supported by the teachers at the school for generalization and application in daily life. You know, I think it is important to do this as teamwork in this way"* (Application of Focused AAC Practices by Speech Language Therapists, Using Different Learning Materials, Family Being a Model, Providing Interactive Communication with the Family) (FF). *"I think the best use is just a little bit more related to the therapist's managerial situation using that material. Regardless of the material used, I can answer such as the therapist's management of activity about that material or about the case group, you know, I think the best practice is the situation in which the therapist feels best, frankly. When we look at the ideal conditions, I think that the therapist's financial resources (laughs), material things must be high, opportunities must be high, or the environment applied must*

Table 4. Opinions of the participants on the case groups they particularly preferred for AAC practices and their reasons

Category	Themes	Frequency (f)
Case groups for which AAC is important	Motor speech disorders	3
	Autism spectrum disorder	9
	Acquired language and speech disorders	5
	Developmental language disorders	2
	Communication disorders because of intellectual disability	1
	Aphasia	7

AAC = augmentative and alternative communication

have a supportive condition along with the environment. In other words, it has to be used around it, it has to be used by itself, after all, this is an education and it has to be an ideal condition to ensure the continuity of education there. I think there must be no such thing as 'I went and used it once, I used it once', let us change it. It has to be sustainable" (Structured Practice Environment, Enhanced Continuous Natural Contextual Language Teaching) (LL). The opinions on current best practice approaches are given in Table 5.

Opinions Regarding Current Working Conditions in AAC Practices

Some examples of participant responses to the themes of this category are as follows. *"Well, not very convenient because we go from one session to the next. You need to make a preparation beforehand in AAC, you have to prepare lists of targeted words, etc. For this reason, I do not have much time like this, 5-10 minutes in between, but the room gets packed, and a family meeting is not possible"* (Lack of Structured Application Environment) (EE). *"I think that I have an ideal environment, in fact, it may be a problem only in terms of providing sufficient resources"* (Lack of Different Materials/Resources) (DD). *"The conditions I work in are actually suitable for AAC practice, but because of the intensity of the cases, I cannot find time to prepare because we have too many sessions"* (Time Limitation) (KK). *"I mean, even if we implement it, families do not understand much or they may have difficulties. I think it would be ideal to have a process where they understand the logic of this business and are also willing to implement it"* (Lack of Family Involvement) (BB). Opinions regarding current working conditions are given in Table 6.

Limitations on the Use of AAC

Some examples of participant responses to the themes in this category are as follows. *"It is probably because of education when we get down to the basics. Therefore, it means that it was not sufficiently dealt with, in other words, at the time of the AAC. While reading this section, AAC was not emphasized much. In other words, the reason why it was not emphasized much may be that its current sample was very small. There may also be results that show that the places where AAC is used are very few or that it is not a very effective method in terms of its statistical status. I think that putting these things forward will put AAC in the foreground, in other words, this method of intervention"* (Lack of Education of Practitioners, Lack of Widely Use of the Application) (AA). *"I mean, those who prevent rather than limit them, especially in the pediatric group, do you have concept knowledge or not? In other words, whether they have general concept skills or not. You have to look at them. A judgment hindering and limiting us. But when we look at the adult geriatric group, I do not think there is a limiting situation. There are many methods. However, when we look at Aphasia within the scope of other acquired lan-*

Table 5. Opinions of participants on current best practice approach on communication and language intervention/use of AAC

Category	Themes	Frequency (f)
Opinions on AAC' current best practice on communication and language intervention/use		
	Structured practice environment	6
	Being a model of the family	2
	Individual approach selection	5
	Implementation of focused AAC practices by speech therapists	1
	Using different learning materials	4
	Providing interactive communication with the family	1
	Enhanced continuous natural contextual language teaching	2

AAC = augmentative and alternative communication

Table 6. Opinions of participants regarding current working conditions in AAC practices

Category	Themes	Frequency (f)
The environment in which AAC is implemented	Lack of structured application environment	7
	Lack of family involvement	1
	Lack of different materials/resources	2
	Time limitation	2

AAC = augmentative and alternative communication

guage disorders, it is one that can be used physically by someone who has accelerated. How can I say? Does the individual have a problem using his other limbs or not, this may affect us in the use of AAC. There is an example of Hawking that we all know. He also uses it, but it is very, very difficult to access, this is a difficult situation in terms of technological production rather than material and non-material possibilities. How can I say that this situation has become widespread as much as possible? The society is more aware of this - not the society - institutions, and managers as well" (Not Widely Use of Application, Lack of Technology Infrastructure System, Lack of Awareness of Institution Managers for Application) (BB). *"Firstly, we did not see AAC in the courses adequately in our undergraduate education. Related to this and afterward, training is not organized very often. I think the biggest reason is the lack of information"* (Lack of Implementation Information) (DD). "*I considered this in terms of family participation, in saying this, you know, it is usually an environment where we work one-to-one with the child. You know, the family also needs to participate in the intervention actively, so in that case, it is not very suitable, you know, where we work, we mostly work one-to-one with the children. Well, we can include families in therapy for a certain period, but I think that this period must be a little longer in AAC. Yes, because of this, it is not very suitable physically. Well, just like this, sometimes children are like that, well, they are sometimes limited in terms of attention, so the participation of the family is constantly changing, you know, these factors affect the situation in general"* (Lack of Structured Application Environment) (FF). *"Firstly, we do not have a lot of knowledge. Since it is not a field, in other words, used very much in Turkey, it is necessary to search the literature, and because there is literature in English or a foreign language, we lack in this regard, so I think it must be supported or on it. Sorry, from a technological standpoint"*. (Lack of Implementation Information) (GG). *"Firstly, I think that there is a shortage of materials, less material diversity, or I cannot organize these materials in line with our case groups, because, as I mentioned before, therapists work very hard in Turkey in terms of time, so they do not have a chance to say that I also produced this or that. For this reason, we are a little bit dependent on foreign materials. You know, instead of producing the more active part within the scope of purchasing something always, I think this is our first biggest situation: having trouble producing things. Another situation is that we can explain this to the families, transfer AAC, and continue this AAC around the environment because you have to give the AAC that you prepared to the family. We need to reproduce the materials. You know, it has to be a suitable material for reproduction. For this reason, a therapist actually creates a certain burden in an extra financial context, so I can say that it actually creates difficulties for us in terms of the material itself, in terms of environment, material, and time"* (Lack of Practical Materials, Practitioners' Time Limits, Family Not Being a Model) (LL). Limitations on the use of AAC are given in Table 7.

Recommendations for Effective AAC Use

Some examples of participant responses to the themes in this category are as follows. *"A detailed explanation and a sample project at first. Sample, or rather an exemplary project. In the past, primary schools used to be exemplary schools, for example, a clinic only on this and sharing the results of the studies. Besides, I think it will be the biggest method that*

Table 7. Opinions of participants on limitations on the use of AAC

Category	Themes	Frequency (f)
Limitations/barriers to the use of AAC	Practitioners' lack of education	11
	Not widely used application	3
	Lack of technology infrastructure system	2
	Lack of practice awareness of institution managers	2
	Lack of structured application environment	1
	Lack of implementation material	1
	Time limitation of practitioners	1
	Limitation of family participation	1

AAC = augmentative and alternative communication

AAC will show itself as an intervention method, in other words, it will be the way" (Sample Case Practices) (AA). *"AAC must be included more in undergraduate education in general"*. (Including SLT Candidates' Course Contents on the Use of AAC in Undergraduate Education) *(BB). "Of course, firstly, courses directly related to AAC can be taught in undergraduate education. Also, training on AAC can be organized for colleagues working in the field, maybe in the form of in-service training. Maybe there is no such option in the institutions we work with, but maybe more AAC-related training can be organized through the association"* (Including SLT Candidates' Course Contents on the Use of AAC in Undergraduate Education, Organizing *In-Service Training for Practice) (DD). "Even if not in workplaces, for example, training can be given among our community. I know that there is training given by more knowledgeable people, but I think I heard it very little, I just could not attend it either. Well. The general progresses in the form of small-scale seminars errr... I think it would be more effective if it was explained more, for example, in congresses or places like this. But I do not know if there are people who are competent in AAC or if there are people who use it actively, maybe it is because of their scarcity, of course, every SLT has to take responsibility at some point"* (Experienced Practitioners in AAC Implementation, Organizing In-Service Training for Implementation) (GG). *"The fact that education is given on AAC in schools can be extended to one semester or even two semesters. In my opinion, if training is organized for this at the same time, yes, I think that the deficiency in this area can be eliminated"* (Including SLT Candidates' Course Contents on the Use of AAC in Undergraduate Education) (KK). *"I think the first one is undergraduate education. During undergraduate education, maybe this is unassisted, errr. Or, maybe within the scope of informing about aided and unaided AAC, maybe case groups can be distributed to the students in the technological devices part, and we can say to these case groups that they must design an AAC board, maybe a communicative board, and I think it may be directed to the students to use it. I think it must be shown. Of course, in terms of this material, let us all say come on, let us design friends, and at least if there is a material cycle that can be given to them, if they have a file, they can be used better in the future, but if we are all SLTs, let us go to the big SLTs, let us graduate SLTs. If we say let us think comprehensively, maybe we can separate a certain framework in congresses, the same language can be spoken in a certain symposium or all together under the association, let us see who we can design better in which case group, what can come out, it may be necessary to inform families since, if family information is missing, how much SLT can be applied? Whether they want it or not, of course, there can be misunderstandings about this AAC. These also must be fixed. So the framework for both the environment, therapists, and undergraduate SLTs' must be handled case-by-case because what we want applies to many case groups, not just one case group. It is necessary to show the frameworks and diversity of this"* (Including SLT Candidates' Course Contents on the Use of AAC in Un-

Table 8. Opinions of participants on recommendations for effective use of AAC

Category	Themes	Frequency (f)
Recommendations for effective AAC use	Case studies	7
	Experienced practitioners for AAC implementation	7
	Including SLT candidates' course contents on the use of AAC in undergraduate education	7
	Organizing practice-oriented in-service training	4

AAC = augmentative and alternative communication, SLT = speech and language therapists

dergraduate Education, Experienced Practitioners on AAC Practice, and Sample Case Studies) (LL). Recommendations for effective use of AAC are given in Table 8.

DISCUSSION

The aim of the research is to reveal the opinions, practices and suggestions of SLTs, who work in various institutions in Turkey and have different demographic characteristics, towards ACC. In this context, how SLTs define ACC, their current practices towards ACC, their views on the importance of ACC and the preferred case groups and reasons for ACC, their best practice understandings towards ACC, current working conditions for ACC practices. their opinions about ACC, the limitations of ACC use, and the recommendations for effective ACC use are described.

Participant opinions differed regarding what could be considered AAC. Their confusion about AAC is perhaps not surprising in light of the variety of definitions in the literature. AAC is defined as any communication method complementing (augmenting) or replacing (providing an alternative) ordinary speaking and/or writing methods when insufficient to cover the requirements of the individual [18]. The opinions of some participants in the present study were considered to reflect this definition, focusing on facilitating understanding and functioning as environmental support.

The American Speech, Language, and Hearing Association defined a clinical practice addressing the requirements of individuals who have significant and complex communication disabilities characterized by impairments in language and speech production and/or understanding, including augmentative and alternative communication, oral and written modes of communication [1]. It is considered that the fact that some of the study participants perceived AAC as a method for providing communication through writing or a different method in cases with limited speech production, was in line with the American Speech, Language and Hearing Association's AAC definition.

Benefiting Status from AAC

Although most of the participants (n =9) said that they did not benefit from AAC in their current clinical practices, a few of them mentioned AAC practices they performed through low or high-technology systems. When talking about PECS and visual supports for applications realized through low-tech systems mentioned here, the use of various artificial intelligence applications for applications performed through high-tech systems was also mentioned. Iacono and Cameron [5] also reported similar applications of SLTs for AAC as vocabulary, preparing graphical symbols, creating low-tech images, and programming high-tech systems.

Opinions on the Importance of AAC

All participants said that they found AAC important for the intervention of communication disorders. Although a small proportion of the participants were aware of the limited studies supporting the effectiveness of AAC for the intervention of communication disorders, the majority seemed to have the impression that the evidence was strong, particularly in facilitating or inhibiting speech. Although there is much debate about how AAC affects speech development, study evidence points to increased speech development in most, if not all, individuals introduced to AAC [19]. There is growing evidence documenting many posi-

tive benefits of AAC, such as improving communication, promoting language development and understanding, increasing participation, and reducing frustration and problem behaviors [20, 21].

Opinions on Preferred Case Groups for AAC

Although most of the participants reported that they found the use of AAC especially important in interventions for Aphasia and Autism Spectrum Disorder (ASD) because of their speech-based language difficulties, some participants said that they were working with dysarthric clients, some with developmental language disorders, communication difficulties because of intellectual disability, and voice disorders and shared that they cared about the use of AAC. This relationship between various AAC practices and ASD is seen in the literature [22-24]. However, individuals who have speech and language disorders also have a wide range of communication requirements and abilities. In this context, there is no typical candidate for AAC. It is already known that AAC can be applied to clients from all age groups, from socioeconomic, ethnic, and racial backgrounds. The unifying characteristics of people who can be accepted as candidates for AAC are their need for adaptive support to communicate effectively because their verbal and/or written communications are temporarily or permanently insufficient to meet all their communication requirements. Also, it is already known that various developmental or acquired conditions can cause significant difficulties in speaking or writing without adaptive support. Common developmental causes of such severe communication disorders include severe intellectual developmental disability, Cerebral Palsy, Down Syndrome, Autism Spectrum Disorder, and developmental apraxia of speech. Acquired medical conditions that result in the need for AAC support include Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis, traumatic brain injury, stroke, high-level spinal cord injury, and several other degenerative cognitive and linguistic disorders [2].

The opinions of the SLTs who participated in the study on the case groups who had communication problems because of Aphasia, ASD, and intellectual disability, which they reported in the literature that they preferred for AAC practices, and these case groups mentioned in the international literature were considered to overlap.

Opinions on the Best Practice Approach to AAC

A variety of opinions were found in the evaluation and intervention approaches reported by the participants, reflecting an implicit understanding of what can be considered current best practice; structured practice environments, use of different learning materials, integrating AAC into everyday situations and interactions, and family involvement. In this context, Iacono [24], Snell [25], and Iacono and Cameron [5] also mentioned similar opinions. It was not understood fully whether participants used such strategies and approaches because they saw it as best practice or because of their evidence-based understanding. It is considered that some practices such as PECS may were preferred because the participants received special training or these were mentioned in speech and language therapy undergraduate/master education.

Opinions on the Current Working Conditions regarding AAC Applications and Limitations on the Use of AAC

Most of the participants expressed their thoughts that when they tried to fulfill all clinical roles and duties, they also did not have enough time during the day to present the best AAC practice to their clients. This was supported by the findings of the study of Beamish and Bryer [26] which showed that time constraints pose a significant challenge for clinicians in their attempts or desire to implement best practices. This obstacle is also mentioned in some other studies conducted on AAC practices [27]). Also, some participants talked about the difficulty of convincing and/or educating clients' families about AAC use. Clinicians currently at risk of burnout are known to face the potential for greater stress, or at least frustration, from their need to address family concerns and other professionals' misconceptions about AAC while remaining sensitive to family requirements and stress [5]. It is reported in the literature that clinicians who provide AAC as a component of speech and language therapy services are likely to benefit better from a family-centered approach with appropriate management and organizational support [28]. An example of such support is the employers' commitment to saving clinicians time by creating positions that are dedicated to the development of AAC systems [5]. Also, some participants mentioned that the current technological equipment is not sufficient to create a favorable setting

for AAC practices. Smith and Connly [29] and McNaughton *et al*. [19] also mentioned a similar situation in their study.

Recommendations for Effective AAC Use

Among the suggestions of the participants in the effective use of AAC are the organization of case study training, the organization of training to be provided by experienced practitioners in AAC practice, the presentation of content on the use of AAC in the practical and theoretical training of SLT candidates, the conduct of individual literature studies and the organization of in-service training. Similarly, McNaughton *et al*. [19] also reported a need for training in existing AAC technology as part of continuing professional development at pre-service and in-service levels. In general, the present literature on AAC and speech and language disorders suggests that clinicians must conduct a systematic review of study evidence to be informed about practices [10, 11]. Considering the resources and time required to conduct systematic reviews, it seems likely that clinicians will need expert assistance, training, and access to electronic literature databases [5].

Limitations and Recommendations for Further Studies

The present study provided qualitative insights into the use of AAC in the intervention of communication disorders from the perspective of SLTs. The results are presented in light of the data obtained from the individual interviews conducted with the participants. Considering the directed nature of the interviews because of their small sample and semi-structured nature, more detailed qualitative studies through individual or focus group interviews with larger sample groups will contribute to a better understanding of issues on AAC practices, such as the participation of clinicians with various experiences in the field. The next step may be to explore the perceptions of other professionals and families regarding AAC, considering the multidisciplinary nature of service delivery to individuals who have communication difficulties and the need to understand family issues to facilitate family-centered processes. The results of the present study point to potential barriers, but direct empirical evidence seems to be lacking. Also, there is a need to determine to what extent the results of the present study reflect the experiences of other SLTs. For this reason, the findings were considered to represent a first step towards developing a more comprehensive quantitative questionnaire in this field. More studies are needed on how government agencies with organizations that establish policies, develop clinical education programs and provide intervention services for communication disorders can contribute to facilitating best practices without overburdening clinicians and families.

CONCLUSION

The results of the present study showed that supporting individuals who might benefit from AAC in the context of intervention services for communication disorders requires great effort, often involves struggling with families, and might threaten overall job satisfaction because of excessive job demands. Despite this, clinicians who participated in the study reported that they strongly believed in AAC and its potential value for individuals who have communication disorders but did not have sufficient self-confidence in their current or developing skills in this field. It was recognized that clinicians need training and support from employers, professional bodies, or government agencies that establish policies and standards to achieve their AAC-related targets. Future studies on relevant issues raised by current and other studies are also necessary in this regard. Although the extreme job demands revealed by the research were interpreted as a valuable result by the researchers, it was thought that this situation was related to the inflexibility of SLTs to new needs and it was realized that this perception should be changed.

Authors' Contribution

Study Conception: İCY, ST; Study Design: İCY, ST; Supervision: İCY, ST; Funding: N/A; Materials: İCY, ST; Data Collection and/or Processing: İCY, ST; Statistical Analysis and/or Data Interpretation: İCY, ST; Literature Review: İCY, ST; Manuscript Preparation: İCY, ST and Critical Review: İCY.

Informed Consent

Written informed consent was obtained from the speech and language therapists who participated in the study.

Conflict of interest

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Appendix 1. Semi-structured interview questions

1. Which patient group/groups do you work with in general?
2. How would you define AAC?
3. Have you used/do you benefit from AAC currently or in your previous intervention attempts?
4. Why do you think AAC is important?
5. In your opinion, for which case group is AAC more important, why?
6. What are your thoughts on how AAC should be the best practice for communication and language intervention/use?
7. Do you think you are working in an environment that allows you to apply current best practices and/or explore approaches in using AAC?
8. What are the limitations/obstacles in the use of AAC? From where?
9. What are your suggestions for a more effective use of AAC?

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The association between umbilical cord coiling index and adverse perinatal outcomes at term pregnancies

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ABSTRACT

Objectives: The umbilical coiling index, calculated by dividing the total coil number to the cord length, is a representative parameter for umbilical cord coiling status. Recent studies have shown that abnormal umbilical coiling index is associated with adverse perinatal outcomes. Here, we aimed to determine this association at term gestation in our population.

Methods: A total of 98 singleton, term pregnant women were included in this prospective study. Demographic, obstetric features and perinatal outcomes of the patients were recorded. Patients were grouped according to the umbilical coiling index as hypocoiled, normocoiled and hypercoiled. Recorded parameters were firstly compared between normocoiled (n = 60) and abnormal coiled (n = 38) groups. Then, they were compared between normocoiled, hypocoiled (n = 20) and hypercoiled (n = 18) groups. Significantly different adverse perinatal outcomes were compared between normocoiled and other groups.

Results: Abnormal coiled group had an higher incidence of low fifth minutes Apgar scores, meconium-stained amniotic fluid, intrauterine growth restriction and acute fetal distress as compared to normocoiled group. No significant adverse perinatal outcome was detected between hypocoiled and normocoiled groups. Intrauterine growth restriction (p = 0.004), low Apgar scores (p = 0.046) and fetal distress (p = 0.038) and meconium-stained amniotic fluid were found to be more common in hypercoiled group than normocoiled ones.

Conclusions: Abnormal umbilical coiling is associated with adverse perinatal outcomes. Hence antenatal measurement of umbilical coiling index could be a useful parameter to determine high-risk pregnancies and can provide close monitoring for fetal well-being.

Keywords: Adverse outcome, perinatal outcome, term pregnancies, umbilical coiling index

The umbilical cord, the connecting tissue between the embryo and placenta, is vital for the well-being of the fetus. It has three blood vessels that provide all the nourishment for intrauterine life. It consists screw-shaped coils defined as the 360-degree spiral courses of vessels around Wharton jelly [1]. Although the main role of coils has not been fully elucidated, it has been claimed that the number of coiling could be related to adverse perinatal outcomes [2, 3].

The umbilical coiling index (UCI), which is calculated by dividing the coil number in the cord to the length of cord, is a representative parameter for umbilical cord coiling status [4]. According to UCI, umbilical cords have been classified as hypocoiled (UCI

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< 10th percentile), normocoiled (UCI between 10-90th percentile) and hypercoiled (UCI > 90th percentile) [5]. In the literature, it has been shown that hypocoiled cords were associated with fetal distress, fetal heart rate abnormalities, low Apgar scores, and meconium-stained amniotic fluid while hypercoiled ones were related to low birth weight, fetal distress, diabetes mellitus, preterm birth, low Apgar scores, and meconium-stained amniotic fluid [1, 4, 6-8].

To the best of our knowledge, there is a few data about the relationship between UCI and adverse pregnancy outcomes in our population. Here, we aimed to determine this association at term gestation.

METHODS

This is a prospective study conducted on a university-affiliated hospital. The present study was approved by the local ethics committee with an approval number of 196. Written informed consent was taken from all the participants. The inclusion criteria were as follows; being at ≥ 37th gestational week, having a singleton pregnancy, and live fetus. After being selected according to the inclusion criteria, a total of 98 term pregnant women were included into the study.

Age, gravida, parity, presence of gestational hypertension (GH), gestational diabetes mellitus (GDM), intrauterine growth restriction (IUGR), meconium-stained amniotic fluid were noted. Also, mode of delivery, delivery week, birth weight, fifth minutes Apgar scores, development of acute fetal distress, and requirement of neonatal intensive care unit (NICU) were recorded. Gestational age was calculated in two ways: the initial day of the last menstrual period for cases with regular menstrual cycles and first-trimester ultrasound for cases with irregular cycles or unknown last menstrual bleeding.

A detailed examination was performed, management of these cases was done due to the universally accepted protocols, and patients were followed up closely during the peripartum period. After delivery of the baby (vaginal or cesarean section), the umbilical cord was tied and cut closer to the placenta. The length of the umbilical cord between the placental end and umbilical stump was measured without stretching. Then, the number of coils was counted. UCI was calculated by dividing the total number of coils by the total length of the cord. Patients were grouped according to the UCI values as hypocoiled (UCI < 10th percentile), normocoiled (UCI between 10-90th percentile), and hypercoiled (UCI > 90th percentile). A total of 60 normocoiled, 18 hypercoiled, and 20 hypocoiled cases were analyzed.

Recorded parameters were firstly compared between normocoiled and abnormal coiled (hypercoiled and hypocoiled) groups. After then, they were compared between normocoiled, hypocoiled and hypercoiled groups. Significantly different adverse perinatal outcomes were compared between normocoiled and other groups.

Statistical Analysis

Statistical analysis was performed by using SPSS Version 22.0. (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.) software. Shapiro Wilk test was used for assessing whether the variables follow normal distribution or not. Variables were reported as mean ± SD or median (minimum: maximum) values for continuous variables and percentage or frequency for categorical variables. Mann Whitney U test was used for two group comparison of non-normally distributed continuous variables while independent t-test was used for normally distributed ones. For comparison of hypocoiled, normocoiled and hypercoiled groups, one way ANOVA and Kruskal Wallis tests were carried out. Chi-square test and Fisher's exact test were performed for the comparison of categorical variables. The level of significance was set at $\alpha = 0.05$.

RESULTS

A total of 98 term pregnant women were included in the study. The study population was grouped into normocoiled (n = 60) and abnormal-coiled (n = 38) groups. The characteristics of normocoiled and abnormal coiled groups were demonstrated in Table 1. There was no significant difference between two groups in terms of age, gravida, parity, cord length, number of coils, the presence of GH, GDM, mode of delivery, delivery week, birth weight, low birth weight, and requirement of NICU. The abnormal coiled group had a higher incidence of low fifth minutes Apgar scores (p = 0.029), meconium-stained amniotic fluid (p =

Table 1. The characteristics of normocoiled and abnormal coiled groups

	Normocoiled (n = 60)	Abnormal coiled (n = 38)	*p* value
Age (years)	28 (19-37)	27 (20-42)	0.384
Gravida (n)	2 (1-5)	2.5 (1-5)	0.952
Parity (n)	1 (0-4)	1 (0-4)	0.688
Cord length (cm)	58 (40-70)	60 (40-70)	0.258
Number of coils (n)	8 (6-11)	7 (4-17)	0.927
GH, n (%)	12 (20)	6 (15.8)	0.797
GDM, n (%)	7 (11.7)	4 (10.5)	1.000
IUGR, n (%)	3 (5)	9 (23.7)	0.010
Mode of delivery, n (%)			0.844
Vaginal delivery	45 (75)	27 (71.1)	
Cesarean section	15 (25)	11 (28.9)	
Delivery week (week)	38 (37-40)	38 (37-40)	0.549
Birth weight (g)	3175 (2250-4100)	3325 (2300-4000)	0.481
Fifth minutes Apgar score < 7, n (%)	8 (13.3)	12 (31.6)	**0.029**
Low birth weight, n (%)	8 (13.3)	8 (21.1)	0.467
Meconium-stained amniotic fluid, n (%)	4 (6.7)	9 (23.7)	**0.034**
Acute fetal distress, n (%)	10 (16.7)	14 (36.8)	**0.043**
NICU requirement, n (%)	11 (18.3)	11 (28.9)	0.328

Data are shown as median (min-max), mean ± SD and n (%). GDM = gestational diabetes mellitus, GH = gestational hypertension, IUGR = intrauterine growth restriction, NICU = neonatal intensive care unit

0.034), IUGR ($p = 0.010$) and acute fetal distress ($p = 0.043$) as compared to normocoiled group.

Patients were also divided into three subgroups as hypercoiled (n = 18), normocoiled (n = 60) and hypocoiled (n = 20) groups. The characteristics of normocoiled, hypercoiled and hypocoiled groups were shown in Table 2. No statistically significant difference was detected with regard to age, gravida, parity, cord length, the presence of GH and GDM, mode of delivery, delivery week, birth weight, low birth weight and NICU requirement. As it was expected, number of coils and UCI were statistically significantly different between three groups. Moreover, the incidence of IUGR ($p = 0.005$), low Apgar scores ($p = 0.036$), acute fetal distress ($p = 0.030$) and meconium stained amniotic fluid ($p = 0.013$) were significantly different between three groups. These significantly different outcomes were compared between two groups (normocoiled- hypercoiled and normocoiled- hypocoiled) and presented in Table 3. The incidence of IUGR was 5% in normocoiled group, 15% in hypocoiled group, and 33.3% in hypercoiled group. This incidence was not significantly different between normocoiled and hypocoiled groups ($p = 0.162$) while it was statistically significantly different between normocoiled and hypercoiled groups ($p = 0.004$). Low Apgar scores were present in 13.3% of normocoiled cases, 30% hypocoiled cases and 33.3% in hypercoiled ones. Significant difference was only detected in the comparison of normocoiled and hypercoiled groups ($p = 0.046$). Similar to those, the incidence of acute fetal distress and meconium-stained amniotic fluid were significantly higher in hypercoiled group as compared to normocoiled group ($p = 0.038$ and $p = 0.008$, respectively). The incidence of acute fetal distress was 16.7% in normocoiled group, 35% in hypocoiled group and 38.9% in hypercoiled group while the incidence of meconium-stained amniotic fluid was 6.7%

Table 2. The characteristics of normocoiled, hypercoiled and hypocoiled groups

	Normocoiled (n = 60)	Hypercoiled (n = 18)	Hypocoiled (n = 20)	*p* value
Age (years)	27.82 ± 4.64	27.72 ± 6.42	26.65 ± 3.62	0.639
Gravida (n)	2 (1-5)	3 (1-5)	2 (1-5)	0.248
Parity (n)	1 (0-4)	1 (0-4)	1 (0-3)	0.401
Cord length (cm)	58 (40-70)	60 (50-70)	60 (40-70)	0.503
Number of coils (n)	8 (6-11)	14 (12-17)	5.5 (4-7)	< 0.001
Umbilical coiling index	0.15 (0.12-0.16)	0.24 (0.23-0.28)	0.10 (0.06-0.11)	< 0.001
GH, n (%)	12 (20)	3 (16.7)	3 (15)	0.934
GDM, n (%)	7 (11.7)	2 (11.1)	2 (10)	1.000
IUGR, n (%)	3 (5)	6 (33.3)	3 (15)	**0.005**
Mode of delivery, n (%)				0.853
Vaginal delivery	45 (75)	13 (72.2)	14 (70)	
Cesarean section	15 (25)	5 (27.8)	6 (30)	
Delivery week (week)	38 (37-40)	38 (37-40)	38 (37-40)	0.726
Birth weight (g)	3175 (2250-4100)	3325 (2300-4000)	3400 (2350-4000)	0.308
Fifth minutes Apgar score < 7, n (%)	8 (13.3)	6 (33.3)	6 (30)	**0.036**
Low birth weight, n (%)	8 (13.3)	6 (33.3)	2 (10)	0.125
Meconium-stained amniotic fluid, n (%)	4 (6.7)	6 (33.3)	3 (15)	**0.013**
Acute fetal distress, n (%)	10 (16.7)	7 (38.9)	7 (35)	**0.030**
NICU requirement, n (%)	11 (18.3)	7 (38.9)	4 (20)	0.193

Data are shown as median (min-max), mean ± SD and n (%). GDM = gestational diabetes mellitus, GH = gestational hypertension, IUGR = intrauterine growth restriction, NICU = neonatal intensive care unit requirement

in normocoiled group, 15% in hypocoiled group and 33.3% in hypercoiled group. No significant difference was detected between normocoiled and hypocoiled groups in terms of acute fetal distress and meconium-stained amniotic fluid ($p = 0.114$ and $p = 0.358$, respectively).

DISCUSSION

The present study revealed that abnormal coiling is related to IUGR, low fifth minutes Apgar scores, higher incidence of meconium-stained amniotic fluid and acute fetal distress. Hypercoiled groups have a higher incidence of IUGR, low fifth minutes Apgar scores, acute fetal distress, and meconium-stained amniotic fluid while hypocoiling was not associated with these adverse outcomes as compared to normocoiled group. No statistically significant relationship was found between GH, GDM, low birth weight, NICU requirement, and abnormal coiling.

The umbilical cord has a vital role in fetal development. It has coils and it was composed of helical-shaped umbilical vessels and Wharton's jelly [8]. Umbilical coils are thought to protect the umbilical cord from external pressure [9, 10]. It has been suggested that abnormal coiling is associated with acute and chronic adverse events such as growth restriction, acute fetal distress, and fetal demise [4]. Abnormal blood flow or thrombus are mostly claimed mechanisms for these adverse events [1].

In 1994, Strong *et al*. [4] defined UCI for umbili-

Table 3. The subgroup comparisons of normocoiled, hypercoiled and hypocoiled groups

	Normocoiled (n = 60)	Hypocoiled (n = 20)	*p* value	Normocoiled (n = 60)	Hypercoiled (n = 18)	*p* value
IUGR, n (%)	3 (5)	3 (15)	0.162	3 (5)	6 (33.3)	**0.004**
Fifth minutes Apgar score < 7, n (%)	8 (13.3)	6 (30)	**0.102**	8 (13.3)	6 (33.3)	**0.046**
Acute fetal distress, n (%)	10 (16.7)	7 (35)	**0.114**	10 (16.7)	7 (38.9)	**0.038**
Meconium-stained amniotic fluid, n (%)	4 (6.7)	3 (15)	0.358	4 (6.7)	6 (33.3)	**0.008**

Data are shown as median (min-max), mean ± SD and n (%). IUGR = intrauterine growth restriction

cal cord coiling, and then Rana *et al*. [5] defined hypocoiled, normocoiled and hypercoiled groups according to UCI. From 1994 to recent years, many study have suggested that abnormal cord coiling is associated with adverse perinatal outcomes. Contrary to these, some studies reported no association between adverse perinatal outcomes and abnormal cord coiling [4, 6, 7, 11-13].

In a meta-analysis searching 24 studies and 9553 pregnant women, hypocoiled cords were reported to be associated with preterm birth, fetal distress, meconium-stained amniotic fluid, low Apgar scores, fetal growth restriction, need for NICU, fetal death and fetal heart rate anomalies [14]. Strong *et al*. [4] reported that hypocoiled cords are associated with aneuploidy, meconium-stained amniotic fluid, and fetal distress. In another study, hypocoiling was found to be associated with low Apgar scores in 130 umbilical cords [15]. Similarly, de Laat *et al*. [7] and Kashanian *et al*. [13] supported the relationship between low Apgar scores and hypocoiling in their study. In a study of Patil *et al*. [16], higher incidence of meconium-stained amniotic fluid was found in addition to low Apgar scores. Some studies have suggested a significant association between hypocoiling and hypertensive disorders [6, 12, 17]. In contrary, Shilpa *et al*. [18] and Mittal *et al*. [19] did not find any relationship between hypocoiling and GHT. Likewise, Shilpa *et al*. [18] did not find any association between hypocoiled cords and GDM. Ezimokhai *et al*. [12] reported significant association between hypocoiling and GDM. Contrary to all these studies, Kumar *et al*. [8] and Ndolo *et al*. [20] reported no significant association between hypocoiled cords and adverse perinatal outcomes. Also, we found no relationship between adverse perinatal outcomes and hypocoiling in our study. We suggest that these conflicting results could be dependent on study population.

The other confounding factor explaining these different results is the timing of UCI measurement. In studies searching UCI by sonography in the early second trimester, hypocoiled cord was found to be associated with IUGR whereas no association was detected in terms of preterm birth, low Apgar scores, meconium-stained amniotic fluid, and abnormal fetal heart rate [1, 21]. According to the mid-second trimester studies, hypocoiled cords were associated with fetal growth retardation, preterm birth, low birth weight, increased NICU admission, and nonreassuring fetal conditions [1, 22]. In the third trimester, fetal growth retardation and interventional delivery were related to hypocoiling [7]. In a study of Kumar [8], UCI was calculated after delivery for term pregnancies and no significant association was found between hypocoiled cords and adverse perinatal outcomes. In our study, we calculated UCI after delivery and found no significant association for adverse outcomes. These differences could depend on the differences in measurements and sample size of the studies.

A meta-analysis revealed that hypercoiled cords are associated with preterm birth, fetal distress, low Apgar scores, meconium-stained amniotic fluid, fetal anomalies, fetal heart rate anomalies, IUGR, and fetal death [14]. Studies searching the relationship between hypercoiling and adverse perinatal outcomes in the mid-second trimester claimed that hypercoiling is related to fetal growth retardation and nonreassuring fetal status [22]. In late second trimester, hypercoiled

cord was not associated with adverse outcomes while it was associated with fetal growth restriction and interventional delivery in the third trimester [1, 7]. In a study performed after delivery at term pregnancies, hypercoiling was found not to be associated with adverse perinatal outcomes [8]. In contrast to this study, we found a relationship between hypercoiling and adverse perinatal outcomes such as low fifth minutes Apgar scores, acute fetal distress, IUGR, and meconium-stained amniotic fluid after delivery at term pregnancies.

Hypercoiling could be related to placental maturation defects and adverse outcomes via reducing pressure in terminal capillaries and altering angiogenesis in the placental bed. Another mechanism of this relationship could be fetal vascular obstruction [23]. Ezimokhai *et al*. [12] found fetal growth restriction to be related to hypercoil cords. The contrary to this study, Devi *et al*. [24] reported no association between fetal growth restriction and hypercoiling. A study searching hypercoiling in IUGR fetuses found no association from Turkey [25]. Similar to fetal growth retardation, acute fetal distress was found to be associated with hypercoiling in previous studies [4, 13, 24, 26]. This condition could be associated with resistance in blood flow [27]. In addition to these, Devi *et al*. [24] found low Apgar scores in hypercoiled cases. Moreover, the relationship between low Apgar scores and hypercoiling was supported by Gupta *et al*. [6], Kashanian *et al*. [13] and Chitra *et al*. [17].

Limitations

The present study has some limitations. First, it has a small sample size and all data were obtained from a single center. Second, sonographic UCI measurement is not present for any trimester of pregnancy. Last, the thickness of Wharton's jelly is associated with adverse perinatal outcomes. Thus, the lack of measurement of Wharton's jelly is another limitation.

CONCLUSION

Abnormal umbilical coiling is associated with adverse perinatal outcomes. Hence antenatal measurement of the umbilical coiling index could be a useful parameter to determine high-risk pregnancies and can provide close monitoring for fetal well-being.

Authors' Contribution

Study Conception: BAB, EZY; Study Design: BAB; Supervision: BAB; Funding: EZY; Materials: BAB; Data Collection and/or Processing: BAB; Statistical Analysis and/or Data Interpretation: BAB; Literature Review: EZY; Manuscript Preparation: BAB and Critical Review: EZY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):970-976 Original Article

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The effect of hypertension on renal functions in patients with acute coronary syndrome

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ABSTRACT

Objectives: In patients with acute coronary syndrome, age, ejection fraction, diabetes, hypertension, and chronic kidney disease (CKD) are regarded as independent risk factors for the development of acute kidney disease (ACD). This research evaluated the glomerular filtration rates (GFR) of acute coronary syndrome patient groups who were hypertensive and those who were not.

Methods: This retrospective analysis comprised 764 patients with acute coronary syndrome who had applied to our institution before coronary angiography. There were two groups created from these patients. In the first group, there were 383 hypertensive patients; in the second group, there were 381 non-hypertensive patients. To assess how well these patients' kidneys were functioning, GFR was determined and compared.

Results: The mean age of the two groups did not significantly differ from one another ($p = 0.053$). The standard lipid measures of total cholesterol, triglyceride, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol levels did not differ substantially between the two groups. The two groups had no discernible difference regarding high-sensitivity C-reactive protein, N-terminal fragment brain natriuretic peptides, creatinine, and thrombocyte levels. Systolic and diastolic blood pressure, as well as diabetes mellitus, were all considerably higher in the hypertensive patients' group $p < 0.001$). The GFR in hypertensive patients was substantially lower (64.83 ± 19.76 vs. 70.71 ± 19.19, $p < 0.001$)

Conclusions: Our research revealed a strong link between hypertension and diminished renal function. This leads us to believe that hypertension may be a separate risk factor for the decline in renal function in acute coronary syndrome patients.

Keywords: Acute coronary syndrome, renal failure, glomerular filtration rate, hypertension

Hypertension occurs in a significant proportion of patients with renal failure due to the reduction of water and sodium excretion in the kidney and the activation of the renin-angiotensin-aldosterone system. Although hypertension increases the frequency of cardiovascular and cerebrovascular events, it may cause renal failure by increasing the progression of kidney disease by causing hyalinization and sclerosis in the afferent arteriole wall. This study aims to examine the effect of hypertension on renal functions in patients hospitalized for acute coronary syndrome [1, 2].

The kidney and the heart coordinate numerous physiological events, including volume balance in the vascular bed, blood pressure, and peripheral tissue per-

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fusion. Thus, dysfunction in the kidney or heart results in the deterioration of the other organ's performance [1]. Important pathophysiological mechanisms underlying this connection include hemodynamic alterations, endothelial dysfunction, oxidative stress, immunological activation, inflammatory process, and activation of the renin-angiotensin-aldosterone and sympathetic nervous systems [2].

Even though approximately 30% of patients with advanced renal failure have hypertensive kidney disease, this rate is believed to be even lower because hypertension is not considered a factor in the development of renal failure. In patients with essential hypertension, the presence of concurrent kidney disease, diabetes, and cardiac ischemia increases the risk of developing kidney failure owing to hypertension. Renal dysfunction in hypertensive patients is a kidney injury that begins or worsens due to uncontrolled systemic blood pressure [3]. The deterioration of kidney function at the same or lower blood pressure levels is defined as the sensitivity to hypertensive kidney injury.

Glomerular filtration rate (GFR) is the most critical indication of kidney function in patients with both standard and impaired renal function [4]. GFR is the total amount of plasma filtered from glomeruli per unit of time in a nephron capable of maintaining its operations. Although a GFR value of 90 or more is considered normal for healthy individuals, this number might vary with age, body mass index, ethnicity, gender, and certain drugs and diets. GFR has a circadian rhythm, with nighttime values 10% lower than daytime [5]. The GFR decreases before the onset of kidney failure symptoms, and there is a clear correlation between the GFR decline and the development of CKD.

The GFR values of hypertensive and non-hypertensive patients with acute coronary syndrome (ACS) were compared in this study.

METHODS

Seven hundred sixty-four patients with ACS who applied to our outpatient clinic between July 2020 and December 2022 were included in this retrospective study. Before coronary angiography, these patients were assessed. Of the 900 patients included in the

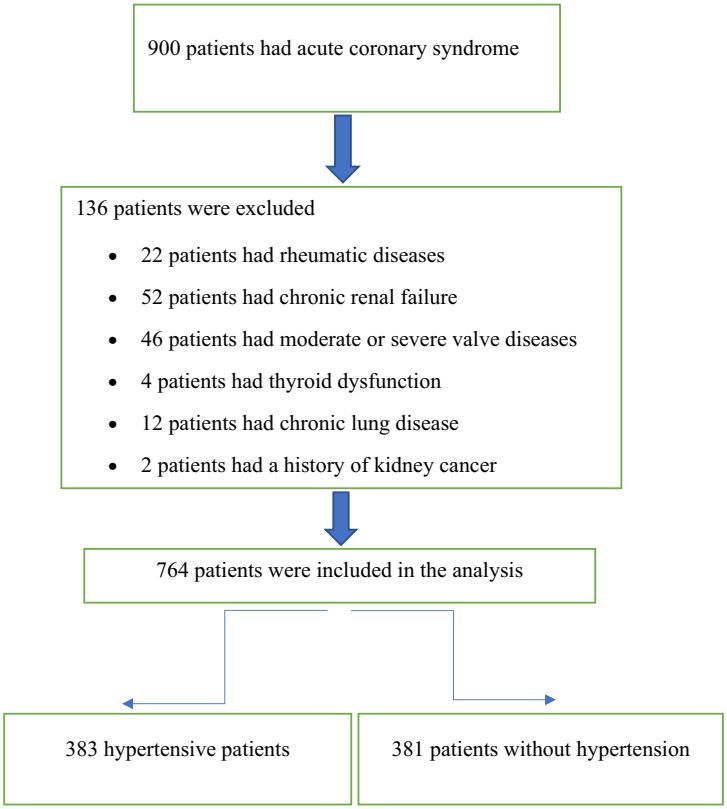

Fig. 1. Study flow chart

study, 136 were excluded due to exclusion criteria. There were 383 hypertensive patients and 381 patients without hypertension (Fig. 1). Patients with rheumatic diseases (rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, etc.), secondary hypertension, electrolyte disorders, cardiac rhythm disorders, chronic renal failure, moderate or severe valve diseases, thyroid dysfunction, chronic lung disease, a history of cancer, and those taking antiarrhythmic, a tricyclic antidepressant, cortisone, antihistamine, and antipsychotic medications were excluded from the study. Systemic disorders were excluded by checking the histories of all patients, completing physical examinations, and examining laboratory tests.

According to World Health Organization (WHO) criteria, the patients included in the study were diagnosed with acute coronary syndrome. According to WHO guidelines, the diagnosis of acute coronary syndrome is made by the presence of ischemic chest pain, ischemic alterations in serial electrocardiograms (ECG), and at least two substantial elevations in blood cardiac markers. All patients with suspected ACS were monitored and followed using ECGs with 12 leads. The type and beginning time of the pain were questioned. The patients who participated in the study had their information (history, history, risk factors, physical examination, ECG findings, laboratory, time of admission to the hospital, and time of treatment) recorded. Patients who arrived with chest pain and were believed to have ACS were given whole blood and biochemistry tests (liver function tests, kidney function tests, cardiac markers, troponin, ck mb). Patients having ST elevation on the admission ECG with supporting clinical or laboratory findings were classified as STEMI. In contrast, those without ST elevation were classified as NSTEMI or unstable angina pectoris based on the presence or absence of myocardial injury. Patients without ST elevation but with elevated biochemical markers on the admission ECG were classified as non-ST-elevation myocardial infarction (NSTEMI); otherwise, they were classified as unstable angina pectoris [6].

According to the guidelines of the American Heart Association/American Society of Cardiology (ACC/AHA) and the European Society of Cardiology, the treatment of the patients was started (ESC). Antihypertensive medication or a history of high blood pressure (systolic blood pressure 140 mmHg or diastolic blood pressure 90 mmHg) were the criteria for the diagnosis of hypertension. Based on previous insulin or antidiabetic medication use or the discovery of increased blood glucose (fasting plasma glucose 126 mg/dL), a diabetes mellitus diagnosis was made [7]. For the diagnosis of dyslipidemia, previous use of antihyperlipidemic medicines or measurements above the values suggested for patient categories in a newly released guideline is based on [8]. The Chronic Kidney Disease Epidemiology Collaboration's (CKD-EPI) formula was used to calculate GFR values.

Statistical Analysis

SPSS 21.0 was utilized for statistical analysis (Statistical Package for Social Science Inc., Chicago, Illinois, USA). The homogeneity and normality of the distribution were evaluated using the Kolmogorov-Smirnov test. Mean and standard deviation was utilized for variables with a normal distribution, whereas number (n) and percentage (%) were used for categorical variables. The median (minimum-maximum) was used to illustrate the descriptive statistics of the variables that were not normally distributed. Pearson's chi-squared or Fisher's exact test was used to compare the categorical variables. Student-T test was used to compare parametric variables in non-categorical variables, while the Mann-Whitney U test was used to compare non-parametric variables. A p-value of 0.05 was judged statistically significant.

RESULTS

Two patient groups with acute coronary syndrome were included in the study. The mean age of 381 non-hypertensive patients was 62.57 ± 10.30 years. The mean age of 383 hypertensive patients was 64.06 ± 10.93 years. The two groups had no significant difference regarding mean age ($p = 0.053$). Clinical and laboratory data for both groups are presented in Tables 1 and 2.

The total cholesterol, glucose, triglycerides, LDL, and HDL cholesterol levels were not significantly different between the two groups. Once again, the two groups had no discernible change in the ranks of hs-CRP, NT-pro-BNP, creatinine, or platelets.

Hypertensive patients had considerably higher

Table 1. Comparison of demographic characteristics of the study population

	Hypertensive Group (n = 383)	Control Group (n =381)	*p* value
Age (years)	64.06 ± 10.93	62.57± 10.30	0.053
Male gender, n (%)	224 (58.5)	307 (80.6)	**< 0.001**
Diabetes, n (%)	105 (27.4)	99 (26)	0.386
Hyperlipidemia, n (%)	150 (39.2)	131(34.4)	0.229
Smoking, n (%)	113 (29.5)	186 (48.8)	**< 0.001**
Systolic blood pressure (mm Hg)	140.21 ± 28.52	118.76 ± 18.17	**< 0.001**
Diastolic blood pressure (mm Hg)	82.59 ± 15.81	73.33 ± 11.49	**< 0.001**
Left ventricular ejection fraction (%)	48.26 + 9.57	45.88 + 11.03	**0.002**

systolic and diastolic blood pressure ($p < 0.001$). In non-hypertensive patients, smoking prevalence was significantly higher ($p < 0.001$). When echocardiography findings were compared throughout patient groups, hypertensive patients had a significantly higher mean ejection fraction ($p = 0.002$). GFR in hypertensive patients was substantially lower (70.71 ± 19.19 vs. 64.83 ± 19.76, $p < 0.001$) than non- hypertensive patients.

Table 2. Comparison of laboratory and echocardiographic values of the study population.

	Hypertensive Group (n = 383)	Control group (n = 381)	*p* value
Creatinine (mg/dL)	1.12 + 0.34	1.10 + 0.28	0.534
Fasting glucose (mg/dL)	139.63 ± 86.30	138.61 ± 71.37	0.482
Total cholesterol (mg/dL)	190.66 ± 54.36	190.22 ± 56.79	0.914
Fasting LDL cholesterol (mg/dL)	117.72 ± 41.77	120.40 ± 44.53	0.396
Fasting HDL cholesterol (mg/dL)	40.48 ± 10.89	40.08 ± 11.43	0.620
Fasting triglyceride (mg/dL)	169.86 ± 113.23	160.37 ± 146.39	0.321
Na (mmol/L)	138.30 ± 2.14	138.70 ± 2.43	0.394
K (mmol/L)	4.31 ±0 .36	4.32 ± 0.37	0.240
Ca (mg/dL)	9.66 ± 0.44	9.80 ± 0.43	0.064
Mg (mg/dL)	2.03 ± 0.24	2.04 ± 0.18	0.874
TSH (mIU/mL)	1.64 ± 0.82	1.68 ± 0.66	0.620
Hemoglobin (g/dL)	14.66 ± 1.87	14.17 ± 1.72	0.724
Leukocyte count (×103/mL)	10.95 ± 3.44	11.19 ± 3.75	0.112
Platelet count(×103/dL)	241.12 ± 78.43	232.49 ± 67.74	0.104
GFR (mL/min.)	64.83 ± 19.76	70.71 ± 19.19	< 0.001
NT-pro-BNP (pg/mL)	1677.23 ± 4067.21	1442.19 ± 4738.88	0.470
hs-CRP (mg/dL)	5.35 ± 4.37	4.92 ± 4.51	0.199

LDL-C = low-density lipoprotein-cholesterol, HDL-C = high-density lipoprotein-cholesterol, hs-CRP = high sensitivity C-reactive protein, NT-pro-BNP = N-terminal fragment brain natriuretic peptides, GFR = Glomerular filtration rate.

DISCUSSION

This study compared GFR values between hypertensive and non-hypertensive patients with acute coronary syndrome. As a result of our research, a significant relationship was found between hypertension and impaired renal function. Regardless of the etiology of kidney disease, there is disagreement about whether hypertension is the primary cause of kidney function loss. It is widely accepted that uncontrolled hypertension accelerates the onset of renal failure, becoming more common as it progresses.

Narrowing of preglomerular arteries and arterioles, as well as enlarged glomerular ischemia caused by decreased glomerular blood flow, are the two main factors that cause renal failure caused by high systemic blood pressure. Another factor is the development of direct hypertensive kidney damage caused by increased systemic blood pressure on the glomeruli. Gradual renal failure begins to develop due to the deterioration of the glomerular structure brought about by the increase in glomerular perfusion [9].

The vascular bed experiences the first physiological changes as the systemic blood pressure rises. The filtration rate increases and the glomerular filtration rate remains constant as the total renal blood flow decreases. The supply vessels constrict to accomplish this, but the uptake narrows less. Selective afferent artery narrowing increases blood flow to some nephrons while maintaining a constant glomerular filtration rate. This type of renal vasoconstriction responds to antihypertensive drugs. The renal vascular response seen in uncomplicated essential hypertension is the narrowing of the afferent arteriolar.

Degenerative changes result in endothelial edema, vascular smooth muscle hypertrophy, enlargement of the internal elastic lamina (due to accumulation of periodic acid-Schiff positive material), focal spasm of the afferent arteriole, and endothelial spasm. The afferent arteriole narrows due to hyalinization caused by focal lumen narrowing. The glomeruli may partially obscure these documented focal vascular changes. This suggests that uncomplicated essential hypertension does not cause significant nephron loss, and the resulting renal failure is unlikely to occur [10].

In the presence of hypertension, vasoconstriction occurs in the preglomerular vascular structure at the border of autoregulation, while the glomerular capillary pressure remains constant. In conclusion, the limits of renal autoregulation and the amount of blood pressure increase affect the renal microvascular system. One mechanism that protects against kidney damage is the widening of renal autoregulation thresholds in systemic hypertension. Severe glomerular damage from hypertensive kidney disease occurs when these autoregulation thresholds are exceeded. Near the upper border of systemic arterial hypertension, remodeling, the thickening of resistant arteries, and rarely glomerular damage are seen. In hypertensive individuals, glomerular injury in the autoregulation range causes prolonged localized glomerular ischemia [11]. The majority of cardiovascular illnesses continue to be the leading cause of morbidity and mortality in CKD patients, according to data from substantial prospective investigations. Even in patients with moderately severe coronary heart disease, it has been reported that decreased renal function increases vascular stiffness [12, 13] The development of vascular damage in atherosclerotic ACS depends on inflammation and oxidative stress [14].

On the other hand, novel risk factors like endothelial dysfunction, hyperphosphatemia, and hyperparathyroidism are highly prevalent and appear to play a more significant role in vascular disease in ACS and end-stage renal disease (ESRD) patients, compared with healthy subjects, in addition to traditional risk factors like diabetes mellitus, hypertension, hyperlipidemia, and advanced age [15, 16].

Renal function and stable ACS have been linked in some studies. According to Goodman *et al*. [17], ACS is typical in young adult patients with ESRD. According to Gradaus *et al*. [18], patients with ESRD experience a faster progression of atherosclerotic ACS than patients with normal renal function.

Studies have shown that the incidence of impaired renal function in patients with acute coronary syndrome ranges from 9 to 19% [19-22]. Poor renal function is an independent risk factor for increased morbidity and mortality in acute coronary syndrome [19, 20].

Many studies have evaluated risk factors for the development of acute kidney disease in patients with ACS, and age, ejection fraction, diabetes, hypertension, and chronic kidney disease are considered inde-

pendent risk factors [21-26].

In our study, the frequency of hypertension and diabetes was higher in the group with acute coronary syndrome with low GFR. Our findings show that lower eGFR values are linked to severe hypertension in patients with acute coronary syndrome. The standard CHD risk factors have no bearing on this connection.

Limitations

The fact that our study was done at a single facility with a small number of patients is its most significant drawback. More blood samples could be obtained from the patients included in our study. Radiological examinations could be used to evaluate kidney functions.

CONCLUSION

The results of our study suggest that hypertension may be an independent risk factor for impaired renal function in patients with acute coronary syndrome. There is a need for extensive randomized controlled studies on this subject.

Authors' Contribution

Study Conception: UU; Study Design: UU; Supervision: UU; Funding: N/A; Materials: N/A; Data Collection and/or Processing: UU; Statistical Analysis and/or Data Interpretation: UU; Literature Review: UU; Manuscript Preparation: UU and Critical Review: UU.

Ethics Committee Approval:

Health Sciences University Tepecik Training and Research Hospital, Non-Interventional Research Ethics Committee Ethics Committee, date: 15.06.2022. Issue no: 2022/06-23

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The possible effect of light exposure reduction via eye patches after the examination for retinopathy in premature infants? An observational study in preterm neonates

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ABSTRACT

Objectives: This study aimed to examine whether infants with and without eye patches differ in terms of vital signs and clinical status after retinopathy of prematurity examination.

Methods: Premature infants hospitalized in study center between June 2021-April 2022 were included. Group 1 is consisted of infants whose eyes were not closed after retinopathy of prematurity examination. Those infants whose eyes were closed eye-patches consisted of group 2. Vital signs were followed for 24 hours following the examination. Demographic, medical and follow-up data were all recorded prospectively. Vital signs were evaluated in accordance with birth week and weight. Pain score was evaluated by Neonatal Pain, Agitation, and Sedation Scale.

Results: Pain scores were found to be lower in group 2 ($p < 0.020$). Although the systolic blood pressure, diastolic blood pressure, and mean blood pressure values of group 2 were found to be lower than group 1, they were within normal limits (all $p < 0.05$). Vomiting was not observed at all in Group 1 ($p = 0.036$). There was no significant difference between the groups in terms of fever, respiratory rate, heart rate, SpO_2, and blood glucose values.

Conclusions: This study showed that using an eye patch in infants after an eye examination reduces pain and increases comfort of infants. Although their pain scores are lower, taking necessary precautions are recommended for these patients in terms of vomiting. Thus, eye patches can be suggested as a non-pharmacological pain-reducing method after get advanced stagnation by the support of more studies with a larger number of participants.

Keywords: Newborn, eye patch, light exposure, retinopathy of prematurity, non-pharmacological method

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Retinopathy of prematurity (ROP) is a disorder of retinal vascular development of preterm infants [1]. The blood supply to the anterior segment of the eye is provided by the hyaloid artery from the 6th week of pregnancy. The hyaloid artery, which originates from the optic nerve, runs along the vitreous and supplies blood supply to the lens and iris surface. The retina is the last organ of the fetus to be vascularized and there is no vascularization before 16 weeks of gestation. Retinal vascularization occurs from the optic disc to the periphery by two mechanisms: vasculogenesis (new capillary formation from endothelial cells) and angiogenesis (new capillary formation as a result of activation, migration and proliferation of endothelial cells from existing venules). The nasal region of the retina is completely vascularized at 36 weeks and the temporal retina at 40 weeks of gestation. [2]. While ROP is predominantly the problem of preterms who are born below 28 weeks in developed countries, severe ROP was reported up to 34 weeks in developing countries [3]. In a multicenter study conducted by the Turkish Neonatal Society in 2014, the frequency of ROP in very low birth weight (BW) preterm infants was found to be 42%, and the frequency of advanced ROP was 8.2%. According to this data advanced stage ROP requiring treatment can be needed for more mature infants with higher GW and BW in our country compared to developed countries [4]. Although the pathogenesis of ROP is not elucidated exactly yet, it is thought to be developed in a two-stage process. Retinal vascularization that started in the intrauterine environment can be paused by any damaging effect in a premature infant. Factors such as prolonged hyperoxia, asphyxia, hypothermia, acidosis, and vitamin E deficiency are possible causes of initial injury. In the early stage of ROP (Phase I), suppression of vascular endothelial growth factor (VEGF) and erythropoietin due to hyperoxia, absence of insulin-like growth factor 1 (IGF-1), poor postnatal growth inhibits normal vascular development [3, 5]. The retina continues to develop, but due to the impaired vascularization, retinal oxygen demand cannot be met resulting in relative hypoxia of retina. Hypoxia encountered by the retina initiates phase II. With the trigger of hypoxia, the levels of mediators such as VEGF, erythropoietin and IGF-I increase and new vessel formation begins. New vessels appear at the vascular-avascular retinal border. Newly formed vessels cluster in the retina and can form a rapidly thickening ridge tissue. New vascularization can lead to leakage and edema formation ending in vision loss, and even retinal detachment [5, 6].

Early detection of ROP can enhance long-term visual acuity [7]. Thus, routine ROP examinations are carried out at certain times which are determined depending on various studies. According to the 2013 recommendations of the American Academy of Pediatrics and the American Academy of Ophthalmology, ROP screening is recommend for all infants who are born with a BW of ≤ 1500 g and/or a GW of ≤ 30 weeks, and infants with a GW above 30 weeks, with a BW of 1500-2000 grams, who required cardiopulmonary support [8]. In the light of national studies, Turkish Neonatal Society guidelines recommended screening all infants born at ≤ 32 weeks, as in the 2006 recommendations of the American Academy of Pediatrics and the American Academy of Ophthalmology [9]. Retinal examination is recommended within 4-6 weeks after babies are born [3]. Physiological changes such as tachycardia, bradycardia, oxygen desaturation, hypertension, and an increase in the frequency of apneic episodes can be seen either during the procedure, and in the following hours in infants undergoing ROP examination [10].

Increased sensitivity of the infant to light due to the dilated pupils before the eye examination, is thought to be effective on the clinical status of the infant. Therefore, the aim of this study is to examine the clinical effects of decreasing the light exposure of infants using eye patches after retinopathy screening in preterm infants.

METHODS

Premature infants hospitalized in Sivas Cumhuriyet University Neonatal Intensive Care Unit between June 2021 and April 2022 were included in this study. Informed consent was obtained from the parents of the patients included in the study, in accordance with the requirements of the Helsinki Declaration of ethical issues. The Ethics Committee of Sivas Cumhuriyet University approved the study (Date: 16.04.2021, Decision No: 2021-04/08). After retinopathy examination, clinical differences were evaluated comparatively between the infants who were exposed to light (Group 1) and infants who were not exposed to light

by using eye patches (Group 2). All patients were followed up for 24 hours after the examination due to the half-life of the drugs used for procedure. No analgesic agents or non-pharmacological methods except swaddling were used in both groups during the follow-up. After the instillation of 0.5% tropicamide and 2.5% phenylephrine drops to bilateral eyes with a 15-minute interval approximately 1-2 hours before the infant's examination, a speculum is used to open the palpebral fissure, and the retina was examined using scleral depression to visualize the periphery. The first examinations of the infants were made by the same ophthalmologist at 31 weeks for infants who were born before 27 weeks and at the postnatal 28th day for infants who were born after 27 weeks. Demographic characteristics (gender, birth weight, gestational week) of the patients were recorded prospectively. The vital signs of the patients included in the study in the first 24 hours after the eye examination were recorded. Vital signs and clinical follow-up data of all infants including body temperature, oxygen saturation, heart rate, blood pressure, blood glucose, pain, respiratory rate, apnea, abdominal distension, vomiting, and resuscitation were all recorded prospectively. The blood pressure values of the patients were evaluated in accordance with the GW and BW according to the guide published by the Turkish Neonatal Society [11]. Pain values were calculated according to the Neonatal Pain, Agitation, and Sedation Scale (N-PASS). N-PASS is a tool used to measure pain in term and preterm infants who experience pain during procedures performed in neonatal intensive care units. The tool uses five physiological and behavioral cues (crying irritability, behavioral state, facial expression, extremities tone, vital signs) with relative validity for measurement [12]. Bradycardia was defined as a heart rate below 100. According to the guidelines of the Turkish Neonatology Association; desaturation was evaluated as SpO_2 below 90. Apnea was defined as the cessation of breathing for 20 seconds or bradycardia with desaturation lasting longer than 10 seconds. Data were obtained by observing all cases 24 hours after retinopathy examination.

Study Population

Infants who were born under 32 weeks and weighed less than 1500 g at birth were included in the study. The infants over 32 weeks, infants whose BW was over 1500 g, those with the genetic disease, heart disease, metabolic disease, lung anomalies, ocular malformations, and infants with conditions that may affect the response to stress were excluded from the study.

Statistical Analysis

The obtained data were evaluated by using the IBM-SPSS (Version 22.0) statistical package program. The percentage, median, and interquartile range 25-75 (IQR 25-75) values were used for descriptive statistics. The Chi-square test was used to compare categorical data, and the Shapiro-Wilk test was used to compare continuous data. Mann-Whitney U test was used for comparisons with categorical groups as it did not show the normal distribution as a result of the normality test. $P < 0.05$ was accepted for statistical significance.

RESULTS

A total of 88 newborn infants were included in this study. While 31.5% (n = 28) of the study group were girls, the median birth weight of the individuals was 860 (780-1250). The median week of delivery of the patients was 27 (25-29), the median APGAR score at 1st and 5th minutes were 5 (4.0-6.0) and 7 (6.0-8.0), respectively (Table 1).

Group 1 included 45 infants, and 43 of them were in Group 2. No correlation was found between the

Table 1. Gender, birth weight, week of birth and APGAR scores of the patients

Gender n (%)	Female	28 (31.8)
	Male	60 (68.2)
Birth weight (g), median (IQR25-75)		860 (780.0-1250.0)
Gestational age (week), median (IQR25-75)		27 (25.0-29.0)
1st. min. APGAR score, median (IQR25-75)		5 (4.0-6.0)
5th. min. APGAR score, median (IQR25-75)		7 (6.0-8.0)

APGAR = Activity- Pulse - Grimace - Appearance – Respiration

Table 2. Comparison of the complications according to the eye closure status of the study groups

Complication		Group 1		Group 2		*p* value
		n	%	n	%	
Apnea	No	45	100.0	42	97.7	0.304
	Yes	0	0.0	1	2.3	
Bradycardia	No	45	100.0	42	97.7	0.304
	Yes	0	0.0	1	2.3	
Tachycardia	No	22	48.9	18	41.9	0.508
	Yes	23	51.1	25	58.1	
Abdominal distention	No	40	88.9	40	93.0	0.500
	Yes	5	11.1	3	7.0	
Vomiting	No	45	100.0	39	90.7	0.036
	Yes	0	0.0	4	9.3	
Resucitation	No	37	82.2	34	79.1	0.078
	Yes	8	17.8	9	20.9	

presence of apnea, bradycardia, tachycardia, abdominal distension, and resuscitation and eye closure. Vomiting was found to be in a significantly higher frequency in Group 2 ($p = 0.036$). Vomiting was observed in 4 (0.09%) of 43 patients (Table 2).

Systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.017$), and mean blood pressure ($p < 0.002$) values were found to be lower in Group 2 compared to Group 1 although they were within the normal limits. Pain values were found to be significantly lower in group 2 ($p < 0.020$). There was no difference between the groups in terms of fever, respiratory rate, heart rate, SpO_2, and blood glucose values. The clinical symptoms and findings according to the eye closure status of the study group are given in Table 3. The systolic, diastolic, mean blood pressure and pain characteristics of the two groups are also shown in the Fig. 1.

DISCUSSION

This study revealed that decreasing the light exposure of infants by using eye patches following the ROP ex-

Table 3. Comparison of clinical and symptoms according to eye closure status of study groups.

Clinical symptoms and findings	Group 1			Group 2			*p* value
	Median	IQR 25	IQR 75	Median	IQR 25	IQR 75	
Fever (°C)	37.00	37.00	37.30	37.10	37.00	37.20	0.446
Respiratory rate (min)	60.00	58.00	62.00	59.00	58.00	60.00	0.226
Heart rate (min)	155.00	148.00	164.00	158.00	154.00	165.00	0.150
SpO_2	94.00	94.00	97.00	95.00	94.00	96.00	0.980
Systolic blood pressure (mmHg)	78.00	69.00	84.00	69.00	65.00	77.00	0.001
Diastolic blood pressure (mmHg)	41.00	39.00	46.80	39.00	33.00	43.00	0.017
Mean blood pressure (mmHg)	53.00	48.00	58.00	46.00	43.00	54.00	0.002
Pain score	1.00	0.00	1.00	0.00	0.00	1.00	0.020
Blood glucose (mg/dL)	80.00	71.00	90.00	80.00	74.00	87.00	0.864

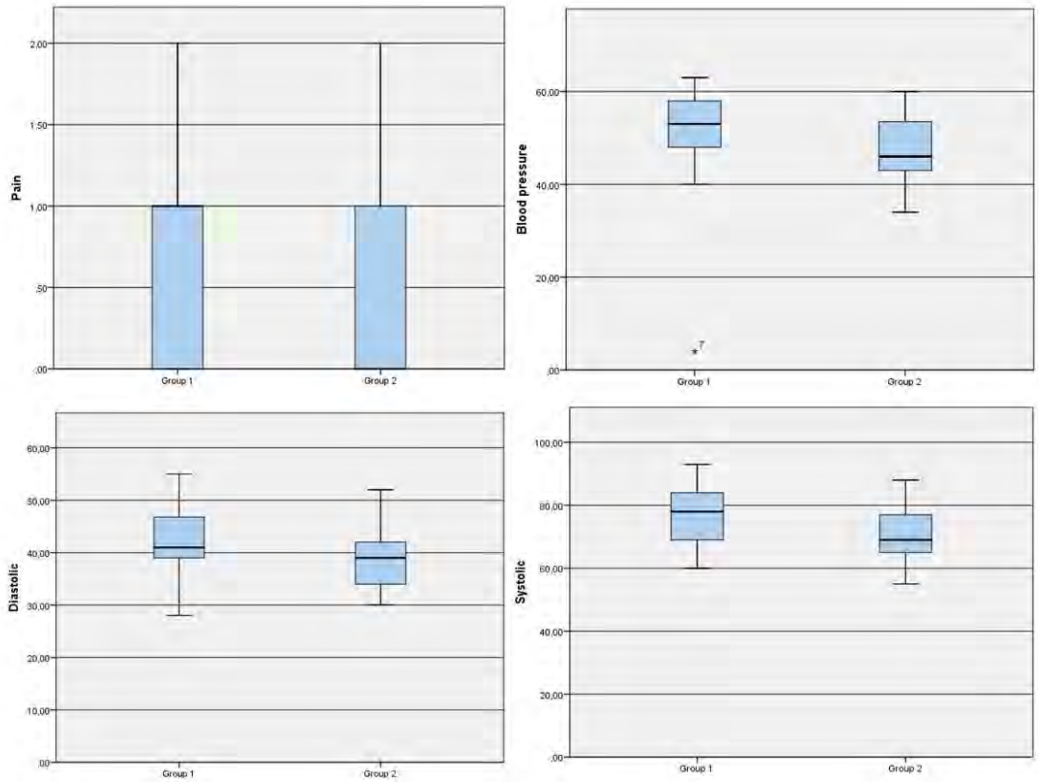

Fig. 1. Comparison of systolic blood pressure, diastolic blood pressure, mean blood pressure, and pain status of the study groups. In group 2, systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.017$), mean blood pressure($p < 0.002$) values were found to be lower than those whose eyes were closed. Pain values were found to be lower in the infants with eye patches ($p < 0.020$).

amination, can reduce the pain due to the procedure. Physiological changes such as tachycardia, bradycardia, desaturation, hypertension, and an increase in apneic episodes can be observed during the ROP examination and in the following hours in infants who are examined for retinopathy [10]. In a study examining the effects of light intensity on the physiological parameters of premature infants, an increase in the intensity of light resulted in elevated heart rate and respiratory rate accordingly followed by an increase in oxygen demand [13]. In this study, we did not detect a significant difference between Group 1 and Group 2 in terms of respiratory rate, heart rate, and SpO_2 values after the examination. In a small randomized clinical study, Szigiato *et al*. [1] showed that in addition to the stress caused by the examination itself, additional stress due to the extreme light sensitivity by mydriasis can be reduced by using eye patches. Since the unequal distribution of infants receiving ventilator support affected the accuracy of the findings, they stated that more comprehensive and prospective studies are needed to increase the reliability of the results [1]. Belda *et al*. [14] found no significant changes in blood pressure and pulse rate in 27 preterm infants after ophthalmological examination, while they detected vomiting in 4%, reflux in 22%, and apnea in 41% of these cases. Similarly, in this study, although systolic blood pressure, diastolic blood pressure, and mean blood pressure values were found to be higher in Group 1 compared to Group 2, blood pressure values were found to be within the normal range. Although no vomiting was seen in Group 1, it was observed in 4 of 43 patients in group 2. This finding could be associated with the relatively small number of participants of this study. However, a clarification can be provided by more comprehensive studies in the future.

The infants who wore eye patches after retinopathy examination had lower pain scores in Group 2 compared to group 1. Since the complications in regard to pain-related stressful events can be common, those infants should be closely monitored for the first 24-48 hours following the ROP examination. Pain re-

duction by using eye patches after ROP examination was found to be useful in a recent study [15]. Likewise, this study revealed that the pain scores of infants who wore eye patches were lower than the ones in group 1. On account of premature infants are more sensitive to painful stimuli, the pain due to the ROP examination was shown to be more severely felt, and its effect was longer [16]. While negative stress increases the oxygen demand of the tissues in infants exposed to pain when the respiratory system cannot meet this need, it can lead to apnea, cardiovascular problems, overloading and bleeding in weak and immature vessels with increased blood pressure, and changes in the level of consciousness. Reducing pain is an important entity for newborn infants. Therefore, the use of an eye patch can be valuable either in preventing intraventricular hemorrhage by reducing the pain. Thus, this can be used as a non-pharmacological method after retinopathy examination. Infants examined with wide-field digital retinal imaging (WFDRI) for ROP screening showed to be more painful and stressed compared with a Binocular İndirect Ophthalmoscope (BIO) [17]. In this study, all infants were examined with BIO, and the patients without eye patches were found to have more pain than the infants whose eyes were closed after the examination. Many pharmacological and non-pharmacological methods are used in infants to reduce pain [18]. Based on this study, using the eye patches can be suggested as a non-pharmacological pain relief method.

Limitations

The limitations of the study can be mentioned as follows. Although the infants are similar in terms of clinical and demographic status, the medications used for infants, treatment modalities, and the genetic factors of the population can not be completely similar between the groups. Besides, vital signs of the infants can be affected by many environmental conditions which can not be recognized. Therefore, we suggest that multidisciplinary studies with larger number of cases are needed to overcome those limitations.

CONCLUSION

This study revealed that using an eye patch in infants after eye examination reduces pain and improves the comfort of infants. However, necessary precautions should be taken for these patients in terms of vomiting. Based on the findings of this study, the use of eye patches can be suggested as a non-pharmacological pain-reducing method after retinopathy examination, however more studies with larger number of participants are required for a better understanding for its action of mechanism.

Authors' Contribution

Study Conception: FK, GT, FK; Study Design: GT, FK, DYY, FK; Supervision: DYY, FK, GT; Funding: SA, EET, FK, GT, ÖŞ; Materials: FK, SA, EET; Data Collection and/or Processing: FK, SA, EET, GT; Statistical Analysis and/or Data Interpretation: DYY, SA, FK; Literature Review: EET, GT, FK, SA; Manuscript Preparation: GT, FK, DYY and Critical Review: DYY, GT, FK, FK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Posttraumatic growth in family members of individuals with methamphetamine use disorder

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ABSTRACT

Objectives: This study aimed to determine post-traumatic growth and its predictors in female relatives of patients with methamphetamine use disorder.

Methods: The volunteers in our study consisted of 80 adult women who were first-degree relatives of male patients diagnosed with MUD. The Post-Traumatic Growth Inventory, the Hospital Anxiety-Depression Scale, the Impact of Events Scale , and the short form of the Coping with Stressful Situations Inventory were given to the relatives of the patients.

Results: In our study, being employed and being married were found to affect task-oriented coping and changes in self-perception positively. Task-oriented coping or seeking emotional support from others predicted higher PTG. Task-oriented coping was positively associated with emotional coping and a change in the philosophy of life. The regression analysis determined that task-oriented and avoidant coping mechanisms were the best predictors of post-traumatic growth.

Conclusions: The findings suggest that environmental and personal factors, such as being married, being employed, and the quality of social relationships that emerge with emotional and instrumental support, influence the experience of life crises. In addition, the duration of methamphetamine uses, the problem's, and the search for solutions were all associated with higher post-traumatic growth. Thus, the results of this study demonstrate that task-oriented coping, seeking emotional support, and the duration of methamphetamine use may contribute to post-traumatic growth.

Keywords: Posttraumatic growth, coping, methamphetamine abusers, family members

Post-traumatic growth (PTG) refers to the positive changes experienced by individuals as a result of an adaptive process during coping with a challenging life event [1]. Although individuals experience negativity in stressful life events, they can develop positive changes by improving their perspectives and recognizing personal and social resources [2, 3]. PTG is also explained as a person's better functioning in certain areas of life after the traumatic event and the person's better revealing of his or her potential [4]. The positive psychological change includes appreciating life, setting new priorities, increasing personal power, identifying new possibilities, strengthening relationships, and spiritual growth [5].

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A recent meta-analysis shows that PTG levels across studies show high heterogeneity, ranging from 10% to 77.3%. Approximately one in two people who experience a traumatic event report moderate to high post-traumatic growth. The same study showed that a long period since the traumatic event was associated with higher PTG in women and youth [6, 7].

PTG can also be considered an adaptive coping strategy for traumatic experiences [8]. Coping, a continuous change in behavior and cognitions to cope with different needs beyond one's competence [9], is a fundamental factor that affect individuals' emotional and behavioral responses to stress. It is also an important factor for post-traumatic growth. In Aldwin's 'transformative coping' model [10], transformative positive coping leads to a higher level of functioning. In the stress-strain-coping support (SSCS) model [11], having a close relative with a methamphetamine abuse disorder creates a long-standing stressful life situation for the family members. In such cases, family members may resort to "coping", such as being active in facing difficulties, solving problems, mediating their destiny, and not being powerless. By responding to buffer, the effects of stress, they may find ways to reduce the tension experienced by themselves or other family members - such as children.

Being a parent or relative of a patient is a traumatic and challenging experience. There are many studies showing the successful adaptation of parents whose children have been exposed to these challenging experiences. In studies, investigating PTG in parents of children with severe illness, PTG was reported as moderate in a significant proportion of parents [12, 13]. Mothers were reported to have higher PTG levels than fathers in a study of the parents with severe illnes [13]. Bellizzi and Blank also [14] found that young women show more post-traumatic growth than older women in a study of individuals with breast cancer survivors [14].

Affected family members use one or more of three coping approaches to deal with the problem: puting up with the behavior (e.g., accept things as they are, inaction, resignation), withdrawal from the relative and the immediate situation (e.g., gaining independence from the problem, becoming involved in other activities), and standing up to or confront the behavior associated with the problem (e.g., set boundaries for unacceptable behavior; protecting other family members, especially children, from the relative's behavior; insisting on the relative seeking treatment; seeking assistance from the police and judiciary) [15, 16].

Methamphetamine use disorder (MUD) is a current social problem that is not only limited to the effects on the life of the individuals with addiction but also affects their family considerably. Social support from relatives is essential in the treatment of addiction. Although there are few studies on post-traumatic growth in substance and alcohol addictions [17, 18], there are no studies dealing with post-traumatic stress and growth variables in the relatives of addicts. In this context, it is thought that the study will make an important contribution to the literature by filling thise gap.

Our study aimed to determine post-traumatic growth and its predictors of female relatives of patients with MUD.

METHODS

The data of this study were collected between 01.12.2021 and 01.07.2022. The volunteers participating in our study consisted of 80 adult women who were first-degree relatives of male patients diagnosed with methamphetamine addiction who applied to the Alcohol and Substance Addiction Treatment Clinic of a Training and Research Hospital outpatient clinic. Volunteers aged 18-75 years were included in the study. The exclusion criteria werw determined as (1) not giving informed consent, (2) iliteracy, (3) having mental retardation, (4) severe psychiatric disorders, (5) systematic diseases, (6) being a relative of individuals with multipl substance or alcohol use disorders.
All participants reviewed the informed consent form and provided written consent. This study was designed in accordance with the 2013 Brazilian version of the Declaration of Helsinki and was approved by the local ethics committee (dated 01.12.2021 and decision number 2011-KAEK-25 2021/12-23). Good clinical practice principles were followed throughout the study.
After their informed consent was obtained, the Post-Traumatic Growth Inventory (PTGI), the Hospital Anxiety-Depression Scale (HADS), the Impact of Events Scale (IES), and the short form of the Coping with Stressful Situations Inventory (CISS-21) were given to the relatives of the patients.

PTGI was developed by Tedeschi and Calhoun

[19] to assess post-trauma growth and self-improvement a person undergoes. The scale is a Likert type, scored between 0 and 5. The range of the scale is 0-5. The higher scores indicates that a person has experienced a high level of growth after a traumatic experience. Its adaptation, validity and reliability study into Turkish language was performed by Kağan *et al*. [20].
IES is a self-report scale measuring the level of post-traumatic stress developed by Horowitz *et al*. [21], later revised by Weiss and Marmar [22] according to DSM-IV post-traumatic stress disorder criteria and adapted into Turkish by Çorapçıoğlu *et al*. [23].
HADS was developed by Zigmond and Snaith [24] to determine the level of anxiety and depression experienced by a person. It was adapted into Turkish by Aydemir *et al*. [25].

CISS-21 [26] measures three dimensions of coping (task-focused, emotion-focused, and avoidance-focused coping) and consists of 21 items. Participants rate each item on a five-point Likert scale (1 = "never" to 5 = "very much") to determine which coping strategies they use for different stressful situations. Boysan [27] carried out a validity and reliability study in Turkish.

Statistical Analysis

Statistical analyses were performed with the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Variables were expressed as the mean ± standard deviation, and categorical variables were expressed as frequency and per cent. Quantitative data were compared with the independent samples t-test and analysis of variance (ANOVA); Pearson correlation analysis was used to measure the relationship between measurement scales. Linear regression analysis with a backward stepwise method was performed to determine the risk factors of PTGI total. A p value of less than 0.05 was considered statistically significant for all tests.

RESULTS

Twelve (15%) of the MUD were assesed in out-patient clinic and hospitalized, and the rest were followed up on an outpatient basis. Regarding daily methamphetamine use, 49 (61.3%) individual used 0.5-1 mg, and 31 (38.8%) used more than 1 g. duration of any substance use 8.47 ± 6.5 days (min: 1 max: 33), age of onset of any substance 20.65 ± 7.05 years (min:13 - max:45), mean age of onset of methamphetamine 25.7 ± 8.25 years (min: 15 - max: 57), duration of methamphetamine use was 3.71± 2.29 days (min: 1 - max: 12). The mean age of the volunteers was 43.36 ± 13.33 years (min: 20 - max: 72). Other sociodemographic data of the volunteers are given in Table.1.

Comparison of Demographic and Clinical Data with Scale Scores

When the scale scores of the employeed and unemloyed individual were compared with the independent samples t-test, the CISS task-oriented scores (p = 0.035) and PTGI Appreciation for life scores (p = 0.017) of the employed individual were found to be significantly higher.

Compared with the independent samples t-test, those whose relatives use 0.5-1 g methamphetamine and 1 g methamphetamine daily, CISS-task-oriented scores of those whose relatives use more than 1 g methamphetamine per day were found to be significantly higher (p = 0.021).

There was a statistically significant difference in IES-hyperarousal scores when the participants' educational status and marital status were compared with the one-way ANOVA test in terms of scale subscores. As a result of the post hoc Tukey test performed to determine significance, the scores of primary school graduates were significantly higher than those of sec-

Table 1. Sociodemographic status of volunteers

		n	%
Education	Primary	43	53.8
	Secondary	15	18.8
	High	16	20.0
	University	6	7.5
	Single	6	7.5
Marital status	Married	67	83.8
	Divorced	7	8.8
Working status	Employed	24	30.0
	unemployed	56	70.0
Living place	Urban	48	60.0
	Town	25	31.3
	Village	7	8.8

Table 2. Scale scores of volunteers

	Minimum	Maximum	Mean	Standard Deviation
HADS anxiety	2.00	15.00	7.262	2.849
HADS depression	4.00	16.00	9.100	2.646
HADS total	8.00	26.00	16.362	4.022
IES intrusion	5.00	28.00	18.387	5.166
IES avoidance	2.00	24.00	12.412	4.905
IES hyperarousal	2.00	21.00	11.900	5.237
IES total	18.00	66.00	42.700	11.259
CISS task-focused	16.00	35.00	26.800	3.820
CISS emotion-focused	14.00	34.00	19.250	3.866
CISS avoidance-focused	7.00	25.00	12.375	4.487
PTGI Changes in Self-Perception	12.00	43.00	28.550	7.479
PTGI Appreciation for life	2.00	23.00	9.675	4.221
PTGI Improved relationships	0.00	16.00	6.650	3.907
Total	19.00	67.00	44.875	11.713

HADS = Hospital anxiety-depression scale, IES-Int = IES- intrusion, IES-T = IES total, CISS-TOC = CISS task-oriented coping, CISS-EOC = CISS emotion-oriented coping, CISS-AOC = CISS Avoidance-oriented coping, PTGI = Post-Traumatic Growth Inventory, PTGI-AL = PTGI Appreciation for life

ondary school graduates ($p = 0.035$) and high school graduates ($p = 0.081$).

There was a statistically significant difference in comparing the participants' marital status and HAD depression scores in the one-way ANOVA test. As a result of the post hoc Tukey test performed to determine the significance, the scores of the singles were significantly higher than those of the married ($p = 0.036$).

There was a statistically significant difference in comparing the participant's marital status and CISS task-oriented scores in the one-way ANOVA test. As a result of the post hoc Tukey test performed to determine the significance, the scores of the married were significantly higher than the singles ($p = 0.004$).
There was a statistically significant difference in the marital status of the participants in terms of PTGI Changes in Self-Perception when compared with the one-way ANOVA test ($p = 0.012$). As a result of the post hoc Tukey test performed to determine the significance, it was understood that the difference was due to the married group. There was also a statistical difference in total PTGI among married people ($p = 0.014$).

HAD depression ($p = 0.018$), CISS task-oriented coping ($p = 0.023$), and PTGI-change in self-perception ($p < 0.001$) were statistically higher than relatives of addicted persons who have been hospitalized.

Relatives of an individual with addiction who did not attempt suicide had higher rates of HAD Depression ($p = 0.021$), CISS Avoidance-oriented coping ($p = 0.037$), and PTGI Appreciation for life ($p = 0.043$) compared to relatives of suicidal individual with methamphetamine abuse. IES-intrusion (p = 0.030), PTGI-Improved relationships ($p = 0.021$), and PTGI-total ($p = 0.024$) were statistically significantly higher in the relatives of those who attempted suicide.
Correlations between age, CISS, PTGI, and IES scores are given in Table 3.

Predictors of PTGI Total Score

To investigate variables that predict PTGI total score, variables that are significantly correlated ($p < 0.05$) with PTGI total score; CISS-task-oriented coping, CISS avoidance-oriented coping, and the variable which fulfils $p < 0.20$ criteria (Pearson correlation analysis revealed a non-significant association between age and PTGI total score [$p = 0.095$] and the

non-significant association between IES avoidance and PTGI total score [$p = 0.136$]) were included in the regression analysis. The backward stepwise method was used in the regression analysis. IES avoidance was excluded in the second step; age was excluded in the third step. Results showed that the regression model was significant (F=11.794; $p < 0.001$). The severity of CISS task-oriented coping, and CISS avoidance-oriented coping accounted for PTGI total score at the rate of 0.215 (R2 = 0.215). When the severity of CISS task-oriented coping, PTGI total score increased by 1.058 units (ß = -1.058; $p = 0.001$) and when CISS avoidance-oriented coping score increased by one unit, PTGI total score increased by 1.004 units (ß = 1.004; $p < 0.001$) (Table.4).

DISCUSSION

Our study to determine post-traumatic growth and its predictors in female relatives of individuals with methamphetamine use, being employed and being married were found to positively affect task-oriented coping and changes in self-perception. The regression analysis determined that task-oriented and avoidant coping mechanisms were the best predictor of post-traumatic growth.

Schaefer and Moos [28] argue in their "conceptual model of positive outcomes of life crises and transitions" that environmental and personal factors influence the experience of life crises through cognitive appraisal processes and coping responses. Individual components include socio-demographic characteristics, self-efficacy, resilience, optimism, self-confidence, adaptability, motivation, health status, and previous crisis experience. Relationships, family, friends and social environment, material resources and other aspects of life constitute the environmental components. Event-related factors include the effects of the severity, the duration, and the timing of the life crisis on the individual. These components, which they consider in their models, are interconnected with feedback loops and affect each other.

We can see some components of this cycle among the findings of our study in which we examined the family members of individual with MUD. For example, being employed and married positively affected task-oriented coping and changes in self-perception. CISS task-oriented scores and PTGI Appreciation of life scores of employed working relatives were significantly higher. The fact that task-oriented coping was one of the best predictors of post-traumatic growth was also consistent with the feedback loops mentioned above. In a systematic review of 39 studies, Linley and Joseph demonstrated that PTG is positively associated with coping strategies such as problem-focused, acceptance, and positive reinterpretation coping [29].

Our finding that changes in the philosophy of life were negatively associated with age is consistent with the literature. One review found that the combined prevalence of moderate to high TSG was higher in those under 60 than in older people [6, 7]. An inverse relationship was found between age and PTG in adults aged 20-70 [30]. Age has been negatively associated with PTG in a range of events throughout life [31, 32].

As we assessed the effects of the traumas, we determined that primary school graduates have higher IES-hyperarousal scores than secondary school, high school and university graduates, that may indicate that the physiological effect of trauma may be higher at lower education levels. Research supports the idea that primary school graduates have higher IES-hyperarousal scores than other education levels. For example, one study showed that among adults aged 55-74, those with less than a high school or college education were more likely to report higher levels of trauma exposure and higher IES-hyperarousal scores than those with higher education levels [33]. Additionally, various studies have found a positive correlation between lower educational attainment and post-traumatic stress disorder [34, 35].

When we evaluated the coping strategies, the CISS task-oriented coping scores, PTGI changes in Self-Perception scores, and PTGI-total scores of the married participants were higher than those of the singles. Single participants have higher HAD depression scores than that of the married. Barsakova and Oesterrich [36] stated that marital status is not directly related to PTG and that the quality of social relationships that emerge through emotional and instrumental support is more valuable. They also suggested that coping strategies, such as a problem-oriented approach or seeking emotional support from others, predicted higher PTG.

In the evaluation of the relationship between the clinical features of the individuals abuse and the variables of the relatives, we determined that the relatives

of individuals with drug addictions who used more than 1 gram of methamphetamine per day who and were hospitalized had higher CISS task-oriented coping scores. Also, HAD depression and PTGI Change in Self-Perception scores were higher in the relatives of individuals with drug addiction who received hospital treatment. Additionally, relatives of those who attempted suicide had higher IES entry, development of PTGI in relationships, and total PTGI. These findings suggest that as the severity of the problem increases, the search for solutions also increases and may lead to more positive results regarding post-traumatic development.

Duration of methamphetamine use was positively associated with CISS-AOC. Family members using the avoidant coping style may have experienced surrender, realizing that nothing can keep their drug-addicted relatives from taking drugs and that the problem is out of their control. In a review, Henson *et al*. [37] suggested that engaged and tolerant-inactive maladaptive coping strategies had a significantly more significant adverse influence on family member's physical health and/or socializing than withdrawal coping strategies. It has been observed that individuals with methamphetamine abuse, who feel that their families tolerate drug use, have had methamphetamine use for many years [38].

The study concluded that task-oriented coping is linked with positive emotional coping and an alteration in a person's philosophy of life. Additionally, emotional coping was positively associated with IES-Total and negatively associated with CISS-Avoidance. The CISS assumes that task-oriented and emotional coping can have positive relationships, while the relationship between task-oriented and emotional coping and avoidance is believed to be negative. Thus, the overall conclusion is that people use a combination of task-oriented, emotional, and avoidance coping when managing stressful situations.

In our study, when we focused on the relationship between trauma and coping strategies, we found that IES-Intrusion was negatively associated with CISS-emotion-focused coping and positively associated with a change in the philosophy of life, consistent with the views of Jones *et al*. [39]. The fact that ruminative thinking by reliving the problem is negatively related to avoidance-focused coping also supports their view. According to the authors, when people face a traumatic event with intrusive rumination, they want to reflect on the trauma for a long time, make sense of what happened to them, and cognitively reprocess their life assumptions. Cognitive processing of trauma can lead to a person's maturation and development, creating an opportunity to change their worldview and develop new perspectives.

In the regression analysis, CISS-TOC and CISS-Avoidance were both highly effective on the PTGI Total Score, indicating that focusing on solving the problem or avoiding the negative effects of the problem is beneficial and contributes to post-traumatic growth.

Limitations

The most important limitation of the study is data reliability since research findings are based on self-report questionnaires. Cross-sectional design does not allow any inference about causality or association. In addition, the sample size is not at a level to ensure the representativeness and generalizability of the findings.

CONCLUSION

The finding of coping mechanisms as a predictor of post-traumatic growth in female relatives of individuals with methamphetamine use in our study shows the importance of appropriate coping mechanisms in the relatives of the patients. Family members should be supported to use adaptive coping strategies to maintain their supportive role. This cooperation will be beneficial in improving the patient's addiction and reducing the risk of relapse.

Authors' Contribution

Study Conception: ÇT, ÖŞ; Study Design: ÇT, GŞ, ÖŞ; Supervision: ÇT, SÜ, ÖŞ; Funding: ÇT, SÜ, ÖŞ; Materials: ÇT, GŞ; Data Collection and/or Processing: ÇT; Statistical Analysis and/or Data Interpretation: ÇT, SÜ, ÖŞ; Literature Review: ÇT, SÜ, GŞ; Manuscript Preparation: ÇT, SÜ and Critical Review: SÜ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Quality and reliability of YouTube videos as a source of information on pulmonary rehabilitation

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ABSTRACT

Objectives: We aimed to evaluate the quality and reliability of the most watched YouTube videos in pulmonary rehabilitation (PR) and to determine the criteria that may be important in the selection of high quality and reliable videos by patients and their relatives.

Methods: We searched for the keywords "pulmonary exercise," "pulmonary rehabilitation," and "pulmonary physiotherapy" on December 12th, 2021. Modified DISCERN (mDISCERN) and Global Quality Score (GQS) were used to assess the quality and reliability of the videos.

Results: Of the 150 videos screened and 76 (50.7%) videos were identified for inclusion. The median mDISCERN score was 2, indicating that most of the videos were of low quality. A statistically significant relationship was found between video reliability classification in terms of video duration, time since upload, number of subscribers, and number of likes ($p < 0.05$). In addition, in terms of video upload source, it was determined that most of the high reliability videos were uploaded by healthcare professionals and most of the low reliability videos were uploaded by independent users ($p < 0.05$). When the videos were compared according to the quality groups subcategory, significant differences were detected in video duration, number of subscribers, average number of views per day, upload sources and mDISCERN scores ($p < 0.05$).

Conclusions: According to the current study results, most of the PR-related videos on YouTube were found to be of poor quality and low reliability. Sharing more videos on social platforms by healthcare professionals in the future may be effective in increasing video quality and reliability.

Keywords: Social networks, social media, patient education, pulmonary rehabilitation

Pulmonary rehabilitation (PR) is a scientifically proven, multidisciplinary and multi-intervention form of rehabilitation for patients with underlying respiratory system diseases and mostly decreased activity levels of daily living due to these diseases, or to provide early intervention without reduction [1]. PR is an essential component of the care of people with chronic respiratory disease [2]. The conservation of effective lung mechanics is closely associated with the proper function of the phases of respiration [3]. PR is important in terms of restoring adequate respiratory functions in patients with impaired lung mechanics. Exercises performed within the scope of PR are effective against pulmonary system disorders by affecting

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both respiratory muscles and airway. PR can be applied not only in chronic obstructive pulmonary disease, but also in asthma, interstitial lung disease, pulmonary arterial hypertension, non-cystic fibrosis bronchiectasis, nonsmall cell lung cancer, and many other respiratory problems [4]. Therefore, PR is important not only for the departments dealing with the rehabilitation and respiratory system, but also for many disciplines, especially the departments dealing with intensive care and rheumatology patients. In addition, PR has become more important with the increase in respiratory system diseases after the Covid-19 pandemic. It is inevitable for every physician to have an idea about PR in the management of many diseases today.

Today, the internet and social media are a part of daily life [5]. Many people use social media on health-related issues as well as in other areas. Especially patients with chronic health problems get information from social media at any stage of their illness, especially diagnosis and treatment.

YouTube is one of the most important social networking sites. YouTube is a free video watch and upload platform that allows users and viewers to watch videos that have been posted, upload new videos, and rate and comment on watched videos [6]. Although this video platform has advantages, it may contain inaccurate and unreliable information [7-9]. So it can be as dangerous as it can be useful.

As far as we've researched the literature, no previous research has looked at the content, quality, and reliability of PR videos on YouTube. Therefore, the primary aim of this study was to evaluate the quality and reliability of YouTube videos regarding PR. In addition, it has been tried to determine which parameters should be paid more attention in finding video sources that give better quality and reliable information.

METHODS

Study Design

This was a descriptive study. On December 12, 2021, the keywords "pulmonary rehabilitation", "pulmonary physiotherapy" "and "pulmonary exercise" were used to search for videos on YouTube (www.youtube.com). These search terms have been selected based on the terms that may be most relevant to PR. Among the scanned videos, the top 50 most watched videos for each keyword were included in the study analysis. Separately, for each keyword, the English language videos were assessed by two researchers experienced in PR. Since many studies examining the quality and reliability of YouTube videos analyze the 50 top-viewed videos, we evaluated the 50 top-viewed videos for each keywords [10-12]. We considered that evaluating the first 50 most watched videos would reflect what most YouTube users opinion. 150 videos were watched in total. Off-topic videos, duplicate videos, videos in a language other than English, and videos whose sound and video quality were too poor to be evaluated were excluded. As a result, 76 videos remained that met the inclusion criteria.

Video Parameters

In all watched videos, the length of the video, the number of views, the upload date, the number of likes, the number of subscribers of the watched channel and the comments number were determined. Total views, likes and comments were divided by the day difference between the date the video was watched by us and it was uploaded to YouTube. In this way, daily values were also evaluated as they would give more reliable results for some parameters.

Video Upload Sources

The video sources were categorized into 3 groups: patient or caregiver, healthcare professionals (physical therapists, physicians, pharmacists, or other healthcare professionals), and independent users (associations, advertisements, or news).

Assessment of Quality

Non-specific educational content quality was assessed using the Global Quality Scale (GQS), a non-validated but widely used score in many similar studies in the literature, that assesses the video quality [13-15]. GQS is a 5-point likert scale. According to this scale, 5 points represent the highest quality content, while 1 point represent the lowest quality content. Results obtained from the scale are indicated as low quality (1-2 points), medium quality (3 points) and high quality (4-5 points) according to quality levels (Table 1) [13].

Assessment of Reliability

The reliability parameter of the YouTube videos was evaluated using modified DISCERN tool (mDISCERN). This scale was found by Charnock *et al*. This scale includes five questions and yes or no options are marked for each video to these questions. While 1 point is given for each "yes" answer on this scale, no point is given to the "no" answer. Therefore, total score can be a maximum of 5 points and a minimum of 0 points, with 5 points indicating the highest reliability and 0 points the lowest reliability. To say a video is high reliability, it must have an mDISCERN score of 3 or more (Table 2) [16, 17].

Ethics committee approval is not required as human or animal subjects were not used in this study and it was done by examining videos that can be accessed by everyone from the internet.

Statistical Analysis

Shapiro Wilk test was used for determine whether the variables follow normal distribution or not. Continuous variables were specified as median (min-max) values. Categorical variables were specified as n (%). Based on the normality test results, the Mann Whitney U test was used for comparisons between the 2 groups, and the Kruskal Wallis test was used if the number of groups were more than 2. Multiple comparisons were performed using the Dunn-Bonferroni approach to identify different group or groups after the Kruskal Wallis test. Pearson chi-square test, Fisher's exact chi-square test or Fisher Freeman-Halton test were used for comparing categorical variables. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) was used for statistical analysis. The level of significance was determined as $p < 0.05$.

RESULTS

General Characteristics

Of the 150 videos screened and 76 videos (50.7%) were identified for inclusion. 11 duplicate videos, 36 off-topic videos, 23 non-English videos, and 4 videos with very poor image and sound quality were excluded. The general features, quality, and reliability scores of the videos are summarized in Table 3.

The Comparison of Reliability Groups

Comparison of video features and upload source by reliability classification is summarized in Table 4. A statistically significant relationship was found between video reliability classification in terms of video duration, time since upload, number of subscribers, and number of likes ($p < 0.05$). Also, according to the video upload source, it was determined that most of high reliability videos were uploaded by healthcare professionals and most of low reliability videos were uploaded by independent users ($p < 0.05$).

The Comparison of Quality Groups

Comparison of video features, reliability scores and upload source by quality classification is summarized in Table 5. There was a significant difference between the quality groups in terms of total video duration ($p = 0.043$) (Table 5). Pairwise comparisons were also made to determine from which group the difference originated. According to the subgroup analysis, total video duration of medium quality videos has been determined to be higher than the total video duration of low quality videos ($p = 0.043$). There was a significant difference between the quality groups in terms of the number of subscribers ($p = 0.006$) (Table 5). Subgroup analysis was performed to determine the source of the difference. According to the subgroup analysis, the subscriber numbers of the channel, where low quality videos are watched, were found to be lower than the number of subscribers of the channel of the channel, where medium quality videos are watched ($p = 0.006$).

There was no significant difference between the quality groups in terms of the median average number

Table 1. Global quality scale

1. Poor quality, poor flow, most information missing, not helpful for patients
2. Generally poor, some information given but of limited use to patients
3. Moderate quality, some important information is adequately discussed
4. Good quality good flow, most relevant information is covered, useful for patients
5. Excellent quality and excellent flow, very useful for patients

Table 2. Modified DISCERN tool

1. Is the video clear, concise, and understandable?
2. Are valid sources cited?
3. Is the information provided balanced and unbiased?
4. Are additional sources of information listed for patient reference?
5. Does the video address areas of controversy / uncertainty?

of views ($p = 0.027$) (Table 5). Subgroup analysis was performed to determine the source of the difference. According to the subgroup analysis, it was determined that the median average number of views of low quality videos was lower than the median average number of views of medium quality videos ($p = 0.038$). here was a significant difference between the quality groups in terms of mDISCERN scores ($p = 0.001$) (Table 5). Subgroup analysis was performed to determine the source of the difference. According to the subgroup analysis, the rate of those with mDISCERN < 3 in the low quality video group is higher than those with mDISCERN < 3 in the medium quality and high quality video groups ($p < 0.001$ and $p < 0.001$, respectively).

A statistically significant difference was found between the quality groups in terms of the videos upload by the patient or caregiver ($p = 0.041$) (Table 5). After the overall significance, subgroup analyzes were performed. However, since the number of units in the groups could not reflect this significance in pairwise comparisons, no significance could be obtained in the subgroup analyzes. There was a significant difference between the quality groups in the videos uploaded by health professionals ($p < 0.001$) (Table 5). Subgroup analysis was performed to determine the source of the difference. According to the subgroup analysis, rate of uploading low quality videos by health professionals was higher than rate of uploading medium and high quality videos ($p = 0.002$ and $p = 0.002$, respectively). A statistically significant difference was determine between the quality groups in terms of videos upload by independent users ($p < 0.001$) (Table 5). Subgroup analysis was performed to determine the source of the difference. According to the subgroup analysis, it was determined that the upload rate of low quality videos by independent users was higher than the upload rate of medium and high quality videos ($p = 0.007$ and $p = 0.007$, respectively).

DISCUSSION

Today, the use of the internet and video sharing sites (especially YouTube) as a guidance is increasing. Although the use of YouTube and other social media networks has a positive effect in the field of health as in other fields, it creates hesitation due to the presence of low quality, unreliable and contradictory information [18, 19]. As far as we have examined in the literature, our current study is the first to examine the quality and reliability of YouTube videos on PR. The information obtained from this study will shed light on future studies. In addition, it will shed light on the situations that physicians should pay attention to when prescribing exercises related to PR to patients at a time when patient education with telemedicine and videos

Table 3. Features, quality, and reliability scores of videos

Source of upload	
Patient or caregiver	10 (13.16%)
Healthcare professionals	35 (46.05%)
Independent users	31 (40.79%)
Video features	
Duration (seconds)	476 (44-2297)
Time since upload (days)	1770 (205-4921)
Number of views	51766 (4115-2315013)
Number of likes	499 (5-31000)
Number of subscribers	5120 (7-3730000)
Number of comments	12 (0-1342)
mDISCERN score	2 (0-4)
Global quality scale	
Low	50 (65.79%)
Medium	13 (17.11%)
High	13 (17.11%)

Data are expressed as n (%) and median(minimum-maximum). MDISCERN = Modified DISCERN tool

Table 4. Comparison of video features and source of upload according to reliability classification

	mDISCERN score < 3	mDISCERN score ≥ 3	*p* value
Video features			
Duration (seconds)	407 (44-1912)	769 (282-2297)	**0.003**[a]
Time since upload (days)	2116 (209-4921)	764 (205-4081)	**0.033**[a]
Number of subscribers	1380 (7-1490000)	10700 (99-3730000)	**0.029**[a]
Average number of views per day	30.70 (1.44-3727.88)	82.90 (1.36-2536.48)	0.119[a]
Average number of likes per day	0.21(0-56.53)	1.02 (0.01-73.17)	**0.028**[a]
Average number of comments per day	0.01 (0-2.16)	0.02 (0-4.99)	0.157[a]
Source of upload			
Patient or caregiver	10 (17.86%)	0	0.055[b]
Healthcare professionals	18 (32.14%)	17 (85%)	**< 0.001**[c]
Independent users	28 (50%)	3 (15%)	**0.006**[c]

Data are expressed as n (%) and median(minimum-maximum). MDISCERN = Modified DISCERN tool
[a]Mann Whitney U Test, [b]Fisher's Exact Chi-Square Test, [c]Pearson Chi-Square Test

are so important.

Most of the videos evaluated in our study were of low quality (65.79%). The median mDISCERN score was 2. In addition, in our current study, it was determined that the majority of the videos were uploaded by healthcare professionals (46.05%) and independent users (40.79%). A study that evaluated YouTube as an information source for narcolepsy, 80 videos were analyzed that met the inclusion criteria. As a result of the study, it was determined that most of the videos were uploaded from sources other than health professionals and the quality of the videos about narcolepsy was low [20]. Ferhatoglu *et al*. [21] stated that online information on cardiopulmonary resuscitation is of low quality and the source and reliability of its content are unknown. The current study showed that, similar to the literature, most of the online videos were of low quality and unreliable. We think this is due to the fact that videos can be shared on YouTube and many other social networking platforms without a control and monitoring mechanism.

D'Souza *et al*. [22] in a study in which they examined the reliability of YouTube videos about epidural analgesia for labor pain, they found mean mDISCERN score of 1.9 (1.3). It has also been found that videos from medical sources have higher mDISCERN scores than videos from other sources. In a study evaluating YouTube videos about epidural injection, it was determined that 22% of the videos were of high reliability and that these videos were upload by medical sources [11]. Study examining YouTube videos about restless legs syndrome, 80 videos were analyzed and 44 (55.0%) videos were found to be reliable. Also misleading videos were found to have a longer mean length than reliable videos ($p = 0.005$). There was no statistically significant difference between other video features and video reliability [23]. In our study, similar to the literature, determined that most of the videos with high reliability were uploaded by healthcare professionals. While most of videos with high reability are uploaded by healthcare professionals, it has been found that many videos with low reability are uploaded by healthcare professionals. This shows us that not all videos uploaded by healthcare professionals are of high reliability. In addition, a statistically significant relationship was found between video reliability and video duration, time since upload, number of subscribers and average number of likes per day. We think that high reliability videos uploaded recently may be related to the increasing interest of health professionals in social media and sharing sites and the uploading of more videos to these platforms by these people. In addition, we think that the high reliability of videos with more subscribers and more likes may be related

Table 5. Comparison of video features, reliability scores and source of upload according to quality classification

	Low quality	Medium quality	High quality	*p* value
Video features				
Duration (seconds)	407 (44-1912)	628 (197-2297)	617 (282-1671)	**0.043**[d]
Time since upload (days)	2116 (209-4921)	1277 (215-3365)	616 (205-4081)	0.133[d]
Number of subscribers	1040 (7-280000)	26400 (20-3730000)	8030 (99-67900)	**0.006**[d]
Average number of views per day	30.70 (1.44-3727.88)	109.50 (2.34-2263.51)	25.58 (1.36-2536.48)	**0.027**[d]
Average number of likes per day	0.18 (0-56.53)	1.28 (0.01-40.43)	0.59 (0.01-73.17)	0.178[d]
Average number of comments per day	0.01 (0-2.16)	0.08 (0-2.13)	0.02 (0-4.99)	0.121[d]
mDISCERN score				
< 3	50 (89.29%)	5 (8.93%)	1 (1.79%)	**< 0.001**[e]
≥ 3	0	8 (40%)	12 (60%)	
Source of upload				
Patient or caregiver	10 (100%)	0	0	**0.041**[e]
Healthcare professionals	15 (42.86%)	10 (28.57%)	10 (28.57%)	**0.001**[b]
Independent users	25 (80.65%)	3 (9.68%)	3 (9.68%)	**< 0.001**[b]

Data are expressed as n (%) and median (minimum-maximum). MDISCERN = Modified DISCERN tool
[b]Pearson Chi-Square Test, [d]Kruskal-Wallis Test, [e]Fisher Freeman-Halton Test

to the increasing awareness of social media users recently.

In a study evaluating the quality of YouTube videos on cancer rehabilitation, a statistically significant relationship was found between quality groups and video upload source, mDISCERN score, and video duration [13]. According to Koçyiğit *et al*. [24] in a study evaluating YouTube videos on COVID-19 and rheumatic disease, they found a statistically significant relationship between quality groups and reliability and daily views. In the same study, no significant relationship was found between quality groups and daily comment and like ratio [24]. In the current study, a statistically significant relationship was found between the quality groups and the video duration, number of subscribers, average views per day, and mDISCERN score. In addition, it was determined that quality groups and video upload sources were related. In our study, it was determined that high quality videos were more common in the high reliability group according to the mDISCERN score, which supports the literature. In addition, the low number of subscribers and the average number of views per day in the low quality group in our study may be an indication that social media platforms have been used more effectively and selectively by users recently. We think that relationship between video duration and quality groups in the current study may be related to the inclusion of a small number of videos in the statistical analysis. Finally, the majority of videos

uploaded by all upload sources were found to be of low quality, even though most high quality videos were uploaded by healthcare professionals. Our results differing from the literature may be due to the fact that we did not review all videos for each keyword and only selected the 50 most viewed videos.

Limitations

This study has several limitations. One of the limitations of this study is the interpretation of English videos only. Other limitation is that videos are evaluated on a certain day. Since YouTube is a constantly watched video sharing platform, its statistical information is constantly changing, so only the current situation at the date and time the video was watched can be determined.

CONCLUSION

In conclusion, exercise therapies are very important treatments for patients with pulmonary system problems. Visual materials and videos guide the patient and caregiver to learn proper exercise methods. In our study, most of the PR-related videos on YouTube were found to be of low quality and unreliable. It may not be the right approach to recommend YouTube to patients for PR due to the abundance of contradictory and misleading videos. Sharing more videos on social platforms by healthcare professionals in the future may be effective in increasing video quality and reliability.

Authors' Contribution

Study Conception: ACE, UE; Study Design: ACE, UE; Supervision: ACE, UE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ACE, UE; Statistical Analysis and/or Data Interpretation: ACE, UE; Literature Review: ACE, UE; Manuscript Preparation: ACE, UE and Critical Review: ACE, UE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):1000-1004 Original Article

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Evaluation of radiologic predisposing factors for greater trochanteric pain syndrome

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ABSTRACT

Objectives: Greater trochanteric pain syndrome (GTPS) is one of the causes of lateral hip pain. We aimed to determine the significance of certain anatomical parameters related to GTPS in imaging tests.

Methods: Data of patients who were treated with glucocorticoid injection for trochanteric bursitis in our clinic between July 2019 and July 2022 for GTPS were analyzed. The control group was constituted of patients without GTPS but with hip and spinal problems who had pelvic computerized tomography (CT) images and undergone robotic-assisted knee arthroplasty. Standard anteroposterior pelvic radiograms, pelvic CTs, or magnetic resonance images were evaluated for anatomical parameters.

Results: Among anatomical parameters, acetabular anteversion, length of trochanter major, and abductor index were significantly different between the patients with and without GTPS. Although mean age was different between the GTPS and control patient groups, age was not found to be correlated with any anatomical parameter. Abductor lever arm length ($p = 0.001$) and abductor index ($p = 0.009$) were found to be correlated.

Conclusions: The length of trochanter major and abductor index were shown to be predisposing anatomical parameters for GTPS.

Keywords: Greater trochanteric pain syndrome, hip pain, acetabular anteversion, abductor index

Greater trochanteric pain syndrome (GTPS) is one of the common causes of intractable lateral-sided hip pain. Greater trochanteric pain syndrome usually arises from gluteal tendons, pertrochanteric bursae and surrounding tissue degeneration [1]. The underlying etiology is inflammation of the trochanteric bursa with repetitive microtrauma. Chronic gluteal tendinopathy is believed to be a result of hypercellularity, irregularity of collagen fibers, increased proteoglycan synthesis, and neovascularization. Studies have shown thatthe production of type I collagen fibers is decreased while type III collagen fibers are increased. This collagen fiber combination results in weakened cross-links between fibers and deterioration of mechanical strength [2].

The prevalence of GTPS in the general population is between 10-20% and its incidence is about 1.8 per 1000 people [3]. Greater trochanteric pain syndrome may be observed in all age groups but most commonly people in their 4th-6th decades are affected while female predominance over males is about 2-3/1 [4].

Research on predisposing factors for GTPS aims

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to define factors correlating with clinical outcomes and specific clinical conditions posing risks for the development of GTPS. We aimed to determine the significance of certain anatomical parameters related to GTPS in imaging tests.

METHODS

Ethics board approval was obtained for this retrospective study. Patients who were between 30-80 years of age and treated with glucocorticoid injection for trochanteric bursitis in our clinic between July 2019 and July 2022 for GTPS were included in the GTPS patient group. Patients with hip osteoarthritis, rheumatoid conditions, history of previous hip surgery, and accompanying low back pain or previous spinal surgery were excluded. The control group was constituted of patients without GTPS but with hip and spinal problems who had pelvic computerized tomography (CT) images and had undergone robotic-assisted knee arthroplasty.

Greater trochanteric pain syndrome diagnosis was established with regard toclinical examination criteria defined by Ege Rassmussen and Fano [5]. Plain radiograms and magnetic resonance imaging (MRI) were used to evaluate gluteal tendinopathy and to exclude other pathologic conditions. Standard anteroposterior (AP) pelvic radiograms were obtained aspatients were lying supine with both lower extremities in 20 degrees internal rotation and toes touching under 10% magnification.

Most lateral points of iliac crests and trochanter majors were marked on both sides and two transverse lines were drawn in AP pelvic radiograms of the GTPS patients. The ratio of length of distance between trochanter majors and iliac crests was calculated as a pelvic-trochanteric index. Anteroposterior pelvic radiograms were also used to calculate femoral offset, femoral neck-shaft angle, trochanter major length, and abductor lever arm length for patients' affected side (Fig. 1). The same measurements were obtained for the control group patients on thei mages of the operated knee and ipsilateral hip to prevent bias. Axial se-

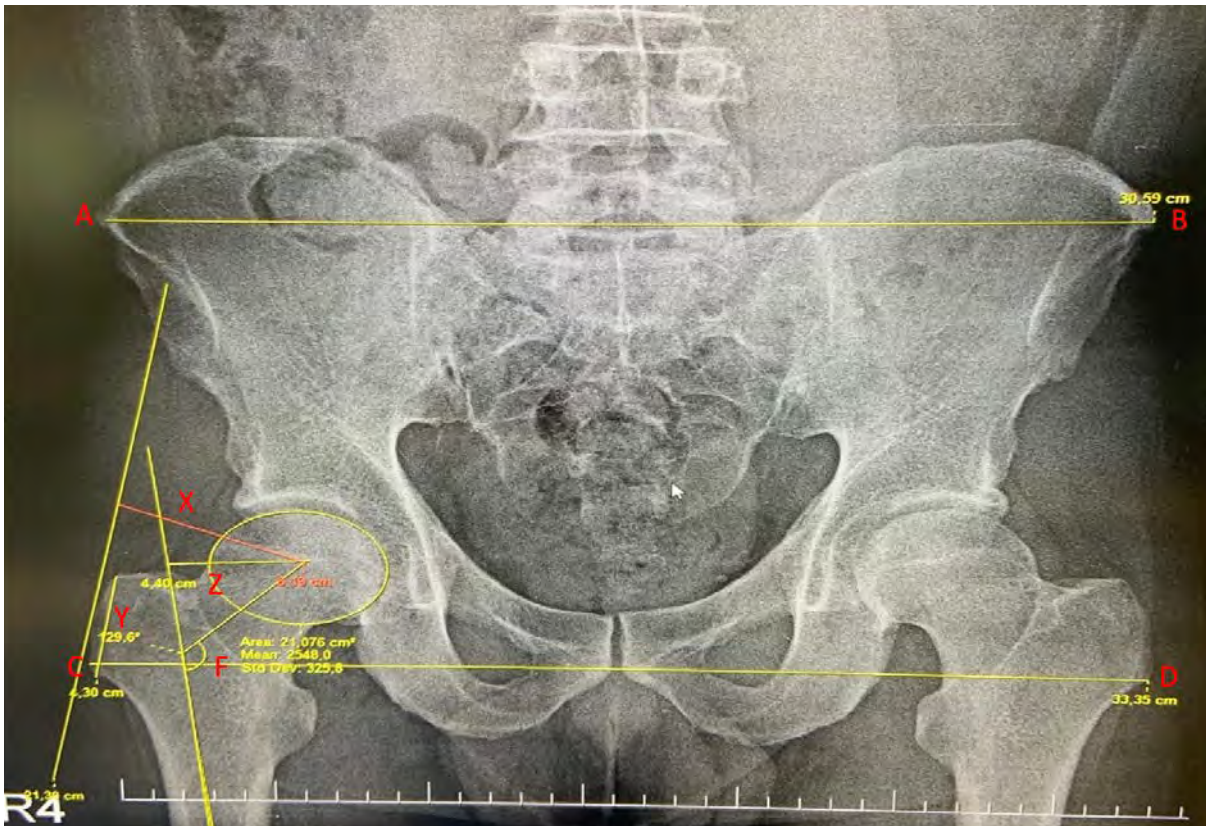

Fig. 1. AB: Distance between most lateral points of iliac crests (cm). CD: Distance between most lateral points of trochanter majors (cm). X: Length of abductor lever arm (cm). Y: Length of trochanter major (cm). Z: Femoral offset.

Table 1. Demographic characteristics of the GTPS and the control patients

	GTPS	Control	*p* value
Age (years), mean ± SD	57.8 ± 13.2	70.1 ± 7.7	**< 0.01**
Sex, n (%)			
Male	6 (20)	2 (6.9)	0.276
Female	24 (80)	27 (93.1)	

GTPS = Greater trochanteric pain syndrome, SD = standard deviation,

ries of MRI of the GTPS patient group was used to determine acetabular anteversion (AA). Pelvic CT images were used to determine AA in the control patient group.

Statistical Analysis

The distribution of numerical parameters was analyzed with the Kolmogorov-Smirnov test. Numerical parameters were expressed as mean (standard deviation). Categorical parameters were expressed as percentages. Numerical parameters of the GTPS and the control patient groups were compared with independent sample t-test. The correlation between parameters was tested with the Pearson correlation coefficient. *P* values lower than 0.05 was accepted as statistically significant.

RESULTS

Anatomical measurements of 30 patients who were treated withcorticosteroid injection for the diagnosis of GTPS were compared with the measurements of 29 patients who had undergone robotic assisted knee arthroplasty. Mean age of the control group was significantly higher (Table 1). Among anatomical parameters, acetabular anteversion, length of trochanter major and abductor index were significantly different between the GTPS and control patient groups (Table 2).

Although the mean age of the control group patients was higher, age was not found to be correlated with any anatomical parameter. Abductor lever arm length ($p = 0.001$) and abductor index ($p = 0.009$) was found to be correlated.

Table 2. Comparison of anatomical parameters

	GTPS	Control	*p* value
Acetabular anteversion (°)	19.0 ± 6.8	22.7 ± 6.1	**0.029**
Distance between most lateral points of iliac crests (cm),	32.6 ± 1.9	31.7 ± 2.6	0.187
Distance between most lateral points of trochanter majors (cm)	34.1 ± 1.6	33.2 ± 2.1	0.63
Pelvic-trochanteric index	0.95 ± 0.04	0.95 ± 0.06	0.741
Femur collum-diaphysis angle (°)	128.2 ± 6.9	127.0 ± 5.4	0.445
Femoral offset (cm)	4.7 ± 0.7	5.0 ± 0.7	0.110
Length of trochanter majör (cm)	4.0 ± 0.4	3.7 ± 0.5	**0.001**
Length of abductor lever arm (cm)	6.1 ± 0.6	6.2 ± 0.7	0.173
Abductor index	0.7 ± 0.1	0.6 ± 0.1	**< 0.01**

Data are shown as mean±standard deviation. GTPS = Greater trochanteric pain syndrome

Pelvic trochanteric index = ratio of length between most lateral points of iliac crests (cm) to length between most lateral points of trochanter majors(cm). Abductor index = ratio of length of trochanter major (cm) to length of abductor lever arm(cm).

DISCUSSION

Greater trochanteric pain syndrome is a complex phenomenon with symptoms that may also be present in many other conditions [6]. The term GTPS has replaced trochanteric bursitis due to various etiologic factors causing posterolateral hip pain. Relation of the iliotibial tract and mid-lumbar dermatomes (L2-L4) to surrounding neural structures as superior and inferior gluteal nerves causes radiation of pain in the posterolateral region [7]. These nerves innervate the femoral neck, three gluteal muscles, and tensor fascia which may be relevant to pain in femoro-acetabular impingement syndrome [8].

Many etiologic factors had been described in the literature for GTPS. These are: repetitive activities, mechanical overload affecting cellular response, erroneous and high-intensity exercise, sedentary lifestyle, fatty infiltration, scoliosis, and limb length discrepancy [1, 2].

Pelvic morphotype was assumed to be a risk factor for lateral hip pain. It was shown that trochanter major is more prominent in people with femoral neck varus, resulting in more pressure on gluteus medius and minimus tendons by iliotibial band causing chronic inflammation [9]. In our study, a comparison of the femoral collum- diaphyseal angle measurements between the GTPS and the control patientshas not yielded significant difference, which does not support the hypothesis.

Govaert *et al*. [10] reported pain-free survival in 7 patients out of 12 patients with intractable GTPS who hadundergonetrochanteric reduction osteotomy with an aimto decreasethe femoral offset. Our data showedno significant difference with regard to femoral offset between the GTPS and the controlpatients. This may be interpreted as femoral reduction osteotomy to decrease femoral offset may have a limited role in the treatment of GTPS.

Another study by Pelsser *et al*. [11] demonstrated that increased acetabular anteversion was related to gluteal tendinopathy and trochanteric bursitis by interfering with the biomechanics of gluteal tendons. In contrast, increased anteversion of acetabulum was observed more frequently in the control group in our study.

Santos *et al*. [12] showed that pelvic trochanteric index was increased in females with GTPS. However, we didn't demonstrate any significant differencewith regards to the pelvic trochanteric index when the GTPS and control group patients or males and females were compared.

Length of trochanter major and abductor index were significantly higher in GTPS patients when compared to the control patients. We hypothesized that; increased length of trochanter major is a predisposing factor for GTPS, independent of the abductor lever arm, as it increases the surface area thus advancing cellular strain and decreasing healing response to microtraumas.

Canetti *et al*. [13] demonstrated that decreased sacral slope was associated with decreased pelvic tilt, and this interferes with gluteal tendon biomechanics. Hence, patients having GTPS also suffer from low back pain [13]. Likewise, in a study on 247 patients with low back pain, Tortelani *et al*. [7] indicated 20% of patients had GTPS simultaneously. In our study, we excluded patients with back pain and history of spinal surgery thus avoidedspinopelvic predisposing factors to have a bias on theinterpretation of theresults.

Limitations

Our study has somelimitations. First of all, it has a retrospective design. Second, as only anatomicalpredisposing factors were inspected, clinical parameters known to be relevant as body mass index or smoking were not evaluated.

CONCLUSION

Length of trochanter major and abductor index have been shown to be predisposing anatomical parameters for GTPS. Similar studies on large patient series may guide the way for possible treatment options.

Authors' Contribution

Study Conception: EŞ; Study Design: EŞ; Supervision: AT; Funding: EŞ, AT; Materials: EŞ, AT; Data Collection and/or Processing: AT; Statistical Analysis and/or Data Interpretation: EŞ; Literature Review: EŞ; Manuscript Preparation: EŞ, AT and Critical Review: AT.

Informed consent

Institutional review board approval was obtained for this cohort study (No: KAEK 2021/12-204). Con-

sent was obtained from the patients who were included in the study.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(5):1005-1014 Original Article

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Surgical, histopathological, and clinical outcomes of parotid gland neoplasms: a 10-year tertiary single-center experience

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ABSTRACT

Objectives: This study aims to contribute to the literature by presenting an overview of a 10-year experience by retrospectively examining the cases with a parotid mass from a tertiary referral center

Methods: Two hundred fourteen patients were diagnosed with a parotid mass in the Otorhinolaryngology Clinic of HSU Izmir Bozyaka Training and Research Hospital between January 2009 and January 2019. Sociodemographic characteristics, diagnostic methods, surgical operations and complications, pathology results, and long-term follow-up results were retrospectively analyzed over the patients' files. SPSS Version 21.0 computer for data analysis.

Results: Of 214 parotidectomies, 140 (75%) were male, 74 (35%) were female, mean age was 55 ± 14 years (15-85 years). The most common diagnoses were 87 (40.7%) pleomorphic adenomas and 48 (22.4%) Wharton tumors. The most prevalent malignant tumors were reported as 18 (8.1%) mucoepidermoid carcinoma. The main type of surgery was superficial parotidectomy, performed in 192 (90%) patients. Facial paralysis was observed in 11 (5.1%) patients as the main postoperative complication.

Conclusions: Considering the histopathological diagnosis of parotid tumors, the stage and grade of the tumor, surgery is generally preferred for treatment. More males are affected than women, especially in the middle ages.

Keywords: Parotid neoplasm, salivary gland, Warthin tumor, pleomorphic adenoma

Salivary gland tumors constitute 3% of all neoplasms in the body and 5-10% of head and neck tumors [1]. The most common period is between the ages of 20-60 and occurs equally in both sexes. Salivary gland tumors originate at rate of 80% from the parotid gland [2]. Although minor salivary gland tumors are generally malignant (65%), 80% of parotid tumors are benign, and 60% of benign tumors are

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pleomorphic adenomas [3]. The appropriate treatment method varies according to the localization and histopathology of the parotid tumor [4]. Usually, partial and total parotidectomy surgery is performed, and sometimes neck dissection, neoadjuvant, and additional treatment methods are combined [5]. Parotid surgery is important as the parotid gland produces saliva, protects the ear canal, and facilitates facial expression [6]. The parotid gland is also a common site for benign and malignant tumors, which can cause discomfort and affect the patient's quality of life. Early diagnosis and treatment are crucial for better outcomes in parotid tumors. The histopathology type of the tumor plays an important role in determining the appropriate treatment and predicting the prognosis [7]. Pleomorphic adenomas, the most common benign tumor, have a low recurrence rate but can have malignant transformation. Other benign tumors, such as Warthin's tumor and oncocytoma, have a low risk of recurrence and metastasis. On the other hand, malignant tumors such as mucoepidermoid carcinoma and adenoid cystic carcinoma have a higher chance of recurrence and metastasis and require aggressive treatment [8, 9].

This study aims to contribute to the literature by presenting an overview of a 10-year experience by retrospectively examining the cases with a parotid mass and evaluating the surgical procedures, histopathological results, complications, and long-term follow-up results.

METHODS

Patients

The study included two hundred fourteen patients diagnosed with a parotid mass in the Otorhinolaryngology Clinic at Izmir Bozyaka Traning and Research Hospital between January 2009 and January 2019. The study was retrospectively conducted with the approval of the Local Ethics Committee with No. 06 (KA-21.11.2018).

Sociodemographic characteristics, comorbid diseases, diagnostic methods, surgical operation type and complications, pathology results, added treatment and surgical procedures, and close and long-term follow-up results were analyzed over the patients' files in a 10-year retrospectively.

Patient Evaluation

All patients with a parotid mass underwent routine otolaryngology and detailed head-neck examinations. Patient's medical history was reviewed, including recent upper respiratory tract infections, chronic diseases such as diabetes mellitus, hypertension, coronary artery disease, smoking, and alcohol use. Patients were also asked about accompanying symptoms such as sweating, difficulty swallowing, and weight or voice changes.

Imaging and Biopsy

After the necessary routine examination and anamnesis information, detailed imaging of the head and neck region was performed on the patients. This included head and neck ultrasonography (USG) covering the thyroid region, and computed tomography (CT) or contrast magnetic resonance imaging (MRI) examinations were performed beforehand. After imaging examinations, a fine needle aspiration (FNA) biopsy was performed on the patients. The histopathological and cytological diagnoses of all parotid gland neoplasms were based on the World Health Organisation (WHO) pathological classification system.

Patient Classification

Patients who underwent surgery were evaluated based on pre-operative, intra-operative, and post-operative data. The effect of parotid masses on a patient's life was determined using prognostic criteria such as survival, remission, and revision. Tumors were divided into groups as primary and secondary, malignant and benign parotid masses, and the groups were compared according to prognostic factors.

Surgical Procedures

The main surgical procedure was the superficial parotidectomy. The patient was placed in a supine position with the head slightly elevated. A curvilinear incision was made in the preauricular area, extending to the neck crease. The skin flap was elevated, and the facial nerve branches were identified and preserved. The parotid gland was then dissected from the surrounding tissue, and the tumor was removed. Hemostasis was achieved, and a drain was placed. The skin was closed with sutures, and a dressing was applied. The patient was transferred to the recovery room in stable condition. The drain was removed on postoper-

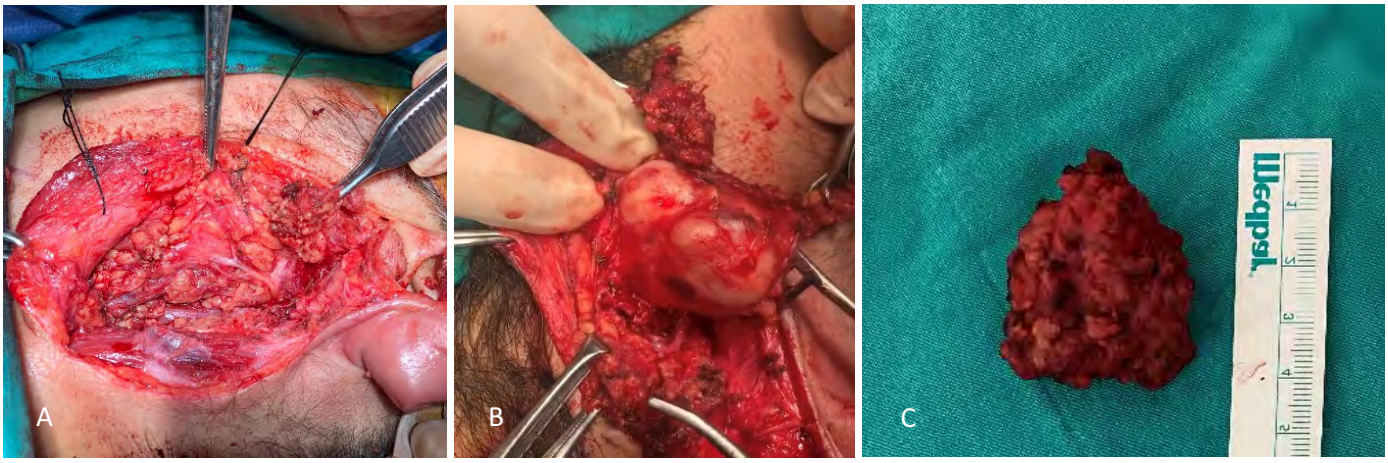

Fig 1. Parotidectomy surgery images (Different cases). (A) Superficial parotidectomy surgery: Facial nerve preserved after completely removing the tumor (female, right side). (B) Superficial parotidectomy surgery: Fixed adeno cystic carcinoma (male, left side). (C) Macroscopic view of the tumor removed from the parotid gland (3×4 cm).

ative day 2, and the patient was discharged on postoperative day 3 with instructions for wound care and follow-up. In need the were performed total parotidectomy and neck dissection (Fig. 1A-C).

Statistical Analysis

'Chi-square test, Fisher's chi-square test, and Fisher–Freeman–Halton test' were used to compare the two groups' recovery levels and evaluate the prognostic criteria. The 'Mann–Whitney U test' was used to calculate the difference between the two groups' numerical measurements and evaluate prognostic criteria based on numerical measurements. SPSS Version 21.0 computer program was used, and a p - value of < 0.05 was considered to be significant

RESULTS

Two hundred fourteen patients were operated on for parotid surgery, and 140 (75%) of the patients had been performed males, 74 (35%) were females, mean age was 55 ±14 years (range: 15-85 years). The most common reason for admission was a mass in the

Table 1. Histopathological examination of the surgical specimens: Benign pararid tumor classification

Histopathological classification	Frequency	Percentage in total	Percentage in benign parotid lesions	Operation type	Additional treatment	Complication
Pleomorphic adenoma	87	41%	51%	70 (85%)	17 (15%)	2
Warthin's tumour	48	22.4%	28%	40 (90%)	8 (10%)	2
Sialadenitis	10	4.7%	5.8%	7 (70%)	3 (25%)	0
Lipoma	9	4.2%	5.2%	9 (100%)	0	0
Inflammation (Granulomatous, non-specific)	8	3.7%	4.67%	8 (100%)	0	0
Basal cell adenoma	5	2.3%	2.9%	3 (60%)	2 (40%)	0
Salivary duct cyst	4	1.9%	2.3%	2 (100%)	0	0
TOTAL	171	80%	100%	145 (85%)	26 (15%)	4

Table 2. Histopathological examination of the surgical specimens: Malign paratid tumor classification

	Frequency	Percent age in total	Percentage in malignant parotid lesions	Superficial parotidectomy	Total parotidectomy	Additional treatment	Complication
Mucoepidermoid carcinoma	18	8.4%	41.9%	2 (11.1%)	16 (88.9%)	N/A	7
Adenoid cystic carcinoma	12	5.6%	27.9%	2 (16,7)	10 (83.7%)	N/A	3
Pleomorphic adenoma ex carcinoma	4	1.9%	9.3%	1 (25%)	3 (75%)	N/A	1
Basal cell carcinoma infiltration	3	1.4%	3.5%	1 (33%)	2 (66%)	0	1
Myoepithelial carcinoma	2	0.93%	4.6%	0	2 (100%)	0	1
Lymphoepithelial Carcinoma	1	0.46%	2.3%	1 (100%)	0	0	0
Malignant melanoma metastasis	1	0.46%	2.3%	0	1 (100%)	0	1
Mammarian analogue	1	0.46%	2.3%	1 (100%)	0	N/A	5
Squamous cell carcinoma metastasis	1	0.46%	2.3%	0	1 (100%)	0	1
TOTAL	43	20%	100%	8 (19%)	35 (81%)	4 (9.3%)	16

N/A = no available

parotid region (80%) with pain by palpation (15%) and some facial nerve dysfunctions (paralyze and paresthesia 5%).

Surgical Evaluation

Superficial parotidectomy was performed in 192 (90%) patients, and total parotidectomy in 22 (10%). One hundred and two (47.7%) patients were right-sided, 112 (52.3%) were left-sided, 4 (1.9%) patients underwent bilaterally in separate sessions and 5 (2.3%) patients underwent revision surgery. Neck dissection was performed in 4 patients due to malignant cytology. The mean follow-up period was 60 ± 19 months (range: 8-108 months).

Histopathological Outcomes

From all groups, 171 (80%) patients were diagnosed with benign, and 43 (20%) were diagnosed with malignant parotid tumors. In the histopathological examination of the surgical specimens of the operated patients, the most common diagnoses were 87 (41 %) pleomorphic adenomas, 48 (22.4%) Wharton tumors. Mucoepidermoid carcinoma 18 (8.4%) and adenoid cystic carcinoma 12 (5.6%) were the most common malignant parotid tumors. There was no gender difference between the histopathological types of malignant tumors. Total parotidectomy was the most common operation type of surgical treatment.

Histopathological classification of the benign tumors and their distribution according to the frequency and operation type of the patients were noted in Table 1. Histopathological classification of the malignant tumors and their distribution according to the frequency and operation type of the patients were reported in Table 2. The bening and malign tumors and their distributions according to age, gender, comorbidity, surgical complication, revision surgery, and additional surgery were noted in Table 3.

Clinical Outcomes

Of the 214 patients included in the study, 171 (80%) had benign tumors and 43 (20%) had malignant tumors. The study found no statistically significant difference between gender, comorbidity (hypertension, diabetes mellitus), cigarette smoking, and the histopathological type of the tumor (benign/malignant) ($p > 0.05$).

Surgical complications were observed in 20 pa-

Table 3. Sociodemographic characteristics of patients with parotid tumors

Data	Total	Benign	Malignant	*p* value
Patient	214	171 (80%)	43 (20%)	**0.003**
Female	87	68 (40%)	19 (45%)	0.785
Male	127	103 (60%)	24 (55%)	0.812
Smoking	53	20	33	0.164
Hypertension	89	41	48	0.06
Diabetes mellitus	71	37	34	0.078
Tumor location (Superficial)	153	145	8	**0.004**
Total	61	26	35	0.089
Age (years)	48 ± 13.4 (15-86)	47 ± 12.6 (15-72)	51 ± 14.8 (18-86)	0.105
Complication	20	4 (0.02%)	16 (37%)	**0.001**
Revision	5	1 (0.05%)	4 (9.3%)	**0.008**
Additional surgery	4	0	4 (9.3%)	**0.009**

Data are shown as mean ± standard deviation or n (%)

tients, with the highest incidence (9.3%, n = 4) observed after total parotidectomy. The most common surgical complication was transient facial nerve palsy in patients who underwent superficial parotidectomy, while complete permanent facial paralysis occurred in 11 (5.1%) patients. Four (1.9%) patients developed Frey syndrome, while hematoma and seroma were observed in 3 (1.4%) and 2 (0.9%) patients, respectively. The study concluded that early drain removal in the postoperative period was the reason for hematoma and seroma formation. Patients with hematoma and seroma in the postoperative site were drained under general anesthesia, followed up with a tight dressing, and experienced complete recovery.

DISCUSSION

Many studies in the literature cover more than ten years of parotid and salivary gland tumors according to various parameters [2, 10-12]. In this study, which compiles ten years of experience, all sociodemographic, pathohistological, surgical, and postoperative information of patients who underwent surgery with a mass in the parotid gland will be discussed respectively.

The presence of a mass in the preauricular or infraauricular area of the patient in the ENT outpatient clinic is a situation that we should approach carefully. Since parotid tumors are usually benign, they appear as a fixed, hard, painless, long-term mobile mass during the examination. In delayed, advanced and malignant masses, complaints are presented as high-stage facial paralysis, hard, fixed mass with pain. Facial nerve palsy and clinically positive cervical lymph nodes are poor prognostic signs [12, 13]. In the clinic, no statistically significant relationship was found between the presentation of the tumor and its histopathology [2, 14].

Many studies have been published on the relationship between age and gender in patients with parotid gland tumors. According to the literature and our patient group, parotid tumor is more common in the 6th decade, although it can occur at any age. The relationship between parotid tumors and gender is still not definitively determined, and the superiority of one over the other has not been established [14-17].

Superficial parotidectomy is a commonly performed surgical procedure in the management of parotid gland diseases, which involves the removal of the glandular tissue located superficially to the facial nerve. On the other hand, total parotidectomy is a more extensive procedure that involves the complete removal of the parotid gland [18-21]. In our study, a

total of 216 patients underwent parotidectomy, with 90% of the patients undergoing superficial parotidectomy and 10% undergoing total parotidectomy. The majority of cases were unilateral, with 47.7% being right-sided and 52.3% being left-sided. A small percentage of patients underwent bilateral parotidectomy in separate sessions, and 5 patients underwent revision surgery. Neck dissection is often performed in patients with malignant cytology, as it helps to remove any metastatic lymph nodes that may be present in the neck [16, 22-24]. In our study, only 4 patients underwent neck dissection due to malignant cytology. The mean follow-up period in our study was 60 ± 19 months, with a minimum of 8 months and a maximum of 108 months. Long-term follow-up is important in patients who have undergone parotidectomy, as it helps to monitor for any recurrence of the disease and to evaluate the overall outcome of the surgical intervention [11, 19, 24].

It is important to note that surgical management of parotid tumors is complex, and the choice of surgical procedure depends on the location and histopathology of the tumor. Complications after parotid surgery can occur and can vary depending on the type and extent of the surgery performed. The incidence of complications can be reduced with meticulous surgical technique, appropriate postoperative care, and patient follow-up. Short- and long-term follow-up is also essential to monitor for tumor recurrence and potential complications [25, 26].

Consistent with the literature, parotid tumor is more common in the 6th decade. Many studies show the relationship between parotid tumors and gender, and the superiority of one over the other has not been determined with a definitive decision [27]. Our study had slight male dominancy for benign and malign parotid lesions groups, which was in concordance with some studies [28]. As can be seen from the statistics, the most common indication for parotidectomy is benign tumors; the most common benign is pleomorphic adenoma and Wharton tumor. The most common malignant tumor is reported as mucoepidermoid carcinoma [29]. When a parotid tumor was suspected on inspection and palpation, this was further examined using ultrasound echo and guided FNAC. In our department, FNAC is performed preoperatively in all patients.

Our patient group had a mean age of 48 ± 13.4 (range 15-86) years for all parotid tumors, 47 ± 12.6 (range 15-72) years for benign lesions, and 51 ± 14.8 (range 18-86) years for malignant tumors. There was no statistically significant difference in age between the groups. Superficial parotidectomy was performed in 192 (90%) patients, and total parotidectomy in 22 (10%). There is no statistically significant difference related with the type of the surgery, recovery and follow up and prognostic factors between the groups. One hundred and two (47.7%) patients were right-sided, 112 (52.3%) were left-sided, 4 (1.9%) patients underwent bilaterally in separate sessions, and 5 (2.3%) patients underwent revision surgery. Neck dissection was performed in 4 patients due to malignant cytology. The revision surgery, malign cytology, neck dissection and chemoradiotherapy, are the pure prognostic factors. The mean follow-up period was 60 ± 19 months (min 8 months; max 108 months). From all groups, 171 (80%) patients were diagnosed with benign, and 43 (20%) were diagnosed with malignant parotid tumors.

In the histopathological examination of the surgical specimens of the operated patients, the most common diagnoses were 87 (41 %) Pleomorphic adenomas, 48 (22.4%) Wharton tumors. Mucoepidermoid carcinoma 18 (8.4%) and adenoid cystic carcinoma 12 (5.6%) were the most common malignant parotid tumors. There was no gender difference between the histopathological types of malignant tumors. Total parotidectomy was the most common operation type of surgical treatment. Histopathological classification of the benign tumors and their distribution according to the frequency and operation type of the patients were noted in Table 1. Histopathological classification of the malignant tumors and their distribution according to the frequency and operation type of the patients were reported in Table 2. The Bening and Malign Tumors and their distributions according to age, gender, comorbidity, surgical complication, revision surgery, and additional surgery were noted in Table 3. The histopathological examination of surgical specimens revealed that 80% (171/214) were benign tumors, while 20% (43/214) were malignant. This is consistent with previous studies that have shown that benign tumors are more prevalent than malignant tumors.

There was a statistically significant difference in the distribution of benign and malignant tumors between the superficial and deep tissues ($p < 0.05$).

Specifically, the majority of malignant tumors (35/43) were found in deep tissue, while the majority of benign tumors (145/171) were found in superficial tissue. This finding suggests that the depth of the tumor may be an important factor in determining its malignant potential.

The presence of complications following surgery was found to be statistically significant ($p < 0.05$), with 16 out of 43 (37%) malignant tumor cases experiencing complications, compared to only 4 out of 171 (0.02%) benign tumor cases. This finding highlights the importance of considering the potential risks and benefits of surgical intervention, especially in cases of malignant tumors.

Age and gender were not found to be statistically significant factors in the distribution of benign and malignant tumors. However, it is worth noting that the mean age of patients with malignant tumors was slightly higher (51 ± 14.8 years) than the mean age of patients with benign tumors (47 ± 12.6 years).
There were no statistically significant differences in the distribution of benign and malignant tumors between patients with a history of smoking, hypertension, or diabetes. However, it should be noted that the sample size for each of these subgroups was relatively small (n = 53, n = 89, and n = 71, respectively), which may have limited the statistical power to detect difference.

Mucoepidermoid carcinoma is a type of malignant tumor that can arise in various parts of the body, including the salivary glands, lungs, and other organs. The reported prevalence of 8.1% suggests that it is a relatively common type of tumor, but the exact incidence and prevalence may vary depending on the population and location being studied [29-33] Treatment for mucoepidermoid carcinoma typically involves surgery to remove the tumor, along with radiation therapy and chemotherapy in some cases. It is important to work closely with a healthcare provider to determine the best course of treatment for each individual case. The finding that mucoepidermoid carcinoma was the most prevalent malignant tumor in our study is consistent with previous research on salivary gland tumors. Mucoepidermoid carcinoma is a type of tumor that arises from the glandular cells in the salivary gland and can be low, intermediate, or high grade in severity. It is the most common malignant tumor of the salivary gland and typically affects adults in their 30s and 40s [30, 31]. Although mucoepidermoid carcinoma is a malignant tumor, the prognosis can vary widely depending on the tumor grade and other factors such as the location and extent of the tumor [33]. Low-grade tumors tend to have a better prognosis than intermediate- or high-grade tumors. Treatment may involve surgery to remove the tumor, radiation therapy, and/or chemotherapy [32].

The reported surgical complications in our study are consistent with previous literature on parotidectomy [34]. Transient facial nerve palsy is a common complication of superficial parotidectomy and occurs due to manipulation of the facial nerve during surgery However, the incidence of complete permanent facial paralysis (5.1%) reported in this study is higher than the reported incidence of 1-3% in previous studies. This may be due to the variability in the surgical technique, skill and experience of the surgeons, or the sample size of the study. [26, 35, 36]. Frey syndrome, which is characterized by gustatory sweating, flushing and swelling in the parotid region, is a common complication after parotidectomy, particularly after superficial parotidectomy. The reported incidence of Frey syndrome (1.9%) is consistent with previous studies [37, 38]. Hematoma and seroma formation are common complications of parotidectomy, particularly after total parotidectomy. Hematoma formation occurs due to bleeding from the surgical site and can result in increased pressure on the facial nerve and compromised blood supply to the surrounding tissues. Seroma formation occurs due to the accumulation of lymphatic fluid in the surgical site, which can lead to swelling and discomfort. Early removal of the drain may increase the risk of hematoma and seroma formation, as reported in this study [39, 40].

Our study highlights the importance of postoperative care and follow-up. Surgical complications were observed in 20 patients, with transient facial nerve palsy being the most common after superficial parotidectomy, and complete permanent facial paralysis being the most common after total parotidectomy. Other complications included Frey syndrome, hematoma, and seroma. It is important to manage these complications properly to ensure a successful recovery. Drain removal should be delayed until appropriate time points, and patients should be closely monitored for any signs of complications in the postoperative period. The table 3 shows that eight patients (19%) experienced complications following surgery,

including four cases of facial nerve palsy, two cases of hematoma, one case of Frey's syndrome, and one case of seroma. The majority of patients (81%) underwent total parotidectomy, while 9.3% required additional surgery. The statistical analysis revealed that the frequency of each type of malignant tumor was statistically significant ($p < 0.05$), while the incidence of complications and additional treatments was not statistically significant ($p > 0.05$). These findings highlight the importance of accurate histopathological examination of surgical specimens to determine the type of malignant tumor and guide appropriate treatment decisions. Logistic regression analysis revealed that none of the sociodemographic characteristics (gender, smoking, hypertension, or diabetes mellitus) were significantly associated with the likelihood of having a malignant parotid tumor ($p > 0.05$).

Limitations

From our projection we had some limitations in our study that can highlight to other researcher in a future. Our limitations are bellow: (1) Retrospective design: The study design is retrospective, which means that the researchers relied on the available medical records and data that may be incomplete or inaccurate. Moreover, the study's outcome is limited by the quality and completeness of the medical records. (2) Single-center study: The study was conducted in a single center, which may limit its generalizability to other populations and settings. The findings may not be representative of the entire population with parotid tumors. (3) Missing data: Some data were missing, such as information on the patients' lifestyle and habits, including smoking and alcohol use, which may be important factors in the development and progression of parotid tumors. (4) Selection bias: The study included only patients who underwent surgery, which may introduce selection bias and limit the generalizability of the findings to patients who did not undergo surgery or received other treatments.

Also from our projection we have a positive aspects and benefits of this manuscript. And they are below: (1) Contribution to the literature: This study adds to the existing body of knowledge on parotid gland tumors and surgical procedures by presenting a 10-year retrospective analysis of cases with a parotid mass. (2) Comprehensive evaluation: The study evaluated various aspects of patient care, including sociodemographic characteristics, comorbidities, diagnostic methods, surgical procedures, pathology results, added treatments, and long-term follow-up outcomes. (3) Large sample size: The study included a relatively large sample size of 214 patients, which increases the generalizability of the findings. (4) Appropriate methodology: The study used appropriate statistical methods to analyze the data and draw conclusions, which enhances the validity of the results. (5) Clinical implications: The findings of this study have important clinical implications for the diagnosis, treatment, and follow-up of patients with parotid gland tumors. The results can help clinicians make more informed decisions and improve patient outcomes. (6) Patient-centered approach: The study emphasized the importance of evaluating the impact of parotid tumors on patients' quality of life and included patient-reported outcomes in the analysis.

Overall, this manuscript provides valuable insights into the management of parotid gland tumors and can help inform clinical practice and future research in this area

CONCLUSION

Surgical intervention remains a primary treatment option for parotid tumors. The choice of surgical approach should be based on the histopathological diagnosis, stage, and grade of the tumor. Proper preoperative evaluation and planning, as well as postoperative care and follow-up, are critical for achieving optimal treatment outcomes and minimizing the risk of complications

Authors' Contribution

Study Conception: AA, AD; Study Design: AA, AD; Supervision: AD, TM; Funding: N/A; Materials: AA, OYA, GYA; Data Collection and/or Processing: AA, OYA, GYA; Statistical Analysis and/or Data Interpretation: AA, OYA, GYA; Literature Review: AY; Manuscript Preparation: AA and Critical Review: AD, TM, OYA, GYA.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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Comparison of human epidermal growth factor receptor 2 and cancer stem cell markers like CD44 and CD133 expressions with clinicopathological parameters in gastric cancer

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ABSTRACT

Objectives: Gastric carcinoma (GC) is the fourth most common cause of cancer-related tumor deaths worldwide. The prognostic significance of CD44, CD133 and human epidermal growth factor receptor 2 (HER2) expression in GC remains controversial. Therefore, we aimed to investigate the relationship of CD44, CD133 and HER2 expression with clinicopathological features in metastatic and non-metastatic GC patients.

Methods: A total of 139 patients with GC (68 with metastasis, 71 without metastasis) diagnosed were retrospectively analyzed. CD44 and CD133 expression were determined by immunohistochemical method in all cases. In addition, HER2 overexpression of the tumor was evaluated in patients with metastatic GC.

Results: The CD133 positivity rate was 90.6% (n = 126) when all cases were considered, and that for CD44 was 84.9% (n = 118). There was no difference in CD133 and CD44 positivity (intensity or density) rates and between the total scores of metastatic and non-metastatic patients with GC ($p > 0.05$). HER2 positivity in metastatic cases was detected in 49 (70.1%) patients by immunohistochemical method. No correlation was found between CD133 total score and age, tumor size or depth, and HER2 scores in metastatic or non-metastatic cases ($p > 0.05$). In the correlation analyzes performed with CD44 scores, only a borderline significant correlation was found between CD44 scores and tumor size (r:0.175; $p = 0.047$) in non-metastatic cases.

Conclusions: We demonstrated associations between CD44/CD133 expression and histological grade in all patients, between CD44 and tumor size in non-metastatic patients, and between HER2 and intestinal type (Lauren) in metastatic patients. The results of this study need to be confirmed by multicenter studies including large case series.

Keywords: Stem cell, CD44, CD133, gastric cancer, HER2

Gastric cancer (GC) (also known as stomach cancer) is the fifth most common cancer in the world, after lung, breast, colorectum, and prostate cancer, and is the fourth most common cause of cancer-

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related death [1]. The incidence of the disease is highest in Eastern Asia (Japan and Mongolia), whereas it is lowest in Northern America, Northern Europe and Africa [1-4].

The incidence and mortality rate of gastric cancer in Turkey has been reported as 14.2 per 100,000 and 12.15 per 100,000, respectively, suggesting that Turkey is one of the countries with the highest incidence of gastric cancer in Europe [2]. In the current WHO classification (2019), gastric carcinomas are classified as adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, undifferentiated carcinoma, and neuroendocrine carcinoma. Gastric adenocarcinoma, the most common type is divided into 5 subtypes: tubular, papillary, poorly cohesive, mucinous and mixed. Signet ring cell carcinoma terminology has been replaced by poorly cohesive carcinoma and is a subtype of adenocarcinoma. However, Lauren's classification of gastric cancers into two major types based on histological features, namely, intestinal (associated with chronic atrophic gastritis and intestinal metaplasia) and diffuse (originates from normal gastric mucosa) types, is more commonly used [5, 6]. The 5-year survival rate in gastric cancer cases is quite low despite aggressive treatments [5, 7].

In recent years, cancer stem cells (CSCs), a subpopulation of cancer cells, have begun to assume increasing importance in cancer studies. It has been shown that CSCs have specific functions such as self-renewal and differentiation as well as differentiation capacities and can acquire tumorigenicity when transferred to an animal host [8]. It has been reported that CSCs affect cancer initiation, progression, metastasis and recurrence, and consequently have a close relationship with the prognosis of the disease [9-17]. Two of the newest and most robust CSC surface markers investigated for GC are CD133 and CD44. CD44 is a principal cell surface glycoprotein for hyaluronic acid and a major component of extracellular matrices. CD44 has been shown to play an important role in adherence to the extracellular matrices, in motility, matrix degradation, proliferation and cell survival [18]. CD133 (also known as prominin-1), a five transmembrane cell-surface glycoprotein, plays a principal role in the maintenance of cell polarity and migration through the interactions of cells with each other [9, 19, 20]. It has been reported that CD133 is associated with a diagnosis of GC [20, 22].

Apart from these markers, human epidermal growth factor receptor 2 (HER2), also known as CerbB-2 or ERBB2 (erb-b2 receptor tyrosine kinase 2), is a proto-oncogene located on chromosome 17q21 that encodes a transmembrane protein with tyrosine kinase activity and is involved in signal transduction pathways, leading to cell growth and differentiation [23]. It has been shown that HER2 is a negative prognostic factor in GC, and HER2-positive tumors are associated with more aggressive biological behavior, higher recurrence frequencies, and decreased survival [23, 24]. There are very few studies evaluating the prognostic significance of CD44, CD133 and HER2, which together are important CSCs markers in GC patients. In addition, some studies have reported conflicting results regarding the effect of these markers on prognosis [11, 21, 24-29]. In this study, we aimed to investigate the relationship of CD44, CD133 and HER2 expression with clinicopathological features of the disease in patients diagnosed with GC in our center.

METHODS

All participants included in this single-center and cross-sectional study were informed about the scope of the study and their informed written consent was obtained. The study was evaluated and approved by the local ethics committee and adhered to the principles laid down by the Helsinki Declaration. One hundred thirty-nine patients with GC, (71 with metastasis and 68 without metastasis) diagnosed and followed up in our hospital between 2016 and 2019, were retrospectively analyzed. Demographic and clinical characteristics, tumor localization, histological type, tumor size, grade and invasion depth (T), lymph node status (N), and metastasis (M) data of all patients were obtained from file records. Histopathological parameters were re-evaluated from the archive slides. Gastric adenocarcinoma was classified as well differentiated, moderately differentiated, or poorly differentiated. All patients were defined as intestinal, diffuse or mixed type according to the Lauren classification [6]. Tumor stage was determined based on the American Joint Commission for Cancer criteria (AJCC 8th edition) [30]. CD44 and CD133 expression were determined by immunohistochemical (IHC) method in all cases.

In addition, HER2 overexpression of the tumor was evaluated in patients with metastatic GC.

Formalin (10%)-fixed and paraffin wax-embedded gastric adenocarcinoma blocks extracted from the archive of the pathology department were prepared for staining with Hematoxylin-Eosin (HE). For IHC staining, 4 µM thick sections were obtained and left for 1 hour at 60 degrees. Sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. The slides were buffered in Tris- EDTA (pH = 9) and then placed in a microwave at full power until the buffer reached boiling point. After that, the microwave temperature was reduced to 40°C and all tissues were left in place for 15 min. Then the slides were removed and left at room temperature for 15 min. Then monoclonal antibody diagnostic kits for CD44 (1: 100 dilution; clone MRQ-13, Millipore Sigma, USA), CD133 (1: 100 dilution; clone D4W4N, Cell Signalling Technology), and HER2 (ready to use;c-erbB-2/HER-2/neu Ab-17 (e2-4001+3B5) Thermo Scientific/LabVision) IHC stains were applied to them respectively.After washing, sections were overlaid with a secondary antibody (VECTASTAIN elite ABC kit Universal; Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature. Sections were incubated in 3.0 % hydrogen peroxide in PBS for 30 min to block endogenous peroxidase activity. The reaction was developed using avidin-biotin-peroxidase complex. The peroxidase reaction was developed with 3-amino-9-ethylcarbazole, and sections were counterstained with hematoxylin. Colon cancer sections were used as a positive control. Negative control sections (isotype control) were incubated with normal mouse serum instead of the primary antibody. HE and IHC sections were examined under a light microscope by two expert pathologists.

Scoring of the cytoplasmic or membranous staining of CD133 and membranous staining of the CD44 proteins were evaluated as semi-quantitative according to the expression percentage and intensity of immune positivity. Intensity scoring was as follows: 0: negative expression; 1: poor intensity; 2: moderate intensity; and 3: strong intensity [31]. The scoring of the expression percentage (extent of positivity) was done according to the percentage of cells showing positive staining as follows: a score of 0 if less than 5%, a score of 1 if it was between 5-25%, a score of 2 if it was between 25-50%, and a score of 3 if it was more than 50%. Tumors were categorized based on the following scores: < 1, negative; ≥ 1, positive. Moreover, the total score was determined from 0 to 6 based on an evaluation of the intensity and the percentage of the expression scores when taken together.

HER2 IHC scoring was evaluated according to the scoring system proposed by Hofmann *et al*. [32] as follows: a score of 0: 0 or < 10% staining in tumor cells; a score of 1: weak or incomplete membranous staining in > 10% of tumor cells; a score of 2: weak-moderate staining in > 10% of tumor cells; and a score of 3: moderate-strong, complete or basolateral staining in > 10% of tumor cells. Scores of 0 and 1 were classified as no or low HER2 expression, while scores of 2 and 3 were evaluated as being HER2 positive (+). Silver enhanced in situ hybridization (SISH) was applied to the samples with +2 and +3 IHC scores. The SISH method was performed according to the manufacturer's protocols for VENTANA HER2 Dual ISH DNA Probe Cocktail (https://www.diagnostics.roche.com). The SISH evaluation was performed by analyzing the HER2 gene and the chromosome 17 centromere signals of at least twenty consecutive cells under a light microscope (with 40× magnification). As a result of this evaluation, samples with a HER2 centromeric probe for chromosome 17 (CEP17) and a ratio ≥ 2 were considered as HER2 positive.

Statistical Analysis

SPSS 26.0 (IBM Corporation, Armonk, New York, United States) program was used in the analysis of the variables. The normal distribution of the data was evaluated with the Shapiro-Wilk Francia test, with the Levene test used to evaluate the homogeneity of variance. In the comparison of the quantitative data of two independent groups, the Independent-samples t-test with the Bootstrap results or the Mann-Whitney U test (with Monte Carlo Simulation technique) was used. In the comparison of more than two groups according to quantitative variables, the Jonckheere-Terpstra test and the Kruskal-Wallis H tests with the Monte Carlo Simulation technique were used and the Dunn's Test was used for Post hoc analyses. In the comparison of categorical variables, the Pearson Chi-square, Fisher exact and Fisher-Freeman-Halton tests with the Monte Carlo Simulation technique were used, and the comparison of column ratios with each other was expressed with the Benjamini-Hochberg corrected *p* -

Table 1. Comparison of anthropometric and clinicopathological features of gastric cancer patients according to metastasis status

	Total (n = 139)	Metastasis		*p value*
		Yes (n = 71)	No (n = 68)	
Age (years)	63 (21-89)	63 (30-86)	61.5 (21-89)	0.820[u]
Gender, n (%)				0.999[c]
Female	38 (27.3)	19 (26.8)	19 (27.9)	
Male	101 (72.7)	52 (73.2)	49 (72.1)	
Tumor type (Lauren), n (%)				**< 0.001[c]**
Diffuse	12 (8.6)	0 (0)	12 (17.6)[A]	
Intestinal	127 (91.4)	71 (100)[B]	56 (82.4)	
Tumor location, n (%)				0.948[ff]
Antrum	58 (41.7)	30 (42.3)	28 (41.2)	
Cardia	33 (23.7)	18 (25.4)	15 (22.1)	
Corpus	30 (21.6)	14 (19.7)	16 (23.5)	
Entire Stomach	15 (10.8)	7 (9.9)	8 (11.8)	
Fundus	3 (2.2)	2 (2.8)	1 (1.5)	
Tumor grade, n (%)				**< 0.001[ff]**
Poor	80 (63.0)	35 (49.3)	45 (80.4)[A]	
Moderate	38 (29.9)	29 (40.8)[B]	9 (16.1)	
Well	9 (7.1)	7 (9.9)	2 (3.6)	
Tumor depth (T), n (%)				**< 0.001[ff]**
T0	2 (1.4)	2 (2.8)	0 (0)	
T1	5 (3.6)	5 (7)	0 (0)	
T2	26 (18.7)	26 (36.6)[B]	0 (0)	
T3	38 (27.3)	38 (53.5)[B]	0 (0)	
T4	68 (48.9)	0 (0)	68 (100)[A]	
CD133 score, n (%)				0.364[c]
0	13 (9.4)	8 (11.3)	5 (7.4)	
1	35 (25.2)	21 (29.6)	14 (20.6)	
2	50 (36.0)	25 (35.2)	25 (36.8)	
3	41 (29.5)	17 (23.9)	24 (35.3)	
CD44 score, n (%)				0.698[c]
0	21 (15.1)	13 (18.3)	8 (11.8)	
1	35 (25.2)	17 (23.9)	18 (26.5)	
2	40 (28.8)	21 (29.6)	19 (27.9)	
3	43 (30.9)	20 (28.2)	23 (33.8)	
CD133 total score	3 (0-6)	3 (0-6)	4 (0-6)	0.117[u]
CD44total score	3 (0-6)	3 (0-6)	3 (0-6)	0.446[u]
Tumor depth (T)	3 (0-4)	3 (0-3)	4 (4-4)	**< 0.001[u]**
Tumor size (cm)	4.5 (0.7-17.5)	4 (0.7-17.5)	5 (2.5-15)	0.229[u]

Data are shown as median (minimum-maximum or n (%).

[u]Mann-Whitney U test(Monte Carlo), [c]Pearson Chi-Square test(Monte Carlo), Post-hoc Test = Benjamini-Hochberg correction

[A]Expresses significance according to the groups without metastasis, [B]Expresses significance according to groups with metastases

value. Kendall's tau-b test was used to analyze the correlations of quantitative variables. While quantitative variables were expressed as mean (standard deviation) and median (Minimum-Maximum) in the tables, categorical variables were shown as n (%). Variables were analyzed at a 95% confidence level, and a *p* - value of less than 0.05 was considered significant.

RESULTS

A total of 139 GC patients, 68 with metastases (median age 63 years; 52 males and 19 females) and 71 without metastases (median age 61.5 years, 59 males, and 19 females) were included in the study. The groups with and without metastases were similar in terms of age, gender, tumor location, and tumor size ($p > 0.05$) (Table 1). In patients with metastatic GC, the frequency of diffuse type, poorly differentiated and T4 stage were significantly higher than those in the non-metastatic group ($p < 0.05$). According to IHC evaluation, CD133/CD44 positivity was 88.7% (n = 63) and 81.7% (n = 58) (respectively). In metastatic cases, CD133/CD44 staining negativity was 7.4% (n = 5) and 11.8% (n = 8), CD133/CD44 positivity was 92.6% (n = 63) and 88.2% (n = 60), respectively ($p > 0.05$). Considering all cases, CD133 positivity (mild/moderate/intense) was 90.6% (n = 126) and CD44 positivity (mild/moderate/intense) rate was 84.9% (n = 118) (Fig. 1a-d). There was no difference in CD133 and CD44 positivity (intensity or density) rates and the total scores of metastatic and non-metastatic patients with GC ($p > 0.05$) (Table 1).

When metastatic cases (n = 68) were evaluated in terms of HER2 IHC positivity, 19 (27.9%) cases had a HER2 IHC score of 0 or 1, 35 (51.5%) cases had a score of 2 and 14 (20.6%) cases a score of 3. The SISH method was applied to cases with a HER2 score of 2 or 3 according to IHC (n = 49), and 26 (53.1%) cases were found to be negative, with 23 (46.9%) cases positive according to the SISH method. HER2 scores determined by the IHC method were divided into two

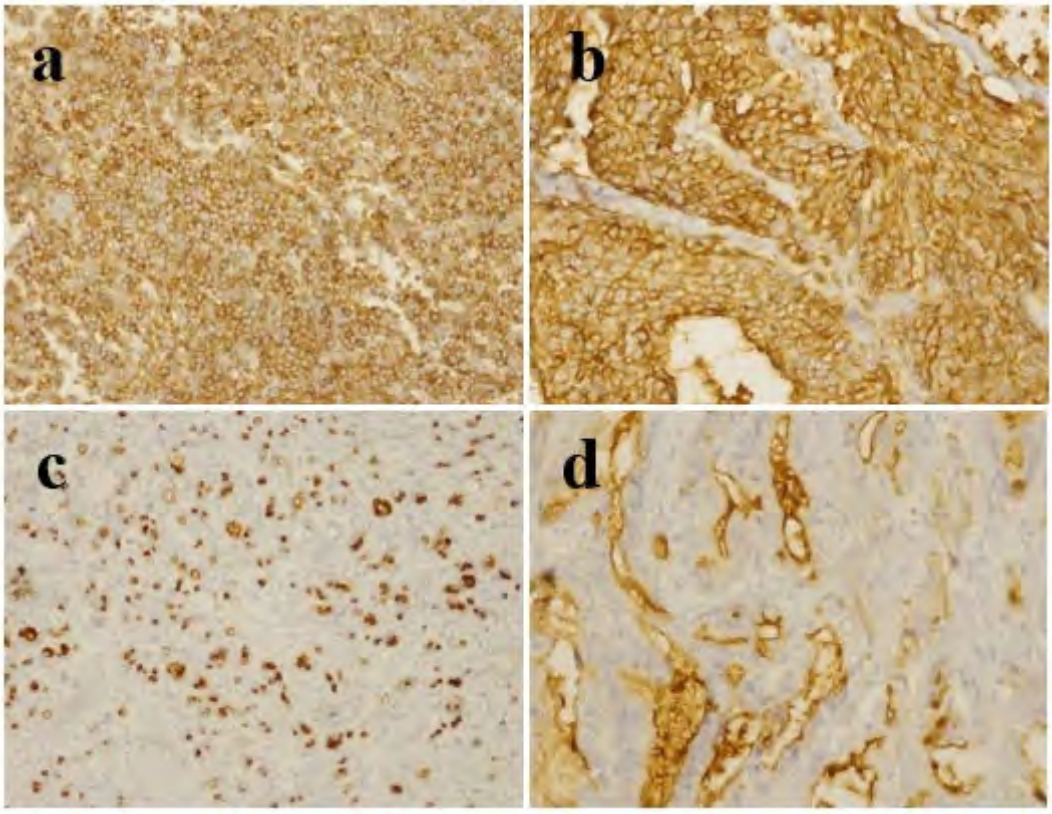

Fig. 1. (a) Membranous strong positivity of CD44 in poorly differentiated gastric carcinoma with ×100, (b) Membranous strong positivity of CD44 in moderately differentiated gastric carcinoma with ×200, (c) Membranous (luminal) staining of CD133 in poorly differentiated gastric carcinoma with ×100, and (d) Membranous (luminal) staining of CD133 in moderately differentiated gastric carcinoma with ×200.

Table 2. Evaluation of HER2 expression evaluated by two different methods in terms of anthropometric and clinicopathological features

	HER2 status (IHC)			HER2 status (SISH)		
	Negative (IHC0/1) (n = 19)	**Positive (IHC2/3) (n = 49)**	***p value***	**Negative (n = 26)**	**Positive (n = 23)**	***p value***
Age(years)	63 (31-80)	59 (21-89)	0.591[u]	58 (21-89)	63 (38-75)	0.194[u]
Tumor Depth (T)	4 (4-4)	4 (4-4)	0.999[u]	4 (4-4)	4 (4-4)	0.999[u]
Tumor size (cm)	5.5 (2.5-11.7)	4.5 (2.5-15)	0.082[u]	4.55 (2.5-15)	4.3 (2.5-10)	0.807[u]
Gender			0.766[c]			0.532[c]
Female	6 (31.6)	13 (26.5)		8 (30.8)	5 (21.7)	
Male	13 (68.4)	36 (73.5)		18 (69.2)	18 (78.3)	
Tumor type (Lauren)			0.294[f]			**0.011[f]**
Diffuse	5 (26.3)	7 (14.3)		7 (26.9)[B]	0 (0.0)	
Intestinal	14 (73.7)	42 (85.7)		19 (73.1)	23 (100.0)[A]	
Tumor location			0.119[ff]			0.155[ff]
Antrum	10 (52.6)	18 (36.7)		12 (46.2)	6 (26.1)	
Cardia	1 (5.3)	14 (28.6)		4 (15.4)	10 (43.5)	
Corpus	4 (21.1)	12 (24.5)		6 (23.1)	6 (26.1)	
Entire stomach	4 (21.1)	4 (8.2)		3 (11.5)	1 (4.3)	
Fundus	0 (0.0)	1 (2.0)		1 (3.8)	0 (0.0)	
Tumor grade			0.552[ff]			0.710[ff]
Poor	13 (92.9)	32 (76.2)		15 (78.9)	17 (73.9)	
Moderate	1 (7.1)	8 (19.0)		4 (21.1)	4 (17.4)	
Well	0 (0.0)	2 (4.8)		0 (0.0)	2 (8.7)	

IHC = immunohistochemical method, SISH = Silver enhanced in situ hybridization

[u]Mann Whitney U test (Monte Carlo), [c]Pearson Chi-Square test (Monte Carlo), [ff]Fisher Freeman Halton Test (Monte Carlo), [f]Fisher exact test (Monte Carlo)

[A]Expresses significance when compared with SISH-negative groups, [B]Expresses significance when compared with SISH positive groups

groups as negative (IHC 0/1) and positive (IHC 2/3). The gender, age, tumor depth, tumor size, tumor type (Lauren), tumor location, and tumor grade characteristics of these two groups were similar ($p > 0.05$). In addition, all of the HER2 positive cases (n = 23, 100%) in the SISH method were of the intestinal type, which was statistically significant according to the distribution of the HER2 negative patients ($p = 0.011$). Age, gender, tumor depth and size, tumor location and tumor grade characteristics of HER2 positive and negative cases regarding the SISH method were similar ($p > 0.05$) (Table 2).

The comparison of CD44 and CD133 scores of both metastatic and non-metastatic GC patients with various parameters are shown in Table 3. CD44 and CD133 scores were similar in terms of gender, HER2 positivity (IHC or SISH methods), tumor type (Lauren) and tumor location ($p > 0.05$). On the other hand, when CD133 and CD44 scores were compared according to disease grade in all patients, they were significantly higher in poorly differentiated cases ($p < 0.05$). CD133 and CD44 scores according to tumor grade in non-metastatic cases and CD133 scores according to disease grade in metastatic cases were similar ($p > 0.05$). CD44 scores in metastatic cases were significantly higher in poorly differentiated cases ($p <$

Table 3. Evaluation of CD44, CD133 and HER2 (evaluated by two different methods) expressions according to the metastasis status of gastric cancer patients

	Total		Non-metastatic		Metastatic	
	CD133 total scores	**CD44 total scores**	**CD133 total scores**	**CD44 total scores**	**CD133 total scores**	**CD44 total scores**
	n / Median (Min-Max)	**n / Median (Min-Max)**	**n / Median (Min-Max)**	**n / Median (Min-Max)**	**n / Median (Min-Max)**	**n / Median (Min-Max)**
Gender						
Female	38 / 3 (0-6)	38 / 3 (0-6)	19 / 3 (2-6)	19 / 3 (0-6)	19 / 3 (0-6)	19 / 3 (0-6)
Male	101 / 3 (0-6)	101 / 3 (0-6)	52 / 3 (0-6)	52 / 3 (0-6)	49 / 4 (0-6)	49 / 3 (0-6)
p value	0.626[u]	0.398[u]	0.197[u]	0.569[u]	0.644[u]	0.517[u]
HER2 (SISH)						
Negative	32 / 3.5 (0-6)	32 / 3 (0-6)	-	-	32 / 3.5 (0-6)	32 / 3 (0-6)
Positive	23 / 3 (0-6)	23 / 4 (1-6)	-	-	23 / 3 (0-6)	23 / 4 (1-6)
p value	0.774 [u]	0.071[u]	-	-	0.774[u]	0.071[u]
HER2 (IHC)						
IHC-0/1	19 / 4 (0-6)	19 / 3 (0-6)	-	-	19 / 4 (0-6)	19 / 3 (0-6)
IHC-2	35 / 3 (0-6)	35 / 3 (0-6)	-	-	35 / 3 (0-6)	35 / 3 (0-6)
IHC-3	14 / 5 (0-6)	14 / 4.5 (2-6)	-	-	14 / 5 (0-6)	14 / 4.5 (2-6)
p value	0.623[j]	0.121[j]	-	-	0.623[j]	0.121[j]
Tumor type (Lauren)						
Diffuse	12 / 3.5 (0-6)	12 / 3.5 (0-6)	-	-	12 / 3.5 (0-6)	12 / 3.5 (0-6)
Intestinal	127 / 3 (0-6)	127 / 3 (0-6)	71 / 3 (0-6)	71 / 3 (0-6)	56 / 4 (0-6)	56 / 3 (0-6)
p value	0.677[u]	0.810 [u]	-	-	0.677[u]	0.810[u]
Tumor location						
Antrum	58 / 3 (0-6)	58 / 3 (0-6)	30 / 3 (0-6)	30 / 3 (0-6)	28 / 3.5 (0-6)	28 / 2.5 (0-6)
Cardia	33 / 4 (0-6)	33 / 3 (0-6)	18 / 3 (2-5)	18 / 3.5 (0-6)	15 / 5 (0-6)	15 / 3 (2-6)
Corpus	30 / 3 (0-6)	30 / 4 (0-6)	14 / 2.5 (0-6)	14 / 4 (0-6)	16 / 3 (0-5)	16 / 3.5 (0-6)
Entire stomach	15 / 3 (0-6)	15 / 4 (0-6)	7 / 1 (0-5)	7 / 4 (0-6)	8 / 3.5 (2-6)	8 / 4.5 (1-6)
Fundus [excluted]	3 / 2 (2-5)[excluded]	3 / 6 (6-6)[excluded]	2 / 3.5 (2-5)[excluded]	2 / 6 (6-6)[excluded]	1 / 2 (2-2)[excluded]	1 / 6 (6-6)[excluded]
p value	0.144[k]	0.359[k]	0.078[k]	0.795[k]	0.294 [k]	0.433[k]
Tumor grade						
Poor	80 / 4 (0-6)	80 / 4 (0-6)	35 / 4 (0-6)	35 / 4 (0-6)	45 / 4 (0-6)	45 / 4 (0-6)
Moderate	38 / 3 (0-6)	38 / 2 (0-6)	29 / 3 (0-6)	29 / 2 (0-6)	9 / 3 (0-5)	9 / 2 (0-6)
Well	9 / 2 (1-6)	9 / 2 (2-6)	7 / 2 (1-4)	7 / 2 (2-6)	2 / 4 (2-6) [excluded]	2 / 3 (2-4)[excluted]
p value	**0.015**[j]	**0.004**[j]	0.054[j]	0.109[j]	0.201[u]	**0.006**[u]
	***P* (Poor-Moderate) = 0.043**	***P* (Poor-Moderate) = 0.004**	-	-	-	-

IHC = immunohistochemical method, SISH = Silver enhanced in situ hybridization

[u]Mann Whitney U Test (Monte Carlo), [k]Kruskal-Wallis Test (Monte Carlo), [j]Jonckheere-Terpstra Test (Monte Carlo); Post Hoc Test: Dun's Test,

0.05) (Table 3).

The results of the correlation analysis of CD133 and CD44 scores and various parameters are shown in Table 4. No correlation was found between CD133 total score and age, tumor size or depth, and HER2 scores in metastatic or non-metastatic cases ($p > 0.05$). In the correlation analyzes performed with CD44 scores, only a borderline significant correlation was

Table 4. Correlation analysis of CD44, CD133 total scores with age, HER2 expression, tumor size and tumor depth

	CD133 total scores		CD44 total scores	
	r	*p* value	r	*p* value
All patients				
Tumor depth (T)	0.082	0.240	0.069	0.325
Tumor size (cm)	0.008	0.893	0.121	0.052
HER2 (IHC)	-0.035	0.726	0.165	0.099
Age (years)	0.003	0.960	0.005	0.937
Non-metastatic patients				
Tumor depth (T)	-0.088	0.384	0.072	0.480
Tumor size (cm)	-0.023	0.793	0.175	**0.047**
Age (years)	-0.030	0.728	-0.042	0.635
Metastatic patients				
Tumor size (cm)	0.025	0.779	0.041	0647
HER2 (IHC)	-0.035	0.726	0.165	0.099
Age (years)	0.052	0.561	0.046	0.609

IHC = immunohistochemical method, Kendall's tau-b Test, r = Correlation Coefficient,

found between CD44 scores and tumor size (r:0.175; $p = 0.047$) in non-metastatic cases (Table 4).

DISCUSSION

Despite medical advances, significant improvements in the prognosis of GC have not been achieved and it still remains a serious public health problem. In recent years, various molecular and histochemical studies have been carried out to investigate the presence of various cell markers in CSCs [the most common markers: CD44, CD133] thought to initiate tumor development, and to be associated with metastasis and disease recurrence- and to consequently identify novel prognostic indicators and targeted biological approaches in the treatment of GC [18-29, 33, 34]. In most of these studies, these markers were considered separately, but in the current study, we evaluated the expression status of these three markers together. In addition, we examined whether there was a difference in the expression of these markers in metastatic and non-metastatic patients and in their relationship with clinicopathological features.

The expression positivity rate of CD44, one of the major components of the extracellular matrix, in GC cells has been shown to vary between 17.7% and 65.0% in various studies [11-13, 27, 35, 36]. In the current study, when all cases were evaluated, the rate of CD44 expression positivity was 84.9% (88.2% in metastatic patientsand 81.7% in non-metastatic patients). The difference in the frequency of CD44 positivity in GC in various reported studies may be related to geographic/racial characteristics, use of different cut-off values, or differences in the CD44 antibodies used. Numerous studies have been conducted on CD44 expression related to clinicopathological features, disease progression and the prognosis of patients with GC. Wakamatsu *et al*. [11] reported that CD44 may be one of the good markers associated with tumor invasion, distant metastasis and survival in patients with GC. In a study by Chen *et al*. [12], it is shown that high expression of CD44 is associated with poor differentiation, the presence of distant metastases, advanced TNM stage and tumor recurrence. In another study conducted by Düzcü *et al*. [15], it was shown that while CD44 was associated with histological grade, intestinal type, lymphovascular and perineural invasion, T-stage, and N-stage, it was not associated with distant metastasis, as in our study. Numerous

studies, including recent systematic reviews and meta-analysis studies, confirm that CD44 overexpression is associated with lymph node invasion, distant metastasis, poor prognosis, tumor size, and poor 5-year survival and as a result, it is suggested that CD44 is one of the most important guiding biomarkers in predicting the poor prognostic outcomes of GC [13, 36-43]. In our study, we found that CD44 expression was associated with poor differentiation and tumor size in our GC patients; however, unlike previous studies, no relationship was found between CD44 expression and tumor type (Lauren), tumor location and T-stage in our patients. On the other hand, we could not perform disease recurrence, prognosis or survival analyzes due to the lack of long-term follow-up of our patients.

CD133 is one of the best known CSC makers and has been shown to be expressed in various cancers including hepatocellular carcinoma, GC, colorectal cancer, pancreatic cancer, and ovarian cancer. Experimental studies suggest that CD133 expression in cancer patients is associated with resistance to various chemotherapeutic agents such as 5-fluorouracil and cisplatin. In addition, it has been shown in some studies that anti-CD133 antibody treatment inhibits the growth of cancer cells and induces apoptosis [44]. Therefore, determining CD133 expression status in patients may be a guide for the use of different treatment modalities. CD133 expression positivity rates in GC cells have been reported as 49.5% [13], 57.4% [46], and 58.4% [19] in various studies. In the current study, when all cases were taken into account, CD133 positivity (mild/moderate/intense) was seen to be 90.6%, and there was no difference in CD133 expression frequency between metastatic and non-metastatic patient groups. The difference in the expression frequency of CD133 may be related to the use of different antibodies in the histopathological evaluation or the use of different cut-off values. Moreover, in many studies, including meta-analysis studies, it has been shown that CD133 overexpression is associated with tumor size, histological grade, intestinal subtype, lymphatic infiltration, vascular invasion, TNM stage, depth of invasion, distant metastasis, tumor recurrence and reduced survival [9, 11-13, 15, 19, 22, 42, 46]. On the other hand, in a study by Sarıcanbaz *et al*. [21], it was shown that CD133 expression was not associated with nodal involvement, tumor size, T-stage, N-stage, and histological grade. In the current study, we demonstrated a relationship between CD133 expression and histological grade, but similar to the study of Sarıcanbaz *et al*. [21]. CD133 expression was not associated with age, tumor depth, tumor size, tumor type (Lauren), and tumor location. These conflicting results indicate the need for further studies with large case series.

It is known that HER2 expression plays a prominent role in the clinicopathological features and the poor prognosis of breast cancer [47]. However, conflicting data have been reported regarding the relationship between HER2-positivity and the clinicopathological features and prognosis of GC [24, 27, 28, 48-50]. In many studies, the rate of HER2 positivity with the IHC method varies between 4.4% and 53.4%, which may be due to geographic differences, tumor heterogeneity, the application of different scoring systems, and dependence on the examining pathologist [24]. In the current study, we found HER2 positivity to be 72.1% in patients with metastasis using the IHC method. In addition, in cases that were found to be HER2 positive by the IHC method, 46.9% of the cases were found to be HER2 positive by the SISH method. In one study, the sensitivity and specificity of the SISH method were reported as 56% and 100%, respectively, and the false-negative rate was 44%, according to the IHC method. These findings indicate that false negativity is very high in the SISH method, which is consistent with the findings of our study [51]. Studies investigating the relationship between clinicopathological features and HER2 expression have reported a close relationship between HER2 overexpression and gender, tumor differentiation, tumor location, tumor invasion, TNM stage, lymph node metastasis, and Lauren classification [48, 50, 52, 53]. In our study, when the SISH and IHC methods were taken into account separately, no relationship between HER2 expression according to the IHC method and demographic and clinicopathological features was found, only between HER2 expression and intestinal type GC in the SISH method, which is in line with the findings of Tanner *et al*. [54], was a relationship found. In addition, similar to our study, some studies reported that HER2 expression is not associated with gender, age, TNM stage tumor differentiation, tumor location, and tumor invasion [27, 28]. In addition, there are studies reporting that disease prognosis is associated with HER2 expression in patients with GC [48, 52, 54]; other studies dif-

fer [27, 28, 50, 55]. It has been reported in a study that the evaluation of HER2 is important because it guides the use of anti-HER2 agents in patients with GC [56]. Since our study did not include long-term follow-up of the patients, its effect on the prognosis could not be evaluated.

Limitations

The strength of this study lies in its evaluation of CD44, CD133 and HER2 expressions together in patients with GC, unlike previous studies, and its investigation of the relationship with clinical features. On the other hand, this study has some limitations. First of all, analysis related to prognosis could not be presented due to the lack of long-term follow-up of the patients. In addition, the number of patients included in the study was relatively small, and the results of subgroup analyzes may have been negatively affected by this. The negative effects of the difference of antibodies used in CD44, CD133 and HER2 measurements in the studies and the use of different cut-off values in determining the positivity of the results were not taken into account.

CONCLUSION

In conclusion, in this study, we investigated the presence of stem cells with CD133 and CD44 in metastatic, non-metastatic cases and in metastatic and HER2 positive cases. We reported associations between CD44 / CD133 expressions and histological grade in all patients, between CD44 and tumor size in non-metastatic patients, and between HER2 (detected by the SISH method) and intestinal type (Lauren) in metastatic patients. In addition, the association of all three markers with age, gender, metastasis, tumor invasion, or tumor location could not be demonstrated. We did not find any relationship between expressions of HER2 and CD44 and CD133. The results of this study need to be confirmed by multicenter studies including large case series.

Authors' Contribution

Study Conception: MG, NB, ST, MB, ZG; Study Design: MG, NB, ST, MB, ZG; Supervision: MG, NB, ST, MB, ZG; Funding: MG, ZG; Materials MG, NB, ST; Data Collection and/or Processing: MG, MB, ZG; Statistical Analysis and/or Data Interpretation: MG, NB, ZG; Literature Review: MG, NB, ST, ZG; Manuscript Preparation: MG, ZG and Critical Review: MG, NB, ZG.

Ethical Statement

The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated.

Informed Consent

Written informed consent was obtained from all participants before the study commenced.

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Conflict of Interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):1027-1033 Original Article

DOI: 10.18621/eurj.1335333 *Plastic, Reconstructive and Aesthetic Surgery*

Immediate reconstruction of nasal alar defects after malignant skin tumor excision without Mohs surgery

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ABSTRACT

Objectives: The National Comprehensive Cancer Network guidelines state that any nasal region with squamous or basal cell skin cancer is at high risk. Although Mohs surgery is the gold-standard procedure for many types of skin cancer, it is not applicable worldwide. A mean of 1.7 Mohs surgery stage is performed in cases of tumors. Nasal obstruction is a problem with Mohs surgery. In this study, we aimed to investigate nasal alar region nonmelanoma malignant skin tumor excision using immediate reconstruction without Mohs surgery.

Methods: Ten patients underwent reconstruction surgery between 2018 and 2022. The inclusion criterion were ulcerated lesions in the nasal alar region measuring less than 1 cm in diameter, the lesions which were suspected either as basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) on dermatoscopic examination, the patients who had intact nasal mucosa during anterior rhinoscopy.

Results: The mean follow-up duration was 26 months. No patient required re-operation because of an excisional biopsy result that involved border proximity. We observed no serious complications or long-term recurrences.

Conclusions: We recommend our algorithm for patients for whom Mohs surgery is not applicable.

Keywords: Malignant skin tumor, Mohs surgery, nasal alar defects, reconstruction

Mohs micrographic surgery (MMS) is the gold-standard technique for treating various cutaneous tumors, and it has numerous advantages [1]. Although it is a successful and cost-effective technique, it is time-consuming and has a tumor-seeding potential with repetitive stages [2-4]. Nose skin tumors require the most stages of MMS relative to other anatomical locations [5]. Furthermore, nasal obstruction commonly occurs after MMS [6, 7]. The nasal alar region has a unique anatomy that contains a thin skin surface, thin wavelike cartilage, and mucosa. Although the reconstruction of such a thin composite structure complicates the aesthetic result, reconstruction after skin tumor resection of its free margin can hamper stable functional conformation. Ultimately, the reconstruction of this region has two main goals: good aesthetic appearance and problem-free nasal breathing.

In this study, we aimed to reduce the number of surgeries and overcome complications related to MMS in the nasal alar region. We investigated nasal-alar region non-melanoma malignant skin tumor excision and immediate reconstruction without MMS in some patients. This study and the proposed algorithm are mostly based on the complications and technique selection.

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METHODS

Ten patients underwent tumor extirpation in the nasal alar region, preserving the nasal mucosa, between April 2018 and December 2022. The inclusion criterion were ulcerated lesions in the nasal alar region measuring less than 1 cm in diameter, the lesions which were suspected either as basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) on dermatoscopic examination, the patients who had intact nasal mucosa during anterior rhinoscopy. The exclusion criterion were lesions with indefinite borders, dermatologic examination of the patient was not compatible with BCC or SCC, lesions which invaded the nasal mucosa during the anterior rhinoscopy.

This study was performed in accordance with the Helsinki Declaration. Ethics committee approval was obtained. All patients signed informed consent form and accepted photographic permission.

Surgical Technique and Plan

A free skin margin of at least 6 mm was planned for the excision of BCC defects and at least 10 mm for that of SCC defects, based on dermatoscopic examination or incisional biopsy. The lesions were excised en bloc in a beveled manner, with the underlying subcutaneous tissue and cartilage. After that, the surgical instruments were changed to avoid tumor tissue seeding.

The posterior auricular skin on the conchal cartilage was incised. The graft border was defined as the graft passing from the anterior surface of the auricle using 27 G needles. We aimed for the cartilage graft positions to meet the following criteria. The first large piece was the lower lateral cartilage (LLC), which should be similar in shape. The position of this large piece should preserve the function of the internal nasal valve. If the cartilage excision includes a part of the upper cartilage, the large piece should be located more

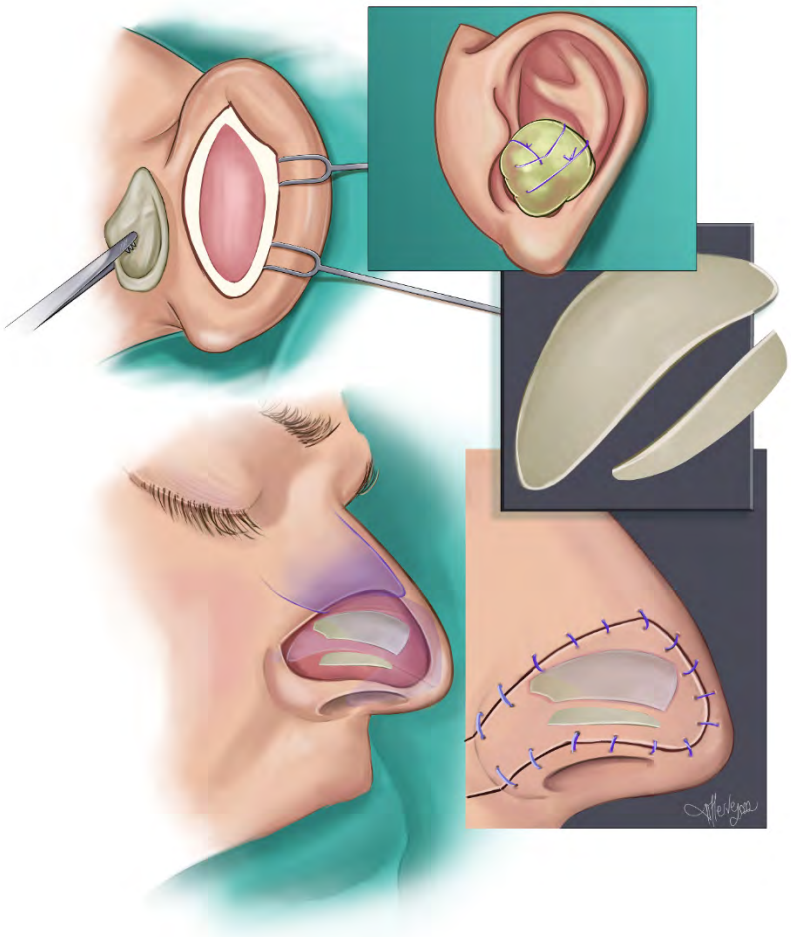

Fig. 1. Illustration of harvesting and insetting the conchal cartilage. This graft has two different surface type. The helical rim side with smooth surface of the graft is used as large piece and the cavum concha side with protuding surface of the graft is small piece (the alar rim graft).

cranially, near the upper lateral cartilage. Second, the small piece is the alar rim graft, which should be located near the alar rim despite the defect being away from it (Fig. 1). A 5/0 PDS (Ethicon, USA) was used to fix the graft onto the nasal mucosa.

The nasolabial flaps were superiorly positioned with transposition or interpolation insetting based on the patients' preference or the distance of the defect from the planned flap. The second stage of the interpolation-type flap was performed after a minimum of three weeks, whereas the other aesthetic operations were performed after a minimum of three months.

Revision surgeries have two main purposes, flap defatting and scar inversion, to resemble natural creases. Curvilinear excision was used to create inverted scars in the foreseen alar crease area that were later inverted.

RESULTS

Our patients included six men and four women, with a median age of 56.4 years. The mean follow-up duration was 26 months. Our algorithm had an average of 1.5 stages per case. Table 1 summarizes patient characteristics.

None of the patients required reoperation because of an excisional biopsy result involving border proximity. At least 6-mm and 4-mm clinically cutaneous tumor-free margins were obtained for patients with SCC and BCC, respectively. Pathological examination revealed no cases of cartilage invasion or base margin contiguity. No patient experienced a recurrence.

No severe complications were observed, such as partial or total flap loss, infection, wound dehiscence, or donor-site morbidity such as hematoma. To prevent hematoma formation, we used bolster dressing for a three-day period in the cartilage donor site (Fig.1). In one patient (Patient 1) with a history of multiple non-melanoma malignant skin tumors on the face, actinic keratosis at the lateral margin was observed after 40 months. Cryotherapy was selected as the treatment of choice. In one patient (Patient 3), the alar rim graft was exposed and removed during the second operation. No functional problems occurred postoperatively.

All patients showed greater aesthetic satisfaction after the second procedure. (Fig. 2). Only the Patient 1 underwent three times to create much more natural nasal appearence. (Fig. 3). Only Patient 7, who did not accept the conchal cartilage graft, was dissatisfied with the functional results of the first operation.

In only one patient (Patient 7), the nasal alar defect was reconstructed using only a nasolabial flap. The patient did not consent to conchal cartilage harvesting and wanted functional nasal airway reconstruction using only a nasolabial flap. The length of the LLC defect was 10 mm. She complained of nasal airway dysfunction immediately after the first operation and

Table 1. Patient summary

Patient number	Age (years)	Sex	Diagnosis	Defect size (cm)	Nasolabial flap insetting type	Total operation (reconstruciton and flap revisions)	Follow-up (months)
1	58	M	BCC	20×18	Transposition	3	53
2	38	F	SCC	25×25	Interpolation	2	45
3	55	M	SCC	30×25	Transposition	2	33
4	72	M	BCC	20×20	Transposition	1	30
5	37	M	SCC	25×22	Transposition	1	25
6	76	F	SCC	28×25	Transposition	1	21
7	56	F	BCC	17×17	Transposition	2	16
8	54	M	BCC	18×15	Transposition	1	16
9	63	M	BCC	19×19	Transposition	1	15
10	55	M	BCC	15×15	Transposition	1	14

M = male, F = female, BCC = basal cell carcinoma, SCC =squamous cell carcinoma

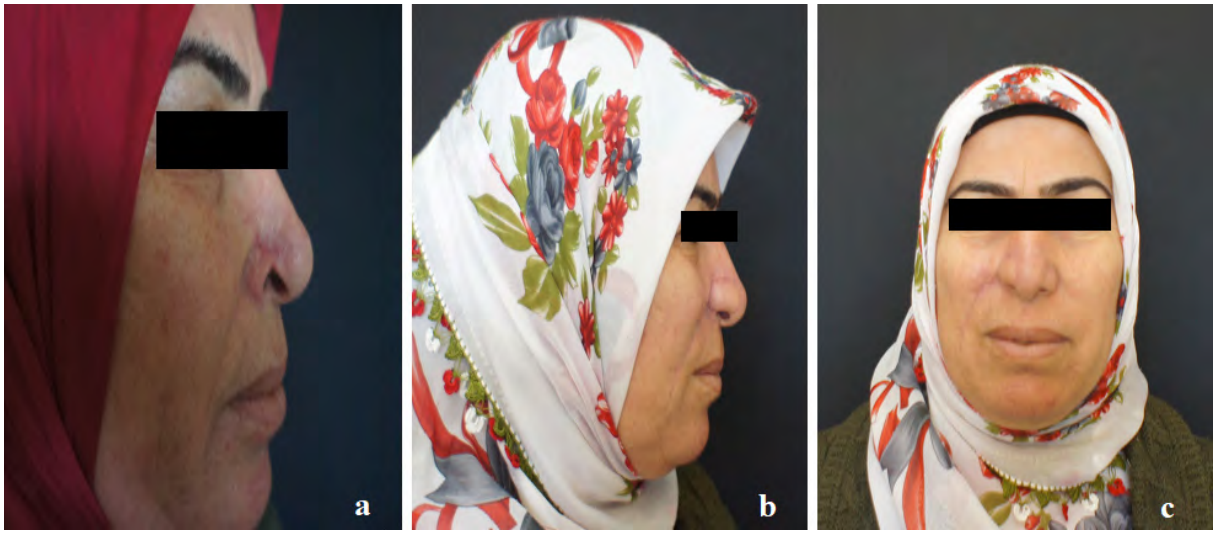

Fig. 2. (a) Patient 2, preoperatively. (b, c) Her left nasal alar region was reconstructed with the interpolation flap insetting type. The photographs show 11 months after second stage interpolation flap.

requested a conchal cartilage graft in the revision surgery performed three months later.

Fig. 4 shows our proposed algorithm.

DISCUSSION

The National Comprehensive Cancer Network guidelines indicate that the heads of any size with SCC and BCC in the nose region are high-risk cases [8, 9]. A study reported that nasal tip skin thickness was 3.32 ± 0.78 mm [10]. Therefore, we hypothesized that en bloc excision that spares the nasal mucosa without MMS provides a safe deep margin when a lesion is ulcerated and not fixed to the LLC and the nasal mucosa is intact.

According to a study, MMS has a 5-year disease-free survival rate of 99.3% [11]. The American College of Mohs Surgery Improving Wisely Quality Collaborative revealed that 2305 physicians practicing MMS had a national average of 1.7 stages per case for the head, neck, genitalia, hand, and foot regions [12]. We had no cases of repetitive surgeries due to skin margins of the specimen after the first excisional

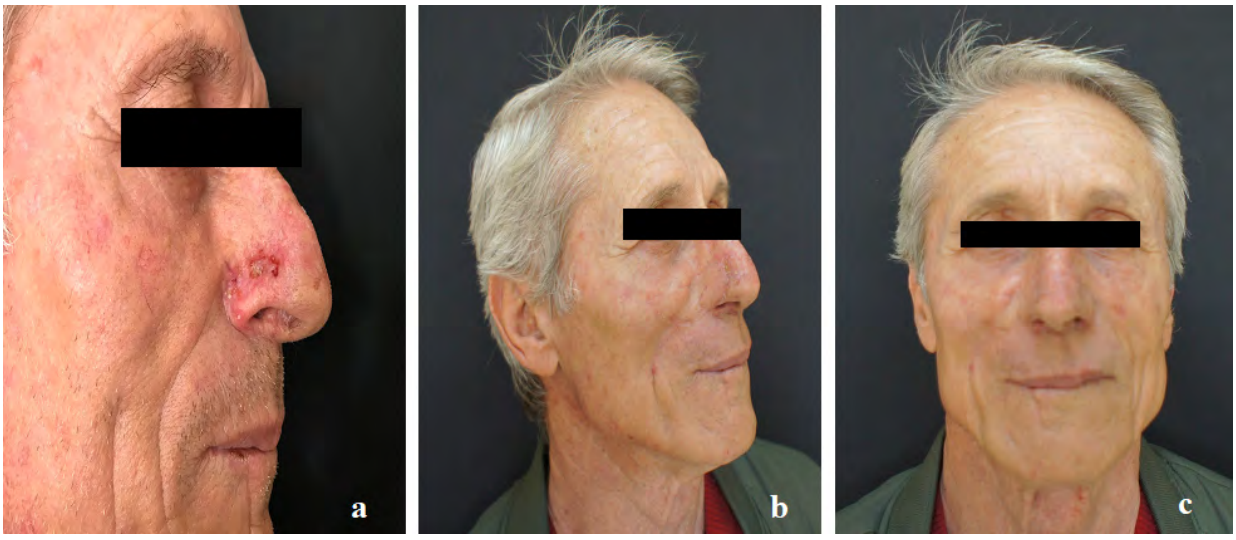

Fig. 3. (a) Patient 1, preoperatively. (b, c) The patient, 30 months after his last operation. Inverted scars like natural creases were created in the second and third stages.

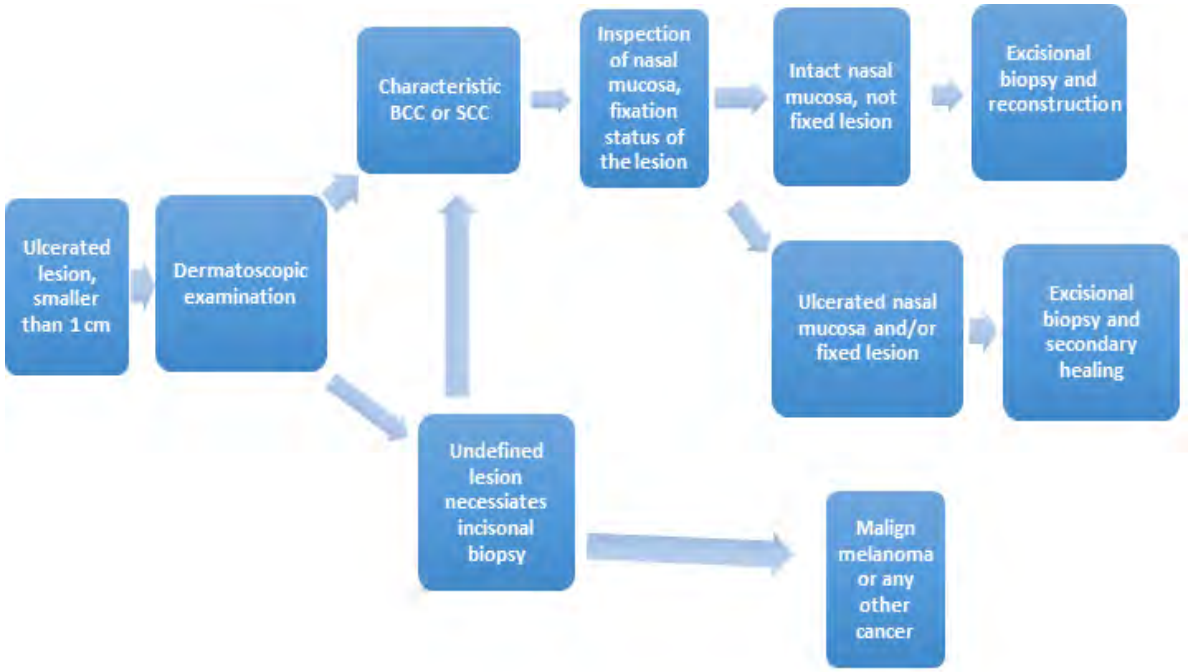

Fig. 4. Our algorithm

biopsy. Our staged surgeries were performed mainly for aesthetic reasons. No cases of functional or aesthetic complaints or donor-site morbidities were present.

The principle "like with like" is the primary goal of every kind of reconstructive surgery. The nasolabial flap contained the most tissue adjacent to the nasal alar defect. Although other well-known options, such as composite grafts, local flaps, regional flaps, prefabricated flaps, and free flaps, for reconstruction of nasal alar defects are available [13-25], the nasolabial flap has good color and texture matching, forming a natural-looking alar contour with minimal donor area scarring.

To maintain a good nasal passage, an appropriate framework should be established that mimics the precise position of the cartilage grafts. The conchal cartilage, as a donor site, has a natural arch of the nasal alar and good elasticity [26-28]. We recommend a plan to maximize the benefit of the conchal cartilage graft (Fig. 1). Inserting a cartilage graft into the mucosal layer provides stable breathing function and resistance to flap weight. None of the patients complained of nasal airway dysfunction after reconstruction using a nasolabial flap or conchal cartilage. We encountered a case where the patient asked for reconstruction without a conchal cartilage graft; the functional result was insufficient, as expected.

One patient experienced alar rim cartilage exposition. We believe this was caused by the suture technique, which concurrently passed through the alar rim skin, cartilage, and nasolabial flaps. We recommend using a skin suture that does not pass through the cartilage (Fig. 1) but fixes the cartilage to the mucosa.

Interpolation-type nasolabial flap insertion was performed in two stages, whereas transposition-type nasolabial flap insertion was performed in one stage. Every patient was informed that the nasolabial flaps may require revision based on the aesthetic results. One patient who underwent interpolation-type nasolabial flap insertion was satisfied with the results and did not need any other revision surgery. However, we believe that patients prefer receiving only a single-stage solution.

Limitations

Our study has some limitations. Our algorithm, without MMS in this region, which is a three-layered thin tissue with different characteristics, is applicable to early-stage BCC and SCC. Nevertheless, cases of early-stage BCC and SCC may have indefinite borders, for which an incisional or excisional biopsy with secondary healing is a wise approach. Although the mean follow-up period was 26 months, 10 patients represented a relatively small sample size.

CONCLUSION

We found that mucosa-sparing nasal alar region excision, including the skin and LLC, is safe for ulcerated non-melanoma skin malignant tumors, and provides a free margin under the malignant tumor. We recommend our colleagues to utilize our algorithm when the gold-standard Mohs surgery is not applicable. However, further studies are needed.

Authors' Contribution

Study Conception: MT; Study Design: MT; Supervision: ÖÖ; Funding: ÖÖ; Materials: MT; Data Collection and/or Processing: MT; Statistical Analysis and/or Data Interpretation: MT; Literature Review: MT; Manuscript Preparation: MT and Critical Review: ÖÖ.

Conflict of interest

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Comparison of frozen section accuracy with final pathology results in early clinical stage of endometrioid type endometrial cancer

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ABSTRACT

Objectives: We aimed to compare the accuracy of the depth of myometrial invasion determined by intraoperative frozen section in the early clinical stage of endometrioid type endometrial cancer, with the result of the final postoperative paraffin section.

Methods: The study was carried out with 102 patients who were diagnosed with type 1-2 endometrioid endometrial cancer in the gynecology clinic of the state hospital between January 2015 and 2019. Retrospective demographic data, clinical characteristics, and pathology results of the patients who underwent surgical staging were recorded.

Results: The mean age of the patients was 59.3 ± 9.1 years and 82.3% of the patients were in the postmenopausal period. The mean age of patients with a depth of myometrial invasion < ½ was lower than myometrial invasion > ½, which was statistically significant ($p < 0.001$). According to the final postoperative pathology results, 93.1% (n = 95) of the cases were diagnosed as FIGO stage 1. The subgroups were 66.7% stage 1a and 26.4% stage 1b. When the stage and grade distribution was made according to the final postoperative pathology result, stage 1a grade 2 endometrial cancer was the most common with a rate of 43.1%. Concordance of the intraoperative and postoperative pathology results for the depth of myometrial invasion was 84.3%, the specificity was 100%, the positive predictive value was 100%, and the negative predictive value was 86.76%.

Conclusions: The accuracy of the intraoperative frozen section in endometrial cancers is quite higher. For this reason, intraoperative pathological examination results are important in terms of minimizing the complications of unnecessary surgery.

Keywords: Endometrial cancer, frozen section, myometrial invasion

Endometrial cancer (EC), which is the most common gynecological cancer seen worldwide, usually occurs in women between the ages of 55-64. Most of the patients present with the complaint of vaginal bleeding at an early stage. As a result, 80% of the cases are diagnosed early at the initial stage and the 5-year survival is 95% [1, 2]. The most common and diagnosed histological type is Type 1 endometrioid adeno-

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carcinoma. They have a better prognosis and usually result from hyperplasia. Type 2 EC is seen at a rate of 10-20%. These tumors are usually high-grade estrogen nonresponsive tumors and, have a poor prognosis. Also, a precursor lesion is rarely detected [3, 4]. One of the biggest problems to be solved in the therapy of EC has been the high-accuracy prediction of preoperative risk. The potential to accurately detect the risk of metastasis and recurrence that may develop during the diagnosis and follow-up of cancer facilitates cancer management. In this way, side effects of unnecessary adjuvant therapy are minimized [5].

Frozen section is defined as intraoperative pathology consultation requested. Although it is generally performed to understand whether the sent tissue is malignant or benign, it is a rapid pathological evaluation method used in cases such as what the disease may be, determination of other necessary examinations for the disease, the size of the tumor, if any, the extent of the tumor, the extent to which the surgery will be expanded, and the determination of the surgical margin [6]. The accuracy rate of frozen examination, which is increasingly used today, in gynecological cancers is between 91.5%-97.4%. Frozen examination in gynecology is mostly used in tumors of ovarian origin, followed by cancers of the endometrium, cervix, and vulva, respectively [7]. Mayo Clinic defines those with low risk of lymph node metastasis as grade 1 histopathology, tumor diameter of 2 cm and below, superficial myometrial invasion (< 1/2), and grade 1-2, on the other hand, described low-risk patients as no myometrial invasion, any grade or superficial myometrial invasion grade 1 [8]. Lymph node dissection has side effects such as prolonging the operation time, increasing the risk of bleeding, impaired lymphatic return, and subsequent edema in the lower extremities [9, 10]. For these reasons, frozen examination becomes very important to be successful, the surgeon and pathologist must cooperate [7].

This work, we aimed to investigate the value and reliability of FS in surgical management by comparing the result of the depth of myometrial invasion (MI) in intraoperative EC with the final pathological outcome.

METHODS

The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical confirmation was obtained by applying to the local commision (Date: 08/01/2020 Decision No: 2011-KAEK -25 2020/01-13). The study was carried out with 102 patients who undertake surgery with the identify of type 1-2 endometrioid EC as a result of endometrial biopsy in Bursa Ali Osman Sönmez Oncology Hospital Gynecology clinic between January 2015-2019. The study was designed retrospectively, no written informed consent form was obtained from patients. Grade 3 EC, no endometrioid type endometrial cancer, presence of extrauterine tumor, and chemotherapy were determined as exclusion criteria. Preoperative histopathological diagnosis in the cases included in the study; was obtained by pathological examination of endometrial material sampled with pipelle and dilation and curettage. Intraoperative frozen section results of cases with grade 1 and /or grade 2, Type 1 and /or Type 2 endometrioid EC as a result of endometrial biopsy were compared with the final postoperative definitive pathological diagnosis. In the operation, abdominal fluid sampling was performed for cytological examination, primarily following the abdominal exploration in all patients. After the hysterectomy was sent to the pathology department of our hospital, a full-thickness tissue sample, including the serosa, was taken from the area that was macroscopically thought to be the deepest of the tumor, and the tissue was frozen in the frozen device. Sections of 0.5-micron thickness were taken. The depth of myometrial invasion was evaluated microscopically by staining with H&E, and verbal and written information was given to the operating room team. The frozen section and PS examination of all materials were performed by two experienced pathologists and the staging system published by FIGO in 2009 was used [11]. All cases; mean age, menopausal status, body mass index, gravida-parities, histological grades, surgical stages, and depth of MI were evaluated. Intraoperative FS results and postoperative final pathology results (FPR) were compared.

Statistical Analysis

The IBM SPSS 22 program was used for data analysis. The conformity of quantitative variables to normal distribution was investigated using the Shapiro-Wilk test. Normal distribution was expressed as mean ± standard deviation, and abnormal distribution was expressed as the median (minimum-maxi-

mum). Qualitative variables are shown as frequency and percentage. During the comparison of independent groups, the sample t-test was used to analyze the normally distributed data, and the Mann-Whitney U test was used for abnormally distributed data. The relationship between qualitative variables was examined by Fisher Exact and Chi-Square analysis. The sensitivity and specificity values of the applications recommended as diagnostic tests were calculated according to the gold standard test. The results obtained as $p < 0.05$ were considered significant.

RESULTS

In this study, the ages of the patients were between 42 and 78 years. Of the cases with grade1 and/or grade 2 endometrioid type EC as a result of the endometrial biopsy, 82.3% (n = 84) patients were in menopause, while 17.7% (n = 18) patients were in the premenopausal period. The complaints of the women were postmenopausal bleeding (PMB) (82.5%), abnormal uterine bleeding (13.7%), and increased endometrial thickness (3.8%) respectively (Table 1). When the cases with endometrioid type EC were evaluated according to the preoperative grade; grade 2 endometrioid type EC was the most common with a rate of 66.6% (n = 68). Grade 1 endometrioid type EC was found to be 33.4 % (n = 34). When the stage and grade distribution of the cases according to the postoperative FPR was made, the most common EC was stage 1a grade 2 EC with a rate of 43.1% (Table 1).

The average age of patients with an MI depth less than ½ was lower than the MI depth > ½ patients (57 [51-62] vs 61 [59-73] years respectively, $p < 0.001$). There was no significant difference between the groups in terms of body mass index (BMI) and MI depth in the FPR. In terms of MI depth measurement, the FPR of 59.5 % (n = 50) of 84 postmenopausal patients was MI < ½, and the FPR of 40.5% (n = 34) patients was MI > ½. Premenopausal 17.7% (n = 18) patients did not have a FPR MI > ½. When the cases divided into 2 groups according to their menopausal status, a significant difference was found in terms of MI depth ($p = 0.012$). Among the cohort number of FIGO Stage 1a patients with an MI depth of < ½ in the frozen evaluation was higher than FIGO Stage 1b patients (66.7%, n = 68) vs (26.4%, n = 27). FIGO stage

Table 1. Clinical features, surgical stage and grade of endometrial cancer patients

Parameter	n	%
Menopause status		
Postmenopausal period	84	82.3
Premenopausal period	18	17.7
Application complaint		
Postmenopausal bleeding	84	82.5
Abnormal uterine bleeding	14	13.7
Endometrial thickness	4	3.8
Preoperative grade		
Grade 1	34	33.4
Grade 2	68	66.6
Postoperative stage and grade		
Stage 1a grade 1	24	23.5
Stage 1a grade 2	44	43.1
Stage 1b grade 1	24	23.5
Stage 1b grade 2	3	2.9
Stage > 1 grade > 2	7	6.9

1a and 1b early-stage patients total constituted 93.1% (n = 95) of the group. Since the final pathology result 6.9 % (n = 7) of the patients in the study was reported as FIGO Stage > 1 and grade > 2, they were not included in the comparison. Final pathology result was consistent with in 63 (92.6%) of 68 patients whose frozen section result was MI depth < ½, while FPR was reported as MI depth >½ 5 (7.4%) patients. At the same time, MI depth was found to be >½ in the FPR of all (100%, n = 27) patients whose MI depth was >½ in frozen examination ($p < 0.001$) (Table 2).

When frozen and final pathology results were compared in terms of accurately predicting the depth of MI, the sensitivity rate was 84.3%, the specificity rate was 100%, the positive predictive value was 100%, the negative predictive value was 92.6%, the false positive rate was 0%, and the false negative rate was 7.4% (Table 3).

DISCUSSION

In all patients with a diagnosis of endometrial cancer who do not have a high surgical risk, primary treat-

Table 2. The relationship of final pathology result with age, BMI, menopausal status and myometrial invasion depth result of frozen section

Parameter	Final pathology < ½ MI	Final pathology > ½ MI	*p* value
Age (year)	57 (51-62)	61 (59-73)	**< 0.001**
BMI (kg/m$^{2)}$	33.78 ± 8.12	33.61 ± 7.1	0.768
Menopause status			**0.012**
Postmenopausal	50 (59.5)	34 (40.5)	
Premenopausal	18 (17.7)	0 (100)	
Frozen section			
MI: no (n = 68)	63 (92.6)	5 (7.4)	**< 0.001**
MI: present (n = 27)	0 (0)	27 (100)	

Data are shown as mean ± standard deviation or median (minimum-maximum) or n (%). BMI = Body mass index, MI = Myometrial invasion

ment may be surgery followed by surgical staging. However, considering the increase in mortality and morbidity that surgical staging will bring to the patient, the benefits and harms of performing complete surgical staging for each patient have begun to be discussed [12, 13]. However, in patients at very high risk for surgery and in the early stages, treatment can be individualized. If primary radiotherapy is in advanced stages, neoadjuvant primary chemotherapy applications may be on the agenda. However, information about the need for postoperative adjuvant therapy and the prognosis of patients with endometrial cancer is only provided by surgical staging [14]. Although endometrial cancers are usually detected between the ages of 50-65, the average age is 60 years. [15, 16]. In our study, the mean age of the patients was 59, which was similar to the literature. The parity of these patients was calculated as 2.4 ± 1.3. In this respect, no data can be presented regarding the effect of nulliparity or infertility on the development of endometrial cancer. In the literature, the average age of incidence of endometrial cancers is over 40 years. In this current study, 82.3% of the cases at the time of diagnosis were in the postmenopausal period, and 17.7% of the cases were in the premenopausal period. Since endometrial cancer is most frequently seen in the postmenopausal period, the most common presenting complaint was also observed as postmenopausal bleeding in our study [6]. Studies have shown that approximately 75% of patients diagnosed with endometrial cancer are at stage 1 [17]. According to the post-operative final pathology results of the cases included in our study, 93.1% (n = 95) were found to be stage 1, 66.7% were reported as stage 1a, and 26.4% as stage 1b. Although full staging of EC is still used for standard surgery, the place of lymph node dissection (LND) for the early stage is controversial. Parameters with high risk for lymph node metastasis (LNM); type II histology has been reported as a deep myometrial invasion (≥ 1/2) and large tumor diameter (> 2 cm). The design of our study was based on early-stage and low-grade EC patients and based on the depth of myometrial invasion [18].

The pathologist has to give the result of intraop-

Table 3. The results obtained when the frozen and final pathology results were compared in terms of depth myometrial invasion

	Sensitivity	Specificity	PPV	NPV	False negative rate	False positive rate
Frozen section evaluation	84.3%	100%	100%	92.6%	7.4%	0%

PPV = Positive predictive value, NPV = Negative predictive value

erative FS within the specified time to minimize the duration of the surgery and possible complications, and therefore only a limited number of tissue sections are taken. At the same time, the pathologist's experience is very important because the location and number of sections taken from the gross examination determine the accuracy of the FS [19]. In the study of Fanning *et al*. [20], the accuracy rate was found to be 95% for myometrial invasion when at least four sections were taken in FS. In our study, at least three sections were taken and the accuracy rate was 84% for the depth of myometrial invasion in FS. Therefore, a learning curve and experience for the pathologist to be used in clinical practice are required to increase the diagnostic accuracy for FS. Furukawa *et al*. [21], reported the specificity of frozen examination in endometrial cancer as 95.9% and the sensitivity as 91.7% in endometrial cancer. In the study of Durdag *et al*. [22], the accuracy of FS in predicting final pathology results was 76.23% for histology, 75.45% for grade, and 85.31% for depth of myometrial invasion. These rates are similar to our study's comparative results for the depth of myometrial invasion. In another study by Mandoto *et al*. [23], the accuracy of FS in predicting the final pathology results was 76.23% for histology and 75.45% for grade. The 85.31% success rate of the frozen section results for myometrial invasion depth supported the 84.3% detection rate of myometrial invasion depth in our study [23].

In the study of Gitas *et al*. [24], with patients with early-stage (FIGO I-II) and low-grade EC, the correlation between the depth of myometrial invasion of FS and the final pathology result was found to be high. The concordance of the frozen section and final pathology results in terms of FIGO stage was 85.2%, the rate of underdiagnosis was 14% and the rate of overdiagnosis was 0.8%. In this current study, the concordance of the FS and final pathology results was found to be 84.3%, while the false positive rate was 0% and the false negative rate was 7.4%, which was significantly lower.

The disadvantage of frozen examination is that it prolongs the operation time by an average of 20-30 minutes depending on the number of sections taken. Apart from the prolongation of the operation time with the waiting time required for the FS result.

Limitations

The most important limitations of our study were that it was designed with a low number of cases, early-stage and low-grade patients, and evaluated only the depth of myometrial invasion. However, the evaluation of EC pathology results by two experienced pathologists in our study increased the accuracy and reliability of the study results.

CONCLUSION

In conclusion, frozen section enables the staging of endometrial cancer intraoperatively with a very high accuracy rate, increasing the surgeon's success in choosing the appropriate operative treatment. This high accuracy of results minimizes unnecessary surgery and potential complications in staging.

Authors' Contribution

Study Conception: LÖ; Study Design: LÖ; Supervision: N/A; Funding: N/A; Materials: N/A; Data Collection and/or Processing: LÖ; Statistical Analysis and/or Data Interpretation: GÖ; Literature Review: GÖ; Manuscript Preparation: LÖ and Critical Review: GÖ.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):1040-1047 Original Article

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Evaluation of arterial stiffness between peritoneal dialysis and hemodialysis in patients with renal replacement therapy

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ABSTRACT

Objectives: The aortic stiffness index beta (ASI-β), calculated non-invasively with the pressure change caused by arterial strain and volume changes on echocardiography, shows a strong correlation with invasive measurements of arterial stiffness. The aim of this study was to compare arterial stiffness and distensibility between peritoneal dialysis and hemodialysis and patients in renal replacement therapy (RRT).

Methods: 108 consecutive patients under RRT (peritoneal dialysis and hemodialysis) were analyzed in cross-sectional and observational study design. The aortic stiffness index beta (ASI-β) was calculated for each group.

Results: The mean age of the patients in the study was 58.2 ± 11.1 years, and 49 (45.4%) of the patients were female and 59 (54.6%) were male. Age, gender, comorbid rates, and levels of blood pressure and heart rate did not differ between the peritoneal dialysis and hemodialysis groups. Blood pressure levels and heart rate. Mean aortic strain (5.6 ± 1.9 vs. 9.4 ± 2.8, $p < 0.001$) and median distensibility (1.5 vs. 2.9 cm, $p < 0.001$) were lower in the peritoneal dialysis group than the hemodialysis group, while median ASI-β (11.6 vs. 6.2, $p < 0.001$) and mean E/e' (10.6 ± 2.9 vs. 9.2 ± 2.3, $p = 0.006$) were higher in the peritoneal dialysis group. The rate of concentric hypertrophy was higher in the peritoneal dialysis group (47.5% vs. 23.5%, $p = 0.005$).

Conclusions: Peritoneal dialysis patients have higher arterial stiffness and lower distensibility levels compared to hemodialysis patients. Therefore, patients with peritoneal dialysis may be more prone to diastolic dysfunction, cardiovascular disease, and events.

Keywords: Aortic stiffness index, renal replacement therapy, cardiovascular disease, peritoneal dialysis, hemodialysis

Patients with end-stage renal disease (ESRD) are predisposed to cardiovascular disease such as left ventricular hypertrophy, heart failure, and acute myocardial infarction [1]. Traditional cardiovascular risk factors like advanced age, diabetes mellitus, smoking, hypertension, dyslipidemia, ventricular hypertrophy, and physical inactivity are common in these patients. ESRD also increases the risk of developing coronary heart disease. Moreover, accelerated atherosclerosis may contribute to the pathophysiology of ESRD [2].

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Arterial stiffness is an important predictor of atherosclerosis, cardiovascular diseases and events [3]. Considered the gold standard, pulse wave velocity (PWV) is a non-invasive, reproducible and simple method of assessing arterial stiffness. [4]. It has been reported that arterial stiffness is increased in patients with renal failure [5]. The type of dialysis has different effects on the cardiovascular system. Peritoneal dialysis (PD) may accelerate atherosclerosis and increase the risk of cardiovascular disease or events because it is associated with the accumulation of excess volume in the body [6]. Previous studies have provided conflicting results that arterial stiffness measured by PWC is similar, low, or high in PD and hemodialysis (HD) patients [7]. There is therefore a need for further research into the impacts of the dialysis type used on arterial stiffness.

The aortic stiffness index beta (ASI-β), calculated in a non-invasive way with the change in pressure caused by arterial strain and volume changes on echocardiography, is highly correlated with invasive arterial stiffness measures [8]. Increased ASI-β has been demonstrated to be an indicator of cardiovascular morbidity and mortality in patients with PD. [9]. The aim of this study was to compare arterial stiffness and distensibility between HD and PD and patients on renal replacement therapy.

METHODS

Study Population

The present study analyzed a total of 108 patients who were receiving renal replacement therapy at the Nephrology Clinic of a tertiary referral hospital and who presented to the Cardiology Clinic for various reasons were evaluated in cross-sectional design. The patients with previous coronary heart disease, myocardial infarction, heart failure, rheumatic diseases, asthma, pulmonary embolism, inflammatory disease, peripheral artery disease, chronic obstructive pulmonary disease, cerebrovascular disease, liver diseases and cancer were excluded from the study.

Informed consent was obtained from the subjects and the study protocol was approved by the local ethics committee of the institute (Decision No: 2022-17/9). The study protocol conforms to the tenets of the Declaration of Helsinki.

Demographic, laboratory and echocardiographic data were collected from all patients. Hypertension was defined as blood pressure > 140/90 mmHg in repeated measurements or use of antihypertensive medication, and diabetes mellitus was defined as fasting plasma glucose ≥ 126 mg/dL or use of antidiabetic medication.

Laboratory Measurements

Blood samples were taken from all patients in the morning after an overnight fast. The levels of hemoglobin (photometrically), platelets (impedance method), highly sensitive C-reactive protein (hs-CRP) (immunoturbidimetric method) were determined. Lipid panels [triglycerides, total cholesterol (enzymatic colorimetric method), high-density lipoprotein (HDL) (homogeneous enzymatic colorimetric method)] and complete blood counts were measured using a Beckman Coulter LH 780 device (Mervue, Galway, Ireland). Low-density lipoprotein (LDL) levels were calculated using the Friedewald formula.

Echocardiography Measurements

Echocardiographic data were obtained by an experienced cardiologist using the Vivid S5-dimensional cardiovascular ultrasound system (General Electric Vingmed, Horten, Norway). Standard American Society of Echocardiography guidelines for images and techniques were followed. ASI-β was used as a substitute indicator of arterial stiffness and calculated in the following way:

$$ASI\text{-}\beta = \ln(SBP/DBP)/[(Asd-Add)/Add]$$

(ASI-β = Aortic Stiffness Index beta, ln = Logarithm Natural BP, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, Asd = Asending Aorta Systolic Diameter, Add = Asending Aorta Diastolic Diameter)

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess the normality of the numerical data. According to the distribution pattern, continuous variables were presented as mean ± standard deviation or median with quartiles (Q1-Q3), and Student's T-test or Mann-Whitney U-test was used for comparisons between groups. Categorical variables were expressed as numbers and percentages, and comparisons between groups were

assessed using chi-squared and Fisher exact tests. Pearson and Spearman correlation analyses were used for the relationship between ASI-β and numerical data. A p value < 0.05 was considered to be statistically significant. All statistical analyses were carried out using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 108 consecutive patients were analyzed in the study, including 49 (45.4%) females and 59 (54.6%) males with the mean age of 58.2 ± 11.1 years. Demographic characteristics did not differ between the PD and HD groups. The rate of spironolactone user was higher in the PD group, for other drug users, there were no significant differences between the groups. Blood pressure levels and heart rate were similar between groups. The detailed demographic and clinical data of the patients were presented in Table 1.

Mean LDL (158.1 ± 19.8 vs. 147.5 ± 25.5 mg/dL, p = 0.026) and median triglyceride (220 vs 1975 mg/dL, p = 0.045) was higher in PD group than the HD group. Other laboratory results did not differ significantly among the groups (Table 2).

Table 1. Distribution of demographic and clinic findings of study populations

Variables	Peritoneal dialysis n = 40	Hemodialysis n = 68	*p value*
Demographic findings			
Age (years)	59.8 ± 11.3	58.7 ± 10.9	0.618
Male gender, n (%)	22 (55.0)	37 (54.4)	0.999
BSA (m^2)	1.8 ± 0.1	1.9 ± 0.2	0.200
BMI (kg/m^2)	24.9 ± 3.0	25.0 ± 3.6	0.842
Smoking, n (%)	22 (55.0)	45 (66.2)	0.306
Hypertension, n (%)	28 (70.0)	43 (63.2)	0.533
Diabetes mellitus, n (%)	10 (25.0)	17 (25.0)	0.999
Hyperlipidemia, n (%)	25 (62.5)	50 (73.5)	0.281
Use of drugs, n (%)			
Beta blocker	18 (45.0)	21 (30.9)	0.152
ACEi	17 (42.5)	23 (33.8)	0.413
CCB	25 (62.5)	33 (48.5)	0.169
Spironolactone	15 (37.5)	9 (13.2)	0.007*
Alpha blocker	4 (10.0)	1 (1.5)	0.118
Number of drugs	2 (0-3)	1 (0-2)	0.957
Duration of CRF, months	46 (29-74)	45 (30-66)	0.573
Clinic finding			
Heart rate (bpm)	73.4 ± 3.6	73.2 ± 3.5	0.741
Systolic BP (mm Hg)	146.5 ± 25.2	143.5 ± 22	0.523
Diastolic BP (mm Hg)	79.1 ± 12.3	81.4 ± 12.6	0.356
Pulse pressure (mm Hg)	67.4 ± 21.2	62.1 ± 16.7	0.154
MAP (mm Hg)	101.6 ± 14.6	102.1 ± 14.4	0.848

Numerical variables were shown as mean ± standard deviation or median (Q1-Q3). Categorical variables were shown as number (%). BSA = body surface area, BMI = body mass index, ACEi = angiotensin converting enzyme inhibitor, CCB = calcium channel blockers, BP = blood pressure, CRF = chronic renal failure, MAP = mean arterial pressure

Table 2. Laboratory findings of study populations

Variables	Peritoneal dialysis n = 40	Hemodialysis n = 68	*p value*
Hemoglobin (g/dL)	13.3 ± 1.4	13.3 ± 1.2	0.796
Leukocyte (×10^3/µL)	5.8 ± 1.5	6.1 ± 2.1	0.300
Neutrophil (×10^3/µL)	3.8 ± 0.7	3.9 ± 1.0	0.509
Platelet (×10^3/µL)	319.3 ± 77.5	312.4 ± 85.1	0.678
HbA1C (%)	5.7 ± 0.8	5.6 ± 0.6	0.508
Glucose (mg/dL)	109 (92-150)	98 (87-121)	0.103
Sodium (mmol/L)	139.2 ± 7.2	136.0 ± 13.5	0.181
Potassium (mmol/L)	4.6 ± 0.4	4.4 ± 0.5	0.173
Calcium (mg/dL)	8.6 ± 0.2	8.7 ± 0.2	0.074
Phosphorus (mmol/L)	4. ± 1.1	4.7 ± 1.2	0.404
Albumin (g/dL)	39.3 ± 6.6	38.4 ± 6.2	0.471
Cholesterol (mg/dL)	252.7 ± 24.3	243.0 ± 30.9	0.092
LDL (mg/dL)	158.1 ± 19.8	147.5 ± 25.5	0.026*
HDL (mg/dL)	40.5 ± 11.0	44.7 ± 14.0	0.103
Triglyceride, mg/dL	220(180-263)	197 (160-236)	0.045*
Hs-CRP (mg/dL)	5 (1.3-9.0)	3 (1.5-7.6)	0.377
Ferritin (ml/ng)	649.2 ± 200.3	705.2 ± 203.7	0.168
Parathyroid hormone (pg/mL)	312 (222-367)	240 (130-345)	0.720

Numerical variables were shown as mean ± standard deviation or median (Q1-Q3). HbA1C = hemoglobin A1C, LDL = low-density lipoprotein, HDL = high-density lipoprotein, Hs-CRP = high-Sensitivity C-Reactive Protein

Mean left ventricle (LV) end diastolic volume, mean LV end systolic volume, mean LV diastolic diameter and mean LV systolic diameter levels were lower in the PD group, while mean interventricular septum diameter and mean posterior wall diameter levels was higher. Mean aortic strain (5.6 ± 1.9 vs. 9.4 ± 2.8, $p < 0.001$) and median distensibility (1.5 vs. 2.9 cm, $p < 0.001$) were lower in the PD group than the HD group, while median ASI-β (11.6 vs. 6.2, $p < 0.001$) and mean E/e' (10.6 ± 2.9 vs. 9.2 ± 2.3, $p = 0.006$) were higher in the PD group. The rate of concentric hypertrophy was higher in the PD group (47.5% vs. 23.5%, $p = 0.005$) (Table 3).

DISCUSSION

This study of dialysis patients without known CAD has demonstrated that ASI-β levels, reflecting arterial stiffness, were significantly higher in patients with PD compared to patients with HD. This also provided new insights, as this is the first study comparing ASI-β as a surrogate of arterial stiffness in patients with PD and HD. ASI-β offer different implications that may allow it to be used as a potential screening tool for high-risk dialysis patients at risk for cardiovascular disease and events.

Approximately 40% of dialysis patients have cardiovascular disease prior to dialysis, and CAD accounts for the majority of hospitalizations for cardiovascular reasons in these patients [10]. An accelerated atherosclerosis process caused by mechanisms such as increased inflammation, oxidative stress, sympathetic activation, and endothelial dysfunction associated with ESRD plays a role in the pathophysiology [2]. Arterial stiffness, an important predictor of both atherosclerosis and cardiovascular events, physiologically increases with age and in-

Table 3. Echocardiography measurements in haemodialysis and peritoneal dialysis patients

Variables	Peritoneal dialysis n = 40	Hemodialysis n = 68	*p value*
LVEF (%)	57.2 ± 3.4	56.6 ± 5.4	0.553
sPAB (mm Hg)	32.8 ± 5.6	32.3 ± 8.4	0.876
LV EDV (mL/m^2)	57.0 ± 8.8	62.8 ± 9.5	0.002*
LV ESV (mL/m^2)	24.4 ± 4.2	27.2 ± 5.4	0.006*
LVDD (mm)	45.9 ± 1.7	47.7 ± 2.8	< 0.001*
LVSD (mm)	25.4 ± 3.1	27.1 ± 3.3	0.009*
IVSD (mm)	11.3 ± 1.1	10.5 ± 1.0	0.001*
PWD (mm)	10.6 ± 1.1	9.8 ± 1.1	0.001*
Aortic strain (×10^2)	5.6 ± 1.9	9.4 ± 2.8	< 0.001*
Distensibility (cm)	1.5 (1.3-2.0)	2.9 (2.5-3.5)	< 0.001*
ASI-β	11.6 (9.1-14.7)	6.2 (5.0-7.3)	< 0.001*
RWT (mm)	0.48 ± 0,04	0.42 ± 0.03	< 0.001*
LV mass index (g/m^2)	106.4 ± 14.5	93.0 ± 19.3	< 0.001*
LAVI (mL/m^2)	32.3 ± 4.5	32.0 ± 4.3	0.708
LV diastolic function			
Peak E velocity (cm/sec)	87.8 ± 18.3	85.4 ± 15.6	0.462
Peak A velocity (cm/sec)	66.2 ± 15.6	68.0 ± 14.1	0.521
E/A	1.4 (1.3-1.7)	1.4 (1.3-1.6)	0.174
DT, ms	180.9 ± 41.8	193.2 ± 49.8	0.192
Peak e' (cm/s)	8.5 ± 1.8	9.6 ± 2.1	0.005*
Peak a' (cm/s)	12.2 ± 2.6	11.4 ± 2.6	0.128
S'	10.2 ± 1.8	10.6 ± 2.1	0.292
E/e'	10.6 ± 2.9	9.2 ± 2.3	0.006*
LV remodeling, n (%)			
Normal geometry	8 (20.0)	32 (47.1)	0.005*
Concentric remodeling	13 (32.5)	16 (23.5)	
Eccentric hypertrophy	0	4 (5.9)	
Concentric hypertrophy	19 (47.5)	16 (23.5)	

Numerical variables were shown as mean ± standard deviation or median (Q1-Q3). Categorical variables were shown as number (%). LVEF = Left ventricle ejection fraction, sPAB = systolic pulmonary artery pressure, LVEDV = left ventricle end-diastolic volume, LVESV = left ventricle end-systolic volume, LVDD = left ventricle diastolic diameter; LVSD = left ventricle systolic diameter, IVSD = interventricular septum diameter; PWD = posterior wall diameter, ASI = aortic stiffness index, RWT = relative wall thickness, LAVI = left atrial volume index, DT = deceleration time

travascular pressure [8]. On the other hand, arterial stiffness is affected by the course of diseases like diabetes mellitus, hypertension, and ESRD, which plays a role in accelerating atherosclerosis [11]. However, the question of which type of dialysis leads to atherosclerosis or cardiovascular disease remains controversial. Previous epidemiological studies reported that the rates of hypertension and diabetes mellitus were similar in patients with HD and PD, whereas rate of CAD was lower in patients with PD and interestingly, PD

patients were associated with increased mortality [12, 13]. In contrast, a recent epidemiological study by Sun *et al*. [14] matched PD and HD patients for age, sex, duration of dialysis treatment, and comorbid conditions, and showed that PD was associated with lower cardiovascular events. These matching parameters play an important role in the acceleration of atherosclerosis, including arterial stiffness [15]. In the current study, there was no significant difference in dialysis duration, as well as demographic and comorbid characteristics between PD and HD patients, but susceptibility to atherosclerosis trended toward PD.

The inconsistency observed in the epidemiological studies mentioned above was also present in the conflicting results of previous studies evaluating arterial stiffness in PD and HD patients [15-18]. In a study measuring PWV in vivo and epigastric artery stiffness in vitro, arterial stiffness was not significantly different in PD and HD patients [16]. In another study, PWV values measured in both the carotid-radial and dorsalis pedis arteries were not significantly different between PD and HD patients. [15]. Similar results were reported in another study of PD and HD patients with similar age and comorbid conditions [17]. In contrast to these studies, increased carotid-femoral PWV has been shown to be associated with increased vascular endothelial dysfunction in PD patients [18]. Another study reported that arterial stiffness measured by brachial- ankle PWC was higher in HD patients [19]. This was supported by the results of a study of serial measurement of brachial- ankle PWV [20]. These inconsistencies between studies may be related to differences in dialysis treatment times between groups, different measurement methods such as carotid-femoral PWV, carotid-radial PWV, OR brachial-ankle PWV, or high measurement values of diagnostic devices [21].

In the current study, ASI-β levels were higher than the range of 4.2-5.3 previously reported only in PD patients [9, 22]. This may be related to the lower mean age range of previous studies. Compared to HD patients, PD patients had higher levels of ASI-β. The type of hemodialysis may contribute to the atherosclerotic process by having different effects on the cardiovascular system. In the early years of treatment, PD, which offers advantages such as hemodynamic stability, continuous ultrafiltration, and the absence of an arteriovenous fistula, may allow prolonged maintenance of residual renal function and diuresis and better fluid and blood pressure control [23]. This is consistent with study results showing no change in serial PWV measurements in PD patients over a 1-year follow-up [24, 25]. In the following periods, with the decrease in peritoneal ultrafiltration capacity and residual diuresis, hypervolemia may develop and blood pressure control may deteriorate [23]. In addition, the presence of higher LDL and triglyceride levels in patients with PD may lead to an exaggerated inflammatory response due to the accumulation of atherogenic lipids and uremic toxins associated with the biocompatibility of the peritoneal fluid [18]. This may lead to an acceleration of atherosclerosis and an increase in the risk of cardiovascular disease or events. [17].

The higher rate of LV concentric hypertrophy of PD patients was consistent with previous studies [26, 27]. LV concentric hypertrophy associated with increased arterial stiffness [28], has been associated with fluid overload in PD [29]. It has been suggested that higher LV myocardial index in PD patients may indicate chronic hypervolemia, which may impair arterial distensibility function [30]. Consistent with this hypothesis, PD patients had low distensibility levels. Volume status, blood pressure, and arterial stiffness/distensibility affect afterload and preload and decrease coronary perfusion. This may lead to diastolic dysfunction [31]. The fact that the E/e' ratio, which is an indicator of diastolic dysfunction, was higher in PD patients supported the susceptibility of these patients to diastolic dysfunction.

Limitations

The main limitation of this study was the small sample size and the single-center design of the study. Another important limitation was the lack of serial measurement parameters, especially arterial stiffness. This may limit the prognostic significance of changes in arterial stiffness in pre-dialysis patients for cardiovascular risk assessment.

CONCLUSION

PD patients have higher arterial stiffness and lower

distensibility levels compared to HD patients. Therefore, patients with PD may be more prone to diastolic dysfunction, cardiovascular disease, and events

Authors' Contribution

Study Conception: TG, DT, SA; Study Design: TG, DT, SA; Supervision: TG, DT; Funding: TG, SA; Materials: TG, SA; Data Collection and/or Processing: TG, DT; Statistical Analysis and/or Data Interpretation: TG, DT; Literature Review: TG, SA; Manuscript Preparation: TG, DT, SA and Critical Review: TG, DT, SA.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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Exploring the role of inflammatory parameters in predicting isthmocele formation following planned cesarean section: a study in patients with a history of one previous cesarean

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ABSTRACT

Objectives: Isthmocele is a hypoechoic area within the lower uterine segment myometrium, resulting from a discontinuation of the myometrium at the site of a previous cesarean scar. The aim of this study was to examine the influence of maternal cellular and inflammatory status prior to Cesarean Section (CS) on isthmocele formation.

Methods: This prospective observational study was conducted in a tertiary hospital and included women with a history of one previous CS. The inflammatory and cellular parameters were collected and ultrasonographic examinations were conducted in the 6th postpartum month and then analyzed. Logistic regression analysis was performed to identify potential factors influencing isthmocele formation.

Results: Of the 106 patients, 31 (29.2%) were diagnosed with isthmocele after one previous CS. There were no significant differences in terms of demographical variables between the groups. However, the duration of CS was significantly longer in the isthmocele group compared to the group without isthmocele (42.58 ± 8.77 vs. 38.42 ± 9.50 minutes, $p = 0.03$). The neutrophil-to-lymphocyte ratio (NLR) was higher and platelet-to-lymphocyte ratio (PLR) was lower in the isthmocele group ($p < 0.001$). Logistic regression analysis revealed that, NLR (OR [odds ratio]: 0.23, 95% CI [confidence interval]: 0.117- 0.473, $p < 0.001$) and PLR (OR: 1.05, 95% CI: 1.027-1.078, $p < 0.001$) were identified as independent predictors for isthmocele formation after planned CS.

Conclusions: Inflammatory markers, such as NLR and PLR, may contribute to the formation of isthmocele in women with a history of one previous CS, shedding light on the underlying pathophysiology.

Keywords: Cesarean scar defect, isthmocele, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

Uterine scar defects, also known as isthmoceles or niches, have gained significant attention in recent years due to their connection to adverse reproductive outcomes and potential clinical implications [1]. Isthmocele refers to a specific type of cesarean scar defect characterized by a localized pouch or indentation

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within the anterior uterine wall. This condition can occur when the healing process after a cesarean section (CS) delivery is incomplete or when the scar formation is faulty, leading to various gynecological symptoms and reproductive complications [2]. Understanding the risk factors associated with isthmocele development is essential for optimizing patient care, guiding prevention strategies, and improving reproductive outcomes. Furthermore, the presence of isthmoceles has been linked to several clinical manifestations, including abnormal uterine bleeding, chronic pelvic pain, infertility, and complications during subsequent pregnancies, such as cesarean scar pregnancy and placenta accreta spectrum disorders [3, 4].

The formation of isthmoceles is believed to have a multifactorial etiology, involving a complex interaction of patient-related, surgical, and obstetric factors [5]. Although the precise mechanisms responsible for isthmocele formation have yet to be fully understood, it is widely accepted that impaired scar healing, insufficient vascularization, and suboptimal tissue repair play a role in their development and persistence [3, 6]. Uterine wound healing is a complex and dynamic process that plays a critical role in the development of isthmoceles. After a CS, the uterine incision goes through a series of intricate biological events, including inflammation, cell migration, extracellular matrix remodeling, and tissue regeneration [7, 8]. The delicate balance between these processes is vital for achieving optimal scar formation and subsequent healing. Disruption of this intricate healing process can result in impaired scar tissue formation and contribute to the development of isthmoceles [6, 8, 9]. Understanding the mechanisms and factors that influence uterine wound healing is crucial for identifying key determinants of isthmocele formation. Previous animal studies have consistently demonstrated the critical roles of neutrophils, platelets, and monocytes in the process of wound healing and tissue maturation [10]. Furthermore, peripheral blood cells, such as neutrophils, lymphocytes, and monocytes, serve as essential biomarkers for evaluating systemic immunity. The ratios between these different cell types, namely the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), have been extensively used as markers for assessing the systemic inflammatory response [11].

This study aimed to investigate the potential impact of maternal cellular and inflammatory status on isthmocele formation.

METHODS

This prospective observational study was conducted at a university-affiliated tertiary hospital from March 2020 to February 2022. The study obtained approval from the local ethics committee (2011-KAEK-25 2019/02-18), and informed consent was obtained from the participating patients, adhering to the principles outlined in the Declaration of Helsinki.

The study focused on women between the ages of 20 and 40 years who had a scheduled CS due to a history of one previous CS. Inclusion criteria consisted of term pregnancies between 37 and 40 weeks, singleton pregnancies, a body mass index (BMI) of 18-25 kg/m2, pregnancy follow-up and delivery at the study hospital, and no known history of isthmocele. Exclusion criteria included emergency CS, multiple previous CSs, connective tissue diseases, a history of gestational diabetes or preeclampsia, uterine anomalies, fetal anomalies, a previous history of isthmocele, smoking, and the use of any medication.

Initially, 217 patients were assessed for eligibility. Demographic characteristics, preoperative laboratory values, intraoperative and postoperative data were recorded for these patients before the planned CS. Ultrasonographic examinations were performed in the 6th month postpartum to evaluate the presence of isthmocele. A total of 106 patients were included in the final analysis after excluding those who underwent CS surgery other than the standard procedure, those who experienced intraoperative complications, those who required additional treatment in the postoperative period, those who needed blood transfusion, those who underwent additional medical or surgical interventions within the 6-month period, and those who were lost to follow-up (Fig. 1).

The CS technique utilized in our hospital follows a standardized approach for all patients. Prior to the procedure, each patient receives intravenous 2 gr cefazolin antibiotic prophylaxis. The surgery begins with a pfannenstiel incision, and the subcutaneous tissue and fascia are separated through sharp dissection. The abdominal cavity is then accessed using a blunt tech-

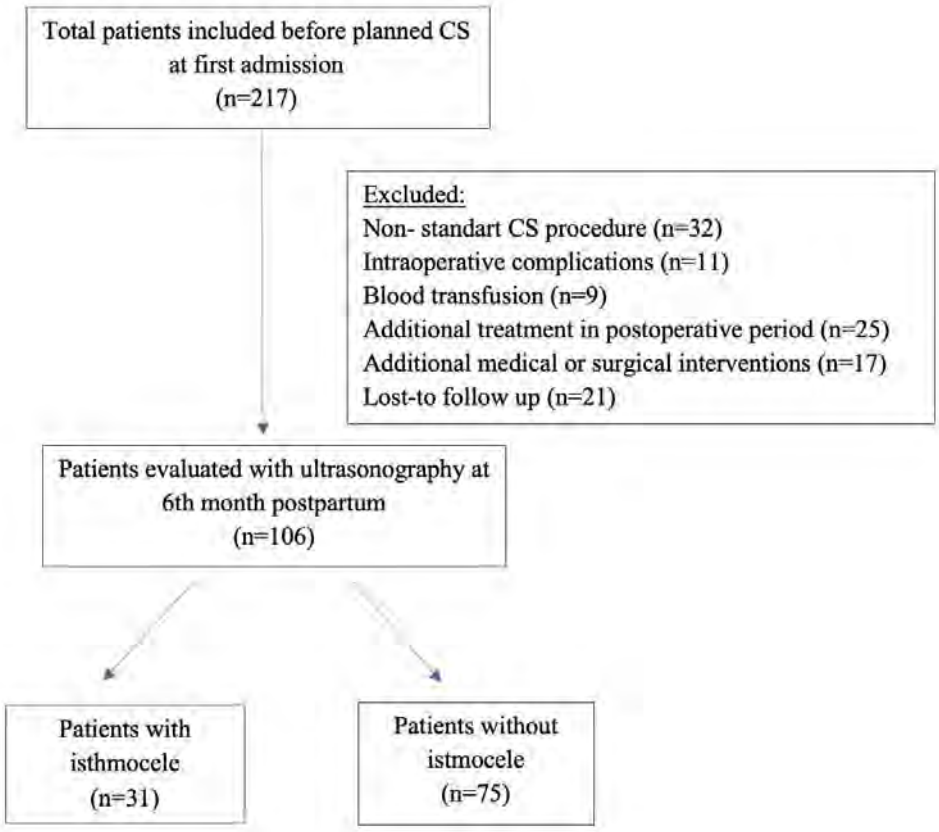

Fig. 1. Flowchart of the study.

nique. For the uterine incision, a lower segment transverse-Kerr incision is made without detaching the bladder. Following the delivery of the baby, the placenta and its attachments separate naturally, and the cervical opening is examined using sterile gloves. The placental bed is cleaned using dry gas, and the uterine scar is closed using a single-layered continuous polyglactin suture. Once bleeding is controlled, the peritoneum is closed with 2/0 polyglactin sutures, and the fascia is closed using a number 1 polyglactin suture. In cases where the subcutaneous tissue thickness is less than 2 cm, the skin is closed subcuticularly using 3/0 sharp prolene sutures, and the wound is dressed without further skin suturing.

In the postpartum 6th month, transvaginal ultrasonography (TVUSG) was conducted by a single doctor (G.G.) to ensure consistency and minimize interobserver differences. Various measurements were taken, including uterine length, width, endometrial thickness, niche-istmocele depth, residual myometrial thickness, and myometrial tissue adjacent to the scar.

Patients with a uterine scar depth of 2 mm or more in the sagittal section of ultrasonography were considered positive for the presence of isthmocele. At the end of the 6th month, the patients were categorized into two groups based on TVUSG findings: those with isthmocele and those without isthmocele. These two groups were then compared in terms of clinical, demographic, cellular, and inflammatory variables. Inflammatory parameters, including the NLR, MLR and PLR, were among the variables considered and analyzed.

Statistical Analysis

The statistical analysis was performed using SPSS version 25.0 (Statistical Package for Social Sciences Inc., Chicago, IL, USA). The normality of the distribution of variables was assessed using the Shapiro-Wilk test. For the comparison of continuous variables between groups, the Mann-Whitney U test was utilized. The chi-square test was employed for categorical variables. Logistic regression analysis was conducted to identify potential cofactors that may influence the formation of isthmocele. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In the study, out of the 106 patients included, 75 (70.7%) did not have an isthmocele, while 31 (29.2%) were diagnosed with an isthmocele. The demographic and clinical characteristics of the patient groups are summarized in Table 1. There were no significant differences between the groups in terms of age, BMI, presence of systemic disease, gestational week, placental position, fetal presentation, and birth weight (Table 1). However, the duration of the CS was longer in the isthmocele group compared to the group without isthmocele (42.58 ± 8.77 vs. 38.42 ± 9.50 minutes, $p = 0.03$) (Table 1). Blood loss, duration of hospitalization, and breastfeeding were similar between the groups.

The laboratory results of the two groups are presented in Table 2. Preoperative hemoglobin (Hb) and fibrinogen values showed no significant differences between the groups. However, the preoperative

Table 1. Comparison of clinical and demographic characteristics and birth follow-up outcomes among patient groups.

	Patients without isthmocele (n = 75)	Patients with isthmocele (n = 31)	*p* value
Age (years)	28.85 ± 4.99	30.06 ± 4.87	0.260
Gravida	2 (2-6)	2 (2-4)	**0.013**
Parity	1 (1-3)	1 (1-3)	**0.015**
BMI (kg/m^2)	29.31 ±2.55	28.88 ± 0.50	0.316
Systemic disease, n (%)			
No	62 (82.7)	28 (90.3)	0.386*
Yes	13 (17.3)	3 (9.7)	
Birth week	38.48 ± 0.50	38.58 ± 0.50	0.348
Placental position, n (%)			
Fundal/lateral	58 (77.3)	21 (67.7)	0.271*
Anterior extending incision	5 (6.7)	1 (3.2)	
Posterior	12 (16.0)	9 (29.0)	
Fetal presentation, n (%)			
Vertex	72 (96.0)	29 (93.5)	0.533*
Breech	2 (2.7)	2 (6.5)	
Podolic	1 (1.3)	0 (0)	
Anesthesia type, n (%)			0.419*
Spinal	65 (86.7)	24 (77.4)	
Combined	3 (4.0)	3 (9.7)	
General	7 (9.3)	4 (12.9)	
Duration of C- section (min)	38.42 ± 9.50	42.58 ± 8.77	**0.030**
Volume of blood loss (mL)	436.80 ± 120.02	424.19 ± 99.89	0.617
Birth weight (g)	3406.40 ± 283.78	3339.83 ± 299.34	0.272
Duration of hospitalization (h)	50.32 ± 7.30	49.54 ± 5.99	0.620
Duration of breast-feeding (days)	187.25 ± 42.73	184.61 ± 39.13	0.542

Values are given mean ± standard deviation or median (minimum-maximum). BMI = body mass index
Mann whitney-U test was performed unless otherwise specified.
$P < 0.05$ was significant. *Chi-square test was used.

Table 2. Comparison of laboratory results among patient groups

	Patients without isthmocele (n = 75)	Patients with isthmocele (n = 31)	*p* value
Preoperative Hb (g/dL)	10.76 ± 1.13	11.20 ± 1.21	0.101
Preoperative WBC ($\times 10^3/\mu L$)	9.81 ± 2.53	9.63 ± 2.13	0.997
Preoperative neutrophils ($\times 10^3/\mu L$)	7.78 ± 1.73	8.43 ± 1.30	0.051
Preoperative lymphocytes ($\times 10^3/\mu L$)	2.02 ± 0.56	1.90 ± 0.63	0.221
Preoperative monocytes ($\times 10^3/\mu L$)	0.54 ± 0.18	0.57 ± 0.20	0.582
Preoperative platelets ($\times 10^3/\mu L$)	323.58 ± 323.12	216.87 ± 29.78	**< 0.001**
Preoperative fibrinogen (mg/dL)	428.81 ± 63.70	445.41 ± 77.17	0.326
NLR	4.12 ± 1.36	4.91 ± 1.67	**0.015**
MLR	0.28 ± 0.12	0.32 ± 0.12	0.183
PLR	183.52 ± 260.21	126.01 ± 40.92	**0.045**

Hb = hemoglobine, WBC = White blood cell, NLR = Neutrophil-to-lymphocyte ratio, MLR = Monocyte-to-lymphocyte ratio, PLR = Platelet-to-lymphocyte ratio

platelet count was higher in the group without isthmocele compared to the isthmocele group (323.58 ± 323.12 vs. 216.87 ± 29.78, $p < 0.001$) (Table 2). The NLR and PLR also exhibited significant differences between the groups. In patients with isthmocele, the NLR was higher, while the PLR was lower compared to those without isthmocele (Table 2).

Logistic regression analysis was conducted to determine factors associated with isthmocele formation, considering age, NLR, MLR and PLR. Among patients with a history of one previous CS, NLR (OR [odds ratio]: 0.23, 95% CI [confidence interval]: 0.117- 0.473, $p < 0.001$) and PLR (OR: 1.05, 95% CI: 1.027-1.078, $p < 0.001$) were identified as independent predictors for isthmocele formation after planned CS (Table 3).

DISCUSSION

The objective of this study was to investigate the cellular and inflammatory factors involved in the development of isthmocele following planned CS in patients with a history of one previous CS. Among the 106 patients included in the analysis, 29.2 % were diagnosed with isthmocele, while 70.7 % did not have isthmocele. The demographic and clinical characteristics of the patient groups were comparable, indicating a similar baseline between the two groups. However, it was observed that the duration of CS was significantly longer in the isthmocele group compared to the group without isthmocele. Interestingly, the laboratory analysis revealed distinct differences between the groups. Specifically, the preoperative platelet count

Table 3. Logistic regression analysis of factor effecting isthmocele presence

Variables	OR	95 % CI	*p* value
Age	1.01	0.907-1.136	0.796
NLR	0.23	0.117-0.473	**< 0.001**
MLR	0.55	0.003-114.318	0.826
PLR	1.05	1.027-1.078	**< 0.001**

NLR = Neutrophil-to-lymphocyte ratio, MLR = Monocyte-to-lymphocyte ratio, PLR = Platelet-to-lymphocyte ratio, Model summary: $R^2 = 0.46$, $p < 0.001$

was significantly lower in patients with isthmocele, suggesting a potential role of platelet dysfunction in the pathogenesis of isthmocele. Additionally, the elevated NLR and decreased PLR observed in the isthmocele group may serve as biomarkers reflecting the inflammatory and immune response associated with isthmocele formation. Logistic regression analysis further identified key factors contributing to isthmocele formation, including NLR and PLR.

The prevalence of isthmocele can vary significantly, ranging from 6.9% to 69%, depending on the study population and methodology employed [1, 12]. It is crucial to acknowledge that the reported prevalence may be subject to change based on the criteria used to define isthmocele. Different diagnostic techniques, such as gel/saline instillation sonohysterography, have been associated with higher prevalence rates of up to 84% [12, 13]. The presence of symptoms can also impact the incidence of isthmocele. Asymptomatic patients with isthmocele may be underestimated, as clinicians may not always recognize it as a potential cause of symptoms, possibly due to a lack of awareness [14]. Furthermore, the prevalence of isthmocele tends to increase with an increasing number of previous CSs [1]. In our study, we observed an incidence of 29.2% for isthmocele in patients with one previous CS. Additionally, it is important to consider the technique used for uterine closure during CS, as it can potentially influence the development of isthmocele. In our study, we specifically utilized one-layer continuous sutures for uterine closure, which may have implications for the occurrence and prevalence of isthmocele.

To the best of our knowledge, this is the first study to investigate the relationship between NLR, MLR, PLR and isthmocele formation. Our findings revealed that higher NLR and lower PLR during the peripartum period were associated with an increased risk of isthmocele formation. It is worth noting that isthmocele formation can be considered as a condition related to inadequate healing of the uterine wound. In normal circumstances, when an injury occurs, neutrophils migrate to the wound site through chemotaxis. They release enzymes to fight infection and remove dead tissue [15]. However, if neutrophils become exhausted, abnormal fibrin fibers can accumulate, delaying wound healing [16]. Increased NLR may indicate impaired healing. Additionally, the balance between platelets and lymphocytes, derived from the same stem cells, is crucial for homeostasis. Abnormal platelet levels caused by systemic inflammation can directly impair wound healing. In cases of abnormal blood cell production, the PLR may temporarily decrease due to faster platelet depletion [17]. Maintaining proper functioning and balance of neutrophils, platelets, and lymphocytes is vital for optimal wound healing and immune response.

In a study investigating wound healing after neck surgery, a higher NLR and lower PLR were found to be associated with postoperative wound complications [18]. Similarly, another study involving pregnant women reported significantly higher NLR and lower PLR in women with preeclampsia compared to healthy pregnancies [19]. The authors of these studies linked these altered levels to impaired inflammation [19]. Another study conducted in people who had elective mesh surgery for groin hernia, high NLR in the preoperative period was found to be significantly associated with postoperative surgical site infection and decrased wound healing [20]. All these mentioned studies show that NLR and PLR are important markers in predicting the course of the inflammatory process. Therefore, it can be inferred that an enhanced inflammatory response during pregnancy could lead to endothelial dysfunction and incomplete wound healing, potentially contributing to the formation of isthmocele.

Limitations

This study has several limitations that should be acknowledged. Firstly, the data was collected from a single center, which may limit the generalizability of the findings to other settings. Secondly, the diagnosis of isthmocele was based solely on TVUSG, without the use of additional diagnostic modalities such as saline infusion sonography or hysteroscopy, which could potentially impact the accuracy of the isthmocele diagnosis. Moreover, the study focused specifically on patients with one previous CS, and it is unclear whether these patients had a previous diagnosis of isthmocele before the current pregnancy. This information could have provided further insights into the relationship between previous isthmocele and its recurrence.

Despite these limitations, there are notable strengths to our study. The prospective nature of the data collection allowed for the accurate assessment of variables and minimized the potential for recall bias. Additionally, the standard surgical methods used for every patient ensured consistency in the surgical approach, reducing the confounding effects of different techniques. Furthermore, the inclusion of patients with comparable demographic characteristics enhanced the validity of the findings. Importantly, this study is the first to evaluate the relationship between inflammatory parameters and isthmocele formation, providing novel insights into the potential mechanisms underlying its development.

CONCLUSION

In conclusion, there was a significant difference in the preoperative NLR and PLR values between patients who developed isthmocele and those who did not. These findings suggest that the inflammatory parameters NLR and PLR may serve as potential biomarkers for predicting isthmocele formation. Further research is needed to validate these findings and explore the underlying mechanisms linking inflammation to inadequate wound healing and isthmocele development. Nevertheless, these results contribute to our understanding of isthmocele pathogenesis and may have implications for risk assessment and preventive strategies in patients undergoing CS with a history of one previous cesarean.

Authors' Contribution

Study Conception: AE; Study Design: AE, NKE; Supervision: NKE; Funding: AE; Materials: AE, GG; Data Collection and/or Processing: GG; Statistical Analysis and/or Data Interpretation: NKE; Literature Review: GG; Manuscript Preparation: AE and Critical Review: NKE.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The effect of glycemic control on sleep quality in type 2 diabetes mellitus

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ABSTRACT

Objectives: There are publications showing sleep quality is impacted in type 2 diabetes mellitus (T2DM) cases. In our study, we planned to assess the prevalence of sleep disorder in these patients compared to society, and investigate whether poor glycemic regulation and increased body mass index (BMI) caused disruption of sleep quality or not.

Methods: Sleep quality was compared between patients followed in our clinic with T2DM (n = 534) for minimum 5 years and a control group (n = 269). Assessment was performed for whether increased glycated haemoglobin (HbA1c) and increased BMI caused an increase in Pittsburgh Sleep Quality Index (PSQI) score or not. Cases with any comorbid disease or drug use affecting sleep quality were excluded from the study.

Results: T2DM patients had higher PSQI points compared to the control group. A statistically significant, very low-level positive correlation was identified between BMI measurements and PSQI scores (as BMI increased, PSQI increased). A statistically significant, very low-level positive correlation was identified between HbA1c measurements and PSQI scores (as HbA1c increased, PSQI increased). HbA1c measurements of those in the good sleep quality group were significantly lower compared to those in the moderate sleep quality and poor sleep quality groups. The BMI measurements in the poor sleep quality group were significantly higher than those in the good sleep quality group.

Conclusions: The sleep quality of T2DM cases was worse compared to the control group, while the increase in HbA1c level further disrupted sleep quality. The increase in BMI is another factor disrupting sleep quality in diabetic patients.

Keywords: Glycemic control, type 2 diabetes mellitus, sleep quality, glycated haemoglobin

Type 2 Diabetes Mellitus (T2DM) is a disease requiring continuous medical care, progressing with increased insulin resistance in the liver and muscles mainly [1]. If appropriate medical treatment is not received or necessary precautions not taken, a variety of microvascular and macrovascular complications develop associated with the disease in the long term. Apart from these well-known and defined complications, there are a range of diseases and situations lowering quality of life in T2DM. Negative changes in sleep quality is one of these.

The daily mean sleep duration recommended for adults is 7-9 hours [2]. Quality sleep assists in preserving mental health, physical health and quality of life. For the initiation and continuation of sleep, functions must occur in many cortical and subcortical brain re-

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gions. To initiate sleep, firstly, there must be cyclical stimuli from the pre-hypothalamus and the ventrolateral preoptic nucleus in the hypothalamus is accepted as playing a role in line with endogenous chemical stimuli. Sleep comprises two periods of rapid eye movement (REM) and non-rapid eye movement (NREM) [3]. Just as disruption in any of these systems may cause sleep disorder, it is necessary for these systems to operate flawlessly for quality sleep.

The circadian rhythm and sleep control important daily physiological situations for metabolic health. All sleep disorders like inadequate sleep, narcolepsy, short sleep, continuous insomnia, sleep apnea, circadian rhythm disruption, and shift work may contribute to metabolic disruption. Sleep disorders and circadian rhythm disorders associated with metabolic irregularity cause visceral fattening by changing the amount and time of food intake and changing and disrupting energy metabolism leading to inflammation, insulin resistance and glucose intolerance contributing to weight gain, obesity and T2DM. The importance of disruptions to the circadian rhythm and sleep disorders is understood by considering the rapidly increasing prevalence of metabolic diseases [4]. We aimed to compare the sleep quality of patients with T2DM diagnosis with the normal population and to research whether there was a correlation between glycemic regulation and sleep disorder frequency.

METHODS

The study was a prospective and controlled study performed between July 2021-October 2021. A total of 803 people, including 534 T2DM cases and 269 non-diabetic controls, who applied to the internal medicine polyclinic were included in the study. The diagnosis of diabetes was based on a previous diagnosis of T2DM or a random plasma glucose level of 200 mg/dL or higher, together with classic features of DM, such as polyuria, polydipsia, polyphagia and weight loss,or a fasting blood glucose level of > 126 mg/dL or glycated haemoglobin (HbA1c) levels of 6.5% or higher. The cases were included in the study with a diagnosis of T2DM for a minimum of 5 years. The control group included non-diabetic patients with similar sex and age distribution to the diabetic group. The demographic data, comorbid diseases patients were questioned and recorded. The Pittsburgh Sleep Quality Index (PSQI) was completed by the researcher, an internal medicine specialist, when consent was obtained from patients.

Exclusion criteria for the study groups were the existence of any comorbid diseases of congestive heart failure, coronary artery disease, chronic obstructive pulmonary disease, obstructive sleep apnea,; insomnia, neurodegenerative disease like alzheimer, dementia, parkinson; terminal period chronic disease or malignancy; receiving any treatment crossing the blood-brain barrier and disrupting sleep patterns (antihistaminic, antiepileptic, antidepressant use etc.), working shift work, Type 1 Diabetes Mellitus and pregnancy. Ethical committee approval was obtained for our study (Decision no: 2021/514/204/17).

Pittsburgh Sleep Quality Index

The PSQI was developed by Buysse *et al*. [5] and subjectively assesses sleep disorders. The PSQI is a scale about sleep difficulties examined in seven groups of subjective sleep quality, sleep duration, sleep latency, habitual sleep activity, sleep disorders, use of sleeping drugs and daytime function disorder. The re-

Table 1. Distribution of descriptive characteristics

	Data
Sex	
Female	465 (57.90)
Male	338 (42.10)
Age (years)	
Mean ± SD	55.82 ± 10.06
Median (Min-Max)	56 (22-89)
BMI (kg/m^2)	
Mean ± SD	29.09 ± 5.12
Median (Min-Max)	28 (19.10-43)
Glucose (mg/dL)	
Mean ± SD	131.72 ± 69.15
Median (Min-Max)	103 (76-490)
HbA1c (%)	
Mean ± SD	6.84 ± 2.07
Median (Min-Max)	6.40 (4.10-18.60)

SD = standard deviation

sponse to each component is given points from 0-3, and the general PSQI points from 0 to 21 are the total of the seven components. According to the points, patients are divided into 3 groups as those with points ≤ 5 in the good sleep quality group; PSQI 6-8 in the moderate sleep quality group and PSQI ≥ 9 in the poor sleep quality group. The PSQI is a subjective sleep quality assessment tool.

Statistical Analysis

For statistical analyses the Number Cruncher Statistical System (NCSS) 2007 program was used (Kaysville, Utah, USA). When assessing study data, descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used. The fit of quantitative data to normal distribution was tested with the Shapiro-Wilk test, graphical investigations and Mann-Whitney U test used for comparisons between two groups. For comparison of quantitative variables without normal distribution in more than two groups, Bonferroni correction, the Kruskal Wallis test and Dunn-Bonferroni test were used. Assessment of correlations between quantitative variables used the Spearman correlation analysis. Statistical significance was accepted as $p < 0.05$.

RESULTS

The research was performed with a total of 803 cases (534 T2DM, 269 controls) including 57.90% females (n = 465) and 42.10% males (n = 338) attending the internal diseases clinic from July 2021 to October 2021. The ages of the cases varied from 22 to 89 years, with mean age identified as 55.82 ± 10.06 years (Table 1).

The sleep duration questioned within the scope of the PSQI was mean 7.05 ± 4.32 hours in the T2DM group, while it was mean 4.76 ± 3.34 hours in the control group. According to groups, there were statistically significant differences in the Pittsburgh scores of cases ($p = 0.001$ and $p < 0.01$).

Points received by cases with T2DM on the PSQI were higher than points received by those in the control group, with the T2DM group having worse sleep quality. According to group, there were statistically significant differences identified between the sleep quality of cases ($p = 0.001$ and $p < 0.01$) (Table 2).

Those in the control group had higher good sleep quality rates, while those in the patient group had higher moderate and poor sleep quality rates. There was a positive (as BMI increased, PSQI score increased), statistically significant, very low level of correlation identified between the BMI measurements of participants with PSQI scores (r = 0.178; $p = 0.001$ and $p < 0.01$).There was a positive (HbA1c increased, PSQI score increased), statistically significant, very low level of correlation identified between HbA1c measurements of participants with PSQI scores (r = 0.217; $p = 0.001$ and $p < 0.01$) (Table 3).

There were statistically significant differences in sleep quality of cases according to BMI measurements ($p = 0.001$ and $p < 0.01$). With the aim of determining the source of the difference, two-way comparisons found the BMI measurements in the group with poor

Table 2. Assessment of PSQI according to groups

	DM patients	Control	*p value*
PSQI score			**[a]0.001****
Mean ± SD	7.05 ± 4.32	4.76 ± 3.34	
Median (Min-Max)	6 (0-21)	4 (1-19)	
Sleep quality, n (%)			**[b]0.001****
Good	237 (44.40)	203 (75.50)	
Moderate	119 (22.30)	42 (15.60)	
Poor	178 (33.30)	24 (8.90)	

PSQI = Pittsburgh Sleep Quality Index, SD = standard deviation

[a]Mann Whitney U Test, [b]Fisher Freeman Halton Test, **$p < 0.01$

Table 3. Correlation of PSQI points with BMI and HbA1c

		PSQI
BMI (kg/m^2)	r	0.178
	p value	**0.001****
HbA1c (%)	r	0.217
	p value	**0.001****

PSQI = Pittsburgh Sleep Quality Index, BMI = Body Mass Index, HbA1c = Glycated haemoglobin glycated haemoglobin r = Spearman Correlation Coefficient

sleep quality were significantly higher compared to those in the group with good sleep quality ($p = 0.001$ and $p < 0.01$). According to HbA1c measurements, there were statistically significant differences identified for the sleep quality of cases ($p = 0.001$ and $p < 0.01$). Two-way comparisons performed with the aim of determining the source of the difference found the HbA1c measurements in the group with good sleep quality were significantly lower compared to those with moderate sleep quality and with poor sleep quality ($p = 0.001$, $p = 0.001$ and $p < 0.01$) (Table 4).

DISCUSSION

Our study researching the effect of T2DM on sleep quality and whether there is a connection between HbA1c and PSQI score indicates that patients with T2DM diagnosis had higher PSQI scores than the control group. Rajendran *et al*. [6] identified mean PSQI score as 7.08 in a study including 120 T2DM cases, and while this situation was associated with poor sleep quality in T2DM, the same study did not observe a correlation between HbA1c and PSQI scores. Cho *et al*. [7] found 49% had poor sleep quality among 614 individuals with T2DM, while HbA1c levels were not associated with poor sleep quality. Telford *et al*. [8] could not form a correlation between HbA1c levels with sleep quality in a study of 279 T2DM cases. However, Tang *et al*. [9] showed weak glycemic control among those with inadequate sleep in a study investigating 551 cases with T2DM diagnosis. A study by Zhu *et al*. [10], similarly, found an inverse correlation between high HbA1c level with sleep quality, with individuals with HbA1c of 7% and above having significantly higher PSQI scores. Bener *et al*. [11] showed that individuals with HbA1c level 7% and below had better sleep quality compared to individuals with levels above 7% in a study investigating 871 T2DM cases. Tsai *et al*. [12] identified a significant correlation between HbA1c level with PSQI score among 46 T2DM cases. They showed a very strong statistically significant correlation between increased HbA1c level and poor sleep quality in T2DM cases. Cappuccio *et al*. [13] showed sleep quality was consistent in predicting T2DM development risk in a meta-analysis including 10 studies and 3586 T2DM cases. As mentioned in the article, while studies by Rajandran *et al*. [6], Cho *et al*. [7] and Telford *et al*. [8] did not show a correlation between poor sleep quality and HbA1c, Tang *et al*. [9], Zhu *et al*. [10], Bener *et al*. [11] and Tsai *et al*. [12] showed a correlation between poor sleep quality and poor glycemic index, as

Table 4. Assessment of sleep quality according to BMI and HbA1c measures

	Sleep Quality			*p value*
	Good	**Moderate**	**Poor**	
BMI (kg/m^2)				**[c]0.001****
Mean ± SD	28.40 ± 5.00	29.32 ± 5.10	30.41 ± 5.15	
Median (Min-Max)	27.20 (19-53)	28.50 (20-47)	30.50 (19-46)	
HbA1c (%)				**[c]0.001****
Mean ± SD	6.55 ± 2.12	7.06 ± 1.96	7.30 ± 1.99	
Median (Min-Max)	5.80 (3.90-16.30)	6.60 (3.90-13.60)	6.80 (4.70-18.60)	

BMI = Body Mass Index, HbA1c = Glycated haemoglobin glycated haemoglobin
[c]Kruskal Wallis Test, **$p < 0.01$

illustrated in our study. Experimental studies support our study results. A variety of studies showed that ≤ 6 hours sleep was associated with glucose intolerance and insulin resistance [14], increased diabetes incidence [15, 16] and high diabetes risk [17]. A meta-analysis investigating 12 studies reported that the pooled probability rate was 1.09 between exposure to shift work and diabetes risk [18]. Buxton *et al*. [19] showed a relative reduction in insulin sensitivity following circadian misalignment and sleep loss in cases with 5.6 hours sleep limitation in 24 hours during experimental studies with 45 cases. T2DM is a disease causing disruption of sleep quality. At the same time, low level of correlation was observed between HbA1c level with PSQI score. The HbA1c values in the group with good sleep quality were identified to be significantly lower compared to the group with poor sleep quality. It appears that sleep quality is disrupted by a significant level after T2DM has developed. These are the most powerful results obtained from our study. Just as there are studies supporting our results, there are publications that are not in line with our results.

Additionally, another result obtained in our study was between poor glycemic control and BMI. There were statistically significant differences observed between sleep quality of cases according to BMI measurements and two-way comparisons to determine the source of this difference identified that BMI measurements in the group with poor sleep quality were significantly high compared to those in the group with good sleep quality. Similarly, a study investigating 1031 cases showed a correlation between poor sleep index and high BMI, with high BMI causing a reduction in sleep duration [20]. A study by Vargas *et al*. [21] including 515 participants identified that 51% of individuals with BMI > 25 kg/m2 had PSQI score above 5moderate or poor sleep quality. In reducing the sleep quality of BMI; increased sympathetic activity, changes in cortisol, leptin and ghrelin levels and insulin resistance have beel suggested as factors explaining this relationship [22].

In our study, the median BMI value was 28 kg/m^2, and T2DM and obesity frequently accompany each other. Just as glycemic outcomes in T2DM may cause disrupted sleep quality, we think increased BMI may contribute to disruption of sleep quality. The results of our study support this idea.

The results of our study are consistent with the general literature results in terms of both T2DM-sleep quality and BMI-sleep quality. Strong aspects of our study are the adequate case numbers, balanced distribution of age and sex in the case and control groups, assessment of patient BMI, exclusion criteria (especially apnea, OSAS, CAD), confirmation of results with two-way statistical comparisons, assessment by a single internal diseases specialist when completing case assessment and PSQI index in the clinical section of the study and standardization specific to the study by using the subjective PSQI index. Apart from experimental studies, no study investigating T2DM-sleep quality included a non-diabetic control group and generally compared the T2DM group with nondiabetic population when investigating the relationships with HbA1c and PSQI score. All these reasons make our study unique and valuable.

Limitations

In spite of being able to assess glycemic fluctuations and especially nighttime hypoglycemia with continuous glucose monitoring (CGM), CGM is not sufficiently common or easily accessible in our country. For this reason, the presence of nocturnal hypoglycemia cannot be excluded. Though it may be considered that some of our patients were controlled according to HbA1c level, a significant portion of these cases experienced proven severe hypoglycemia. We think glycemic fluctuations reduce sleep quality. OSAS is generally an obesity problem, and our study excluded those with diagnosis of cases describing apnea. Though not a practical approach, exclusion of OSAS among our cases without a polysomnographic study by a chest diseases expert is a limitation.

CONCLUSION

In conclusion, we identified a positive low level significant correlation between HbA1c level with PSQI score. Our T2DM cases had significantly high PSQI scores, and poor sleep quality. Diabetic patients should be questioned about sleep quality and those with advanced sleep disorder should be assessed in detail. It should be considered that low sleep quality is both a cause and an outcome in uncontrolled DM patients.

After better glycemic control in diabetic patients with regulated treatment, repeated sleep scales may further explain this topic.

Authors' Contribution

Study Conception: ZK, BB, SA, ÖK, NA; Study Design: ZK, BB, SA, ÖK, NA; Supervision: ZK, BB, SA, ÖK, NA; Funding: ZK; Materials: ZK; Data Collection and/or Processing: ZK; Statistical Analysis and/or Data Interpretation: ZK; Literature Review: ZK; Manuscript Preparation: ZK and Critical Review: ZK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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Is semantic feature analysis effective when applied intensively? A randomized pilot study with non-fluent aphasic individuals

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ABSTRACT

Objectives: In this study, it was investigated the effects of intensive aphasia treatment applied to individuals with non-fluent aphasia.

Methods: Sixteen patients diagnosed with non-fluent aphasia were included in the study and were randomly divided into two groups. The therapy interventions were one day per week for the eight patients in the first group, for a total of 8 hours in 2 months (standard intervention). For the eight patients in the second group, therapy was applied for a total of 48 hours in 2 months, for one hour per day, six days a week, excluding Sundays (intensive intervention). Participants were tested using the Turkish aphasia test (ADD), Aphasia Impact Scale-21 (AIQ-21), and Boston Naming Test (BNT) before starting the treatment (pretest), after the treatment (posttest), and one month after the treatment ended (follow-up).

Results: At the end of the treatments, a significant increase in ADD and BNT scores and a significant decrease in AIQ-21 scores were observed in both groups. Although there was a change in the follow-up test, the scores were still significantly different than the pretest scores. The rate of improvement in test scores of group II patients who received intensive aphasia treatment was superior to the group I patients.

Conclusions: Intensive application was superior to once-weekly aphasia treatment, and post-treatment improvement continued for at least one month after the treatments.

Keywords: Non-fluent aphasia, semantic feature analysis, intensive therapy, therapy effectiveness, randomized controlled trial

Aphasia is the loss of the ability to use speech and language skills as a result of lesions in the speech and language regions in the brain [1]. Aphasia usually occurs after a stroke and can lead to isolation, passivity, and depression, i.e., secondary psychological and psychiatric symptoms, due to deficiencies in using language and poor communication [2, 3].

The primary goal in the treatment of patients with aphasia is management and maximizing patients' language and communication skills, activities, and participation. Recently, positive results have been obtained with the collaboration of speech and language therapists, patient with aphasia, and their relatives and caregivers [4]. In a meta-analysis investigating the

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effectiveness of speech and language therapy for patients with aphasia, it was revealed that speech and language therapy benefited language production, functional communication, and comprehension of people with aphasia according to the results of the 27 studies reviewed by [4]. Although the optimal treatment intensity for aphasia rehabilitation is unknown, treatment intensity is very important for effective and efficient aphasia rehabilitation, in a study investigating the effectiveness of treatment intensity in the rehabilitation of patients with aphasia [5, 6]. Bhogal *et al*. [6] reported that more intensive therapy in less time (8.8 hours of treatment per week for 11.2 weeks) had more positive results than less therapy over a longer period (2 hours per week for 22.9 weeks). Recently described Semantic Feature Analysis (SFA) is a word retrieval process that acts by reinforcing disrupted semantic networks [7]. The SFA treatment protocol uses a "feature analysis chart" that includes action, group, use, location, features, and associations. Numerous studies have demonstrated that SFA has positive effects, especially in naming [8-10].

In this study, we aimed to investigate the results of SFA administered at two different intensities to individuals with aphasia. Turkish aphasia test (ADD), Aphasia Impact Questionnaire -21 (AIQ-21), and Boston Naming Test (BNT) were applied before starting the therapy, after the therapy, and one month after the therapy to evaluate the results of the therapies.

METHODS

Participants

Ethics approval for this randomized controlled trial was received from the Bahcesehir University Clinical Research Ethics Committee. Using the GPOWER 3.1 program, it was determined that the minimum sample size required to provide the power of the test (1-β) = 0.90 and the effect size 0.80 was 15 people, 8 people in each group. The study included 16 patients with a previous stroke and were diagnosed with non-fluent aphasia in MRI imaging and language-speech skills evaluations. Inclusion criteria for the study were not having any additional neurological disease diagnosis, having Turkish as their native language, being at least a high school graduate, being between 40-65 years old, and being able to understand the instructions given. Exclusion criteria were defined as having severe sequelae due to previous neurological diseases, being younger than 40 or older than 65, having a history of using psychoactive substances other than tobacco, having been diagnosed with mental retardation, having a history of head trauma with loss of consciousness, brain tumor, history of neurosurgery and/or intracranial implant, and being pregnant or using birth control pills. Participants determined according to the inclusion and exclusion criteria were listed and divided into two groups of eight, each using the computer-assisted randomization technique.

The interventions were applied at Bahçeşehir University, Speech and Language Therapy Unit. After the participants were determined for the study, a test before intervention (pretest) was used. The treatment design was inspired by Stahl *et al*. [11]. The therapy interventions were one day per week for the eight patients in the first group, for a total of 8 hours in 2 months (standard intervention). For the eight patients in the second group, therapy was applied for a total of 48 hours in 2 months, for one hour per day, six days a week, excluding Sundays (intensive intervention). The same therapist performed therapy interventions. The test after intervention (posttest) was applied after the therapy interventions, and one month after the therapy sessions were completed, the follow-up test was applied. Another speech and language therapist performed the pretest, posttest, and follow-up tests. Thus, we tried to eliminate the bias effect. Demographic In-

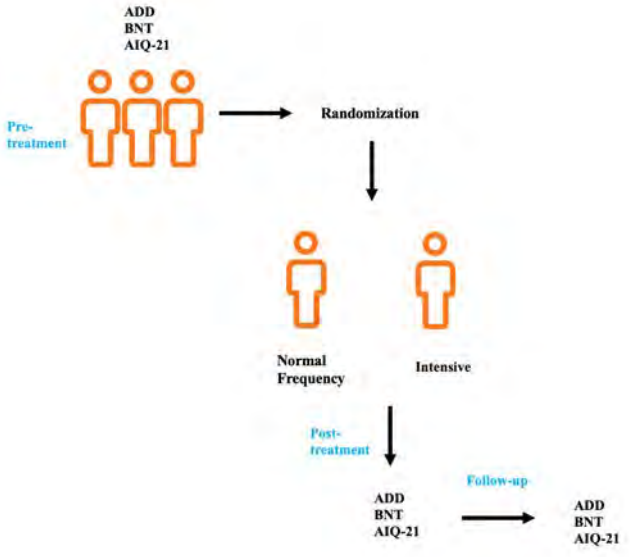

Fig. 1. Study design

formation Form, ADD, AIQ-21, and BNT were applied to the participants in the study (Fig. 1).

Randomization and Masking

The trained therapist completed baseline assessments before randomizing participants using a computer-generated block randomization sequence (permutated block of six, with 1:1 randomization to two groups to achieve an overall ratio of 1:2 via Research Electronic Data Capture (REDCapTM) [12]. Randomization was performed by the baseline assessor, who was not otherwise involved in the trial and participants were stratified by aphasia severity determined by the WAB-R (AQ) score. Participants, family members, and outcome assessors were not informed of group allocation, and all participants and trial staff were asked to refrain from discussing the treatment received. Only the therapist wrote in medical notes per healthcare standards and all research documentation was stored separately in a secured location to avoid unblinding. The blinded outcome assessor was not involved in the participants' stroke care and was not permitted to ask participants about treatment received during follow-up assessments. Only treating therapists were unblinded to treatment allocation.

Semantic Feature Analysis Treatment

In this analysis, a verbal word-picture matching task was designed to determine the framework and target of the treatment to be applied and to evaluate the treatment results. Each picture set in the designed 100-word-picture matching included four separate images, three distractors, and one target image. A target of 20 new words was studied in each session. After completing the targeted 100 words, 25-word repetition and generalization studies are carried out in order in the follow-up sessions. Photographs or color drawings of natural objects were used as picture stimuli. The target images were selected from different categories: clothing, objects (accessories), vehicles, body parts, and food and beverages. The three distractors in the target image were designed as semantic distracters, phonological distractors, and familiarity distractors. Previously, Tunçer [13] dealt with words in 6 categories: animals, vegetables and fruits, vehicles, clothes, body parts and furniture in his research with various age groups. In this study, the word frequency tables reported by Tunçer [13] were taken into account and words with high, medium and low usage frequency and functionality in daily language were used.

In the verbal word-picture matching task, participants were asked to identify the target picture in their picture set by verbally requesting, "Show me the _." Selected target images were randomly presented to the participants in three separate sessions over a week. Targets detected incorrectly on two or more occasions were selected for use in the treatment program.

SFA sets were prepared on a white A4 (29.7 × 21.0 cm) sheet. In the middle of the page was a large photograph or colored illustration (14 × 11 cm) of the target item. Around this photograph were written four pairs of printed words representing semantic features. One of the items in each semantic trait pair was consistent with the treatment goal, while the other was a distractor. Semantic features were presented in pairs to enable the participant to actively participate in semantic processing by making a correct and informed decision. The semantic properties comprised six options: Category, Use, Action, Properties, Location, and Associations.

Treatment

Pretreatment

The verbal word-picture matching task, as previously stated, was performed in three consecutive but separate situations over the course of one week. Before each treatment, word-picture matching tasks were presented to the participant in random order, and no feedback was provided as to whether the participant's response was correct. ADD, BNT, and AIQ-21 tests were administered to all participants the day before the therapy, and the scores obtained by the participants were recorded.

Treatment

The therapies were administered for the following eight weeks. Patients in the first group received eight therapy sessions once a week, one hour a day. The patients in the second group received a total of 48 therapy sessions of one hour a day, six days a week. In each session, the participant was asked to say what the middle image in each picture was. For each item, questions were asked around the image: Category: "This is a...", Usage: "used for...", Action: "What to do with

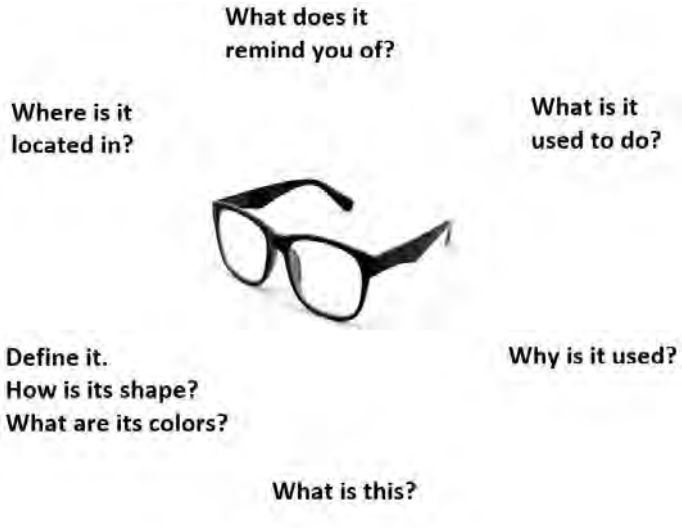

Fig. 2. Semantic Feature Analysis (SFA) Therapy Material (Turkish Language)

it?", Attributes: "Describe how," Location: "Where do we use it?", Relationship: "reminds me of…"

If the participant still has not named the image, they are told what it is and then asked to repeat it back. In the process of naming the target concept and forming the meaning network related to the concept, clues can be given to the participants to express the semantic features of the target concept verbally and they can be guided to benefit from the diagram. After the participants name it, the practicing clinician maps the semantic feature to the appropriate box and directs them to create relevant sentence structures (Fig. 2-Turkish version of the therapy material).

Post-Treatment

ADD, BNT and AIQ-21 tests were performed again on the participants one day after the completion of the therapy process. Thus, the effectiveness of the therapy was evaluated. One month after completion of the treatment phase, a follow-up evaluation was performed to determine whether changes in comprehension persisted long-term following treatment discontinuation.

Tests

Turkish Aphasia Test (ADD)

ADD was developed by Toğram and Maviş [14] for individuals with brain injury (a) to determine their performance in all language areas, (b) to diagnose aphasia, and (c) to help select appropriate therapy targets. Toğram [15] conducted a validity, reliability and standardization study for ADD applied to healthy individuals with stroke. ADD consists of 8 subsections that evaluate language and speech characteristics. These evaluation tests are spontaneous language and speech, auditory comprehension, repetition, naming, reading, grammar, speech, action and writing subsections. A high score indicates the effective use of language and speaking skills. Only the first 4 sections of the test were used and their total score is 162. The first 4 sections of the test are the sections that evaluate primary language and speaking skills such as speaking fluency, auditory comprehension, repetition, and naming. The remaining sections are sections that assess reading and writing skills in an academic context. The language and speaking skills of the participants are currently being evaluated, and they cannot be successful in writing tasks because their right side is paralyzed. For this reason, parts of advanced academic skills are not included. The reliability coefficients of the subsections of the ADD test are between 0.94 and 0.99, and the reliability coefficient for the overall test is 0.99 [15].

Boston Naming Test (BNT)

BNT, developed by Kaplan *et al*. [16], is currently the best-known neuropsychological tool for evaluating language skills, including object naming and word retrieval. BNT is used for neuropsychological evaluation in children, adults, and elderly individuals with different clinical pathologies such as communication disorders, aphasia, dementias, or brain lesions.

Aphasia Impact Questionnaire-21 (AIQ-21)

AIQ-21 is an aphasia-friendly scale that is administered face-to-face to individuals with aphasia and aims to evaluate the quality of life of individuals [17]. In the scale, there are a total of 21 items consisting of 3 sub-sections, namely communication, participation, and emotional state. The use of a large font, the fewest texts and simple pictures in the entire scale, and the repetition of the word "this week" at the beginning of each question, aim to support the aphasic individual to understand the scale more easily. In addition, the positive question sentences used in some questions aim to instill the thought of "you have positive things to do."

All questions in the scale inquire about how the

last one week of the aphasic individual has been, and the person is asked to rate the difficulty experienced between 0-4, with 4 being the worst and 0 being the best. The problems experienced by individuals with aphasia in this process are in the person's inwardness. Since the primary purpose of interventions and approaches is to maximize the participation of the individual, the perception of quality of life in the scale is derived directly from the answers of the individual.

Validity and Reliability of the Intervention

The scales which were developed by the researcher used in this study calculated the reliability of the tests. Cronbach's Alpha reliability coefficient was considered in these calculations. The reliability of the scales used in the research was previously ensured. A speech and language therapist carried out all pretest, posttest, and follow-up test interventions 7 years of experience, and all these interventions were recorded via a recording device. The whole process from the beginning to the end was recorded, steps such as answering and scoring were fully controlled, and any errors were prevented from entering the process. Inter-observer (evaluator) reliability intervention was used for the data obtained from the pretest, posttest, and follow-up tests. In the interventions, the score made by the researcher for the recordings was compared with the score made by the independent observer, and the consistency between the observers was checked. The higher agreement means the more reliable scoring. Moreover, the existence of an agreement between the researcher and the independent observer can be interpreted as the researcher measuring the target behaviors. A calculated agreement of 80-100% indicates that the agreement between the researcher and the other independent observer is reliable [18].

In the research, pretest, posttest, and follow-up test applications were recorded with a voice recorder, and 25% of the data randomly selected from the data was listened to carefully by two independent expert language and speech therapists. The relevant tests were scored and transferred to the data collection forms. Scoring was done according to the application protocol of the related tests, and reliability was calculated by using the intra-class correlation calculation technique, considering the evaluation scores of two independent experts. While calculating the reliability, the formula "Reliability = (Agreement) / (Agreement + Disagreement) × 100" was used [18]. The compatibility between these scores was checked, and the inter-observer reliability was calculated (Table 1). The "Therapy Applications Evaluation Form" developed by the researcher was used to show the application reliability of the study. In this form, there are a total of 7 questions consisting of a 5-point Likert structure (1-Lowest, 5-Highest). This form is intended for the observer to evaluate the therapist and therapy process for the language therapy method applied to participants with aphasia. Therapist gives the participants enough information about the sessions, the therapist follows the relevant therapy process, the therapist follows the known clue steps in the naming process, the suitability of the method used by the therapist, the suitability of the material used by the therapist, the therapist adjusting the therapy time correctly, and both observers gave almost the highest score to all items related to the appropriateness.

Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, descriptive statistics were given as mean, standard deviation, frequency, and percentage. The conformity of the quantitative data to the normal distribution was tested with the Shapiro-Wilk test and graphical examination. Repeated measures ANOVA was used for within-group comparisons of normally distributed quantitative variables and Bonferroni-adjusted pairwise comparisons were used for pairwise compar-

Table 1. Inter-observer agreement values

Test	Before Intervention	After Intervention	One month After Intervention
ADD	94%	94%	99%
BNT	98%	91%	92%
AIQ-21	88%	86%	98%

isons. Student's t-test was used to compare the difference in questionnaire scores according to the frequency of therapy. Statistical significance was accepted as $p < 0.05$.

RESULTS

Demographic and clinical details of the 16 participants included in the study are given in Table 2. Based on independent sample t-tests, the randomization procedure did not result in significant differences between Group I and Group II regarding age, education level, employment status, months after disease onset, and marital status (Table 3).

Changes in ADD, A-21, and BNT scores over time according to repeated measures ANOVA is given in Table 4. Pretest ADD mean score was 70.25 ± 4.58, BNT mean score was 21.06 ± 4.75, and AIQ-21 mean score was 76.43 ± 1.54. In the posttest performed after therapy, the mean ADD score increased to 97.43 ± 18.61, the mean BNT score increased to 39.25 ± 4.83, and the mean AIQ-21 score decreased to 66.00 ± 3.26. The change in all three tests was statistically significant ($p < 0.05$). In the follow-up tests performed one month after the treatment, the ADD total score was 93.75 ± 16.31, the BNT total score was 36.56 ± 5.03, and the AIQ-21 total score was 67.62 ± 3.77. Although the decrease in ADD and BNT and the increase in AIQ-21 were statistically significant compared to the posttest, the scores in the follow-up tests were still substantial compared to the pretest (Table 4).

A mixed ANOVA method was used to compare ADD, AIQ-21, and BNT changes according to treatment frequency (Table 5). Student t-test was used to compare changes in ADD, AIQ-21, and BNT scores according to treatment frequency after mixed ANOVA (Table 4). The 36.87 ± 5.55 point increase in the ADD score after the intensive intervention was significantly higher than the 17.50 ± 4.85 point increase in the ADD score after normal treatment ($t(14) = -2625$; $p = 0.022$) (Fig. 3). The increase in BNT score after normal intervention (16.12 ± 1.55) was similar to the increase in BNT score after intensive intervention (20.25 ± 1.55) ($t(14) = -1.887$; $p = 0.081$) (Fig. 4). The reduction rate in AIQ-21 score (13.37 ± 0.82) after the intensive intervention was significantly higher than the decrease rate in AIQ-21 score (7.50 ± 0.56) after normal inter-

Table 2. Demographic and clinical characteristics of the patients from the two groups

Group	Patient ID	Age	Sex	Education level	Time post-onset (months)	Aphasia Type	Hand Dominance	Paralysis	Severity
Group I	N6	51	M	University	11	Non-fluent	Right	Non-paralysis	Moderate
	N2	49	F	High school	17	Non-fluent	Right	Paralysis	Moderate
	N5	64	M	University	14	Non-fluent	Right	Non-paralysis	Mild
	N13	61	F	University	13	Non-fluent	Left	Paralysis	Mild
	N4	49	M	High school	19	Non-fluent	Right	Non-paralysis	Mild
	N10	58	F	High school	11	Non-fluent	Right	Non-paralysis	Moderate
	N16	57	F	High school	9	Non-fluent	Right	Non-paralysis	Moderate
	N8	45	M	High school	10	Non-fluent	Right	Non-paralysis	Moderate
Group II	N12	50	M	University	13	Non-fluent	Right	Paralysis	Mild
	N7	63	F	High school	14	Non-fluent	Right	Paralysis	Mild
	N11	59	F	University	14	Non-fluent	Left	Non-paralysis	Mild
	N14	56	M	High school	15	Non-fluent	Right	Non-paralysis	Moderate
	N9	53	F	High school	18	Non-fluent	Right	Non-paralysis	Moderate
	N3	52	M	University	11	Non-fluent	Right	Non-paralysis	Moderate
	N15	46	F	High school	12	Non-fluent	Right	Non-paralysis	Moderate
	N1	45	M	University	9	Non-fluent	Right	Paralysis	Moderate

Table 3. Comparison of demographic characteristics of patients in groups after randomization

		Group 1 (n = 8)	Group II (n = 8)	*t*	*p value*
Age (years)		54.25 ± 6.69	53.00 ± 6.18	0.388	0.704
Diagnosis time (months)		13.00 ± 3.50	13.25 ± 2.71	-0.160	0.876
Gender	Male	4 (50.0%)	4 (50.0%)	0.000	1.000
	Female	4 (50.0%)	4 (50.0%)		
Employment status	Yes	5 (62.5%)	5 (62.5%)	0.000	1.000
	No	3 (37.5%)	3 (37.5%)		
Educational status	High School	5 (62.5%)	4 (50.0%)	-0.475	0.642
	University	3 (37.5%)	4 (50.0%)		
Marital status	Married	7 (87.5%)	7 (87.5%)	0.000	1.000
	Single	1 (12.5%)	1 (12.5%)		

Data are shown as mean ± standard deviation or n (%). ᵗStudent t test

vention (t(14) = 5.582; p = 0.00004) (Fig. 5). Considering the difference between the follow-up test scores and the pre-test scores according to the frequency of treatment applied, the changes in ADD (t(14) = -3.18; p = 0.007) and AIQ-21 (t(14) = 3.793; p = 0.002) were significant. It was observed that the change in BNT (t(14) = -1.429; p = 0.175) was not statistically significant (Table 6).

DISCUSSION

In this study, the results of the modified SFA treatment method, which was applied to 16 patients diagnosed with aphasia, were compared in two different treatment intensities. In the posttest performed after both modified SFA treatments, significant changes were determined in ADD, BNT, and AIQ-21 total scores compared to the pretest. One month after the end of therapy, although there was a significant change in the follow-up test scores compared to the posttest, the follow-up test results were still statistically different from the pretest results. However, the test results of the patients to whom we applied intensive intervention were found to be significantly different from those who received the normal intervention.

Cognitive therapy and speech and language therapies are applied to people who develop aphasia after stroke to improve their communication skills. However, the issue still under discussion is the duration and intensity of speech and language therapy to be administered. However, recent studies have reported that intensive speech and language therapy is beneficial in patients with aphasia [19-21]. Especially in people with chronic aphasia, significant improvements in language skills have been achieved as a result of a training intensity of 5-10 hours per week [6]. Two different intensive therapies were compared by Stahl *et al*. [11]. The 30 patients included in the study were randomly divided into two groups and one of the groups was administered ILAT three days a week and four hours a day (total of 48 hours) for four weeks, while the other group was administered ILAT three times a week and two hours a day (total 24 hours) for four weeks. Stahl *et al*. did not detect any difference in therapies at the end of four weeks, but they demonstrated that prolonging the treatment period by even two weeks contributed to the improvement in chronic aphasia. In another study, Mohr *et al*. [22] documented that ILAT administered to individuals with chronic aphasia was effective not only on language recovery but also in depression symptoms in chronic aphasia.

SFA is a method that contributes to the process of verbalizing the semantic features of the targeted items by the aphasic person. SFA has been reported to be effective in different types of aphasia [7-10]. It has been observed that SFA gives positive results in both group and discourse therapies [23, 24]. In his study examin-

Table 4. Comparison of Turkish aphasia test (ADD), Aphasia Impact Scale-21 (AIQ-21), and Boston Naming Test (BNT) scores

	Pretest	Posttest	Follow-up test
ADD			
Spontaneous speech, language, and cognition assessment	9.06 ± 1.52	12.87 ± 1.92[a]	12.81 ± 1.79[a]
Spontaneous speech	1.93 ± 0.85	5.18 ± 3.03[a]	5.18 ± 3.03[a]
Understanding commands	7.00 ± 1.03	7.31 ± 0.79	7.18 ± 0.83
Understanding Yes / No questions	7.62 ± 1.36	8.18 ± 1.06	7.87 ± 1.02
Understanding objects	10.75 ± 0.93	10.75 ± 0.93	10.31 ± 1.19
Understanding the categories	8.62 ± 1.54	8.62 ± 1.54	8.31 ± 1.62
Understanding the details within the category	7.18 ± 1.22	7.81 ± 1.79	7.00 ± 1.96
Simple sentence matching	3.43 ± 1.75	4.43 ± 1.50[a]	4.06 ± 1.23
Complex sentence matching	3.81 ± 2.07	5.43 ± 2.36[a]	5.43 ± 2.36[a]
Repetition	4.37 ± 1.31	7.81 ± 3.74[a]	7.50 ± 3.48[a]
Categorical naming	0.31 ± 0.47	1.25 ± 1.00[a]	1.25 ± 1.00[a]
Naming by looking at the picture	3.31 ± 2.79	7.62 ± 5.03[a]	7.37 ± 4.51[a]
Noun naming	0.81 ± 1.10	4.81 ± 3.08[a]	4.37 ± 2.72[a]
Action naming	2.00 ± 1.31	5.31 ± 2.75[a]	5.06 ± 2.32[a]
Total score	70.25 ± 4.58	97.43 ± 18.61[a]	93.75 ± 16.31[a, b]
BNT	21.06 ± 4.75	39.25 ± 4.83[a]	36.56 ± 5.03[a, b]
AIQ-21			
Communication	21.37 ± 1.08	16.12 ± 1.70	17.31 ± 1.81
Participation	15.68 ± 0.47	11.00 ± 1.63[a]	11.31 ± 1.74[a]
Emotional state	39.37 ± 1.25	38.87 ± 1.08	39.00 ± 1.09
Total score	76.43 ± 1.54	66.00 ± 3.26[a]	67.62 ± 3.77[a, b]

Data are shown as mean ± standard deviation. Repeated measures ANOVA, [a]$p < 0.05$ compared with pretest, [b]$p < 0.05$ compared with posttest.

ing the effectiveness of the SFA, Boyle [24] observed that 16 of 17 participants improved their naming skills with pictures, according to the meta-analysis results of Efstratiadou *et al*. [25], it was determined that the duration of SFA applied was between 2 weeks and 12 weeks and varied between 315 minutes and 1500 minutes in 21 separate studies with a total of 55 participants. It has been reported that weekly treatment sessions are between two and four sessions, and the duration of the sessions varies between 45 minutes and 120 minutes; the most frequently applied session duration is one hour [26]. In the same meta-analysis, 45 participants (81.82%) found improvement in the naming of the trained items, and it was documented that 32 participants (58.18%) continued to name the trained items [26].

In this study, intensive intervention and normal intervention of SFA were compared. When comparing the results, ADD, BNT and AIQ-21 tests were used. In the pretest performed before starting the treatments, ADD, BNT, and AIQ-21 scores of both groups were similar. When the posttest results after eight weeks of treatment were compared with the pretest results, it was found that the ADD and BNT scores increased significantly, and the AIQ-21 score decreased significantly. According to these results, it can be said that the therapy applied in both groups was effective on language skills. However, when the differences in the

Table 5. Comparison of Turkish aphasia test (ADD), Aphasia Impact Scale-21 (AIQ-21) and Boston Naming Test (BNT) according to the frequency of treatment

		Pretest	Posttest	Follow-up Test
ADD				
Spontaneous speech, language, and cognition assessment	Group I	9.25 ± 1.83	12.37 ± 1.50	12.37 ± 1.18
	Group II	8.87 ± 1.24	13.37 ± 2.26	13.25 ± 2.25
Spontaneous speech	Group I	1.87 ± 0.83	4.87 ± 2.23	4.87 ± 2.23
	Group II	2.00 ± 0.92	5.50 ± 3.81	5.50 ± 3.81
Understanding commands	Group I	6.87 ± 0.99	7.25 ± 0.88	7.12 ± 0.83
	Group II	7.12 ± 1.12	7.37 ± 0.74	7.25 ± 0.88
Understanding Yes / No questions	Group I	7.62 ± 0.51	7.62 ± 0.51	7.37 ± 0.74
	Group II	7.62 ± 1.92	8.75 ± 1.16	8.37 ± 1.06
Understanding objects	Group I	10.87 ± 0.64	10.87 ± 0.64	10.62 ± 0.91
	Group II	10.62 ± 1.18	10.62 ± 1.18	10.00 ± 1.14
Understanding the categories	Group I	8.25 ± 1.83	8.25 ± 1.83	7.87 ± 1.64
	Group II	9.00 ± 1.19	9.00 ± 1.19	8.75 ± 1.58
Understanding the details within the category	Group I	6.62 ± 0.74	6.62 ± 0.74	5.75 ± 1.48
	Group II	7.75 ± 1.38	9.00 ± 1.77	8.25 ± 1.58
Simple sentence matching	Group I	3.75 ± 1.03	4.50 ± 1.06	4.12 ± 0.83
	Group II	3.16 ± 2.29	4.37 ± 1.92	4.00 ± 1.60
Complex sentence matching	Group I	4.37 ± 1.99	5.87 ± 1.55	5.87 ± 1.55
	Group II	3.25 ± 2.12	5.00 ± 3.02	5.00 ± 3.02
Repetition	Group I	4.50 ± 1.19	7.12 ± 3.27	6.87 ± 3.09
	Group II	4.28 ± 1.48	8.50 ± 4.27	8.12 ± 3.94
Categorical naming	Group I	0.37 ± 0.51	0.62 ± 0.74	0.62 ± 0.74
	Group II	0.25 ± 0.46	1.87 ± 0.83	1.87 ± 0.83
Naming by looking at the picture	Group I	3.87 ± 2.99	6.00 ± 5.12	5.50 ± 4.30
	Group II	2.75 ± 2.65	9.25 ± 4.68	9.25 ± 4.13
Noun naming	Group I	0.87 ± 1.24	3.37 ± 2.87	2.87 ± 2.41
	Group II	0.75 ± 1.03	6.25 ± 2.71	5.87 ± 2.23
Action naming	Group I	2.37 ± 1.59	3.62 ± 1.59	3.50 ± 1.41
	Group II	1.62 ± 0.91	7.00 ± 2.67	6.62 ± 1.99
Total score	Group I	71.50 ± 5.23	89.00 ± 15.16	85.37 ± 11.83
	Group II	69.00 ± 3.74	105.87 ± 18.71	102.12 ± 16.43
BNT	Group I	20.75 ± 3.05	36.87 ± 5.40	34.62 ± 4.56
	Group II	21.37 ± 6.23	41.62 ± 2.82	38.50 ± 4.98
AIQ-21				
Communication	Group I	21.75 ± 0.70	17.50 ± 1.06	18.50 ± 1.30
	Group II	21.00 ± 1.30	14.75 ± 0.88	16.12 ± 1.45
Participation	Group I	15.75 ± 0.46	12.12 ± 1.45	12.37 ± 1.59
	Group II	15.62 ± 0.51	9.87 ± 0.83	10.25 ± 1.16
Emotional status	Group I	38.75 ± 1.28	39.12 ± 0.83	39.50 ± 0.53
	Group II	40.00 ± 0.92	38.62 ± 1.30	38.50 ± 1.30
Total	Group I	76.25 ± 1.16	68.75 ± 1.03	70.37 ± 2.19
	Group II	76.62 ± 1.92	63.25 ± 2.12	64.87 ± 2.90

Data are shown as mean ± standard deviation. Mixed ANOVA, [a]$p < 0.05$ compared with pretest, [b]$p < 0.05$ compared with posttest

Table 6. The differences between the posttest and follow-up test results and the pretest results are according to the groups.

		Group I	Group II	t	*p value*
ADD	Post – Pre	17.50 ± 4.85	36.87 ± 5.55	**-2.625**	**0.022**
	Follow up – Pre	13.87 ± 3.75	33.12 ± 4.73	**-3.186**	**0.007**
BNT	Post – Pre	16.12 ± 1.55	20.25 ± 1.55	-1.887	0.081
	Follow up – Pre	13.87 ± 1.60	17.12 ± 1.60	-1.429	0.175
AIQ-21	Post – Pre	-7.50 ± 0.56	-13.37 ± 0.82	**5.582**	**0.0004**
	Follow up – Pre	-5.87 ± 1.05	-1.75 ± 1.12	**3.793**	**0.002**

Data are shown as mean ± standard deviation. [t]student t test.

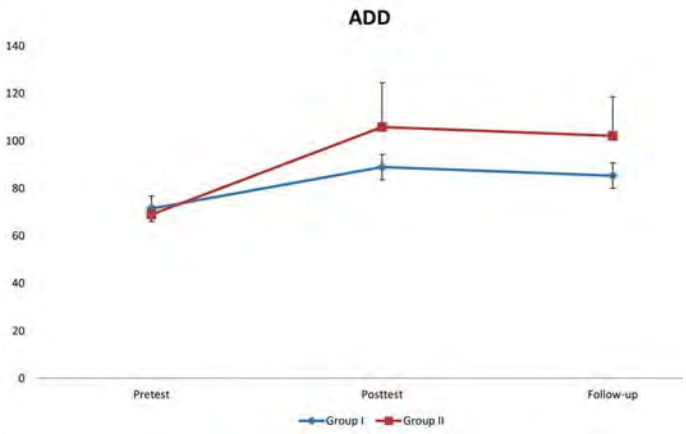

Fig. 3. Comparison of Turkish aphasia test (ADD) administered to the participants according to the frequency of treatment. Group I, Aphasic patients receiving therapy for one hour a day, once a week for eight weeks (total 8 hours); Group II, Aphasic patients receiving therapy for one hour a day, six days a week for eight weeks (total 48 hours).

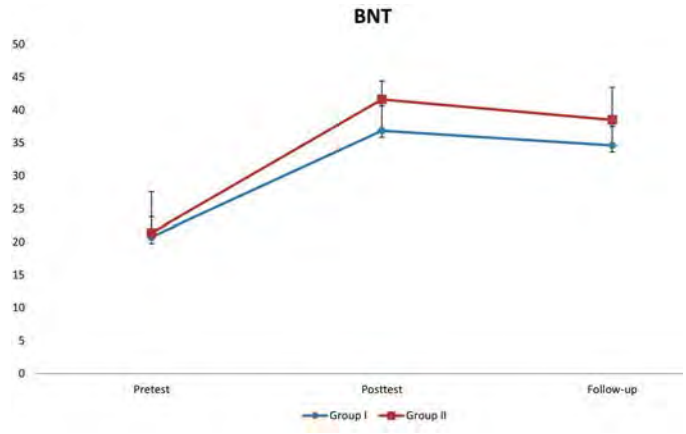

Fig. 4. Comparison of Boston Naming Test (BNT) administered to the participants according to the frequency of treatment. Group I, Aphasic patients receiving therapy for one hour a day, once a week for eight weeks (total 8 hours); Group II, Aphasic patients receiving therapy for one hour a day, six days a week for eight weeks (total 48 hours).

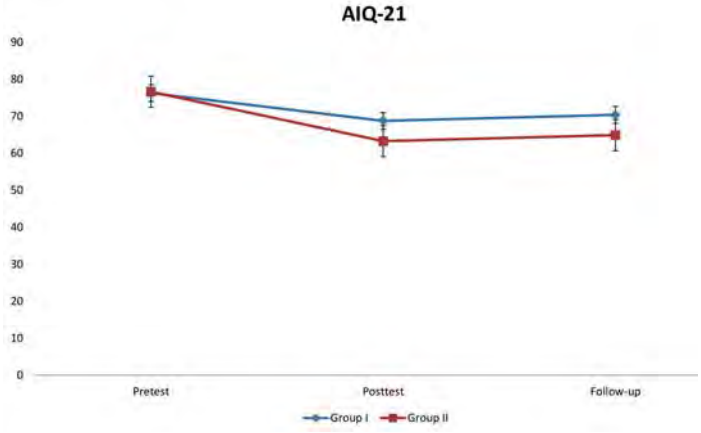

Fig. 5. Comparison of Aphasia Impact Questionary-21 (AIQ-21) administered to the participants according to the frequency of treatment. Group I, Aphasic patients receiving therapy for one hour a day, once a week for eight weeks (total 8 hours); Group II, Aphasic patients receiving therapy for one hour a day, six days a week for eight weeks (total 48 hours).

test results of both groups were compared, the intensive intervention was more effective than the normal intervention. Obtaining different treatment results from studies on SFA may be due to different treatment durations, dosages, and the total amount of treatment. In the previous meta-analysis, it was reported that SFA was applied for a maximum of 1500 minutes so far. In our study, a total of 2880 minutes of SFA was administered in the intensive intervention group. The results of our study are consistent with both studies in which SFA is effective in aphasia patients and the results of studies showing that intensive speech and language therapy is more effective.

How much the acquired skills are maintained is as important as the effectiveness of speech and language therapy applied to patients with aphasia. It has been documented that the gains obtained from training on word retrieval were maintained one month after the treatment [27]. Breitenstein *et al*. [19] reported that the effects of intensive treatment applied to individuals with chronic aphasia were maintained after six months. In another study by Meinzer *et al*. [27], they determined the stability of the effect of intensive restraint-induced aphasia treatment at 6-month follow-up. In this study, we performed a follow-up evaluation one month after the end of treatment. Although follow-up evaluation results varied from the posttest, they were still significantly different from pretest results. These results show that the gains from the therapy we applied are maintained one month after the treatment.

The strength of the study is that it is the first study to compare both the normal intervention and the intensive intervention of SFA. In addition, working with individuals with chronic aphasia is important in terms of demonstrating the effectiveness of the treatment applied. The limitations of the study are that it was single-centered and did not have long-term results. The results of our study should be supported by multicenter studies, more participants, and longer-term studies.

Limitations

The study consisted of only individuals with non-fluent aphasia and was limited to only the specific group in order to eliminate the effect of different types of aphasia on recovery. At the same time, only chronic individuals were included, and spontaneous recovery processes were excluded, and education level and pre-therapy history were not considered when determining the participants. Furthermore, no neuropsychological evaluations were made after the applications.

CONCLUSION

According to the results of our study, intensive intervention treatment was superior to normal intervention of SFA. In this study, individuals with chronic aphasia benefited from both SFA interventions. Therefore, it is very important to include aphasic patients in therapy processes (especially in intensive intervention SFA), even if they are in the chronic phase.

Authors' Contribution

Study Conception: İCY; Study Design: İCY; Supervision: İCY; Funding: İCY; Materials: İCY; Data Collection and/or Processing: İCY; Statistical Analysis and/or Data Interpretation: İCY; Literature Review: İCY; Manuscript Preparation: İCY and Critical Review: İCY.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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Anemia and COVID-19

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ABSTRACT

Objectives: Coronavirus disease-19 (COVID-19) is an infective-inflammatory disease that mainly affects the lungs. Hematological symptoms such as thrombocytopenia, decreased eosinophil and lymphocyte counts are quite common and are of prognostic importance. Although it is known that the presence of anemia generally increases the severity of respiratory diseases, there is little data on the prevalence and importance of anemia in COVID-19. In this study, our aim is to evaluate the clinical features of patients with anemia in COVID-19 infection and to investigate the relationship between the presence of anemia and the prognosis of the disease.

Methods: This retrospective, observational study included 353 patients who presented to our pandemic reference hospital between 15.04.2020 and 15.05.2020 and were diagnosed with SARS-CoV-2 infection confirmed by real-time reverse transcription polymerase chain reaction (PCR) test and typical clinical symptoms.

Results: Our study included 167 female and 186 male patients. The mean age was 54.54 ± 18.28 years (range 19-99). One hundred forty-eight (41.93%) patients had anemia. In patients with anemia, age was higher than others ($p < 0.001$). The percentage of women was significantly higher in the anemia group ($p < 0.001$). Comorbidities were observed more in the anemia group. The percentages of intensive care stay ($p = 0.003$) and mortality ($p = 0.001$) were significantly higher in the anemia group compared to the group without anemia. Logistic regression analysis was performed to determine the important risk factors of death. We found patients with high age ($p = 0.001$), high red cell distribution width-coefficient of variation (RDW-CV) levels ($p = 0.009$), high D-dimer levels ($p = 0.012$) and high ferritin levels ($p < 0.001$) have higher risk of death. Anemia was found to be non-significant.

Conclusions: Anemia is frequently observed in patients with severe COVID-19 disease and low hemoglobin values at presentation are thought to be associated with a worse prognosis. Being more sensitive to the hemoglobin levels of COVID-19 patients is important for early recognition of the high-risk patient group and for successful patient management. However, in our study, the presence of anemia was found to be effective in mortality in univariate analysis, but not in multivariate analysis. According to the multivariate analysis of this study, advanced age, high D-dimer, high ferritin and RDW-CV determine death.

Keywords: COVID-19, anemia, iron metabolism, mortality

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Coronavirus Disease (COVID-19) is a new viral disease with high mortality and infectivity, which has spread to many countries around the world since the end of 2019 and seriously affects the elderly, people with chronic diseases such as diabetes, cardiovascular disease and hypertension [1, 2]. Although most patients infected with COVID-19 have a mild clinical course, up to 20% of patients are hospitalized mainly for pneumonia. Some of these patients who require hospitalization are followed in the intensive care unit and may need mechanical ventilation support [3, 4]. In such severe COVID-19 infections, a hyperinflammatory state characterized by an increase in inflammation markers such as interleukin-6 (IL-6), C-reactive protein (CRP) and ferritin is observed [5, 6]. Age is an important risk factor in mortality. It is known that mortality rates due to COVID-19 infection are higher in the geriatric population [7, 8]. It was observed that high ferritin levels were associated with disease severity, development of acute respiratory distress syndrome (ARDS) and death [9, 10].

Iron metabolism and the presence of anemia are thought to play an important role in coronavirus disease, the course of the disease, and the progression to multiple organ dysfunction syndrome [11]. Hemoglobin is involved in oxygen transport, and the decrease in hemoglobin concentration leads to a decrease in oxygen carrying capacity and arterial oxygen content. As a result, anemia facilitates hypoxia and can lead to end-organ ischemia [12, 13]. The presence of anemia has been associated with the adverse clinical course of many diseases [14, 15]. Previous studies have reported that anemia often increases the severity of respiratory tract diseases and is associated with poor outcomes and increased mortality in patients with community-acquired pneumococcal pneumonia [16-199. Therefore, it is very important to understand the relationship between anemia, iron metabolism and the prognosis of COVID-19. Studies have reported that the more severe course of COVID-19 disease is associated with low hemoglobin levels and anemia is a risk factor for serious disease [20].

In our study, we aimed to evaluate the prevalence of anemia in hospitalized COVID-19 patients and the effect of the presence of anemia on the clinical course of COVID-19 patients. In particular, we evaluated on the differences between anemic and non-anemic patients, the severity and type of anemia. We hypothesized that low Hgb levels were associated with a worse course of COVID-19 disease. In order to contribute to the early recognition of disease severity, we tried to reveal the dynamic relationship between anemia and COVID-19 severity, need for intensive care and mortality. However, the presence of anemia was not found to be associated with mortality in multivariate analysis. Along with anemia, we also evaluated other prognostic laboratory measurements that may be effective in the course of the disease.

METHODS

Patients and Laboratory

This retrospective study included 353 patients who presented to our pandemic reference hospital between 15.04.2020 and 15.05.2020. Patients were diagnosed with SARS-CoV-2 infection confirmed by typical clinical symptoms and real-time reverse transcription polymerase chain reaction (PCR) test and received inpatient treatment. These patients were diagnosed with COVID-19 in accordance with the ‘interim‘ guide issued by the Ministry of Health of the Republic of Turkey. The demographic characteristics of the patients, biochemical, hematological and inflammatory parameters, length of hospital stay, mortality and intensive care status were obtained by scanning their electronic medical records. People who were PCR negative, younger than 18 years old, outpatients and whose data could not be reached were not included in the study. The study protocol was approved by the Bursa Yüksek İhtisas Clinical Research Ethics Committee (Date:10.06.2020, Decision no: 2020/06-07]. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study was also approved by the Turkish Ministry of Health.

PCR samples were taken from the patients. The samples were evaluated by an experienced team in the microbiology laboratory. Only patients with positive PCR tests were included in the study. Blood samples taken from the patients at the time of admission were analyzed in the laboratories of our hospital with fully automatic tests. Laboratory evaluations including routine blood tests, coagulation profiles, inflammation profiles, liver function, cardiac function and kidney function were performed. Laboratory findings were then extracted from the clinical information system.

Definition of Anemia and Iron Status

Based on the laboratory data obtained, we evaluated the prevalence and pathogenesis of anemia in patients infected with COVID-19. We determined the relationship between the presence of anemia and other clinical and laboratory findings in patients. In our study, anemia was defined according to the World Health Organization (WHO). Hemoglobin < 13 g/dL in males and < 12 g/dL in females was accepted as anemia. Anemia was classified as severe anemia (hemoglobin < 8 g/dL), moderate anemia (hemoglobin 8-10.9 g/dL), and mild anemia (hemoglobin 11-12.9 g/L in men and 11−11.9 g/dL in women) [21]. In addition, anemic patients were classified according to the mean corpuscular volume value (MCV). These were morphologically classified as microcytic (MCV < 80), normocytic (MCV: 80-100), and macrocytic (MCV > 100) anemias. Iron deficiency (ID) was defined as transferrin saturation (TSAT) < 20% in combination either with serum ferritin < 100 µg/L (absolute ID) or serum ferritin > 100 µg/L (functional ID) [16, 22].

Statistical Analysis

All analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). Histogram and Q-Q plots were used to determine whether variables are normally distributed. Data are provided 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. Normally distributed variables were analyzed with the independent samples t test. Non-normally distributed variables were analyzed with the Mann Whitney U test. Categorical variables were analyzed with the chi-square tests or Fisher's exact tests. Logistic regression analysis (forward conditional method) was performed to determine significant risk factors of death. $p < 0.05$ values accepted as statistically significant results.

RESULTS

Three hundred and fifty-three patients (167 women and 186 men) were included in this study. The mean age was 54.54 ± 18.28 years (range 19 to 99). One hundred and forty-eight (41.93%) patients had anemia. Age was significantly higher in patients with anemia compared to the others ($p < 0.001$). The percentage of women in the anemia group was significantly higher than in the non-anemia group ($p < 0.001$). The percentages of diabetes mellitus, hypertension, heart disease, atrial fibrillation, dementia, and kidney disease were significantly higher in the anemia group than in the non-anemia group ($p < 0.005$). Lymphocyte count, GFR, serum iron levels and transferrin saturation were significantly higher in the no anemia group than in the anemia group ($p < 0.001$). Red cell distribution width-coefficient of variation (RDW-CV), sedimentation, CRP, D-dimer and troponin levels were significantly higher in the anemia group than in the no anemia group ($p < 0.001$). In the anemia group, the rates of staying in the intensive care unit and death were found to be significantly higher than in the non-anemia group (Table 1).

Sixty-eight (45.95%) patients had mild anemia, 59 (39.86%) patients had moderate anemia and 21 (14.19%) patients had severe anemia. The percentage of women in the moderate and severe anemia group ($p = 0.034$) was significantly higher than in the mild anemia group. Renal diseases percentage ($p = 0.016$) was significantly higher in the moderate and severe anemia group than in the mild anemia group. RDW-CV, sedimentation, D-dimer and troponin levels were significantly higher in the moderate and severe anemia group than in the mild anemia group ($p < 0.005$). GFR, serum iron level and transferrin saturation were significantly higher in the mild anemia group than in the moderate and severe anemia group ($p < 0.005$). According to the severity of anemia, there was no significant difference between the percentages of intensive care stay and death.

Forty-seven (31.76%) patients had microcytic anemia and 101 (68.24%) patients had normocytic and macrocytic anemia. Age was significantly higher in normocytic and macrocytic groups compared to microcytic group ($p < 0.001$). The percentage of women in the microcytic group was higher than in the normocytic and macrocytic groups ($p = 0.002$). Heart disease and renal disease percentages were significantly higher in the normocytic & macrocytic group than in the microcytic group ($p < 0.005$). Glomerular filtration rate (GFR), RDW-CV and iron binding capacity were significantly higher in the microcytic group than in the normocytic and macrocytic group ($p < 0.001$). Lymphocyte count, sedimentation, C-reactive protein

Table 1. Summary of patients' characteristics and laboratory measurements with regard to presence of anemia

	Anemia			
	Absent (n = 205)	**Present (n = 148)**	**Total (n = 353)**	***p* value**
Age (years)	50.20 ± 16.32	60.54 ± 19.18	54.54 ± 18.28	**< 0.001**
Sex				
Female	72 (35.12%)	95 (64.19%)	167 (47.31%)	**< 0.001**
Male	133 (64.88%)	53 (35.81%)	186 (52.69%)	
Comorbidity	53 (25.85%)	70 (47.30%)	123 (34.84%)	**< 0.001**
Hemoglobin (g/dL)	13.88 ± 1.11	10.27 ± 1.89	12.36 ± 2.32	**< 0.001**
D-Dimer (µ/mL)	0.54 (0.30-1.00)	1.04 (0.51 - 2.24)	0.67 (0.35-1.45)	**< 0.001**
Fibrinogen (mg/dL)	446.38 ± 163.44	484.39 ± 174.92	463.17 ± 169.33	**0.073**
Ferritin (ng/mL)	151.90 (86.81-286.40)	151.30 (49.30- 471.20)	151.30 (72.89-365.80)	0.947
Transferrin saturation (%)	18.33 (13.52-26.02)	13.54 (8.97-20.03)	16.22 (11.31-24.34)	**< 0.001**
Ferritin / Transferrin saturation ratio	8.93 (4.53-20.29)	9.40 (4.01-28.42)	9.22 (4.20-21.62)	0.583
Stay in ICU (days)	4 (1.95%)	14 (9.66%)	18 (5.14%)	**0.003**
Length of stay in hospital (days)	7 (5-11)	7.5 (5-13)	7 (5-12)	0.242
Mortality	6 (2.93%)	18 (12.16%)	24 (6.80%)	**0.001**

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.

(CRP), D-dimer, troponin, blood urea nitrogen (BUN), creatinine, serum iron, ferritin levels, transferrin saturation, ferritin / transferrin saturation ratio and length of stay in hospital were significantly higher in the normocytic and macrocytic group than in the microcytic group ($p < 0.005$). According to the MCV groups, there was no significant difference between the percentages of intensive care stay and death (Table 2).

Two hundred and twenty-seven (64.31%) patients had iron deficiency. The percentage of women in the iron deficiency group was significantly higher than the non-iron deficiency group ($p = 0.005$). RDW-CV was significantly higher in the iron deficiency group than in the no iron deficiency group ($p < 0.001$). Lymphocyte ($p = 0.004$) counts was significantly higher in the non-iron deficiency group than in the iron deficiency group. In terms of iron deficiency, there was no significant difference between the percentages of stay in the intensive care unit and death.

Logistic regression analysis was performed to determine the important risk factors of death. Significant factors of the univariate analysis were included in the multivariate analysis with forward conditional method. We found patients with high age ($p = 0.001$), high RDW-CV levels ($p = 0.009$), high D-dimer levels ($p = 0.012$) and high ferritin levels ($p < 0.001$) have higher risk of death (Table 3). Other variables included in the multivariate model, comorbidity ($p = 0.266$), eGFR ($p = 0.672$), anemia ($p = 0.304$), moderate and severe anemia ($p = 0.475$), WBC ($p = 0.074$), CRP ($p = 0.073$), serum iron ($p = 0.170$), iron binding capacity ($p = 0.346$), transferrin saturation (p = 0.114) and ferritin / transferrin ratio ($p = 0.413$) were found to be non-significant.

Patients with ≥ 75 age have 5.208-fold higher risk of death than the other patients (odds ratio [OR]: 5.208, 95% confidence interval [CI]: 2.220 - 12.213, $p < 0.001$). Patients with ≥ 15 RDW-CV level have 3.872-fold higher risk of death than the other patients (OR: 3.872, 95% CI: 1.654 - 9.068, $p = 0.002$). Pa-

Table 2. Summary of characteristics and laboratory measurements of the patients with regard to anemia types

	Anemia		
	Normocytic and Macrocytic (n = 101)	**Microcytic (n = 47)**	***p* value**
Age (years)	64.54 ± 18.09	51.94 ± 18.79	**< 0.001**
Sex			
Female	56 (55.45%)	39 (82.98%)	**0.002**
Male	45 (44.55%)	8 (17.02%)	
Comorbidity	52 (51.49%)	18 (38.30%)	0.187
Hemoglobin (g/dL)	10.39 ± 1.98	10.01 ± 1.67	0.226
D-Dimer (µ/mL)	1.35 (0.64-2.37)	0.61 (0.36-1.05)	**< 0.001**
Fibrinogen (mg/dL)	494,63 ±.169,81	461,26 ± 186,38	0.350
Ferritin (ng/mL)	263.90 (86.57-554.00)	59.72 (21.00-139.10)	**0.001**
Transferrin saturation (%)	16.33 (10.36-24.44)	10.00 (7.04-13.57)	**< 0.001**
Ferritin / Transferrin saturation ratio	15.36 (4.51-31.64)	6.13 (2.12-10.83)	**0.010**
Stay in ICU (days)	11 (11.22%)	3 (6.38%)	0.549
Length of stay in hospital (days)	9 (5-13)	6 (5-9)	**0.026**
Mortality	15 (14.85%)	3 (6.38%)	0.231

Data are provided 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.

Table 3. Significant risk factors of death, logistic regression analysis

	Univariate		**Multivariate**	
	OR (95% CI)	***p* value**		***p* value**
Age, years	1.067 (1.036-1.099)	**< 0.001**	1.119 (1.049-1.194)	**0.001**
Sex, male	2.299 (0.929-5.691)	0.072		
Comorbidity	6.400 (2.469-16.591)	**< 0.001**		
eGFR	0.959 (0.946-0.973)	**< 0.001**		
Anemia	4.592 (1.776-11.874)	**0.002**		
Anemia				
Mild anemia	2.073 (0.567-7.577)	0.270		
Moderate and severe anemia	7.035 (2.598-19.049)	**< 0.001**		
Anemia				
Normocytic& macrocytic anemia	5.785 (2.171-15.413)	**< 0.001**		
Microcytic anemia	2.261 (0.545-9.392)	0.261		
Ferritin	1.002 (1.001-1.003)	**< 0.001**	1.004 (1.002-1.006)	**< 0.001**
Transferrin saturation	1.028 (1.000-1.056)	**0.048**		
Ferritin / Transferrin saturation ratio	1.027 (1.010-1.044)	**0.002**		
Iron deficiency	1.118 (0.465-2.691)	0.803		

OR = Odds ratio, CI = Confidence interval

tients with ≥ 500 ferritin level have 15.896-fold higher risk of death than the other patients (OR: 15.896, 95% CI: 5.543 - 45.586, $p < 0.001$).

DISCUSSION

In this study, the data of 353 patients who received inpatient treatment in our hospital due to COVID-19 infection were evaluated retrospectively. Our results report that anemia is common in patients with COVID-19 infection. The presence of anemia causes poor clinical conditions and the need for intensive care. Comorbidities were observed more frequently in patients with anemia. However, anemia was not found to be effective in predicting mortality in multivariate analysis. In the study, it was observed that anemic patients were at high risk for severe inflammatory responses and were older. While there is a positive relationship between the severity of anemia and female gender, presence of renal disease and elevation in coagulation parameters, there is no significant difference between the intensive care unit needs and death rates. In the multivariate analysis, it was found that elderly patients with high RDW-CV, D-dimer and ferritin levels had a higher risk of death.

One of the most important determinants of the oxygen carrying capacity of the blood is the hemoglobin concentration. Low hemoglobin levels result in an inability to support increased peripheral tissue oxygen needs due to hypermetabolic conditions during infection, especially in populations at high risk of complications and death. A meta-analysis showed that regardless of age, gender and cardiovascular disease, anemia caused an increased risk of all-cause death and cardiovascular death by 41% and 33%, respectively [23]. It has been reported that the presence of anemia is associated with poor outcomes and increased mortality in respiratory tract diseases, as it increases the severity of the disease [18, 19]. It was found that the presence of anemia in chronic obstructive pulmonary diseases was associated with a 2.6-fold increased risk of mortality [24, 25]. In a study involving 191 patients with COVID-19, the frequency of anemia, Zhou *et al*. [5] reported as 15%. In our study, this rate was higher as 41.9%. The reason for this may be the inclusion of more severe patients who require hospitalization in our study. The correct prevalence of anemia remains uncertain in this patient group.

Few studies have so far examined the direct relationship between clinical and laboratory features, anemia and disease severity in patients with COVID-19. In patients with anemia, it is important to pay attention to the clinical features as well as the severity of the anemia. Taneri *et al*. [11] reported that hemoglobin levels were significantly lower in patients with severe COVID-19 infection in a meta-analysis and that the prognosis and severity of COVID-19 patients may be associated with low hemoglobin levels. In the studies, a significant trend towards lower hemoglobin values was observed with the worsening severity of COVID-19 [10, 26]. We could not find a significant relationship between the severity of anemia and the need for intensive care and mortality.

Changes in anemia and iron homeostasis are quite common in hospitalized patients. Tao *et al*. found that anemia, which was diagnosed based on hemoglobin measured within the first 24 hours after admission was strongly associated with progression (27). Since iron metabolism is associated with biomarkers and increased mortality at baseline, it is thought to contribute to the risk classification of patients. Zhou *et al*. [5] reported in a study that COVID-19 patients with anemia were more susceptible to death (26% vs 11%, p = 0.009).

There is a decrease in hemoglobin level in acute inflammation due to many complex mechanisms. Inflammation observed in COVID-19 infections can lead to iron homeostasis changes by keeping iron in macrophages and reducing its absorption from the intestine [28]. As a result, circulating iron levels decrease and hemoglobin production decreases. The cytokine-mediated inhibition of erythropoiesis decreases the biological activity of erythropoietin and shortens its erythrocyte half-life. And this leads to the development of inflammation anemia (AI) [22]. In our study, we observed that inflammation-related indicators (e.g., D-dimer, CRP, sedimentation) were higher in the anemia patients group. In addition, we found a significant increase in coagulation markers and inflammatory markers in patients with moderate to severe anemia.

One of the most common causes of anemia during infections is iron deficiency anemia. Iron requirements are essential for maintaining hemoglobin synthesis. In our study, iron deficiency was not found to be associ-

ated with the need for intensive care and mortality on its own. However, iron homeostasis disorders and the presence of anemia are important clinical determinants for risk stratification of patients infected with COVID-19 and it can guide the clinical management of those at highest risk, especially [29]. Accurate criteria must be established to define iron deficiency and iron-restricted erythropoiesis in COVID-19, particularly in the "hyper-inflammatory" stage of the disease. Changes in iron homeostasis may contribute to the pathogenesis of severe COVID-19 infection, with mechanisms that need to be resolved by future research.

There is a close relationship between the levels of ferritin and inflammation markers of macrophage activation and lung damage [30]. Studies have shown that increased risk of death in COVID-19 is associated with high ferritin levels [6, 9, 109. Hyperferritinemia has been suggested to be associated with iron toxicity and end organ damage in COVID-19 [31, 32]. Chronic disease anemia due to underlying comorbidities may also occur in patients. Also, the prevalence of comorbidities such as cardiovascular disease, hypertension or chronic kidney disease is higher in anemic patients [5]. In our study, it was found similar to the literature. In addition, patients with more than one comorbidity are expected to be more likely to be anemic and to be transferred to the intensive care unit when their physical condition worsens. Advanced age is an important risk factor for anemia. The prevalence of anemia rises to 12% in people aged 65 and over, and up to 47% in nursing home residents [15, 33, 34]. In our study, it was found that being older than 75 years increases the risk of death approximately 5 times.

Similar to the literature, only the prognostic significance of RDW was observed among the hematological parameters in our study. Increased RDW is an important risk factor for mortality. Previous studies have also shown that high RDW are associated with heart disease, sepsis, and more severe COVID-19 infection and increased mortality rates [35, 36]. Although the underlying mechanisms of the association between increased RDW and critical disease and mortality are unclear [37].

Limitations

This study includes a retrospective analysis of COVID-19 patients. Since there was no prospective study, iron metabolism variables could not be fully evaluated. In addition, cases with laboratory parameters were included in the study, which may lead to a possible selection bias for patients. Laboratory parameters of the patients were taken at the first admission and were determined according to the diagnosis of anemia, hemoglobin levels at the time of admission and iron parameters. Pre-application values, how long the anemia has existed, its follow-up values and the course of the anemia have not been mentioned. However, the exact cause and duration of anemia remains uncertain. It is difficult to confirm whether patients have chronic disease anemia.

CONCLUSION

Our results provides evidence that anemia is common in patients with COVID-19 requiring hospitalization. Low hemoglobin at admission is associated with a worse prognosis. Given the impact of anemia on quality of life and disease prognosis, the problem cannot be ruled out. Early recognition of those at risk and allergy to earlier and more aggressive medical intervention are important. Given the high costs, the risk of side effects and the shortage of blood supply, which have become a more serious problem during the COVID-19 pandemic, studies should be directed towards efforts to reduce the prevalence and severity of anemia. Our systematic review highlights important gaps in the presence of iron biomarkers and anemia other than ferritin in the prognosis of COVID-19. It shows that hemoglobin and ferritin levels vary according to the severity of COVID-19, as well as the presence of age, gender and comorbidity among COVID-19 patients. We hope that accurate diagnosis and effective treatment of the causes of anemia together with newly emerging treatment strategies will reduce the clinical burden of anemia in COVID-19. However, future prospective studies are still needed to confirm the effect of anemia on COVID-19 outcomes, management of patients and whether low hemoglobin levels predict mortality in these patients.

Authors' Contribution

Study Conception: VG; Study Design: VG, SA; Supervision: VG, SA; Funding: N/A; Materials: VG; Data Collection and/or Processing: AE, MY; Statistical

Analysis and/or Data Interpretation: SE; Literature Review: VG; Manuscript Preparation: VG, SA and Critical Review: SE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Evaluation of ultrasound-measured gastric volume and content in type 2 diabetes mellitus patients undergoing elective surgery: a prospective observational study

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ABSTRACT

Objectives: Delayed gastric emptying create a risk of pulmonary aspiration during anesthesia. We aim to assess the antral cross-sectional area (CSA) and gastric volume using ultrasound techniques and to investigate the relationship between these variables and both the duration and regulation of type 2 diabetes mellitus (DM).

Methods: Gastric volume was estimated by measuring the antral CSA in the supine and right lateral decubitus (RLD) positions in 80 patients. The antral content was qualitatively classified according to Perlas et al. (grades 0, 1, and 2), and gastric volume was computed using a previously described formula. The presence of solid content or > 1.5 mL/kg fluid in the stomach was classified as indicative of a full stomach.

Results: The mean duration of diabetes among the subjects was 9.4 ± 3.7 years. The mean fasting duration was 10.2 ± 2.1 hours for solids and 2.5 ± 0.7 for liquids. Twelve of the 80 patients exhibited grade 2 stomach. Age ($p = 0.005$), Body mass index ($p = 0.001$), solid fasting duration ($p = 0.027$), and supine and RLD CSA ($p < 0.001$ for both) were significantly associated with full stomach. A history of ≥ 8 years of diabetes ($p < 0.001$) and peripheral neuropathy ($p = 0.005$) was identified as a risk factor for a full stomach.

Conclusions: Despite adherence to standard fasting protocols, 15% of the type 2 DM patients were identified with a 'full stomach' condition. Preoperative ultrasound assessment of gastric contents in patients with type 2 DM, especially with long-standing diabetes (≥ 8 years) and with peripheral neuropathy is recommended. The findings of this study necessitate additional investigation to support the conceptualization of specific guidelines for diabetes to mitigate the risk of pulmonary aspiration.

Keywords: Diabetes mellitus, gastric volume, ultrasound, surgery, pulmonary aspiration, delayed gastric emptying

Diabetes Mellitus (DM), which is prevalent in approximately 25% of surgical patients, has generated significant interest in residual gastric volumes after adequate pre-anesthesia fasting. Patients with DM, particularly those suffering from gastropathy linked to autonomic dysfunction, are predisposed to delayed gastric emptying, rendering them more vulnerable to an increased risk of aspiration than their

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healthy counterparts [1, 2]. It is worth noting that roughly 30-50% of patients with long-standing DM exhibit significantly prolonged gastric emptying time, as evidenced by radioisotope examination [3, 4].

Perioperative pulmonary aspiration of gastric contents is a severe and destructive complication associated with substantial postoperative morbidity and mortality [5]. The reported incidence of gastric aspiration fluctuates between less than 0.1% and 19%, with aspiration pneumonia accounting for 9% of all anesthesia-related fatalities [6]. Aspiration-related complications may include pneumonitis, pneumonia, and hypoxia, potentially escalating to respiratory failure, acute respiratory distress syndrome (ARDS), and ultimately, death [7].

To minimize the risk of perioperative aspiration, fasting guidelines have been established by the American Society of Anesthesiologists (ASA) [8] and the European Society of Anesthesiology [9]. However, these guidelines are primarily designed for healthy individuals undergoing elective surgery and may prove unreliable for patients with concurrent conditions that affect gastric emptying. Contemporary preoperative guidelines recommend at least 2 hours without liquids, 6 hours for non-fatty solid food, and 8 hours of fasting for fatty or high-calorie foods [10] to limit residual gastric volume and the subsequent risk of aspiration. Nonetheless, devastating aspiration events can still occur [11], partly because of the limitations in quantifying or eliminating hazardous gastric contents prior to anesthesia.

In recent years, Gastric Ultrasound (GU) has emerged as a point-of-care ultrasound (POCUS) technique and has rapidly gained traction within the anesthesiology domain. The GU is used to inform decisions before anesthesia induction, particularly in cases where fasting status is ambiguous or in emergency scenarios where surgery is vital [12]. Being simple, readily available perioperatively, noninvasive, radiation-free, and easy to perform at the bedside, POCUS has become a favored tool for gastric content and volume assessment [13].

The primary objective of the present study was to meticulously measure the antral cross-sectional area (CSA) and analyze fasting gastric volume in patients with type 2 DM. The secondary aim was to evaluate the association between gastric content and volume and the duration and regulation of diabetes in patients with type 2 DM scheduled for elective surgery. The insights derived from this study could contribute valuable information to the existing body of knowledge regarding risk factors and prevention strategies for aspiration in diabetic surgical patients.

METHODS

Study Setting

This prospective, unicentric observational study was conducted using a cohort of 80 patients scheduled for elective surgeries in a tertiary hospital from August 2019 to August 2020. Clearance from the Institutional Ethics Committee was obtained prior to the commencement of the study (2011-KAEK-25 2019/08-22). In the preoperative zone, written informed consent was obtained from all participants following a detailed briefing about the nature and purpose of the study.

The inclusion criteria were male and female patients aged between 35 and 75 years, with a minimum of 6 years of documented history of type 2 DM and classified under the ASA physical status II-III. Exclusion criteria were defined to eliminate patients taking medications known to affect gastric motility, those diagnosed with chronic renal failure, liver cell failure, hypothyroidism, obesity (BMI > 30 kg/m^2), connective tissue disorders, non-diabetic autonomic-neurological diseases, chronic opioid usage, gastrointestinal motility disorders and malignancies, prior surgeries involving the gastrointestinal and respiratory systems, current pregnancy, and those designated for emergency surgical procedures.

All patients included in the study were required to fast overnight, with a minimum fasting period of 8 h from their last meal. A comprehensive medical history was collected from each participant, focusing on details pertaining to the diagnosis and duration of diabetes, postprandial plasma glucose concentrations, and medication history. Creatinine levels and glomerular filtration rate (GFR) were obtained from hospital electronic health records, ensuring that the information did not exceed one month of age. Recent hemoglobin A1c concentration within the preceding 3 months was also recorded or, in its absence, a blood sample was collected on the day of the procedure for analysis. Peripheral neuropathy was identified and defined based on

scores exceeding 2 on the Michigan Neuropathy Screening Instrument (14). Notably, no premedication was administered to the patients, and Nil per os (NPO) status, along with the fasting duration for both solids and liquids, was meticulously assessed and documented.

Ultrasound Examination Technique and Assessment

Gastric Ultrasound Examination Procedure

Gastric Ultrasound (GU) examination was conducted in the recovery room within the operation theatre complex, prior to the patient being transferred to the operating room. An anesthesiologist (AD) with 13 years of experience in anesthesia performed a minimum of 50 training GU examinations. The study was executed using a portable US machine (MyLab30; Esaote, Italy) equipped with a curved array low-frequency (2-5 MHz) probe set in the abdominal scan mode.

For optimal antral visualization, the patient was first scanned in the supine position, followed by the right lateral decubitus (RLD) position, with the head of the bed elevated to 45° to maximize sensitivity (15). The transducer was carefully aligned in the sagittal plane in the epigastric region, with the left lobe of the liver anteriorly and the head or body of the pancreas posteriorly serving as reference points. The antrum, distinguished by its characteristic multi-layered wall, was scanned above large abdominal vessels, such as the aorta or inferior vena cava (2). A still image of the antrum was captured between peristaltic contractions, allowing for both qualitative (type of content) and quantitative (volume) assessments of gastric content. Notably, the anesthesiologist performing the GU measurement was not blinded to the patient's identity and did not influence the anesthesia method or any subsequent procedures.

Qualitative Assessment

Qualitative assessments were performed according to the appearance of the contents visualized using ultrasound. The classifications are as follows.

Empty Antrum: If the walls of the antrum were detected in close proximity to each other or displayed a "bull's eye/target pattern," the antrum was considered empty [16, 17].

Clear Liquids: Clear fluids, such as normal gastric secretions, water, or tea, manifested as an anechoic (black) appearance on the US.

Liquids with Gas Bubbles: The presence of multiple gas bubbles yielded a "starry night" appearance [2].

Solid/Particulate Matter: In the initial phase, solids or particulates presented a "frosted glass" pattern, where multiple ring-down artifacts obscured the posterior antrum. As this progressed, the solid content appeared to be increasingly heterogeneous, particulate, and hyperechoic [2, 16].

The sonographic appearance of the gastric antrum was systematically categorized, based on the grading system defined by Perlas *et al*., as grades 0, 1, and 2. These grades represent an empty antrum, fluid detected solely in the RLD position, and antral fluid visible in both the supine and RLD positions, according to the perspective obtained in both positions (14).

Quantitative Assessment

Quantitative assessment was conducted after the qualitative analysis of the antrum, with a focus on calculating the Cross-Sectional Area (CSA). Measurements were performed at the level of the aorta or inferior vena cava by employing an ultrasound machine in both the supine and RLD positions.

The traditional two-diameter method served as the framework for this calculation, which entailed measuring two perpendicular diameters of the antrum, namely the craniocaudal (CC) and anteroposterior (AP) dimensions, extending from the serosa to the serosa. The formula developed by Perlas *et al*. was used for this computation [14].

Upon completion of the preoperative GU examination, the quantitative residual gastric volume was ascertained for each patient by employing the formula delineated by Perlas *et al*. (18): Gastric volume (ml) = (27 + 14.6 x RLD CSA (cm2) − 1.28 x age (years). Aspiration risk was then evaluated qualitatively using the antral grading system (14) and further classified according to the scheme devised by Van de Putte and Perlas (2):

(a) Low Aspiration Risk

This category encompassed patients with an empty antrum or those with a gastric residual volume less than 1.5 mL/kg.

(b) High Aspiration Risk

This category included patients presenting with solid contents or those with a gastric residual volume exceeding 1.5 mL/kg.

This comprehensive quantitative assessment, coupled with qualitative evaluation, facilitated a detailed understanding of gastric contents and volumes, thereby allowing for tailored risk stratification regarding aspiration in the perioperative setting.

Statistical Analysis

Statistical analysis in this study was methodically structured and executed to ensure accurate and robust conclusions. Descriptive statistics were employed, with categorical data presented as frequencies and percentages, and continuous variables presented as mean values plus or minus standard deviation. Comparative analyses for categorical data were undertaken using the Chi-square test and Fisher's exact test, as applicable. For continuous variables, the Independent Samples T-test was used to compare single measurements across distinct groups, illuminating any significant differences. The interrelationships among various measurements were explored through Pearson correlation analysis, which helped identify and quantify the strength of the associations between different variables. A threshold of $p < 0.05$ was predetermined as the level of significance, ensuring that any p - values below this cut-off were indicative of statistical significance. All statistical computations were meticulously performed using the SPSS 20 software package, allowing for rigorous data processing and analysis, thus underpinning the credibility of the study's findings.

RESULTS

The study enrolled 80 patients. The demographic and surgical data are presented in Table 1. The sex distribution included 55.0% females and 45.0% males, slightly favoring females. The average patient age was 61 ± 9 years (range, 35-75 years). The types of surgeries performed were general surgery (27.5%), neurosurgery (27.5%), urological surgery (18.8%), orthopedic surgery (15%), gynecological surgery (12.5%), and others (11.3%). Cardiovascular comorbidities, such as hypertension and coronary artery dis-

Table 1. Demographic, baseline and preoperative characteristics of the patients

	Data
Age groups (year)	
35-45	5 (6.3)
46-55	20 (25.0)
56-65	28 (35.0)
66-75	27 (33.8)
Age (years)	61 ± 9
Sex	
Female	44 (55.0)
Male	36 (45.0)
BMI (kg/m^2)	
Normal weight (20-24.99)	12 (15.0)
Overweight (25-29.99)	68 (85.0)
Height (cm)	168 ± 9
Weight (kg)	76 ± 8
ASA	
II	48 (60.0)
III	32 (40.0)
Section to be operated	
General surgery	22 (27.5)
Neurosurgery	12 (15.0)
Urology	15 (18.8)
Orthopedic surgery	12 (15.0)
Gynecology	10 (12.5)
Ear, nose, throat	8 (10.0)
Plastic surgery	1 (1.3)
Comorbidities	
Diabetes mellitus	80 (100.0)
Hypertension	47 (58.8)
CAD	26 (32.5)
COPD	10 (12.5)
Astma	5 (6.3)
Others	2 (2.5)
Fasting time (hour)	
6-10	48 (60.0)
11-15	32 (40.0)
Fasting time (solid) (hour)	10.2 ± 2.1
Fasting time (liquid) (hour)	2.5 ± 0.7
Postprandial glucose concentration (gr/dL)	141.4 ± 35.3
HbA1c (%)	7.94 ± 2.11
Duration of diabetes (year)	
< 8 year	32 (40.0)
≥ 8 year	48 (60.0)
Duration of diabetes (year)	9.4 ± 3.7

Data are shown as mean ± standard deviation or number (percent). BMI = Body mass index, ASA = American Society of anaesthesiologists, HbA1c = Hemoglobin A1c, CAD = Coronary artery disease, COPD = Chronic obstructive pulmonary disease

Table 2. Qualitative grade of assessment of gastric content in type 2 diabetic patients

(n = 80)		n	%
Qualitative assessment of gastric content			
	Grade 0	25	31.3
	Grade 1	43	53.8
	Grade 2	12	15.0

ease, were common, present in 58.8% and 32.5% of patients, respectively. Average fasting durations were 10.2 ± 2.1 hours for solids and 2.5 ± 0.7 hours for liquids. The mean hemoglobin A1c concentration was 7.94 ± 2.11. A 40.0% of the patients had diabetes for less than 8 years, and 60.0% for 8 years or more, with a mean duration of 9.4 ± 3.7 years (Table 1).

Ultrasound grading variations are presented in Table 2. Qualitative ultrasound evaluations indicated that 31.3% were grade 0, 53.8% were grade 1, and 15% were grade 2 in the antrum (Table 2).

The association between gastric volume and gastric content with respect to hour is shown in Table 3. Five patients had remarkable amounts of clear liquid (> 1.5 ml/kg), while seven had solid content, despite adhering to traditional fasting guidelines. The mean volume was significantly lower for those who fasted 6-10 hours compared to 11-15 hours ($p = 0.045$), with no significant difference between liquid fasting times and full stomach instances (Table 3).

The mean supine CSA was 6.40 ± 2.52 cm^2, RLD CSA was 7.57 ± 2.75 cm2, and the mean RLD gastric volume was 58.63 ± 37.62 ml.

This study investigated the risk factors for a full stomach in diabetic patients and divided them into groups based on the ultrasound grade. Differences were not significant for sex, ASA physical status, fasting blood sugar, hemoglobin A1c value, or GFR ($p > 0.05$), but significant differences were found for mean age, BMI, solid fasting time, and CSA in both positions ($p < 0.001$). The number of full-stomach instances was significantly higher in patients with neuropathic complaints ($p = 0.005$). ROC curve analysis identified a threshold value of 8 years for diabetes duration, predicting a higher likelihood of a full stomach for those aged ≤ 8 years ($p < 0.001$) (Table 4) (Fig 1).

The mean BMI was higher in the full stomach group (27.70 ± 1.90) than in the empty/intermediate stomach group (26.09 ± 2.13). However, no statistically significant correlation was found between BMI and the measured antral CSA or gastric volume.

Table 5 shows the parameters of the threshold value, which further enhances the understanding of the factors contributing to the observed results (Table 5).

The results provided a comprehensive analysis of the relationship between various factors and gastric content and volume in patients with diabetes undergoing elective surgery. The findings of quantitative and qualitative assessments, correlations, and subgroup analyses have contributed to the understanding of the factors affecting the risk of aspiration in this population. These results have implications for anesthetic planning and patient care, emphasizing the need for individualized fasting guidelines to minimize the risk of aspiration, especially in patients with diabetes.

Table 3. Correlation of gastric content and volume with respect to fasting hours

	Gastric volume	Grade 2	
		Solid	Liquid > 1.5 mL/kg
Fasting time (hour)			
6-10	51.77 ± 37.36	5 (71.4)	2 (28.6)
11-15	68.91 ± 36.18	2 (40.0)	3 (60.0)
***p* value**	**0.045** [a]	0.558 [b]	
Fasting time (liquids) (hour)		2.6 ± 0.8	2.6 ± 0.9
***p* value**		0.954 [a]	

Data are shown as mean ± standard deviation or number (percent).
[a]Chi-square test, [b]Independent samples T test

Table 4. Diabetic patients' characteristics between those with empty /intermediate stomach and full stomach

	Empty /intermediate stomach (Grade 0-1)	Full stomach (Grade 2)	*p value*
Gender			0.396[a]
Female	12 (27.3)	32 (72.7)	
Male	13 (36.1)	23 (63.9)	
Age (year)	57 ± 8	63 ± 9	**0.005**[b]
BMI (kg/m^2)	26.09 ± 2.13	27.70 ± 1.90	**0.001**[b]
ASA			
II	18 (37.5)	30 (62.5)	0.140[a]
III	7 (21.9)	25 (78.1)	
Postprandial glucose concentration (gr/dl)	135.7 ± 35.9	144.0 ± 35.1	0.332[b]
HbA1c	7.32 ± 1.54	8.22 ± 2.28	0.079[b]
Peripheral neuropathy			
Yes	7 (17.1)	34 (82.9)	**0.005**[a]
No	18 (46.2)	21 (53.8)	
GFR	92.51 ± 19.23	84.29 ± 18.26	0.070[b]
Duration of diabetes(year)			
< 8 year	23 (71.9)	9 (28.1)	**0.005**[c]
≥ 8 year	2 (4.2)	46 (95.8)	
Fasting time (solid) (hour)	9.5 ± 1.8	10.6 ± 2.2	**0.027**[b]
Fasting time (liquid)(hour)	2.4 ± 0.7	2.6 ± 0.7	0.293[b]
CSA Supine (cm^2)	4.72 ± 2.32	7.16 ± 2.24	**< 0.001**[b]
CSA RLD (cm^2)	5.54 ± 2.34	8.49 ± 2.42	**< 0.001**[b]

Data are shown as mean±standard deviation or number (percent). BMI = Body mass index, HbA1c = Hemoglobin A1c, GFR = Glomerular filtration rate, CSA = Cross-sectional area, RLD = Right lateral decubitus

[a]Chi-square test, [b]Independent samples T-test, [c]Fisher test

DISCUSSION

This prospective observational study ascertained that approximately 15% of patients with type 2 DM exhibited a full stomach post-adherence to the conventional preoperative fasting guidelines set by the ASA. Notably, parameters such as age, BMI, duration of solid fasting, and CSA values in both supine and RLD stances were significantly elevated in these individuals. Our data also highlighted an increased likelihood of a full stomach among patients with diabetes exhibiting peripheral neuropathy and those diagnosed with type 2 DM for eight years or more.

Currently, diabetes affects approximately 25% of patients undergoing surgical intervention worldwide [19]. This metabolic disorder is a substantial risk factor that complicates anesthesiologic management. A primary concern is the threat of pulmonary aspiration, given the predisposition of diabetic patients towards full stomachs as a consequence of autonomic gastropathy [20]. Camilleri *et al*. [21] identified that nearly half of the patients with long-standing diabetes experience delayed gastric emptying, a salient concern. Although clinicians typically obtain the NPO status from patients, its accuracy is debatable, especially in high-risk individuals, potentially leading to an ele-

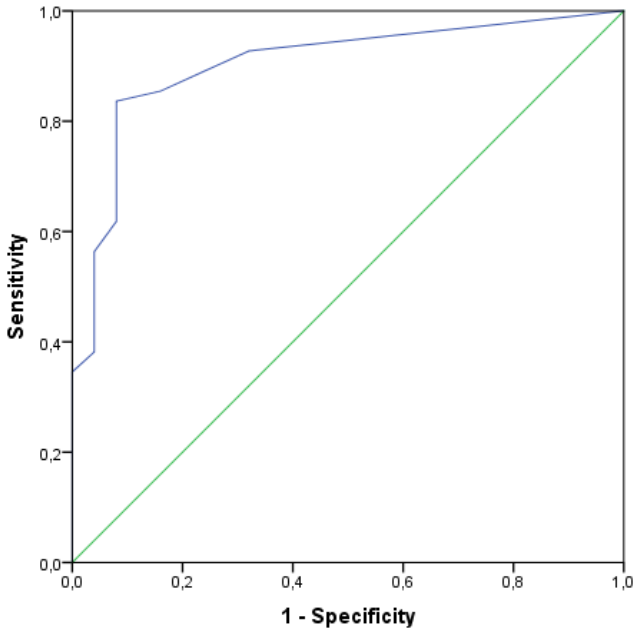

Fig 1. Determination of a threshold value for prediction of diabetes duration (ROC curve).

vated aspiration risk.

Associations between delayed gastric emptying in patients with diabetes and complications such as retinopathy, nephropathy, and autonomic neuropathy have been established in the literature [22, 23]. Earlier studies have also underscored the centrality of autonomic and enteric neuropathies in the onset of diabetic gastroparesis [21]. Our findings echo these observations, with an elevated prevalence of full stomach in patients with peripheral neuropathy. Such insights suggest a potential need for extended preoperative fasting durations in this patient subset, underscoring the need for rigorous studies to corroborate our postulates.

Although diverse techniques for stomach content evaluation exist, from paracetamol absorption to radiolabeled diet evaluations [17, 24], their utility during the preoperative phase remains questionable, often due to reliability and convenience concerns. Modern ultrasound technologies enable clinicians to detect hazardous stomach contents with noninvasive precision. Such tools enable the personalization of aspiration risk assessments at point-of-care and streamline anesthetic approaches [24]. Moreover, the adoption of POCUS enhances real-time decision-making before anesthesia administration, reduces perioperative complications [25], and assists in monitoring the gastric antrum and volume thresholds [26]. The learning curve for anesthesiologists in GU evaluations is also notably brief [27].

In our investigation, the differentiation between empty and full stomachs was based on Perlas's qualitative grading system, coupled with antral CSA measurements in the RLD position [14]. Qualitative ultrasound analyses of diabetic patients revealed 31.3% with grade 0 in 53.8%, grade 1, and grade 2 in 15 %. Aligning with Chaitra *et al*.'s [28] findings, we found that 15% of patients with type 2 DM, following current preoperative fasting recommendations, remained at high regurgitation risk, amplifying pulmonary aspiration concerns under general anesthesia (GA). It is noteworthy to juxtapose these findings with

Table 5. Results of ROC curve analysis

	Threshold value	SEN	SPE	PPV	NPV	AUC	%95 CI	*p value*
Duration of diabetes	8 year	83.6	92.0	95.8	71.9	0.904	(0.832-0.976)	**< 0.001**

SEN = Sensitivity, SPE = Specificity, PPV = Positive predictive value, NPV = Negative predictive value, AUC = Area under the curve, %95 CI = %95 Confidence Interval

those of Sabry *et al*. [29] and Chaitra *et al*. [28], who reported a 60% and 28.4% prevalence of full stomachs, respectively. When GA is essential for a patient with full stomach, rapid sequence induction and tracheal intubation are recommended [22].

Patients with diabetes had larger residual GV and antral CSA [12, 30]. Similarly with the study by Sharma *et al*. [31], the mean GV was found to be 58.63 ± 37.62 ml and the mean calculated supine CSA was 6,40 ± 2,52 cm2, the calculated RLD CSA was 7.57 ± 2.75 cm2. In line with the literature, when we compared the CSA and gastric volumes regarding the fasting times, patients who had fasted between 11-15 hours had greater gastric volumes than patients who had fasted between 6 and 10 hours. Therefore, there is no relationship between secure gastric enviroment and prolonged fasting. Other studies stated that with prolonged fasting, due to increased secretion of gastric acid, there is a decrease in the pH of gastric content (pH < 2.5) [32, 33]. Two of our patients who fasted more than 6 hours and three who fasted for more than 10 hours had significant liquid on their stomach (> 1.5 mL/kg). Of the seven patients who had solid gastric contents five have fasted for more than 11 hours and two for 6-10 hours. This would implicit slow gastric emptying.

The mean age of our study cohort was 61 ± 9 years, spanning between 35 and 75 years, which aligns with existing literature [1, 22, 29, 31]. This skew towards older ages is unsurprising given the propensity for type 2 DM to manifest later in life, coupled with our inclusion criterion necessitating a minimum diabetes duration of six years.

Our data also revealed a statistically significant elevation in BMI for those with a full stomach (27.70 ± 1.90) compared to their counterparts (26.09 ± 2.13). However, no correlation was discerned between CSA values in either the supine or RLD position and gastric volumes, potentially because the study excluded obese patients. Since we did not include patients with BMI>30 kg/m2 in our study, this may have been the case. Sharma *et al* found that as the BMI increased from 25-35 there were significantly rise in the gastric CSA in both supine and RLD positions [31].

While several studies have posited varied conclusions regarding the impact of hemoglobin A1c and blood glucose levels in patients with type 2 DM [22, 34-36], our study found no discernible relationship between postprandial glucose and hemoglobin A1c concentrations.

In alignment with the extant literature, our participants exhibited a mean fasting duration of 10.2 ± 2.1 hours. As stated by other studies, predicting the timing of the surgery is often accurate and surgical schedule is habitually subject to change [1, 28, 31]. Current trends in the medical community are leaning towards more liberal preoperative fasting protocols [37, 38]. Consequently, our findings urge anesthesiologists to exercise increased vigilance in patients with long-standing diabetes. The existing guidelines lack targeted preoperative recommendations for this demographic, signifying an urgent research gap that future prospective studies must address.

Limitations

As this was an observational and single-center study, the generalizability of our findings is limited. Different centers may have varied patient demographics, practices, and equipment, which can influence outcomes. Our study exclusively involved patients with type 2 DM, which did not provide insights into patients with type 1 DM. Given that individuals with type 1 DM often have a longer exposure to complications associated with the disease, the prevalence of gastroparesis might be more pronounced than in those with type 2 DM. The study did not consider or control for the influence of different diets on gastric emptying or residual volumes. Dietary habits can significantly influence the gastric motility and emptying time. The mean fasting interval observed in this study was approximately 10 h. Owing to the hectic conditions of our operating room schedules, it was challenging to have strict control over fasting duration in the preoperative phase. Our study exclusively utilized US to assess gastric residual volume, without comparing its efficacy or accuracy with other existing methods. This limits our ability to validate the accuracy or superiority of US as an evaluation tool. None of our patients with type 2 DM had diabetic nephropathy or was categorized as obese. Both uremic patients and those with obesity are known to exhibit elevated gastric residual volumes. This exclusion may have provided a skewed perspective regarding the prevalence of delayed gastric emptying in the broader diabetic population. Given these limitations, future research endeavors should aim to be multi-centered, involve a diverse pa-

tient cohort, including those with type 1 DM, obesity, and diabetic nephropathy, and perhaps incorporate various methods for gastric volume assessment. Such studies would offer a more comprehensive understanding of the factors that delay gastric emptying in patients with diabetes.

CONCLUSION

The findings of our study underscore the crucial fact that fasting for more than 6 to 10 h does not automatically equate to an empty stomach in patients with type 2 DM. There was a noticeable increase in antral CSA and, consequently, fasting gastric volume, as measured by POCUS in the RLD position, particularly in individuals afflicted with type 2 DM. This could potentially necessitate alterations in the anesthesia plans.

As a significant consideration for clinical practice, anesthesiologists should be aware of the importance of evaluating the gastric antrum with POCUS prior to induction, especially in diabetic patients who have had the disease for eight years or more, or those with peripheral neuropathy. Such an assessment is not merely a procedural formality but an eminent necessity in ensuring patient safety and optimal management.

There is a compelling need for comprehensive studies with larger and more diverse sample sizes, including patients with multiple comorbidities such as obesity, chronic renal disease, and advanced liver disease. Such research endeavors could elucidate a stronger correlation between gastroparesis and the diverse factors that contribute to delayed gastric emptying in patients with DM scheduled for elective surgeries under general anesthesia. By doing so, the medical community can hope to refine preoperative guidelines and anesthesia protocols, further enhancing the safety and effectiveness of surgical interventions for this particular patient population.

Authors' Contribution

Study Conception: AD, MD; Study Design: MD, AD; Supervision: AD, MD; Funding: FG, AO; Materials: ANB, AO; Data Collection and/or Processing: FG, AD, AO; Statistical Analysis and/or Data Interpretation: AO, ANB; Literature Review: FG, ANB; Manuscript Preparation: AD; AO and Critical Review: AD, MD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Evaluation of infections in patients with kidney and liver transplantation

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ABSTRACT

Objectives: Infection is a frequent complication of organ transplantation and is associated with significant morbidity and mortality.

Methods: Patients who had liver and kidney transplants between 2011 and 2022, who were hospitalized in our hospital, and who were consulted for infectious diseases were retrospectively analyzed from hospital records.

Results: Of the patients included in the study, 9 (28%) were female, 23 (72%) were male, and the mean age was 33.7 ± 11.3 years. Patients had congestive heart failure (87.2%, n = 28), hypertension (43.7%, n = 14), and chronic obstructive pulmonary disease (21.8%, n = 7). Twenty (62.5%) kidney transplant recipients and 12 (37.5%) liver transplant recipients were seen within ten years. The most common infections were urinary tract infection in 8 (25%) patients and pneumonia in 11 (34.3%). The other infections were gastrointestinal infections such as diarrhoea, bloodstream infections and COVID-19 and Cytomegalovirus. Culture-isolated organisms in 20 (62.5%) of the 32 patients admitted with infections. The microbiological data were notable for some unusual and opportunistic pathogens, including one case of acute cytomegalovirus viremia. Severe sepsis had been seen in six (18.75%) out of 32 patients with documented infections.

Conclusions: Infection prevention has become a cornerstone of modern transplantation medicine due to the significant incidence of post-transplant infectious complications resulting from improved immunosuppressive therapies and surgical procedures.

Keywords: Liver transplantation, renal transplantation, infectious disease, post-transplant infections

For patients with failing organs such as kidneys, liver, heart, lung, and pancreas, solid organ transplantation (SOT) is the treatment of choice worldwide. According to the data of the Ministry of Health in our country, 17,406 liver transplants and 42,277 kidney transplants were performed until August 2023 [1]. Over the past few decades, survival rates for SOT recipients (SOTRs) have improved as a result of developments in surgical procedures and immunosuppressive regimens. These vulnerable patients are affected by in-

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vasive infectious diseases, which are more severe and involve a more diverse range of agents than in the general population [2]. Risk factors that predispose to infections in transplant recipients can be classified as those present in the recipient or donor prior to transplantation and secondary to intraoperative and post-transplant events. The timing of specific infections after SOTRs is generally predictable regardless of which organ is transplanted. The majority of clinically significant infections occur within the first 180 days; Individual pathogens typically emerge at stereotypical times after transplantation. However, the time of onset of some pathogens may be affected by the use of prophylactic strategies, changes in immunosuppression, or the need for additional surgical intervention. When assessing potential causes of infection in SOT recipients, it is useful to divide periods of risk into three main ranges to assess which pathogens are most likely: (a) early (0-30 days post-transplant); (b) medium (30-180 days) and (c) late (more than 180 days). However, this evaluation based on time is not absolute. Some infections may occur in the post-transplant period, while others may occur outside of the usual risk periods. However, consideration of these time intervals provides a useful framework for approaching a patient with post-transplant fever and guides the initial differential diagnosis [3].

Retrospective cohort studies describing the epidemiology, clinical and outcome of the infections in the SOTRs are limited [3,4]. Infections are one of the leading causes of death in SOTRs. However, little is known about the incidence, epidemiology and clinical significance of infectious diseases in this population.

Vaccination, surgical prophylaxis, universal coverage, preemptive or pre-symptomatic treatment, targeted therapy, education, and avoidance are all preventive strategies that have been used in SOT recipients.SOT patients are a challenging group of patients with multiple etiologies due to the underlying immunosuppression. It is important for patient survival to be aware of the infections that can occur in this patient group. The prevention, diagnosis, and treatment of infectious diseases make important contributions to clinical organ transplantation. The emergence of transplantation infections as a specialty of infectious diseases has paralleled the prolongation of organ transplantation, prolongation of allograft and patient survival, and increasingly effective immunosuppressive agents [4].

This study aimed to evaluate and discuss common infectious diseases in patients with a history of liver or kidney transplantation and infectious diseases. All patients in our study include late-stage transplantation infections.

METHODS

Patients older than 18 years of age who underwent liver and kidney transplantation between 2011 and 2022 and were hospitalized in our hospital due to heart failure and for whom infectious diseases consultation was requested were retrospectively analyzed from hospital records. Demographic information, intensive care unit records, types of infection, microbiological investigations, other laboratory data, and radiological examinations of 32 consecutive patients were obtained.

The study was approved by Kosuyolu Yüksek İhtisas Training and Research Ethics Committee (Decision No: 2023/06/682, Date: 04/04/2023).

Statistical Analysis

Mean ± standard deviation, median (minimum, maximum), frequency, and ratio values were used in determining the descriptive statistics of the data.

RESULTS

Of the patients included in the study, 9 (28%) were female, 23 (72%) were male, and the mean age was 33.7±11.3 years (range: 17-66 years). Our patients' median age was 60 years. Fourteen patients (14%) over 65 years of age were observed. Patients had similar comorbidities. Patients had congestive heart failure (CHF) (87.2%, n = 28), hypertension (43.7%, n = 14), and chronic obstructive pulmonary disease (21.8%, n = 7) (Table 1).

In this retrospective study, 20 patients (62.5%) of kidney transplant recipients and 12 (37.5%) of liver transplant recipients were seen within ten years. The most common infections were urinary tract infections (UTIs) in 8 (25%) patients and pneumonia in 11 (34%) patients. The other infections were gastrointestinal infections such as diarrhoea, bloodstream infections and

Table 1. The characteristic of 32 transplant patients

Characteristic	Data
Age (years) (mean ± SD)	33.7 ± 11.3
Gender, n (%)	
Male	23 (72)
Female	9 (28)
Type of transplant, n (%)	
Kidney	20 (62.5)
Liver	12 (37.5)
Hospital admissions, n (%)	
Non-ICU	15 (46.5)
Direct to ICU	17 (53.5)
Primary sites of infection, n (%)	
Pneumonia	11 (34.3)
Urinary tract infection	8 (25)
Bloodstream infection/Bacteremia	6 (18.7)
Enteritis	3 (9.2)
COVID-19	3 (9.2)
CMV	1 (3.1)
Co-morbidities, n (%)	
CHF	28 (87.2)
DM	3 (9.2)
HT	14 (43.7)
COPD	7 (21.8)

ICU = intensive care unit, CHF = Congestive Heart Failure, DM = Diabetes Mellitus, HT = Hypertension, COPD = Chronic obstructive pulmonary disease, CMV = *Cytomegalovirus,* SD = standard deviation

Table 2. Classification and percentage of organisms from patients

Organism Isolated	Strain (n = 20) n (%)
Escherichia coli	5 (25%)
Enterococcus spp.	2 (10%)
Streptococcus pneumoniae	4 (20%)
Staphylococcus aureus	4 (20%)
Klebsiella pneumoniae	2 (10%)
Cytomegalovirus	1 (5%)
Candida albicans	2 (10%)

COVID-19 and *Cytomegalovirus* (CMV). All patients were found to have an infection. Culture positivity was found in 20 (62.5%) of 32 patients who presented with infection (Table 2).

The microbiological data were noteworthy for unusual and opportunistic organisms, including one instance of acute CMV viremia.

Severe sepsis and organ failure occurred in six (18.75%) of the 32 patients with documented infections. The patients had more than one accompanying comorbidity. Four (66%) of these six patients were diagnosed with cardiovascular dysfunction, four (66%) with acute renal dysfunction, and three (50%) with severe metabolic acidosis. With 17 (44%) patients, CHF was the predominant reason for the intensive care unit admission. As a result, six (18.7%) patients died after hospitalization. In our study, the pneumococcal vaccination rate was calculated as 9.3%. All of our patients wer late transplant patients (2 years-23 years). There were eight (25%) patients between 2 years and 10 years, 20 (62.5%) patients between 10 and 20 years and 4 (12.5%) patients over 20 years.

DISCUSSION

Despite significant improvements in patient and graft survival in the post-transplant period, it remains a significant cause of morbidity and mortality. To prevent graft rejection, transplantation patients are lifelong immunocompromised hosts due to immunosuppressive drug regimens. This excessive immunosuppression increases the potential for infections with common and opportunistic pathogens. The early signs of infection may be mild because of a suppressed inflammatory reaction to the infection, and these infections can be fatal in this state [5, 6]. In our study, six of thirty-two patients had a mortal course.

The majority of clinically significant infections are seen in the first 180 days. Six months after transplantation, most recipients are clinically stable, and immunosuppressive therapies are reduced. However, community-acquired infections are more common in this period [7, 8].

This is expected due to the high number of renal transplant patients seen and their high incidence of

UTIs. Many authors report that the frequency of UTI is higher in the early years after transplant, especially in the first year (74 %), while the frequency of UTI decreases to around 35 % in the second year and further to 21 % in the four years after transplant. In the later period, while immunosuppression continues, infections specific to the general population, such as UTIs with community pathogen aetiology, are more frequent [9]. In a large retrospective cohort study by Abbott *et al*. [10], the cumulative UTI rate after kidney TX was found to be 60% for women and 47% for men after four years. Only 17% of patients develop early UTIs in the first three months. Over 70% of all UTIs following renal transplantation are caused by Gram-negative organisms, with *Escherichia coli* being the most common organism in the general population (30 to 80%) [11, 12]. *E. coli* (54.2%) was the most common organism isolated from infected patients in the study [10].

In our study, we found *E. coli*, *Enterococcus* spp and *Candida albicans* as common Bloodstream infections (BSI) are the leading causes of SOT deaths and illnesses. While pathogens, gram-positive bacteria, are the most common cause of BSIs, Gram-negative bacteria are more common in renal transplant recipients and are primarily associated with UTIs [13, 14]. In our patient group, six patients were followed up due to bacteraemia and the source of the disease was observed to be the lung and urinary system. In the blood cultures of our patients, *E. coli* was found in 4 patients, *Staphylococcus aureus* in 1 patient and *Enterococci* with *Candida albicans* in 1 patient. The literature has reported that urinary tract infections and lower respiratory tract infections due to ventilation have emerged as the source of bacteremia, as in our study [15]. Sepsis is a severe complication of SOT. One study found that transplant patients were admitted to the intensive care unit for life-threatening complications. 24% of these were urinary tract infections [16-18]. Chuang *et al*. [19] Reported that 90% of transplant recipients who died of sepsis had UTI. The primary focus of infection in 2/3 of our patients who died due to sepsis was the urinary system.

Diarrhoea is common after transplantation, with a prevalence of 20% to 50% in solid organ transplant recipients, and the infectious agents are similar to those in non-transplant populations, but the presentation can be more severe. Diarrhea in this population can lead to dehydration, increased drug toxicity, organ rejection, and death [20, 21]. Immediate stool culture and toxicology are essential in such cases. *Clostridium difficile* infection (CDI) incidence rates in transplant recipients vary from 1% to 23%. The most critical risk factor for developing CDI is antibacterial exposure. Risk factors specific to the SOT population include age> 55 years, the use of anti-thymocyte globulin, retransplantation and the type of organ transplanted. The highest CDI rate is found in liver transplant recipients [22]. In our study, three patients had gastroenteritis. In two of these three patients, CDI caused diarrhoea in our transplant recipients.

The COVID-19 pandemic caused everyone to apply to the hospital, and many were hospitalized and treated. The rate of COVID-19-related hospitalization was higher in the solid organ transplant patient group than other patients [23]. Several comorbidities have been studied which impact the severity of COVID-19 disease. SOT patients have been identified as a risk group for COVID-19. This is due to their chronic immunosuppressive therapy. In a systematic review, age, post-transplantation time and comorbidity were variably identified as independent risk factors for mortality or disease severity.

SOT recipients show similar humoral and cellular immune responses after COVID-19 infection. SOT recipients have a decreased immune response after two doses of the SARS-COV-2 mRNA vaccine [24]. We conclude that the overall outcome was similar to the general population, based on another review and meta-analysis [20]. There were 3 COVID-19 patients in our study, 1 of whom died.

Prophylaxis after SOT is effective in the prevention of CMV infection during the period when patients are on antiviral therapy. Therefore, after the end of prophylaxis, CMV infection is usually observed [20]. Adult CMV-seronegative recipients transplanted from CMV-seropositive donors are the highest-risk group [25].

In another study, 37% of patients developed late-onset CMV infection on average 67 days after discontinuation of prophylaxis (range 1-475 days) and 244 days after transplantation (range 150-655 days) [26]. Considering that CMV disease occurred in our patient two years after transplantation, it would not be wrong to define our patient in the classification of late-onset primary CMV infection.

During the study period, %53 SOT were admitted to our ICU, of which 40% were admitted for sepsis, %26 for pneumonia, %40 for urinary tract infection, one for COVID-19, and one for acute gastroenteritis.

Limitations

The primary limitation of this study is the retrospective methodology. The patients are older than 18 years of age have had transplantation in external centers and have been hospitalized in our hospital for various reasons. Therefore, they were evaluated only with infectious diseases consultation.

CONCLUSION

Infection prevention has become a keystone of modern transplantation medicine due to the significant incidence of post-transplant infectious complications from improved immunosuppressive therapies and surgical procedures. This is essential to decrease the morbidity and mortality directly attributable to infection and reduce the burden of infection-related indirect effects contributing to impaired long-term allograft survival.

Authors' Contribution

Study Conception: SDK, GE; Study Design: SDK, GE; Supervision: SDK, GE; Funding: SDK, GE; Materials: SD, GE; Data Collection and Processing: SDK, GE; Statistical Analysis and Data Interpretation: SDK, GE; Literature Review: SD, GE; Manuscript Preparation: SD, GE, MD, YUK and Critical Review: SD, GE, MD,YUK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):1099-1104 Original Article

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Prognostic value of the leuko-glycemic index in coronary chronic total occlusion patients

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ABSTRACT

Objectives: Inflammation parameters are related to the prevalence and mortality of coronary artery disease (CAD). We aimed to evaluate the prognostic value of the leuko-glycemic index (LGI) and determine mortality in patients with chronic coronary total occlusion (CTO).

Methods: A total of 546 patients were evaluated in the study. All-cause death was the primary endpoint. The leuko-glycemic index was calculated from the blood samples at admission and patients were divided into 3 groups according to their LGI levels. Kaplan-Meier survival curves were performed and logistic regression analyses was used for all multivariable analysis.

Results: The mean age of the study population was 63.1 ± 11.1 years and 70.3% were male. Median follow-up time 58.2 ± 22.4 months. The mortality rate was 33.6% in the high LGI group and significantly higher compared to the other group. In multivariable analysis, LGI (OR: 1.05, 95% CI: 1,0-1.2; $p = 0.02$) and age (OR: 1.07, 95% CI: 1.04-1.11; $p = 0.001$) were found as predictors of all-cause death.

Conclusions: The study revealed that high LGI is associated with all-cause death in CTO patients and LGI was a predictor of all-cause death.

Keywords: Leuko-glycemic index, coronary total occlusion, mortality, coronary artery disease

Coronary artery disease (CAD) is still the leading cause of mortality and morbidity all over the world [1]. Coronary total occlusion (CTO) is a complex coronary lesion, and it is a condition of 100% angiographic occlusion for 3 months or more [2]. It is detected in an average of 10-30% of coronary angiographies [3]. The presence of CTO has been associated with recurrent myocardial infarction, cardiogenic shock, heart failure, and sudden death [4-6]. Parameters or findings that predict clinical events in patients with CTO have always been the subject of research.

Identifying high-risk patients with CAD may help clinicians take preventive measures. Inflammatory markers, which are elevated in the primary response to ischemic heart disease, may be helpful in estimating the extent of ischemic damage [7]. It has been found in previous studies that inflammation parameters are

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closely related to the prevalence and mortality of CAD [7, 8]. Systemic immune-inflammation index, neutrophil-lymphocyte ratio, and other additional hematological parameters have been investigated in coronary artery patients with CTO and acute coronary syndrome patients and have been associated with adverse cardiac events [8-11].

The leuko-glycemic index (LGI) is an easy-to-calculate, noninvasive simple indicator of inflammation that includes parameters directly related to inflammation, such as glucose and leukocyte count. LGI is an inflammation parameter studied in patients with acute coronary syndrome and is associated with poor clinical outcomes and coronary artery prevalence [12-14]. There are no studies examining the relationship between LGI and mortality in CTO patients. Therefore, the study aims to investigate the relationship between LGI and mortality in CAD patients with CTO.

METHODS

Our study is a retrospective and observational study and includes patients with CTO in at least one vessel after CAG between 2014 and 2020. Angiograms were analyzed by the independent experienced operators. Chronic inflammatory disease, nonregulated diabetes mellitus, hemolytic disease, malignancy, and active infectious disease were excluded.

The electronic database of the hospitals was used to acquire the baseline characteristics and medical histories of the patients. After exclusions, a total of 546 patients enrolled in the study. The study protocol was approved by the ethics committee (Dicle University) with the date 04/03/2021 and number 181.

The laboratory parameters of the patients were obtained from blood samples taken at the admission. All-cause deaths were determined as the primary endpoint.

The leuko-glycemic index (mg/dl.mm3) was calculated by multiplying the fasting blood glucose (mg/dL) and leukocyte count (on admission) in mm3 divided by one thousand [7].

Statistical Analysis

The continuous variables were presented as mean ± standard deviation or median interquartile range (IQR) (25-75%). Normally distributed data were compared using one-way ANOVA, and non-normally distributed data were compared using the Kruskal-Wallis test. The categorical variables were examined using Fisher's exact test and are shown as the number of cases with percentages. The relationship between LGI and mortality was investigated using multivariate logistic regression analysis. The survival of groups was analyzed using Kaplan-Meier methods. A $p < 0.05$ was considered statistically significant.

RESULTS

A total of 546 patients were included in the study. The mean age of the study population was 63.1 ± 11.1 yearsand 70.3% were male. The patients were divided into 3 groups according to their LGI levels as follows: < 1082 (mg/dL.mm3) in group 1 (low), 1082-1688 (mg/dl.mm3) in group 2 (intermediate), and > 1688 (mg/dL.mm3) in group 3 (high). White blood count, platelet, CRP (c-reactive protein), triglyceride, and glucose levels were higher in patients in group 3 compared to other groups, while glomerular filtration rate (GFR) and ejection fraction (EF) were lower than other groups. Patients in group 3 had a higher prevalence of hypertension (HT), diabetes mellitus (DM), and all-cause mortality. The demographic and laboratory findings of the groups are shown in Table 1.

In multivariate regression analysis, age (odds ratio (OR): 1.07, 95% CI: 1.04-1.11; $p = 0.001$) and LGI (OR: 1.05, 95% CI: 1,0-1.2; $p = 0.02$) were found as independent factors for all-cause mortality. The multivariate regression analysis is given in Table 2.

The log-rank test was used to quantify the significance of the differences between the Kaplan-Meier curves, and it revealed that group 3 had a greater incidence of all-cause death. (Fig. 1).

DISCUSSION

In this study, we found that high LGI was associated with all-cause mortality in CTO patients. In the multivariate analysis, we showed that LGI and age were independent predictors of all-cause mortality. The relationship of CTO with some inflammatory parameters has been studied before, but the relationship between LGI and CTO is examined for the first time.

There is a long-known relationship between CAD

Table 1. Baseline demographic and laboratory parameters of groups

	Tertile 1 (n = 276)	Tertile 2 (n = 134)	Tertile 3 (N =136)	*p* value
Age (years)	62.3 ± 10.9	63.3 ± 11.1	63.5 ± 11.3	0.73
Male, n (%)	215 (77.8)	95 (70.8)	81 (59.5)	**0.001**
HT, n (%)	75 (27.2)	49 (36.6)	70 (51.4	**0.001**
DM, n (%)	25 (9.1)	36 (26.9)	50 (36.7)	**0.001**
Smoking, n (%)	76 (27.5)	43 (32.1)	31(22.8)	0.23
CKD, n (%)	13 (4.7)	6 (4.5)	12 (8.8)	0.18
All-cause death, n (%)	55 (19.9)	30 (23.4)	46 (33.8)	**0.007**
MI, n (%)	90 (32.6)	42 (31.3)	49 (36)	0.69
Follow-up period (months)	58 ± 23.2	57 ± 23.7	59 ± 24.5	0.1
EF (%)	50.6 ± 10.4	49.2 ± 11.4	46.4 ± 11.1	**0.003**
WBC count ($\times 10^3/\mu L$)	7.8 ± 1.8	10.3 ± 2.4	11.9 ± 3.6	**< 0.001**
Hemoglobin (g/dL)	13.6 ± 1.8	13.7 ± 1.7	13.3 ± 2.1	0.15
Platelets ($\times 10^3/\mu L$)	236.3 ± 78.5	269.7 ± 84.9	272.8 ± 91.3	**< 0.001**
Glucose (mg/dL)	102 ± 21.6	136.7 ± 36.8	220 ± 87.3	**< 0.001**
Albumin (g/dL)	3.6 ± 0.43	3.5 ± 0.52	3.5 ± 0.49	0.08
GFR ($mL/min/1.73m^2$)	86.5 ± 23.8	86.1 ± 24.3	76.6 ± 24.3	**< 0.001**
LDL (mg/dL)	106.3 ± 38.8	106.5 ± 40.2	106.8 ± 44.2	0.98
Triglyceride (mg/dL)	160 ± 79.6	182.6 ± 88.3	205.5 ± 120.2	**0.001**
Total cholesterol (mg/dL)	177.6 ± 47.7	180.5 ± 47.3	185.7 ± 52.7	0.27
CRP (mg/L)	1.4 ± 0.7	2.7 ± 1.1	4.9 ± 1.5	**< 0.001**
LGI ($mg/dL.mm^3$)	783.9 ± 182.3	1343.5 ± 168.2	2775.3 ± 980.2	**< 0.001**
SYNTAX score	15.3 ± 6.3	15.8 ± 5.9	17.9 ± 5.5	**0.03**

Data are shown as mean ± standard deviation or number (percent). HT = hypertension, DM = diabetes mellitus, MI = myocardial infarction, CKD = chronic kidney disease, EF = ejection fraction, WBC = white blood cell, GFR = glomerular filtration rate, LDL = low density lipoprotein, CRP = C-reactive protein, LGI = leuko-glycemic index, SYTNAX = Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery

and inflammation [15, 16]. It has been found in many previous studies that the indicators of inflammation in the blood are high in patients with stable CAD and acute coronary syndrome [17, 18]. An increase in inflammatory markers is associated with free oxygen radical release, coagulation cascade activation, an increase in platelet aggregation and an increase in infarct size [19]. One of the main indicators of inflammation in the blood is the leukocyte count. It has been found that a high leukocyte count is closely associated with cardiogenic shock, heart failure, and in-hospital mortality in acute coronary syndrome patients [20, 21].

The inflammatory process affects glucose metabolism through some mediators and also increases the blood glucose level in acute states independent of DM. Inflammation and hyperglycemia accelerate the atherosclerotic process with an additive effect [22]. As a result of the HORIZONS-AMI study, it was determined that a high glucose level at admission was associated with 1-month and 3-year mortality [23]. In addition, it has been shown in previous studies that a high blood glucose level is associated with no reflow and adverse cardiac events [24].

Since LGI includes leukocyte count and glucose level, it can be an inflammation parameter that can help predict prognosis in cases of acute cardiac events

Table 2. Univariable and multivariable regression analysis for determine the predictor of all-cause mortality

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age	1.06 (1.04-1.09)	**0.001**	1.07 (1.04-1.11)	**0.001**
Gender	0.87 (0.68-1.34)	0.53		
HT	0.58 (0.34-0.77)	**0.001**	0.68 (0.36-1.30)	0.25
DM	0.66 (0.43-1.03)	0.058		
HL	0.92 (0.29-2.91)	0.89		
CKD	0.27 (0.12-0.56)	**0.001**	0.72 (0.2-2.28)	0.61
Smoking	0.99 (0.64-1.55)	0.95		
CRP	1.11 (1.02-1.22)	**0.008**	1.05 (0.96-1.14)	0.29
LGI	1.04(1.0-1.1)	**0.01**	1.05 (1.0-1.2)	**0.02**
SYNTAX score	0.98 (0.95-1.2)	0.40		

HT = hypertension, DM = diabetes mellitus, CKD = chronic kidney disease, CRP = C-reactive protein, LGI = leuko-glycemic index, SYTNAX = Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery

or stable coronary artery disease. It has been found that high LGI is closely associated with short and long-term prognosis and 1-year mortality in patients with acute myocardial infarction [13, 14]. CTO is a complex CAD, and the presence of CTO is associated with poor cardiovascular events. The relationship between CTO and inflammation parameters has been the subject of research before. In a study, it was found that a high leukocyte count at admission was associated with high cardiovascular risk in CTO patients [11]. Demir *et al*. found that the systemic immune-inflammatory index, which consists of many blood parameters, is closely associated with an increased risk of all-cause death in CTO patients [25]. In our study, similar to these studies, we found higher leukocyte count, CRP, and all-cause mortality in the high LGI group, and we

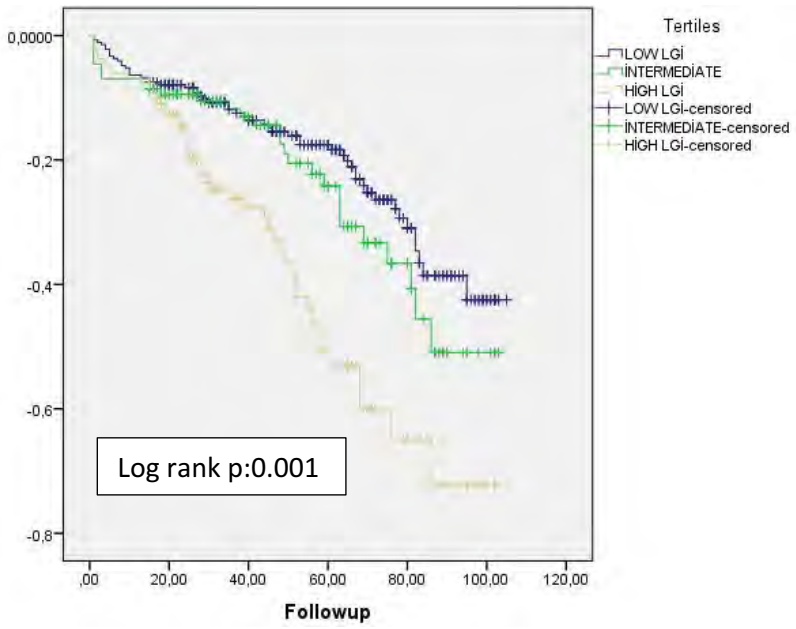

Fig. 1. Kaplan-Meier analysis of groups according to the survival.

also determined that LGI is an independent predictor of mortality.

Studies have shown that hyperglycemia is associated with poor cardiovascular events and a poor prognosis in CTO patients [26, 27]. Song *et al*. found that triglyceride-glucose index and stress hyperglycemia were associated with a poor prognosis in CTO patients [27]. In our study, triglyceride and glucose levels were found to be higher in the high LGI group compared to the other groups. In addition, mortality was higher in the high LGI group compared to the other groups.

Limitations

First of all, its retrospective nature and relatively small number of patients are important limitations. For the blood parameters used for LGI, values were taken at the time of admission to the hospital, and averaging the values during hospitalization or follow-up may affect the results. Evaluation of previously known inflammation parameters and comparison with LGI would have reinforced the effectiveness of our study. Multicenter studies with a higher proportion of patients are needed for risk assessment in CTO patients.

CONCLUSION

In this study, we determined for the first time that high LGI is associated with mortality in CTO patients. We also found that LGI was an independent predictor of all-cause death. This simple, noninvasive parameter can help identify high-risk CTO patients and help them be treated more effectively and followed up more closely.

Authors' Contribution

Study Conception: TP, MÖ, BB, SFA, MD, BA; Study Design: MÖ, BA; Supervision: SFA; Materials: TP, SFA; Data Collection and/or Processing: MÖ, SFA, MD; Statistical Analysis and/or Data Interpretation: BA, BB, TP; Literature Review: TP, BA; Writer: TP, BA; Manuscript Preparation:TP, SFA and Critical Review: BB

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Acute poisonings requiring intensive care in childhood and a hidden threat, suicide attempts: a single-center experience

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ABSTRACT

Objectives: Very few studies have been conducted to identify the conditions that cause poisoning in pediatric patients needing intensive care, both by age group and toxic agent factor. This study will support the development of strategies for poisoning prevention measures by comparing the data in our region with other data in the world.

Methods: This study is a single-centered, retrospective study. The baseline status of acute poisoning was defined in pediatric patients aged one month to 18 years who required intensive care hospitalization between November 2017 and March 2022.

Results: There were 148 patient admissions due to acute poisonings (5.2% of all admissions, 69.6% females, median age: 13.6 months). Our study revealed that acute poisoning in children is caused mainly by pharmacological (88.5%), oral intake (97.3%) and at home (85.8%). It was observed that intoxication peaked at two different ages; the first peak was at preschool (33.1%), and the second peak was at adolescence (58.7%). In the univariate analysis, females (odds ratio [OR] = 4.1), adolescents (OR = 167.6), psychiatric drug users (OR = 55.5), and multiple drug intoxications (OR = 3.6) were associated with more suicides. Being adolescents and using psychiatric medication contributed significantly to suicide attempts in multivariate analysis (OR = 145.3 and OR = 37.9). None of our patients died.

Conclusions: Preventing both poisoning and suicide attempts is the most critical priority. However, we suggest prevention strategies should be strengthened even if mortality is not observed. Furthermore, our study shows that suicide attempts are very likely to be repeated, especially if an underlying psychiatric illness exists.

Keywords: Acute poisoning, children, pediatric intensive care unit, self-poisoning, suicide attempts

Poisonings are a predictable and preventable public health problem. Drug, chemical, or herb-related poisonings can cause multi-organ failure and even death in childhood. The World Health Organization (WHO) states that poisoning in children is one of the top five causes of death from unintentional injuries [1]. According to the 2020 data from the American Association of Poison Control Centers (AAPCC), pub-

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lished annually, 58% of the over 3 million poisoning calls made to the poison center are made for children, with children under six years of age having the highest exposure [2]. Today's rising drug use rates in adults and children raise the risk of poisoning, particularly in pediatric patients. We discovered that the small number of patients and forensic cases were only mentioned in acute poisoning studies in Bursa province and that epidemiological data were not revealed in such a large study. The present research is the first study in Bursa province on acute poisoning cases requiring pediatric intensive care.

Suicide refers to a person's action to end their life willingly; it can be done by hanging, jumping from a height, poisoning, or injury with a firearm [3]. This study did not include only suicidal attempts with toxic substance intake. However, our data show that the probability of suicide is significantly higher if the poisoned person is a female and/or an adolescent if the poisoning is done with multiple drugs, and if there is a history of psychiatric drug use. Therefore, with this study, we wanted to raise awareness to reduce child deaths, hospitalizations, and acute poisoning-related costs and not waste precious life-saving intensive care beds with predictable and preventable acute poisonings.

METHODS

This is a retrospective study on acute poisoning patients admitted to the medical/surgical pediatric intensive care clinic at Bursa Yuksek Ihtisas Training and Research Hospital between November 2017 and March 2022. Following the approval from the local ethics committee (2011-KAEK-25 2022/08-01), data collection was initiated in accordance with the principles of the Helsinki Declaration.

Patients were admitted to the intensive care unit for respiratory failure, cardiovascular dysfunction, arrhythmia, seizures, or loss of consciousness. Alternatively, if the toxic substance they were exposed to was risky in terms of content or dose in relation to the conditions listed above, they were admitted to PICU.
Patients' demographic characteristics (sex, age, race, presence of concomitant disease), substance content and number (one or more), route of exposure (oral, inhaler, cutaneous), time of hospitalization in intensive care clinic, specific antidote treatment if used, supportive intensive care treatments, stay in intensive care and total hospitalization duration, and outcomes were recorded by scanning the files. Age groups were defined as follows: infant (1 month-12 month), preschool (1-5 years), school age (6-11 years), and adolescent (12-18 years). All patients are older than 1 month and younger than 19 years. The study excluded patients with drug side effects, anaphylaxis, burns, food poisoning, chronic poisoning, and data deficiency. A patient with multidrug poisoning was considered a single case.

The forms of poisoning were divided into five: unsupervised intake, suicidal intake, recreational intake, therapeutic error, and others (snake bite, mushroom poisoning). The factors causing acute poisoning were classified as pharmaceutical and non-pharmaceutical factors. Pharmaceutic drugs consisted of analgesics (paracetamol, anti-inflammatory, myorelaxant), antimicrobials (antibiotics, antivirals, antifungals), neuroactive drugs (antidepressants, antipsychotics, antianxiety, antiepileptics), antihypertensives and antidiabetics. Non-pharmaceuticals included alcohol, stimulants, pesticides, corrosives, snake bites, inedible vegetable oils, and mushroom poisonings.

Statistical Analysis

The SPSS 15v Chicago IL program was used in data analysis. A p - value of $p < 0.05$ was considered significant. When presenting qualitative data, frequency and percentage values are given, mean and standard deviation when presenting quantitative data, and median and minimum-maximum values when the data is heterogeneously distributed. Normality tests (Kolmogorov-Smirnov and Shapiro-Wilk) were used to test the data distribution. Chi-square test and, if applicable, Fisher's Exact and post hoc Z test were used to compare qualitative data. In the second step, Multivariable analysis was used to analyze the patients' poisoning characteristics since the significant features at the 0.05 level in the Bivariate analysis were suitable for a Binary logistic regression model.

RESULTS

Between November 2017 and March 2022, 2820 patients aged one month to 18 were admitted to PICU,

with 148 (5.2%) patients due to acute poisoning. All patients were mostly female (69.6%), and the most common exposure was at home (85.8%). The exposures were frequent (97.3%) in the form of ingestion. None of our patients died. The factors were pharmacological in 131 (88.5%) patients.

In Table 1, the sociodemographic characteristics of the patients hospitalized due to intoxication are given in detail. There was no difference in terms of sex based on age groups ($p > 0.05$ for all groups). Separately for the male and female sex groups, the preschool group has a significantly higher number of cases than the infant group, and the adolescent group has a significantly higher number of cases than the other three groups ($p < 0.05$). There were no statistical differences between the infant group and school group, school group, and preschool group (Chi-square post hoc Z test, $p > 0.05$). Figs. 1a and 1b show the sex distribution by age group and the causes of poisoning.

The mean age of all cases was 10.4 ± 6.2; the median is 13.6 years. Unsupervised intakes are most common in the preschool group, accounting for 88.5% of all unsupervised intake cases ($p < 0.0001$; median: 2.6). Intentional recreational intakes were all in the adolescent group, limited in number; there were only 3 cases (100%), and it was not significant ($p > 0.05$). The adolescent group accounted for 96.3% (n = 78) of the suicide cases, and they were significantly higher ($p < 0.0001$; median: 15.4). The age group distribution of therapeutic error cases is balanced. 130 (87.8%) of the acute poisoning patients were caused by pharmacological agents. The poisoning factors of patients who were poisoned for pharmacological and non-pharmacological reasons are given in Table 2.

In Table 3, the data of the patients poisoned for suicide are given in detail. According to the univariate analysis in Table 3, females are more associated with suicide in cases of intoxication (odds ration [OR] = 4.1). Intoxications in adolescents are associated with suicide 167.6 times more than in other age groups. While cases in the morning are 3.3 times more likely to be a suicide, cases in the evening are less likely to be a suicide than all cases in the other parts of the day

Table 1. Sociodemographic variables of hospitalized children with acute poisoning

Variables*	Data
Age (year)	13.6 (9.9-11.1)
Gender (male/female), n (%)	45(30.4) / 103 (69.6)
Setting home/other, n (%)	127 (85.8) / 21 (14.2)
Intensive care duration (day)	1.0 (1.7- 2.0)
Outpatient clinic duration (day)	3.0 (3.5-4.7)
Exitus/ discharge, n (%)	0 (0) / 148 (100)
Substance pharmacological/ Non	131 (88.5) / 17 (11.5)
Season winter/ spring/ summer/ autumn, n (%)	27 (18.2) / 49 (33.1) / 38 (25.7) / 34 (23.0)
Route of intake (oral/ inhaler/ dermal), n (%)	144 (97.3) / 1 (0.7) / 3 (2.0)
Drug use in anamnesis (Yes/ No), n (%)	93 (62.8) / 55 (37.2)
Psychiatric drug in anamnesis (Yes/ No), n (%)	38 (25.7) / 110 (74.3)
Additional disease (Yes/ No), n (%)	59 (39.9) / 89 (60.1)
Psychiatric disease, n (%)	52 (35.1) /96 (64.9)
PICU (Level 2/ Level 3), n (%)	62 (41.9) / 86 (58.1)
Glasgow coma scale	13.0 (12.6-13.1)
Former suicidal attempt (Yes/ No), n (%)	20 (13.5) / 128 (86.5)
Race (Turkish/Refugee), n (%)	139 (93.9) / 9 (6.1)

Continuous data were expressed as median (25-75 percentile). PICU = pediatric intensive care unit

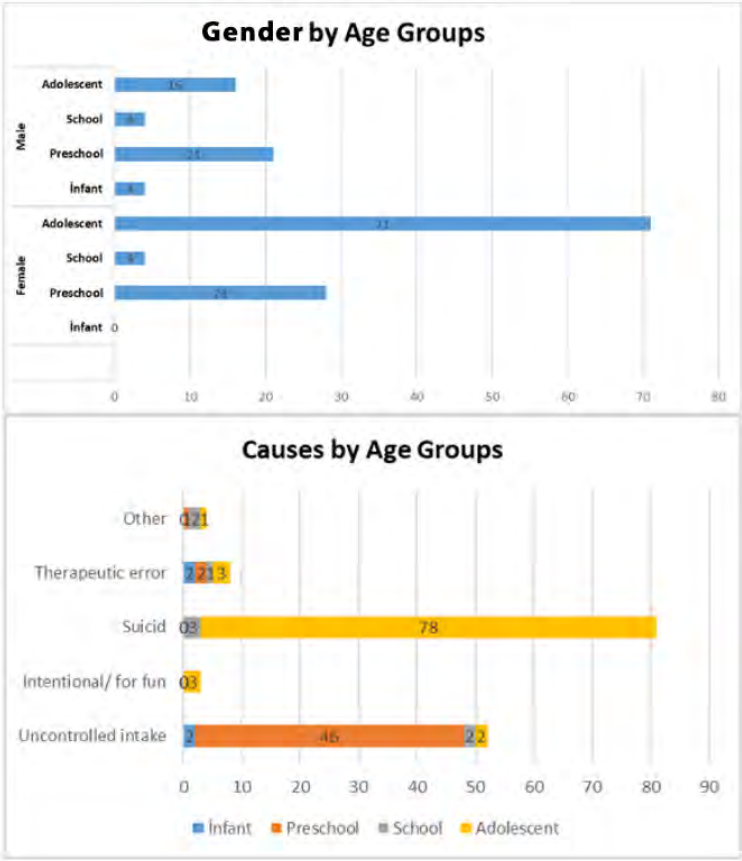

Fig. 1. **(A) Gender distribution by age groups. (B) Causes of poisoning by age groups**

(OR = 0.4). In cases that occurred on Fridays, the suicide rate was significantly lower than on other days (OR = 0.3). When psychiatric drug and non-drug users are compared, the probability of drug-using cases being suicidal is stronger (OR = 55.5). If multiple drugs cause the case of intoxication, the probability of suicide is 3.6 times higher compared to toxicity with only one drug. A local (Turkish) case is more likely to be a suicide than an immigrant case (OR = 5.2). In 20 of the suicide cases, there were previous histories of suicide attempts.

Since the findings in Table 3 were also appropriate for a binary logistic regression model, multivariate analysis was used in the second step, and the model was found to be significant. Again, being an adolescent and using psychiatric medication contributed significantly to the model (OR = 145.3 and OR = 37.9).

The most common drug used in drug poisoning was neuroactive drugs (n = 71). Analgesics were used with the second frequency (n = 48). Exposure to drug categories by age and sex is shown in Table 4. In 59 patients, multiple drug exposure was present.

Most patients (n = 119, 80.5%) were followed up with supportive care. Patients were given N-acetyl cysteine (n = 6), NaHCO3 (n = 6), invasive mechanical ventilation (n = 7), vasoactive drug (n = 7), and snake antiserum (n = 1) based on their needs. In addi-

Table 2. **Distribution of poisoning causes**

	Frequency	Percent
Pharmaceutical	130	87,8
Substance use	5	3,3
Muriatic acid	4	2,7
Pesticide	3	2,0
Snakebite	2	1,4
Alcohol	2	1,4
Thinner	1	0,7
Mushroom poisoning	1	0,7
Total	**148**	**100**

Table 3. Univariable and multivariable analysis of suicide

Suicid	Yes n (%)	Total	Univariable analysis OR	Univariable analysis 95% CI	Multivariable logistic analysis OR	Multivariable logistic analysis 95% CI
Gender						
Male	14 (31.1)	45	1			
Female	67 (65.0)	103	4.1*	1.9-8.7	2.7	0.6-11.9
Age category						
Infant	0 (0.0)	4	0.4*	0.4-0.5		
Preschool	0 (0.0)	49	0.2*	0.1-2.8		
School	3 (37.5)	8	0.5	0.1-2.1		
Adolescent	78 (89.7)	87	167.6*	43.4-646.4	145.3*	28.0- 753.8
Location						
Own Home	67 (54.0)	124	1			
Other	14(58.3)	24	1.2	0.5-2.8		
Time of exposure						
Morning	8 (61.5)	13	3.3*	1.0-10.5	1.3	0.2-11.2
Afternoon	27(69.2)	39	0.9	0.5-1.9		
Evening	20 (38.5)	52	0.4*	0.2-0.7		
Night	26 (59.1)	44	1.2	0.6-2.4		
Days						
Monday	13 (59.1)	22	1.2	0.5-3.1		
Tuesday	15 (65.2)	23	1.7	0.7-4.2		
Wednesday	11 (61.1)	18	1.3	0.5-3.7		
Thursday	13 (54.2)	24	1.0	0.4-2.3		
Friday	7 (30.4)	23	0.3*	0.1-0.8		
Saturday	7 (43.8)	16	0.6	0.2-1.7		
Sunday	15 (68.2)	22	1.9	0.7-5.1		
Pandemic Measures	14 (63.6)	22	1.5	0.6-3.9		
Psychiatric History	37 (97.4)	38	55.5*	7.3-419.5	37.9*	2.1- 686.5
Substance						
Pharmaceutical	73 (55.7)	131	0.7	0.3-1.9		
Multi drug	43 (72.9)	59	3.6*	1.8-7.3	1.3	0.3-5.6
Race						
Refugee	1 (11.1)	9	1			
Turkish	80 (57.6)	139	5.2*	0.8- 33.0	13.7	0.9- 200.1
Total	**81 (54.7)**	**148**				

*Asterisk indicates $p < 0.05$

Table 4. Distribution of pharmacological drugs by age groups and gender

	Age n (%)				Gender n (%)		Total
	Infant	Preschool	School	Adolescest	Male	Female	
Neuroactive	1 (1.4)	17 (23.9)	4 (5.6)	49 (69.0)	22 (31.0)	49 (69.0)	71
Analgesic	0 (0.0)	11 (22.9)	2 (4.2)	35 (72.9)	11 (22.9)	37 (77.1)	48
Cardioactive	0 (0.0)	10 (50.0)	0 (0.0)	10 (50.0)	2 (10.0)	18 (90.0)	20
Hormones	0 (0.0)	3 (33.3)	0 (0.0)	6 (66.7)	3 (33.3)	6 (66.7)	9
Antimicrobial	0 (0.0)	0 (0.0)	1 (12.5)	7 (87.5)	3 (37.5)	5 (62.5)	8
Gastrointestinal	1 (20.0)	2 (40.0)	0 (0.0)	2 (40.0)	2 (40.0)	3 (60.0)	5

tion, renal replacement therapy was applied to two patients with isoniazid and metformin intoxication. Since our hospital did not have a pediatric psychiatry inpatient service, the patients were transferred to the pediatric service.

DISCUSSION

Acute poisoning in pediatric patients is still a significant public health problem. An international study of pediatric poisonings from 20 countries and eight different global regions discovered significant epidemiological differences by region [4]. Therefore, we wanted to raise awareness about acute poisoning in critically ill patients in Bursa province to reduce child deaths, hospitalizations, and costs, as well as not waste precious life-saving intensive care beds with predictable and preventable acute poisonings.

This study is a special report on acute childhood poisoning requiring intensive care. During the study period, acute poisonings accounted for 5.2% of total patient admissions in the PICU, where medical/surgical patients were followed. This is approximately half the rate determined by some studies [5]. The majority of our cohort consisted of females (n =103, 69.6%). However, with age, not only females but also males increased in number (Fig. 1b). In the childhood age group, poisoning cases peak at two different ages [5-7]. The first peak was during the preschool period between the ages of 1-6, and the second peak was during the adolescence period between the ages of 12-18 (Fig. 1b).

The pattern of poisoning in children varies according to age and sex. According to the American Association of Poison Control Centers (AAPCC) annual National Poison Data System (NPDS), while the majority of poisonings in infants are caused by unintentional intakes or therapeutic errors[8], in adolescents, they are caused by deliberate intakes [2]. However, 64% of poisonings occur in girls between the ages of 13-19, and 64% are due to deliberate intake [2]. In many studies, the preschool age group is thought to be the most frequently poisoned age group due to a desire to explore the environment and imitate adults [9-11]. In our study, exposures in the preschool age group are primarily unintentional, with 88.5% being unsupervised intakes and suicide attempts occurring in the adolescent period (Figs. 1a and 1b). The most common place of exposure was home (85.8%) and was consistent with the available literature [2, 8]. Although many studies emphasize that the poisoning is often caused by unintentional exposure in the preschool period, it is thought-provoking that our study is also compatible with the literature. Unfortunately, this result shows that the public is still not sufficiently aware of poisonings.

It is known that in our country, it is easy to access health services and medicines since health expenditures, particularly for those under the age of 18, are completely under the guarantee of the state. Therefore, we believe that medicines are stocked at every home, and enough sensitivity is not shown in terms of properly storing them at home. 131 (88.5%) of acute poisoning patients were caused by pharmacological agents. The most common drug used in drug poisoning was neuroactive drugs (n = 71). Analgesics were used

with the second frequency (n = 48).

The poisoning factors for 18 patients who were poisoned due to non-pharmacological reasons are given in the Table 2. In our region, where agriculture is an essential source of income, only three patients were hospitalized with pesticide poisoning during these 3.5 years. Intentional recreational purchases, although not statistically significant, were all in the adolescent group, limited in number. There were only 3 cases. Unfortunately, some acute poisonings are also caused by a therapeutic error or incorrect application [12]. In our study, the distribution of therapeutic error cases according to age groups was balanced. Eight of our cases were poisoned due to therapeutic error.

No specific treatment was applied to 80.4% of the patients admitted to the PICU. All patients were given symptomatic treatment, and monitored their vital signs. Even if the patients were asymptomatic, the hospitalization decision was made considering the toxic factor and the amount they were exposed to. When the published literature on this subject is reviewed, it can be seen that death is usually an undesirable outcome [2, 5, 13, 14]. As far as we are aware, our study is one of the few in which no deaths were reported. Health measures were taken for four patients. 2 patients were in the child welfare institution and were followed up. All patients were transferred to the ward after they were examined and followed up by a child psychiatrist.

Suicide refers to a person's action to end their life willingly; it can be done by hanging, jumping from a height, poisoning, or injury with a firearm [3]. Suicide is the second most common cause of adolescent death [15]. As emphasized by WHO, unfortunately, suicidal ideations and attempt rates in adolescents have increased worldwide over the years [15]. In this study, most of the suicide cases were composed of adolescents and females. In our study, 96.3% (n = 78, median age: 15.4) of the suicide attempt cases were in the adolescent group. Today, the incidence of depressive moods in adolescents is increasing. Antidepressant intake is known to encourage suicide [16]. Suicide attempts, in our opinion, are facilitated by patients' easy access to antidepressant drugs prescribed to them. In our cohort, almost all of our patients were using psychiatric drugs. Every patient admitted to our clinic with a suicidal attempt necessarily receives a child psychiatry expert opinion.

At least one psychiatric disorder exists in adolescents who have attempted suicide [18]. In the case series of 11 children who died by suicide in Zambia, nine died by hanging, and two died by organophosphate poisoning [17]. None of these cases were prone to behavioral disorder, anxiety, or depression [17]. These cases, in our opinion, did not have psychiatric diagnoses because their families neglected them. So they wanted to die, and they died. In a study on 6483 adolescents aged 13 to 18 conducted in 2013, the suicidal ideation rate was 12.1%, and the suicide attempt rate was 4.1% [17]. Moreover, at least 80% of suicidal adolescents received psychiatric treatment [18]. In our study, when the cases using psychiatric drugs were compared with the cases not using psychiatric drugs, the probability of suicide was 55.5 times stronger for the case using it.

In 2004, the U.S. Food and Drug Administration (FDA) requested that a warning be included in antidepressant drug product catalogs that may increase the risk of suicidal ideation and behavior, particularly during dose changes [19]. However, it is known that the risk of suicide attempts increases in those who have a psychiatric illness [20]. In 2008, the FDA requested that the drug catalogs be updated again, emphasizing that mood disorders and severe psychiatric disorders are major causes of suicide [21].

In a survey that investigated suicidal behavior in adolescents with the participation of 41 schools and 6020 students in the United Kingdom, 7% of the participants emphasized that they had intentionally harmed themselves in the previous year [22]. A Canadian study compared 20471 adolescents aged 10 to 19 who had their first self-poisoning episode within the previous 12 years to a control group of over 1 million people with no such history [23]. Suicide risk was 30 times higher within the first year after the first self-poisoning episode than in the control group [23]. Children who had attempted suicide had a high risk of repeating the attempt. Unfortunately, in our study cohort with 81 suicide attempts, 20 patients had attempted suicide before.

Our study aimed to draw attention to all acute poisoning cases in patients between 1 month and 18 years old. However, since the number of suicides is increasing in the childhood age group, especially in adoles-

cents, it was concluded that stricter child psychiatry controls for patients with comorbidities and especially psychiatric diagnoses, as well as increased interventions to take health measures by contacting social services, should be implemented.

According to the American Association of Poison Control Centers (AAPCC) National Poison Data System (NPDS) annual report, at least 80% of children younger than 13 were managed by phone outside of a health facility where the exposure occurred [2]. According to the 2020 data from the National Poison Information Center published by the Republic of Turkey Ministry of Health, 9.14% of phone calls were made by family members and 5.09% by the exposed person. As a result, increasing the frequency with which families in Turkey use poison control centers will reduce unnecessary health expenditures. In Turkey, families should be educated on how to avoid poisoning, and if there is a suspicion of poisoning, the telephone information system should be used more actively. Thus, by reducing unnecessary emergency applications, better quality emergency services will be provided, and unnecessary health expenditures will be eliminated, thereby contributing to Turkiye's economy.

According to a study in Sweden that compared suicide attempts among refugees and Swedes by forming groups of approximately 5 million people each, suicide attempts among refugees were significantly lower than among Swedes [24]. In our study, which lasted approximately 3.5 years, the total number of patients hospitalized in our unit was 2820, with 658 (23.3%) of these patients being Syrians. However, regarding exposure to intoxication, while our cohort consisted of 148 patients, 9 (6%) were Syrian. Only one patient's poisoning in this group was caused by a suicide attempt. Suicidal intoxication was rarely observed in Syrian patients; we believe it is a survival instinct in war societies that have fled their homeland.

Limitations

Our study has some limitations. Only patients with acute poisoning who required intensive care were included in the study. It excludes patients discharged and sent home from the emergency department, admitted to the wards, or died before being taken to the hospital. It is a single-center study, and multi-center studies are needed.

CONCLUSION

It would be beneficial to increase the number of studies aimed primarily at preventing acute poisonings, which are a major cause of morbidity and mortality in children. Increased drug use in adults and children raises the risk of poisoning, particularly in pediatric patients. We recommend that families in Turkey be educated on poisoning prevention methods regularly through public health campaigns and that the ways of protection from poisoning be explained. The phone information system should be used more frequently if poisoning is suspected. Of course, focusing on the psychological safety of children, especially adolescents, will significantly reduce suicide attempts.

Authors' Contribution

Study Conception: AO; Study Design: AO, SK; Supervision: SK; Funding: N/A; Materials: N/A; Data Collection and/or Processing: AO; Statistical Analysis and/or Data Interpretation: MS; Literature Review: AO; Manuscript Preparation: AO and Critical Review: AO, SK, MS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Availability of Data and Material

The datasets utilized and analyzed during the present study are not publicly available but from the corresponding author on reasonable request; however, restrictions apply to the availability of the data due to privacy protection laws.

Ethics Approval and Consent to Participate

We initiated the study after the approval of the local ethics committee. The study received ethical approval (protocol number 2011-KAEK-25 2022/08-01) by Bursa Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committe. Informed consent was not applicable due to study design (retrospective). All methods were performed in accordance

with the ethical standards in the Declaration of Helsinki and its later amendments or comparable ethical standards.

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Comparison of nutritional adequacy in adult patients with acute respiratory distress syndrome with and without veno-venous extracorporeal membrane oxygenation: a single-center experience

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ABSTRACT

Objectives: Limited data is available regarding nutrition practices for patients with acute respiratory distress syndrome (ARDS) who are also receiving veno-venous-extracorporeal membrane oxygenation (VV-ECMO). The aim of the study was to describe the nutritional status of patients receiving VV-ECMO and compared with those who did not.

Methods: Patients (> 18 years-old) diagnosed with ARDS who received VV-ECMO (≥ 72 hours) were included in this retrospective study. The daily achievement of an energy target (%) and average protein intake during 2 weeks after initiation of VV-ECMO were calculated. Adequate feeding was defined as achieving 80-110% of the calculated target. The duration before initiating parenteral (PN) and enteral nutrition (EN), feeding route, length of intensive care, and hospital stay were evaluated. Data was compared between groups.

Results: In this study, 24 patients were included, of whom 12 received VV-ECMO. EN was started in a median 1.5 and 1 day in the VV-ECMO and non-ECMO groups, respectively. In the VV-ECMO group, 75% of the patients could achieve nutritional adequacy (> 80% energy goal) and 83.3% in the non-ECMO group ($p = 0.615$). PN being required in 4 (33.3%) patients who received VV-ECMO and 3 (25%) patients who did not ($p = 0254$). Ten of all patients experienced inadequate EN because of hemodynamic instability (n = 3), prone position (n = 4), gastric distension (n = 2) and diarrhea (n = 1).

Conclusions: VV-ECMO was not an obstacle for adequate nutrition, but prone position and hemodynamic instability were common causes of enteral feeding interruptions and inadequate energy delivery.

Keywords: Acute respiratory distress syndrome, veno-venous-extracorporeal membrane oxygenation, nutrition, enteral, parenteral, critically ill

Acute respiratory distress syndrome (ARDS) is a life-threatening condition characterized by poor oxygenation and non-compliant lungs. This disorder is associated with capillary endothelial injury and diffuse alveolar damage [1]. For treating ARDS, extracorporeal membrane oxygenation (ECMO) may be required as well as ventilation with low tidal volume, prone position, and high positive end expiratory pres-

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sure (PEEP) applications. ECMO may be a promising method for treating ARDS in the future [2]. Although the main treatment strategies for ARDS are respiratory support and treatment of the underlying disease, supportive care is necessary. The pro-inflammatory response and related hypercatabolism in ARDS can cause significant nutritional deficiency, and nutritional support should not be ignored in critically ill patients with ARDS [3].

Mechanically ventilated patients are at a high risk of malnutrition, and malnutrition can cause respiratory muscle weakness, prolonged mechanical ventilation (MV), and length of stay (LOS) in intensive care unit (ICU) [4, 5]. In addition to MV administration, patients who need venovenous ECMO (VV-ECMO) are the most severely ill patients with prolonged ICU-LOS and increased nutritional support [6]. Full calorie and protein nutritional support is essentially recommended by Extracorporeal Life Support Organization (ELSO) for patients undergoing ECMO [7]. In the absence of detailed guidelines on nutrition for ECMO patients from ELSO, healthcare professionals may have adopted different approaches and practices based on their individual judgement, experience, and the availability of evidence from other sources [3, 8].

Despite the concerns that ECMO administration will cause gut barrier dysfunction and allow bacterial translocation, studies suggest that enteral nutrition (EN) is well tolerated in patients undergoing ECMO. Additionally, reporting of EN related adverse events is rare in these studies [9-11]. Macgowan *et al*. [6] reported that adequate energy and protein delivery is possible in patients receiving VV-ECMO support. Hardy *et al*. [12] also stated that VV-ECMO was not a barrier to nutritional adequacy in COVID-19 patients. This variation reflects the need for further research and consensus in order to provide standardized guidelines and best practices for implementing optimal nutrition to patients undergoing VV-ECMO [6, 12].

The nutritional support can be challenging in patients with ARDS who receive VV-ECMO. This is likely because VV-ECMO can cause gastrointestinal disturbances, such as nausea, vomiting, and diarrhea. These disturbances can make it difficult to provide patients with adequate nutrition through EN. Therefore, it is important to monitor critically ill patients who receive VV-ECMO for nutritional problems. Early identification and intervention can help to prevent complications and improve outcomes. In addition, there are a few studies comparing nutritional adequacy and problems in critically ill patients who received VV-ECMO to those who did not.

This study aimed to describe the nutritional care, adequacy of nutrition, and clinical outcomes of providing nutritional support and to compare these in patients with ARDS who received VV-ECMO with those who did not.

METHODS

A retrospective observational study of adult patients with ARDS receiving and not receiving VV-ECMO was undertaken on our mixed medical and surgical ICUs. This study was approved by the Hospital's Medical Ethics Committee (The decision number is 2022-15/2) and conducted in accordance with the principles of the Declaration of Helsinki.

Participants and Study Groups

Our hospital has a total of 189 adult intensive care beds, 61 of which are under the responsibility of anesthesiologists and intensive care specialists. Patients (age > 18 years) with severe ARDS receiving VV-ECMO (≥ 72 h) between January 1, 2021 to October 31, 2022 were included in the study. Patients with severe ARDS who did not receive VV-ECMO between the same period were included as the control group. ECMO patients were analyzed while forming the control group, and they were formed from patients with similar age, gender and comorbidities. The diagnosis of severe ARDS was made according to the Berlin Definition [13]. Patients were excluded if there was not any documentation about calculated nutritional targets. Pregnant women, end-stage cancer patients, and patients who stayed in the ICU for less than 24 hours were also excluded from the study.

Nutrition Support Protocol

All patients in the study received nutritional therapy according to our ICU's standard protocols. Our ICU's standard protocols are based on the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines [14]. Medical nutrition therapy is considered for all patients staying in the ICU for more than 48 hours. Our aim is to begin nutritional therapy

through a nasogastric (NG) tube within 24 hours of the patient's admission to the ICU. Afterwards, individualized nutritional goals were calculated by the ICU dietitians and physicians within 48-72 hours. In patients who do not tolerate full-dose EN during the first week in the ICU, the safety, and the benefits of initiating parenteral nutrition (PN) are being evaluated on an individual basis. Since we have no indirect calorimetry, a simple equation based on weight is used for energy calculation. While the energy target is 20-25 kcal/kg/day in the acute disease period, it is calculated as 25-30 kcal/kg/day in the anabolic period. While hypocaloric nutrition is preferred in the early stage of acute disease, nutritional support is increased by 80-100% of the calculated energy after 3 days. Daily protein requirements are estimated to be at least 1.3 g/kg considering the actual body weight. If the patient's body mass index (BMI) is > 25 kg/m^2, ideal body weight is considered in target protein and energy calculations.

Data Collection

Data were collected for each patient's sex, BMI, the number of days of ECMO support, the number of invasive mechanical ventilation (IMV) days, length of ICU, and hospital stay were collected via an electronic medical record and nurse patient follow-up forms. The severity of critical illness was calculated using Acute Physiology and Chronic Health Evaluation (APACHE) II and NUTrition Risk in Critically ill (NUTRIC) score on the day of ICU admission. Additionally, the NUTRIC score was calculated when the patients were intubated. The degree of organ failure was evaluated using by the Sequential Organ Failure Assessment (SOFA) score. In the VV-ECMO group, the SOFA score was calculated at admission to the ICU and on the 1st day of ECMO. In the non-ECMO group, the SOFA score was calculated at admission to the ICU. The daily achievement of the energy target (in percentage) and the daily average protein intake were calculated during the first 2 weeks after the initiation of VV-ECMO, or until death if it occurred within 2 weeks. In patients who did not receive VV-ECMO, daily achievement (%) of the energy target and mean daily protein intake were calculated for 2 weeks after intubation, or for the period until death if the patients died within 2 weeks. Underfeeding was defined as < 80% of the target of energy or protein intake for that day, and overfeeding was defined as receiving > 110% of energy targets. Propofol was not included in the energy calculation because only 1 of the patients included in the study received short-term propofol infusion.

The primary outcome is whether we can give adequate calories to patients receiving VV-ECMO compared with those who do not. We also determined the reasons for hypocaloric underfeeding and discontinuation of EN and outcome of patients.

Statistical Analysis

The data were analyzed using SPSS software (version 20.0) and Graphpad Prism 8. The descriptive statistics are presented as number, percentage, mean ± SD, and median with minimum-maximum value. The normal distribution of the data of the numerical variables was evaluated using the Shapiro-Wilk normality test. Comparisons between groups were performed with a t -test for variables with normal distribution and the Mann–Whitney U test for variables without normal distribution. The relationships between categorical data were evaluated using the chi-square test. Friedman test was used to compare the data at different individual time points. To determine which pairs are different, a pairwise comparison test was carried out. $P < 0.05$ was considered statistically significant.

RESULTS

In this study, 24 patients were included, of whom 12 received VV-ECMO (Fig 1). The patients were divided into two groups, received VV-ECMO (10 men; mean age, 46.4 years) and not received VV-ECMO (7 male; mean age, 51.8 years). The mean APACHE II, SOFA and NUTRIC scores in all patients were 12.71 ± 6.59, 4.71 ± 1.57 and 2.63 ± 1.58, respectively. The median value of PaO2/FiO2 (P/F) ratios was 76.96 ± 21.78. There was no difference between groups in terms of age, gender, ICU scores, and P/F ratio. The mean duration between intubation and start to VV-ECMO was 6.83 ± 11.12 days. The mean duration of ECMO support was 13.08 ± 4.03 days. Two patients (16.6%) were weaned off ECMO.

Mechanical ventilation duration was longer in the

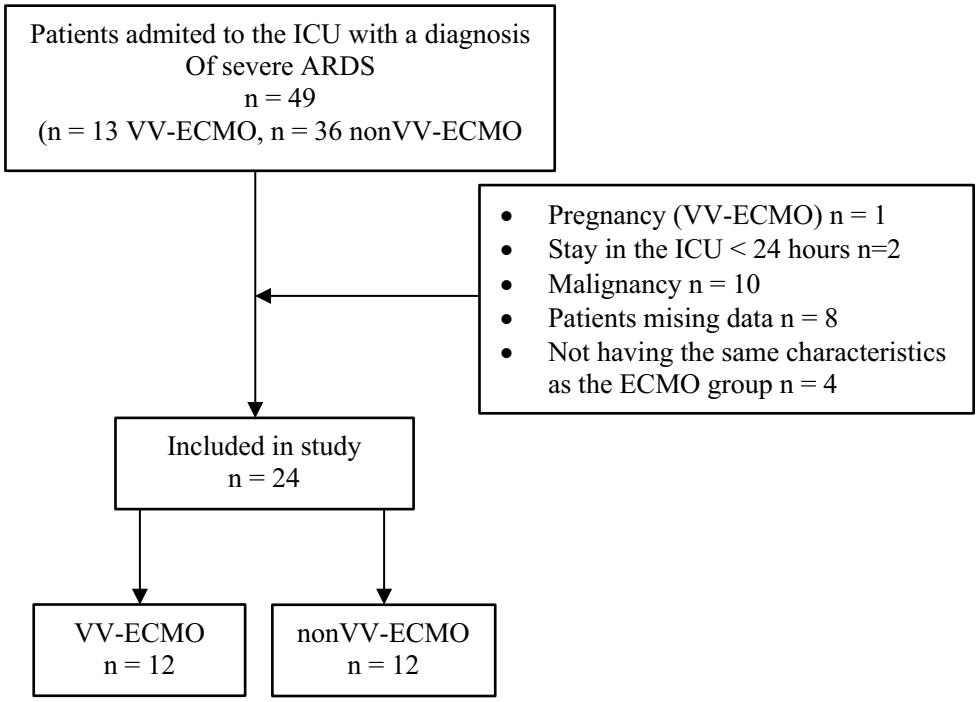

Fig. 1. Flow diagram of the study.
ICU = Intensive Care Unit, VV-ECMO = Veno-Venous Extra-Corporeal Membrane Oxygenation.

VV-ECMO group than non-ECMO group ($p = 0.045$). The length of stay (LOS) in the ICU was 34.42 ± 24.33 days in the VV-ECMO group and 20.33 ± 10.5 day in the non-ECMO group ($p = 0.079$). Mortality was high in both the groups (83.3% in VV-ECMO group, 91.7% in non-ECMO group). The baseline characteristics and ICU treatments of both groups are summarized in Table 1.

Nutrition Support

Continuous EN was first administered to all patients by the NG route. The median time to start enteral feeding was 1 days in all patients, and the mean time to reach 80% of the daily calorie goal was 3.92 ± 3.13 days. In the VV-ECMO group, the mean target calories and protein were 1583 ± 158.59 kcal/day and 100.42 ± 10.75 gr/day, respectively. The mean target calories and protein were similar in both groups ($p = 0.915$ and $p = 0.931$, respectively). In the VV-ECMO group, 75% of the patients could achieve nutritional adequacy (> 80% energy goal) and 83.3% in the non-ECMO group ($p = 0.615$). The average energy delivered to the VV-ECMO group was 73% of the targeted energy. However, the average energy delivered to the non-VV ECMO group was 82% of the targeted energy. There was no significant difference between groups ($p = 0.233$) (Fig. 2). In the VV-ECMO group, the energy delivery rate increased from 1st day's 31% to 67% of their nutritional goal on the third day. By the end of 1st week 82% of the nutritional goal was reached. The increase in energy delivery from day 1 to day 7 was significant ($p = 0.043$). On the other hand, in the non-ECMO group, the energy delivery rate increased from 1st day's 49% to 92% of their nutritional goal by the third day. By the end of 1st week 83% of the nutritional goal was reached. Although the targeted energy level was reached, the increase in energy delivery from day 1 to day 7 was not significant ($p = 0.393$) (Fig. 3).

When we analyzed the protein targets, although they were similar in both groups, target protein (> 80% protein goal) could be given in only 6 (50%) patients in both groups. The protein delivery percentage was below the targeted level in both groups (Figs. 2 and3). The enteral feed was administered polymeric in 23 patients and olymeric diet in 1 patient. Most diets contained 1 kcal/mL. The used polymeric products was 500 ml which contained an average of 26 g of protein.

Table 1. Baseline characteristics of all patients and comparison of groups

	All patients (n = 24)	VV-ECMO Group (n = 12)	non-ECMO Group (n = 12)	*p* value
Age (years)	49.08 ± 12.37	46.42 ± 11.53	51.83 ± 13.1	0.294
Gender, n (%)				
Male	17 (70.8)	10 (83.3)	7 (58.3)	0.178
APACHE II score	12.71 ± 6.59	12.33 ± 8.47	13.08 ± 4.31	0.787
SOFA score	4.71 ± 1.57	4.75 ± 1.71	4.67±1.49	0.9
NUTRIC score	2.63 ± 1.58	2.5 ± 1.78	2.75 ± 1.42	0.708
BMI (kg/m^2)	27.62 ± 3.10	27.27 ± 2.9	27.98 ± 3.36	0.587
Duration between admission to ICU and intubation (days)	6 ± 4.37	7.33 ± 4.84	4.67 ± 3.55	0.139
Duration between onset of symptoms and intubation (days)	11.92 ± 7.98	15.3 ± 8.04	8.5 ± 6.55	**0.033**
PaO2/FiO2- ICU on admission	76.96 ± 21.78	76.33 ± 25.03	77.58 ± 19.09	0.892
PaO2/FiO2- First day of intubation	70.67 ± 18.02	72.58 ± 13.79	70.67 ± 18.02	0.773
Co-morbidites, n (%)				
Diabetes mellitus	6 (25)	5 (41.7)	1 (8.3)	0.059
Hypertension	8 (33.3)	4 (33.3)	4 (33.3)	1
Treatments in ICU, n (%)				
CRRT	6 (25)	5 (41.7)	1 (8.3)	0.059
Vasopressors	16 (66.7)	10 (83.3)	6 (50)	0.083
Proning, n (%)	8 (33.3)	1 (8.3)	7 (58.3)	**0.009**
IMV (days)	18.29 ± 11.42	18.5 (6-45)	11 (7-24)	**0.045**
ICU (days)	27.38 ± 19.68	34.42 ± 24.33	20.33 ± 10.5	0.079
Hospital satay (days)	35.29 ± 27.87	44 ± 35.87	26.58 ± 13.13	0.129
Mortality, n (%)	21 (87.5)	10 (83.3)	11 (91.7)	0.537

Data are shown as mean±standard deviation or median (minimum-maximum or number (percent). VV-ECMO = Veno-Venous Extra-Corporeal Membrane Oxygenation, APACHE = Acute Physiology and Chronic Health Evaluation, SOFA = Sequential Organ Failure Assessment, NUTRIC = NUTrition Risk in Critically ill, BMI = Body Mass Index, = ICU Intensive Care Unit, CRRT = Continuous Renal Replacement Therapy, IMV = Invasive Mechanical Ventilation

When we examined the reasons of inadequate EN application, the most common reason was the prone position. Calories received during proning were reduced in 4 (33.3%) patients in the non-ECMO group. Hemodynamic instability was detected in 2 patients in the VV-ECMO group and in 1 patient in the non-ECMO group. Hemodynamic instability was considered if the shock was uncontrolled and the hemodynamic and tissue perfusion targets were not achieved with vasopressors/inotropes (MAP < 65 mmHg, lactate > 2 mmol/L, signs of insufficient tissue perfusion). While upper digestive intolerance was not detected in any patient in the non-ECMO group, it developed in 2 (16.7%) patients in the VV-ECMO group. Vomiting was not observed in these 2 patients although the gastric residual volume (GRV) was >500 mL. Parenteral nutrition was used to complement nutrition requirements in case of underfeeding with EN in 4 patients in the VV-ECMO group and 3 patients in the non-ECMO group. The nutritional interventions of the patients are shown in Table 2.

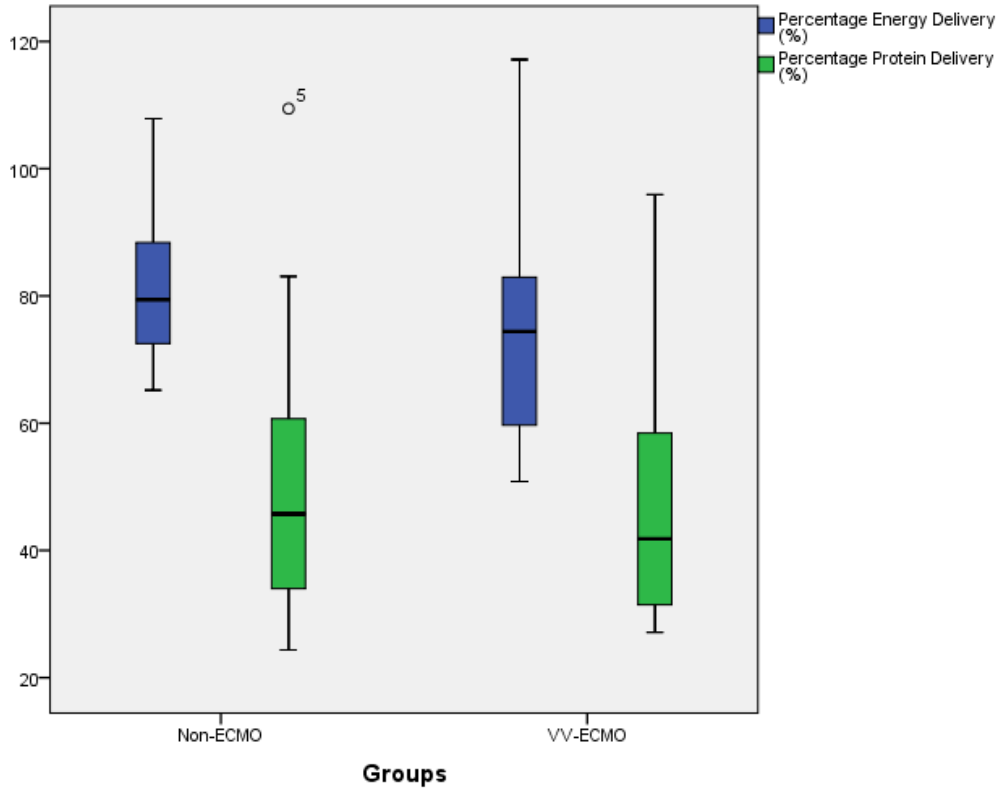

Fig. 2. Energy and protein delivery.
Figure shows the percentage delivery of total energy and the protein. Box plot indicates mean values. Whiskers indicate minimum and maximum values. No statistical significance between groups according to the percentage delivery of total energy and the protein (p = 0.233 and p = 0.735 respectively).

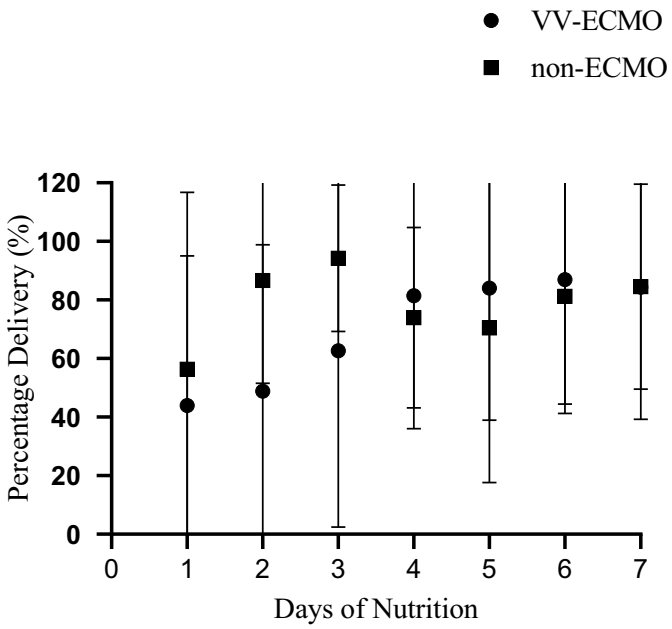

Fig. 3. Daily energy delivery for the first 7 days.
Figure shows the daily percentage delivery of total energy (including all sources) for the first 7 days. Box plot indicates mean values. Whiskers indicate minimum and maximum values. VV-ECMO = Veno-Venous Extra-Corporeal Membrane Oxygenation.

Tablo 2. The nutritional interventions by groups

	All patients (n = 24)	VV-ECMO Group (n = 12)	non-ECMO Group (n = 12)	*p* value
Time to start EN (days)	1 (1-4)	1.5 (1-4)	1 (1-2)	0.303
80% of measured energy targets (days)	3.92 ± 3.13	4.58 ± 3.77	3.25 ± 2.30	0.308
Energy target (kcal)	1587.50 ± 184.89	1583 ± 158.59	1591 ± 215.146	0.915
Protein target (g)	100.21 ± 11.46	100.42 ± 10.75	100.0 ± 12.61	0.931
Max. protein delivery (days)	6.67 ± 2.74	6.33 ± 3.37	7 ± 3.07	0.564
Max. energy delivery (days)	7.21 ± 2.93	7.33 ± 2.80	7.08 ± 3.17	0.84
Patients achieving 80% of their energy goal, n (%)	19 (79.2)	9 (75)	10 (83.3)	0.615
Patients achieving 80% of their protein goal, n (%)	12 (50)	6 (50)	6 (50)	1
Time to start PN (days)	5 ± 2.12	5.40 ± 2.51	4.5 ± 1.73	0.563
Parenteral nutrition, n (%)	7 (29.2)	4 (33.3)	3 (25)	0.254
Reasons for inadequate EN, n (%)				0.119
Hemodynamic instabilit	3 (12.5)	2 (16.7)	1 (8.3)	
Pronin	4 (16.6)	0	4 (33.3)	
Gastric distention	2 (8.4)	2 (16.7)	0	
Diarrhea	1 (4.2)	1 (8.3)	0	

Data are shown as mean ± standard deviation or median (minimum-maximum or number (percent). VV-ECMO = Veno-Venous Extra-Corporeal Membrane Oxygenation, EN = Enteral Nutrition, PN = Paranteral Nutrition

DISCUSSION

In this study, we described the nutritional practices in patients with ARDS supported by VV-ECMO at a single center. We think that VV-ECMO was not an obstacle for adequate nutrition provision in critically ill patient. However, the prone position was a frequent reason for enteral feed interruptions and inadequate energy delivery. To our knowledge, our study is one of a limited number of studies describing nutritional adequacy and problems in critically ill patients, which compares a cohort receiving VV-ECMO with a non-ECMO cohort.

Nutrition guidelines recommend early EN in critically ill patients unable to maintain adequate intake [14, 15]. Physicians may be reluctant to initiate enteral feeding because of concerns about complications such as delayed gastric emptying and non-occlusive mesenteric ischemia. In our study, enteral nutrition was started in the early period (< 48 hours) in the VV-ECMO group, as in the non-ECMO group. Additionally, the time to reach 80% of the calculated energy was similar in both groups. Besides, the percentage of energy supply on the 7th day in both groups was quite satisfactory (Group VV-ECMO 73%, group non-ECMO 81%). In the VV-ECMO group, 9 (75%) patients achieved of their energy goals and 10 (83.3%) in the non-ECMO group. Lukas *et al*. [16] detected that the mean nutritional adequacy during ECMO support was 55%, which was lower than that of general ICU patients. On the other hand, in the study by Hardy *et al*. [12] the rate of achieving the energy goal was high (81%) in patients receiving VV-ECMO similar to ours. In another study, it was reported that 80% of 102 patients who underwent VA-ECMO tolerated EN [17]. In our study, the finding of similar energy delivery with enteral feeding in VV-ECMO compared with the non-ECMO cohort is promising. However, the target protein was reached in 50% of the patients in both groups. Weijs *et al*. [18] found that ICU patients with

1.2-1.5 g/kg/day delivered protein had reduced 28-day mortality. Compher *et al*. [19] reported that the odds of death decreased by 6.6% with each 10% increase in protein intake. Although we cannot attribute the high mortality rate in our study solely to protein deficiency, we think that insufficient protein intake may be a contributing factor to high mortality. A reason for inadequate protein support was the low protein content of the enteral products used and the lack of products to provide additional protein support in our hospital.

Enteral nutrition was initiated using the gastric route as recommended in the guidelines [14, 15]. In addition, NG feeding is the most common route for nutritional support reported in studies of patients undergoing ECMO [3,17]. The administration of continuous versus bolus feeds is an important matter to address. Current studies suggest that bolus and continuous enteral feeding can achieve the same goal in critically ill patients without an increase in side effects in either of these pathways [20-22]. In this study, continuous EN was administered to all patients through the NG route.

The best timing to prescribe supplemental PN remains debated. The ESPEN 2019 guidelines recommended that those who do not tolerate a full dose of EN during the first week in the ICU should be considered for additional PN [14]. In our study, PN was initiated in approximately 5.4 days in the VV-ECMO group and approximately 4 days in the non-ECMO group. PN support was applied in 33.3% of the patients in the VV-ECMO group and 25% in the non-ECMO group. In the literature, the reported use of PN in patients receiving ECMO support ranged from 4% to 30% [3]. In our study, the use of PN was similar to the literature.

Patients undergoing ECMO may have circulatory shock, requiring vasoactive agents. While guidelines on nutritional support in critically hemodynamically stable patients share a common recommendation, there is no consensus in hemodynamically unstable patients [14, 15]. Although critical care providers may be reluctant to initiate EN early in circulatory shock, recent randomized controlled trials have shown that early initiation of low-dose EN is associated with improved clinical outcomes [23]. In this study, early low-dose EN was initiated in 3 patients with hemodynamic failure (VV-ECMO group 2 patients, non-ECMO 1 patient), and no adverse complications related to nutrition were detected.

Studies have highlighted increased GRV as one of the most common causes of EN interruption during ECMO [10, 25]. Mentec *et al*. [25] found that 49 (32%) of critically ill patients had an increase in GRV after a median EN duration of 2 days. In addition, sedation and use of catecholamines before and during EN have been reported to be risk factors for increased GRV. Increased GRV was detected in 2 patients in the VV-ECMO group, and vomiting was not observed in these patients.

In our study, the most common reason for the interruption of EN in the non-ECMO group was the prone position. EN volumes were considerably lower in these patients, resulting in underfeeding. Saez de la Fuente *et al*. [26] reported that EN was not associated with an increased risk of gastrointestinal complications in critically ill patients with severe hypoxemia receiving mechanical ventilation in the prone position. However, Reignier *et al*. [27] documented an incidence rate of 82% for the development of EN intolerance in the prone position. Studies supporting the safety of EN in the prone position are needed.

In studies evaluating the safety, tolerability, and results of EN during ECMO, the rate of development of intestinal ischemia has been reported to be quite low [11, 24, 28-31]. These studies have suggested that intestinal ischemia is associated with disease severity, and it has been reported that these patients have high APACHE II and SOFA scores [24, 29]. We did not detect bowel ischemia in any patient in our study. In our study, the patients were in young population who received VV-ECMO due to hypoxemia and the severity of illness scores (APACHE and SOFA) were low.

The mortality rate was high in our study. The Berlin Definition was developed in 2012, which categorizes ARDS as mild, moderate, or severe based on the degree of hypoxemia [13]. When our patients were evaluated according to the Berlin Definition, they were in the severe ARDS classification. The P/F ratios of patients in both groups were < 100 mmHg. Previous studies were reported that ICU and hospital survival decreased when ARDS severity increased [32, 33].

Limitations

Our study compares nutritional support in patients with and without VV-ECMO. However, the limitations of our study include being a single-center retro-

spective study and having a small number of patients. Another limitation is that the energy needs of the patients are calculated by the dietitian and clinician, and no indirect calorimetry was used.

CONCLUSION

ICU stays prolonged in patients undergoing ECMO. Additionally, these patients have more severe organ dysfunction. Considering these conditions, although the risk of acquiring malnutrition increases, guideline recommendations for the nutrition of patients undergoing ECMO are insufficient. Our results suggest that adequate energy and protein delivery is possible for most patients during VV-ECMO. Stronger randomized controlled trial-level evidence is needed to provide adequate nutritional care in patients undergoing ECMO.

Authors' Contribution

Study Conception: GÇ; Study Design: GÇ, NKG; Supervision: GÇ, NKG; Funding: N/A; Materials: N/A; Data Collection and/or Processing: GÇ; Statistical Analysis and/or Data Interpretation: GÇ; Literature Review: GÇ, NKG; Manuscript Preparation: GÇ, NKG and Critical Review: GÇ, NKG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):1124-1128 Original Article

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Clinical evaluation of treatment with hook plate in patients with acromioclavicular joint dislocation

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ABSTRACT

Objectives: Treatment of acromioclavicular joint dislocations vary. In this study, we aim to examine the functional results of patients who underwent hook plate fixation due to dislocation of acromioclavicular joint.

Methods: We retrospectively observed 21 patients who had been treated with hook plate due to dislocation of acromioclavicular joint. At follow up, Constant-Murley scoring system was performed for shoulder function evaluation, while radiological results were performed by X-ray.

Results: Seventeen of these patients were male and four were female. Eleven of these patients had Rockwood type 5 joint dislocation and ten had Rockwood type 3 joint dislocation. The mean age was 36.7 ± 13.37 years (range: 19-61 years). The mean follow-up period was 27.3 ± 19.3 months (range: 10-59). Constant-Murley shoulder scoring was excellent in 18 patients (93.6) and good (82.5) in 3 patients. While one patient had wound site infection and one patient had plate broken, no one had any neurological damage. Except for the failed implant, plate removal was not performed because the patients did not have plate-related complaints. At follow-up, it was observed that the acromioclavicular joint was in the reduced position on direct radiographs.

Conclusions: We observed good clinical and functional results for the treatment of acromioclavicular joint dislocation with hook plate treatment. The hook plate method is a safe and effective method in the acute treatment of type 3-5 injuries according to the Rockwood classification

Keywords: Acromioclavicular joint, dislocation, Rockwood classification, hook plate

The acromioclavicular (AC) joint is damaged in approximately 9% of the shoulder girdle injuries [1]. AC joint damage often occurs as a result of falling on the open and adducted arm indirectly or direct trauma to the lateral side of the shoulder.

The Rockwood classification is generally used to define the type of dislocation and determine treatment algorithms for AC joint dislocations [2]. According to the Rockwood classification, AC joint injuries are divided into six subgroups. The degree of injury and the patient's functional expectations are the primary indicators when choosing the treatment method of the patients. While type 1 and 2 injuries are followed with conservative methods, surgery is recommended for type 4-6 injuries. For type 3 injuries, the optimal treatment method for type 3 injuries is controversial [3].

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Various surgical procedures are available for AC joint dislocation such as coracoclavicular (CC) fixation, TightRope® fixation, hook plate, coracoacromial ligament transfer, AC or CC reconstruction [4].

In our study, we retrospectively evaluated the functional results of 21 patients who underwent hook plate due to AC joint dislocation.

METHODS

In our study, we examined 21 patients who applied to our clinic with the complaint of post-traumatic shoulder pain between November 2016 and February 2022 and treated with hook plate after AC joint dislocation was detected. Diagnosis made by direct radiography and MRI was used for Rockwood classification (Fig. 1). The patients included in this study were acute injuries of type 3-5 according to the Rockwood classification, older than 18 years of age, had no history of shoulder trauma or surgery to the shoulder region, and were not accompanied by a clavicle fracture.

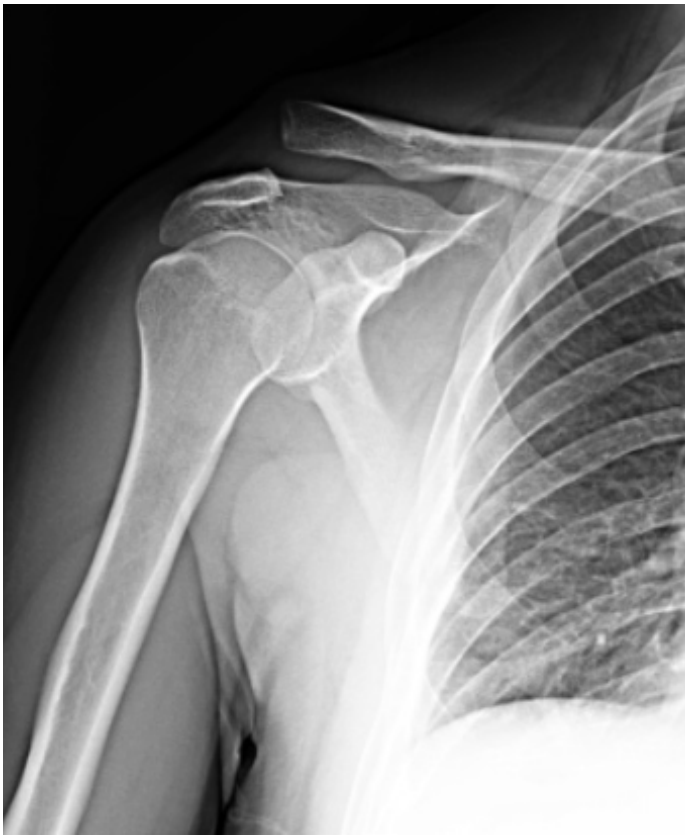

Fig. 1. **X-ray image of acromioclavicular joint dislocation.**

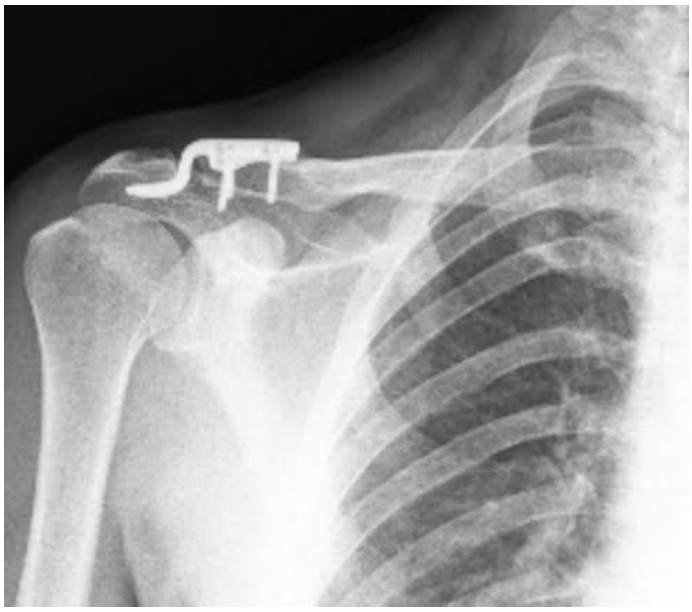

Fig. 2. **Postoperative x-ray image**

Surgical Technique

All of the patients were treated by the same surgical team and with the same surgical method. A skin incision of approximately 4 cm was made over the AC joint in beach-chair position under general anesthesia for all patients. The AC joint was exposed. After the joint reduction, we placed the tip of the hook plate under the acromion and fixation was made with three screws (Fig. 2). Joint reduction was confirmed after control imaging with scopy. Since no sign of impingement was detected in the intraoperative examination, the operation was terminated.

Patients were followed up with velpau bandage for 3 weeks after surgery. At the end of 3 weeks, the patients were allowed to use their shoulders in daily activities and rehabilitation was started. All movements were allowed at the end of the postoperative 6th week. While radiological results were evaluated by X-ray in the routine follow-up of the patients, Constant-Murley shoulder scoring was used for functional results.

The study was approved by the local Ondokuz Mayıs University Clinical Researchs Ethics Committee (Date: 2020, Decision Number: B.30.2.ODM.0.20.08/09-73-102). In this prospective study, all procedures and practices are in accordance with the ethical standards of the national/ institutional research committee and the 1964 Helsinki declaration. Informed consent was obtained from all patients.

RESULTS

Twenty-one patients who had AC joint dislocation were treated with hook plate. 17 of these patients were male and 4 were female. The mean age of the patients was 36.7 ± 13.37 years (range: 19-61 years). Eleven of the patients had right AC joint dislocation and ten of them had left AC joint dislocation. Demographic data are shown in Table 1. Eleven of these patients had Rockwood type 5 and ten had Rockwood type 3 AC joint dislocations.

The mean follow-up period was 27.3 ± 19.3 months (range: 10-59). While radiological results were evaluated by x-ray, Constant-Murley shoulder scoring was used for functional results. Constant-Murley shoulder scoring was excellent in 18 patients (93.6) and good (82.5) in 3 patients.

During the follow-ups, superficial wound infection was observed in 1 patient, and broken plate was observed in 1 patient after falling again, and no neurological damage was observed in any patient.

DISCUSSION

While AC joint dislocation is frequently seen in young athletes, it can also be seen in patients exposed to direct trauma [2]. According to the Rockwood classification, conservative methods are preferred in type 1-2 injuries and surgical methods are preferred in type 4-6 injuries, while the treatment of type 3 injuries is still controversial. While some authors recommend conservative treatment in patients with type 3 injuries, there are also authors reporting good clinical results of patients followed by surgical method [3, 5-8].

Surgical treatment methods vary [4]. In our study, we applied hook plate to patients with type 3-5 injuries according to the Rockwood classification, and we evaluated the clinical results of these patients retrospectively. Radiological evaluation was evaluated using direct radiographs and functional results were evaluated using Constant-Murley scoring. Complications which developed during the follow-up were also recorded.

Table 1. Characteristics of the patients

Characteristics	Data
Age (years)	36.7 ± 13.37 (19-61)
Gender (female/ male)	4/17
Side (right/ left)	11/10
Follow-up period (months)	27.3 ± 19.3 (10-59)
Complication	2 (9%)

In the evaluation of 21 cases, we found the Constant-Murley shoulder score to be excellent in 18 patients (93.6) and good (82.5) in 3 patients. Our results were compatible with the literature [9-12]. We evaluated isolated AC joint dislocations in our study. There are also studies comparing the results of hook plate and other methods in the treatment of AC joint dislocation. Xin Pan et al. [13] compared the results of tight rope and hook plate application in the treatment of AC joint dislocation in their meta-analysis study. According to their study, both TightRope and hook plate techniques were effective in relieving dislocation pain and improving AC joint function, and they found Constant Murley scores similar to our study.

Surgical treatment of AC joint dislocation with hook plate is relatively easy.It requires small incisions and minimal dissection, and its use is increasing. In addition to the treatment of AC joint dislocation, hook plate can also be used in distal clavicle fractures [7, 14]. With this method, the biomechanics of the AC joint are preserved in both AC joint dislocation and fracture treatment, allowing some degree of early mobilization after surgery [15]. Beside these, there are publications reporting that hook plate use may cause bone erosion, osteolysis and subacromial impingement in the postoperative period, as well as general complications such as nonunion, infection and implant failure [16-18].

After AC joint dislocation surgery, superficial wound infection has been reported in the literature at rates of 0-53%. [19, 20]. In this study, a superficial wound infection was detected in one patient, which was consistent with the literature, and healing was achieved after follow-up with oral antibiotics and wound dressing.

There are publications reporting broken plate after hook plate treatment [11, 21]. In one of our patients, implant failure (broken plate) occured after falling on the same shoulder again. On this case, plate was re-

moved. The AC joint was found to be stable in the intraoperative examination and scopy images.

In a cadaver study, it was shown that hook plate fixation allows physiological clavicular movement without deformation and shows similarity to the stiffness of the natural AC joint [22]. This study suggests that hook plates can be left in place in asymptomatic patients. In our study, apart from the patient who developed implant failure, we did not perform implant removal because other patients did not have any complaints. Thus, we thought that while avoiding the risk of loss of reduction after removal of the implant, we could avoid anesthesia-related risks and possible local complications due to additional surgery to be performed for removal.

Limitations

This study has some limitations. It was conducted in a single center and the number of patients was relatively low. Comparisons could be made with other surgical methods with larger series.
ith other surgical methods with larger series.

CONCLUSION

In conclusion, we think that the hook plate method is a safe and effective method in the acute treatment of type 3-5 injuries according to the Rockwood classification. With the hook plate method, early functional treatment is possible and we can prevent limitations in postoperative shoulder function. We think that the risks of re-dislocation and a second surgical procedure can be avoided by not removing the plate in patients who do not have active complaints related to plate. However, larger series studies are also needed.

Authors' Contribution

Study Conception: İB; Study Design: HSC; Supervision: DK; Funding: N/A; Materials: AMY; Data Collection and/or Processing: HÇ; Statistical Analysis and/or Data Interpretation: AY; Literature Review: İB, HÇ, AY, HSC, AMY, DK; Manuscript Preparation: İB, HÇ and Critical Review: AY, HSC.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(5):1129-1134 Original Article

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A new adjunct in the differentiation of encephalitis and meningitis after negative cerebrospinal fluid culture: systemic inflammatory immune index

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ABSTRACT

Objectives: Encephalitis and meningitis can have serious and potentially life-threatening consequences. This study aims to investigate whether the effects of systemic immune inflammation index (SII) and neutrophil/lymphocyte (N/L) ratio on differential diagnosis, severity, and clinical outcomes are superior to each other in patients diagnosed with encephalitis and meningitis in the emergency department.

Methods: Patients aged 18 years and older who presented to the adult emergency department of the hospital and were diagnosed with meningitis or encephalitis between January and December 2022 were included in the study. Patients under 18 and those with missing data in their files were excluded from the study. N/L ratio, SII values, and other associated parameters were compared between the group with mortality and the group who survived both diseases.

Results: There were significant differences in neutrophil, lymphocyte, N/L ratio, SII, and C-reactive protein (CRP) values between meningitis and encephalitis patients. N/L ratio and SII values were significantly lower in encephalitis patients than in meningitis patients. There was no significant difference in any of the parameters between surviving and deceased patients.

Conclusions: Simple calculable ratios such as SII and N/L ratio can be a supportive parameter in the differential diagnosis of the disease. However, it has been observed that using these indices is not a useful tool in determining the severity and prognosis of patients with encephalitis and meningitis.

Keywords: Systemic inflammatory immune index, neutrophil-lymphocyte ratio, encephalitis, meningitis

Encephalitis and meningitis are infectious diseases that can cause severe and potentially life-threatening outcomes for the central nervous system [1, 2]. Early diagnosis and treatment can reduce the risk of death and the likelihood of long-term neurological complications [2, 3]. Recognition of symptoms, physical examination, and relevant laboratory tests are important for rapid diagnosis. Cerebrospinal fluid (CSF) analysis, CSF culture, polymerase chain reaction (PCR) tests, and other imaging techniques can also be

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used to confirm the diagnosis [2, 4].

The neutrophil/lymphocyte (N/L) ratio indicates the inflammatory response [5]. In central nervous system infections such as meningitis and encephalitis, the N/L ratio can provide valuable information about the severity and prognosis of the disease. Studies have shown that the N/L ratio is effective in diagnosing and determining the prognosis of infectious diseases such as meningitis and encephalitis [6, 7]. A high N/L ratio indicates an increase in the severity of infection and a worsening prognosis [5].

The systemic immune-inflammation index (SII) is commonly used in many infectious and non-infectious diseases, such as cancer [8, 9]. It is calculated using the formula 'SII = P × N/L' based on the complete blood count N, L, and platelet (P) counts [9]. SII has been reported to play an important role in the early diagnosis of diseases and in providing valuable information about the severity and prognosis of infections [9-11]. In cases where the lumbar puncture is contraindicated or unsuccessful, decision-making for patient management can be difficult for clinicians.

This study investigates the impact of the SII and N/L ratio on early differential diagnosis and prognosis in patients diagnosed with encephalitis and meningitis in the emergency department. This study aims to determine whether SII and N/L ratios are superior to each other in the differential diagnosis, severity, and clinical outcomes of meningitis and encephalitis cases.

METHODS

Study Design and Setting

This study was a single-center retrospective cross-sectional study conducted in the emergency medicine clinic of a tertiary care hospital located in a metropolitan area with an approximate population of 4.5 million. The study received approval from the Izmir Katip Celebi University Non-Interventional Clinical Research Ethics Committee (Decision No: 0012, Date: 26.01.2023).

Study Population

Patients aged 18 years and older who presented to the adult emergency department of the hospital and were diagnosed with meningitis or encephalitis between 01 January 2022 and 31 December 2022 were included in the study. Patients under 18 and those with missing data in their files were excluded from the study.

Data Collection and Processing

The participants were divided into two groups according to their diagnoses of meningitis and encephalitis. Demographic characteristics (age and gender), initial laboratory test results including neutrophil, lymphocyte, and platelet counts, as well as the calculated N/L ratio and SII, C-reactive protein (CRP), blood urea nitrogen (BUN), creatinine, sodium, potassium, pH, lactate, bicarbonate values from arterial blood gas analysis, bacterial growth in CSF culture, patients' level of consciousness at the initial examination (alert, confused, comatose), clinical diagnosis (meningitis, encephalitis), hospital admission or intensive care unit (ICU) admission status, and patient outcomes were recorded from the medical records.

Outcome Measures

To determine the superiority of the N/L ratio and SII values in differential diagnosing meningitis and encephalitis. On the other hand, to predict the severity and clinical outcomes of the disease, these parameters were compared with other variables that could be associated with disease severity and clinical outcomes. Additionally, the N/L ratio, SII values, and other as-

Table 1. Demographic distribution of the cases included in the study

	Meningitis	Encephalitis	*p* value
Age (years)	62 ± 18	58 ± 19	0.283
Gender, n (%)			0.069
Female	11 (30.6)	32 (49.2)	
Male	25 (69.4)	33 (50.8)	

Data are shown as mean ± standard deviation or n (%)

sociated parameters were compared between the group with mortality and the group who survived both diseases.

Statistical Analysis

Data obtained in the study were analyzed using IBM SPSS Statistics for Macos, Version 26.0. Armonk, NY: IBM Corp. Categorical variables were expressed as numbers and percentages, while numerical variables were expressed as mean and standard deviation when presenting the descriptive statistics. Shapiro-Wilk test was used as the normality test. Since the data did not follow a normal distribution, the Mann-Whitney U test was used to compare two group means. The chi-square test was used for comparisons of categorical variables. A p - value of < 0.05 was considered statistically significant. Results were presented with a 95% confidence interval.

RESULTS

Of the 101 included cases, 65.4% were diagnosed with encephalitis. When examining the gender distribution, 69.4% of meningitis patients were male, and 30.6% were female. In contrast, 50.8% of encephalitis patients were male, and 49.2% were female. There was no significant difference in gender distribution (p = 0.069) (Table 1).

The laboratory values and SII, N/L ratio of the cases included in the study are presented in Table 2. There were significant differences in neutrophil, lymphocyte, N/L ratio, SII, and CRP values between the meningitis and encephalitis groups. Neutrophil and CRP levels were significantly higher in the meningitis group compared to the encephalitis group (respectively 14.4 ± 9.1 vs. 9.9 ± 5.3, p = 0.012, 81 ± 92 vs. 45 ± 69, p = 0.008). Lymphocyte levels were significantly higher in the encephalitis group compared to the meningitis group (1 ± 0.6 vs. 1.6 ± 2.1, p = 0.014). N/L ratio and SII values were also significantly lower in the encephalitis group compared to the meningitis group (N/L ratio: 10.6 ± 12.1 vs. 20.4 ± 21.7, p = 0.002; SII: 2749 ± 4101 vs. 5557 ± 7712, p = 0.015). Other laboratory values (platelet, BUN, creatinine, sodium, potassium, pH, lactate, bicarbonate) did not show significant differences between meningitis and encephalitis groups.

Table 3 compares clinical characteristics such as level of consciousness, CSF culture results, and outcomes of the cases included in the study. There was no significant difference in the level of consciousness

Table 2. Laboratory values and SII, N/L ratio of the cases included in the study

	Meningitis	Encephalitis	*p* value
Neutrophil (×10³/µL)	14.4 ± 9.1	9.9 ± 5.3	**0.012**
Lymphocyte (×10³/µL)	1 ± 0.6	1.6 ± 2.1	**0.014**
Platelet (×10³/µL)	242 ± 95	247 ± 82	0.645
N/L Ratio	20.4 ± 21.7	10.6 ± 12.1	**0.002**
SII	5557 ± 7712	2749 ± 4101	**0.015**
CRP (mg/dL)	81 ± 92	45 ± 69	**0.008**
BUN (mg/dL)	23 ± 19	22 ± 12	0.480
Creatinine (mg/dL)	1.43 ± 1.64	1.22 ± 0.7	0.826
Sodium (mEq/L)	136 ± 7	136 ± 8	0.997
Potassium (Eq/L)	4 ± 0.8	4.1 ± 0.6	0.801
pH	7.39 ± 0.09	7.40 ± 0.09	0.790
Lactate (mmol/L)	2.5 ± 2.9	2.2 ± 2.5	0.812
Bicarbonate (mEq/L)	24.1 ± 4.6	24.9 ± 4.4	0.554

Data are shown as mean±standard deviation. SII = Systemic immune-inflammation index, N/L = neutrophil/lymphocyte, CRP = C-reactive protein, BUN = Blood urea nitrogen

Table 3. Consciousness, CSF culture results, and outcome patterns of the cases included in the study

	Meningitis	Encephalitis	*p* value
State of consciousness, n (%)			0.699
Alert	17 (47.2)	24 (36.9)	
Confused	15 (41.7)	32 (49.2)	
Closed	4 (11.1)	9 (13.8)	
Bacterial CSF culture, n (%)			**< 0.001**
Positive	24 (66.7)	8 (12.3)	
Negative	12 (33.3)	57 (87.7)	
Hospital admission, n (%)			0.220
Service	25 (69.4)	34 (52.3)	
ICU	11 (30.6)	31 (47.7)	
Outcome, n (%)			0.947
Survive	29 (80.6)	52 (80)	
Exitus	7 (19.4)	13 (20)	

CSF = Cerebrospinal fluid, ICU = Intensive Care Unit

between meningitis and encephalitis cases ($p = 0.699$). However, there was a significant difference in bacterial CSF culture results ($p < 0.001$), with higher positive results in meningitis cases. There was no significant difference between hospital admission service and intensive care unit (ICU) admission ($p = 0.220$). Lastly, the survival rates were similarly high in meningitis and encephalitis cases (80.6% and 80%,

Table 4. Comparison of surviving and exitus cases included in the study

	Survive	Exitus	*p* value
Age (years)	61 ± 18	60 ± 19	0.919
Neutrophil (×10^3/µL)	11.2 ± 6.2	12.7 ± 10.3	0.905
Lymphocyte (×10^3/µL)	1.2 ± 0.7	2.1 ± 3.8	0.743
Platelet (×10^3/µL)	242 ± 81	256 ± 107	0.695
N/L Ratio	13.5 ± 13.6	16.1 ± 26.2	0.461
SII	3465 ± 4538	4907 ± 9348	0.633
CRP (mg/dL)	52 ± 72	79 ± 101	0.305
BUN (mg/dL)	21 ± 14	25 ± 17	0.461
Creatinine (mg/dL)	1.34 ± 1.21	1.13 ± 0.6	0.264
Sodium (mEq/L)	136 ± 8	135 ± 7	0.313
Potassium (mEq/L)	4.1 ± 0.7	3.8 ± 0.8	0.086
pH	7.40 ± 0.07	7.37 ± 0.151	0.453
Lactate (mmol/L)	2 ± 1.9	3.5 ± 4.4	0.403
Bicarbonate (mEq/L)	25.3 ± 3.7	22 ± 6.3	0.076

Data are shown as mean ± standard deviation. N/L = neutrophil/lymphocyte, SII = Systemic immune-inflammation index, CRP = C-reactive protein, BUN = Blood urea nitrogen

respectively, $p = 0.947$).

Table 4 compares the demographic and laboratory values of the survived cases and those with an exit. There were no statistically significant differences between the two groups regarding age, neutrophil, lymphocyte, platelet, N/L ratio, SII, CRP, BUN, creatinine, sodium, pH, and lactate values ($p > 0.05$). However, the two groups had no significant difference regarding potassium and bicarbonate values ($p = 0.086$ and $p = 0.076$, respectively).

DISCUSSION

Encephalitis and meningitis are serious infectious diseases of the central nervous system that require early diagnosis and treatment. Meningitis is caused by inflammation of the meninges (the brain membranes), while encephalitis is caused by direct inflammation of the brain tissue. Therefore, meningitis patients tend to have a higher level of inflammation [4].

In this study, the N/L ratio and SII values were higher in meningitis patients than in encephalitis patients. Similarly, although the N/L ratio and SII values of patients with an exit were higher than survivors, they did not reach statistical significance. When we look at the reasons for the high values in meningitis, it is associated with the higher inflammation seen in meningitis, inflammation of the brain membranes, and increased inflammatory cells in the affected area [1, 3]. The brain membranes act as a protective barrier, preventing inflammatory cells in the blood and other immune cells fighting the infection from entering the brain tissue. Therefore, it is more difficult for the bacteria or virus to penetrate the brain tissue, and if the blood-brain barrier falls, the inflammatory response is more severe.

In this study, it was observed that the rate of negative CSF culture was higher in encephalitis patients, with 87.7% of the patients having negative CSF cultures. In contrast, the positive CSF culture rate was higher in meningitis patients. These results indicate differences in CSF culture results between encephalitis and meningitis. In encephalitis, inflammation is generally limited to the brain tissue, and unlike meningitis, there is no infection in the cerebrospinal fluid (CSF) [2, 12]. Therefore, encephalitis is often associated with negative CSF culture.

On the other hand, in meningitis, the infection spreads directly to the CSF, and cultures are used to detect bacteria or viruses in the CSF. The positivity of CSF cultures is an important factor in diagnosing meningitis. However, there are also meningitis cases with negative CSF cultures associated with pathogens that cannot be detected by CSF cultures or incorrect culture collection or processing techniques [3, 4]. The difference in CSF culture results between encephalitis and meningitis and the possibility of negative CSF culture in meningitis infections directs the clinician to other supportive parameters in the differential diagnosis. Using SII and N/L ratios is a cost-effective and rapid parameter in this context. SII and N/L ratios may serve as supportive parameters in the differential diagnosis to validate false-positive or false-negative results in CSF examination. Additionally, they can be a valuable aid in diagnosing the disease in cases where the lumbar puncture is contraindicated or unsuccessful.

The inflammation caused by meningitis can lead to higher CRP levels due to increased bacterial load in the CSF [3, 4]. On the other hand, in encephalitis cases, as the main cause of inflammation is a viral infection, CRP levels are usually lower or within the normal range [2, 11]. In this study, CRP levels were significantly higher in meningitis than in encephalitis cases.

The demographic results indicate no significant difference in age and gender distribution between meningitis and encephalitis patients. This result is an expected finding and suggests that age and gender are evenly distributed for conditions where the blood-brain barrier is compromised [12]. Finding results compatible with the literature ensures the reliability of our study.

Limitations

Our study's retrospective and single-center nature is a major limitation, and therefore, the results cannot be generalized to the entire population. Another limitation of the study is the need for more consideration of the presence of neurological sequelae when evaluating disease severity and clinical outcomes.

CONCLUSION

Simple calculable ratios such as SII and N/L ratio can

be a supportive parameters in the differential diagnosis of the disease. Knowing SII and N/L ratios can help to start treatment early. However, using these indices is not useful in determining the severity and prognosis of patients with encephalitis and meningitis. However, these results need to be confirmed by further research.

Authors' Contribution

Study Conception: CA, ESB; Study Design: EK; Supervision: ESB, EK; Funding: N/A; Materials: OSÇ, CA; Data Collection and/or Processing: OSÇ, EK; Statistical Analysis and/or Data Interpretation: ESB; Literature Review: CA, EK; Manuscript Preparation: MGE, ESB and Critical Review: ESB.

Conflict of interest

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Evaluation of the gender effect in operated prolactinomas

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ABSTRACT

Objectives: To investigate the differences between the characteristics of disease presentation and treatment outcomes on the basis of gender in patients with operated prolactinoma.

Methods: Prolactinoma patients who underwent endoscopic transsphenoidal surgery at Istanbul University-Cerrahpasa, Neurosurgery clinics between 2013-2023 were included in this study. Surgical indications, secondary treatments, clinical, demographic, biochemical, radiological findings, and pathological data were analyzed. Data were compared between the gender groups.

Results: Thirty-two men and 28 women were included in the study. The mean age of the men was 44 years and that of the women was 29 years. While men were more likely to have decreased libido, women were more likely to have menstrual irregularities ($p < 0.001$). The tumor was larger in men ($p = 0.001$), presenting with a more frequent suprasellar invasion ($p = 0.001$) and cavernous sinus invasion ($p < 0.001$). Pituitary hormone deficiency ($p < 0.001$) and visual field defects ($p < 0.001$) occurred more frequently in men.

Conclusions: Male prolactinoma patients tend to have more invasive and larger tumors. Men are less likely than women to go into remission with surgery. This difference in presentation may be due to indistinct symptoms in male patients and late diagnosis.

Keywords: Prolactinoma, remission, sex, surgery

Prolactinomas are the most common functional pituitary adenomas [1]. Oligomenorrhea and galactorrhea in women, decreased libido and infertility in men are the most common symptoms [2]. Less commonly, the adenoma is discovered incidentally by a neuroimaging result or when looking for symptoms of a pituitary mass effect, such as a visual field defect, followed by a secondary prolactin measurement [3]. Dopamine agonists are recommended as first-line therapy for prolactinomas due to their proven high efficacy [4]. Surgical removal of the adenoma is recommended as second-line therapy but as first-line therapy in selected cases: (i) resistance to dopamine agonists; (ii) presence of intolerance to dopamine agonists; (iii) immediate or progressive neurological deficit; (iv) patient preference [5]. The preferred method for surgical removal of the adenoma is endoscopic transsphenoidal surgery (ETSS) [6].

There is a significant difference in the prevalence of prolactinoma according to gender and age [7].

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While the sex ratio between females and males for prolactinomas is 10:1 between the second and the fifth decade of life, it decreases to 1:1 after that [7]. One explanation for this situation is the increased expression of estrogen receptors in prolactinomas [8]. Previous studies investigated whether this sex selection influences the occurrence of the disease as well as the efficacy of the applied treatments and reported the negative influence of the male sex on surgical outcomes [9,10]. Similarly, in another previous study, we found that male gender was associated with failure in surgical remission in prolactinomas [11]. This study aimed to compare the characteristics of patients with prolactinoma treated with ETSS at a tertiary center by sex and to obtain data that shed light on the reasons for sex selection in this disease.

METHODS

This single-center, retrospective study was conducted at the Pituitary Center of a tertiary care university hospital and approved by the Research Ethics Committee of Istanbul University-Cerrahpasa. Patient data were coded and stored anonymously.

Study Design and Procedure

Patients with prolactinoma who underwent ETSS in the Department of Neurosurgery, Istanbul University-Cerrahpasa, between 2013 and 2023 were studied. Inclusion criteria were (i) a definite pathological diagnosis of prolactinoma; (ii) adult patients. Exclusion criteria were (i) patients who underwent surgery for a sellar mass but who did not have prolactin immunostaining; (ii) patients with positive immunostaining for both prolactin and other hormones (mixed or plurihormonal pituitary adenoma); (iii) medically treated prolactinoma patients; (iv) patients with missing follow-up data.

Demographic data, type and duration of medical treatment, reasons for the decision to operate, prolactin level at presentation, tumor size before surgery, presence of suprasellar extension, presence of cavernous invasion, Hardy and Knosp stages, type of resection during surgery, complications, pathology results, postoperative prolactin level, prolactin level at last visit, postoperative radiotherapy, and ongoing medical treatment after surgery were collected from all participants. In the final analysis, all these data were evaluated by two gender groups.

Statistical Analysis

The statistical analyses in this study were conducted using the Statistical Package for the Social Sciences (SPSS) software, specifically version 21.0. To assess the normality of the data, the Kolmogorov-Smirnov test was employed. Continuous variables were presented as mean ± standard deviation (SD) or medians with interquartile range (IQR) if the data distribution was not normal. For comparing means between groups with normally distributed data, Student's t-tests or analysis of variance (ANOVA) were utilized. In cases where the data did not follow a normal distribution, medians were compared using the Mann-Whitney U test or Kruskal-Wallis test. Correlation coefficients between continuous variables were calculated using Spearman's rank order or Pearson correlation tests. To compare frequencies, Pearson's chi-square test or Fisher's exact test was employed. The significance level was set at $p < 0.05$, and all results were evaluated with a 95% confidence interval.

RESULTS

A total of 60 patients treated surgically for prolactinoma were included in this study. Thirty-two were men and 28 were women. The most common admission symptom in males was decreased libido (75%), and the most common symptom in females was oligomenorrhea (71.4%). The characteristics of patients at preoperative presentation and comparison of tumor pathologies are shown in Table 1. The median age at surgery was 44.3 years in men and 29.1 years in women ($p < 0.001$). Median prolactin levels in men at diagnosis were 919 ng/mL, significantly higher than in women at 127 ng/mL ($p = 0.004$).

Men were found to have significantly larger tumor sizes at surgery ($p = 0.001$), higher suprasellar extension rate ($p = 0.001$), and higher prevalence of cavernous sinus invasion ($p < 0.001$) compared with women. There was no statistically significant difference between the two groups in the distribution of tumors in the micro, macro, and giant adenoma categories ($p = 0.110$). A comparison of the patient's preoperative Modified Hardy-Wilson grades (sellar

Table 1. Comparison of preoperative findings and pathological features

	Male	Female	*p* - value
Age (year), mean (range)	44.3 (18-72)	29.1 (16-58)	**< 0.001**
Prolactin at diagnosis (ng/mL), median [IQR]	919 [455-3829]	127 [75-191]	**0.004**
Preoperative prolactin (ng/mL), median [IQR]	305 [23-700]	100 [49-147]	**0.043**
Maximal tumor diameter (mm), median (range)	27.5 (10-65)	14 (4–61)	**0.001**
Preoperative visual field deficiency, n (%)	24 (75)	4 (14.3)	**< 0.001**
Tumor size category, n (%)			
Micro	1 (3.1)	3 (10.7)	0.110
Macro	24 (75)	24 (85.7)	
Giant	7 (21.9)	1 (3.6)	
Suprasellar extension, n (%)	24 (75)	9 (32.1)	**0.001**
Cavernous sinus invasion, n (%)	26 (81.3)	12 (42.9)	**< 0.001**
Intraoperative adenoma structure, n (%)			
Cystic	7 (21.9)	8 (28.6)	0.551
Hemorrhagic	4 (12.5)	5 (17.9)	
Solid	16 (50)	13 (46.4)	
Mixt	5 (15.6)	2 (7.1)	
Ki -67 labeling index, mean ± SD	2.3 ± 2.1	2.7 ± 1.7	0.464
Sparse granular adenoma structure, n (%)	23 (71.9)	16 (57.1)	0.260

IQR = Interquartile range, SD = Standard deviation

destruction grade), Modified Hardy-Wilson stages (extrasellar expansion stage), and Knosp grades are given in Table 2. The Ki-67 labeling index in pathology reports was, on average, slightly higher for women than for men, 2.70 and 2.26, respectively, but this was not statistically significant ($p = 0.464$).

The assessment regarding pituitary hormone deficiency before surgery is shown in Table 3. The presence of at least one hormone deficiency was significantly more frequent in male than in female patients ($p < 0.001$). Data on patients treated preoperatively with dopamine agonists and the duration and dosage of medical treatments are shown in Table 4. The most common reason for surgery in men was the presence of an immediate/progressive neurologic deficit; in women, the most common reason for surgery was drug resistance (Table 4).

Most patients did not experience postoperative complications. No leakage of cerebrospinal fluid was observed postoperatively in any of the patients. Persistent diabetes insipidus, meningitis, vascular complications, and patient death did not occur. Evaluation of surgical outcomes and follow-up data are shown in Table 5. The gross overall resection rate and surgical remission rates evaluated at the third postoperative month were significantly higher in women ($p = 0.001$). The remission rates evaluated at the last visit were similar in both groups ($p = 0.097$). However, remission with medication was significantly higher in men ($p < 0.001$).

DISCUSSION

In this study, gender differences in prolactinoma presentation, treatment outcomes, and disease progression were investigated. Male patients had higher prolactin levels, larger tumors, more pituitary hormone deficiency, more tumor compression findings, and lower surgical remission rates at diagnosis. Women tended to report at a young age and with irregular menstruation. Men, on the other hand, presented at older ages

Table 2. A comparison of the patients' preoperative Modified Hardy-Wilson grades (sellar destruction grade), modified Hardy-Wilson stages (extrasellar expansion stage), and Knosp grades

	Male	Female	*p* value
Modified Hardy-Wilson grades, n (%)			**< 0.001**
1	0 (0)	5 (17.9)	
2	5 (15.6)	14 (50)	
3	5 (15.6)	4 (14.2)	
4	22 (68.8)	5 (17.9)	
Modified Hardy-Wilson stages, n (%)			**0.001**
A	13 (40.6)	21 (75)	
B	4 (12.5)	3 (10.7)	
C	0 (0)	0 (0)	
D	8 (25)	4 (14.3)	
E	7 (21.9)	0 (0)	
Knosp grades, n (%)			**0.002**
0	5 (15.6)	12 (42.8)	
1	4 (12.5)	8 (28.6)	
2	4 (12.5)	5 (17.9)	
3	7 (21.9)	2 (7.1)	
4	12 (37.5)	1 (3.6)	

and with loss of libido.

Prolactinomas are more common in females [12]. However, there were more male patients in this series. In our center, surgery is performed in selected cases of prolactinoma patients. Immediate/progressive neurological deficits were the most important surgical indication and were significantly higher in male patients. Moreover, in other series, women tended to be 10 years younger at the time of diagnosis [12, 13]. This is also the case in our series. The difference in the frequency of the disease between the sexes might be related to the differences in clinical presentation. The clinical manifestation of hyperprolactinemia in men and women can be explained by the fact that men are more prone to symptoms such as low libido and erectile dysfunction, which are more insidious and often underestimated clinically and/or present later for sociocultural reasons. Therefore, men may seek medical attention much later than when symptoms appear [13]. Our results were also in this direction. Because the most common symptoms in men and women were decreased libido and menstrual irregularities, respec-

Table 3. Evaluation of pituitary hormone deficiency before surgery

	Male	Female	*p* value
At least one pituitary hormone deficiency before surgery, n (%)	29 (90.6)	9 (32.1)	**< 0.001**
Thyroid-stimulating hormone deficiency, n (%)	16 (50)	5 (17.9)	**0.031**
Adrenocorticotropin hormone deficiency, n (%)	11 (34.4)	5 (17.9)	0.290
Gonadotropin hormones deficiency, n (%)	25 (78.1)	4 (14.3)	**< 0.001**
Growth hormone deficiency, n (%)	4 (12.5)	3 (10.7)	0.758

Table 4. Preoperative medical treatment and reasons for the operation

	Male	Female	*p* value
Medical treatment with a preoperative dopamine agonist, n (%)	28 (87.5)	20 (71.4)	0.187
Duration of preoperative medical treatment (months), median [IQR]	3 [1-12]	12 [1-30]	0.057
Preoperative medical treatment maximum dose*, mean ± SD	1.86 ± 1.25	1.6 ± 1.4	0.498
Indications of the operation, n (%)			
Dopamine agonist resistance	11 (34.4)	13 (46.4)	0.047
Dopamine agonist intolerance	5 (15.6)	3 (10.7)	
Patient preference	3 (9.4)	9 (32.2)	
Immediate/progressive neurologic deficit	13 (40.6)	3 (10.7)	

*Maximum DA dose data used are given as equivalent doses in cabergoline (mg/week). IQR = Interquartile range, SD = Standard deviation

tively. As opposed to an actual incidence, women may be thought to be affected more frequently and at an earlier age because of more pronounced clinical signs and symptoms. Previously autopsy studies found that the prevalence of postmortem prolactinomas was similar in men and women [14]. However, these are hypotheses and causality cannot be established with certainty.

We found that men had significantly larger tumors compared with women and therefore more mass effect-related symptoms, such as visual disturbances. In addition, cavernous sinus invasion, suprasellar spread, Hardy and Knosp stages, which are clinical markers of advanced and aggressive tumors, were significantly higher in men. These results are consistent with other series and the literature [10, 15-18]. In general, the differences in tumor size and aggressiveness have been attributed to the fact that the tumor is detected later in men. It has also been argued that differences in tumor biology are a cause. Studies have claimed that male tumors have a higher number of Ki-67 staining, which can be attributed to greater tumor size and aggressiveness [17-19]. However, our results did not differ between males and females in terms of the Ki-67 labeling index.

We observed that the male patients in our population had higher prolactin levels at diagnosis than the female patients. It has been previously shown in the literature that males have shorter symptom duration before surgery, higher preoperative serum prolactin levels, and more drug-resistant diseases [15-18, 20, 21]. In this series, women had tumors resistant to

Table 5. Surgical results and follow-up

	Male	Female	*p* value
Gross total resection, n (%)	11 (34.4)	21 (75)	**0.001**
Postoperative first-week prolactin (ng/mL), median [IQR]	56 [3-421]	9.5 [2.4–27.8]	**0.011**
Last prolactin (ng/mL), median [IQR]	18 [5-86]	17 [9-34]	0.184
Surgical remission, n (%)	11 (34.4)	25 (89.3)	**< 0.001**
Recurrence, n (%)	5 (15.6)	4 (14.3)	0.831
Postoperative secondary treatments, n (%)			
Dopamine agonist, n (%)	27 (84.4)	7 (25)	**< 0.001**
Radiosurgery, n (%)	3 (10.7)	1 (3.6)	0.175
Reoperation, n (%)	4 (12.5)	1 (3.6)	0.244
Remission at last visit, n (%)	27 (84.4)	28 (100)	0.097

IQR = Interquartile range.

dopamine agonist therapy, which was often the reason for surgery. In this case, we can attribute the earlier diagnosis in women to the fact that medical treatment was initiated before the disease was complicated. Therefore, they were less likely to need emergency surgery. And they were more often operated on when medical treatment was tried and did not work.

In our study, there were also differences between the two sexes in terms of surgical outcomes. Men were found to have significantly lower gross resection rates, lower surgical remission rates, and a higher need for continued medical treatment with a dopamine agonist after surgery. These results are consistent with previous reports showing lower remission rates in men than in women [10, 20]. This is because the postoperative remission rate is likely related to tumor size and invasiveness. Microprolactinomas without cavernous sinus invasion have been reported in the literature to have higher remission rates [22].

Limitations

This study has several limitations. Our study was not selected from all patients with prolactinomas but from patients who had surgery for their prolactinomas; this may explain some of the differences in results from previous studies by introducing selection bias. Patients in our tertiary referral center may be more advanced and complicated because patients are more often treated initially in external centers. For the same reason, the number of our patients with microprolactinoma was significantly lower. In addition, the lack of molecular and genetic studies that could explain the differences between the sexes prevents us from making further comments.

CONCLUSION

In this study, we found that male prolactinoma patients who underwent surgical treatment differed significantly from female prolactinoma patients in larger tumor size, higher prolactin levels, higher frequency of suprasellar invasion, and higher frequency of cavernous invasion. Men were less likely than women to have a complete resection at their surgery and to remain in drug-free remission after surgery. In addition, men were more likely to lose libido, while women were most likely to have menstrual irregularities. All of these findings suggest that diagnosis is delayed in men and that outcomes can be improved if an early diagnosis is made. However, further research at the molecular level is needed to understand gender-based differences.

Authors' Contribution

Study Conception: DÖ, AND, NT; Study Design: DÖ, AND, NT; Supervision: NT; Funding: DÖ, AND, NT; Materials: DÖ, AND, NT; Data Collection and/or Processing: DÖ, AND; Statistical Analysis and/or Data Interpretation: DÖ, AND, NT; Literature Review: DÖ, AND, NT; Manuscript Preparation: DÖ, AND, NT and Critical Review: DÖ, AND, NT.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Statement of ethics

The study adhered to the ethical principles for medical research involving human participants described in the World Medical Association's Declaration of Helsinki. The Ethics Committee of Istanbul University-Cerrahpasa approved the study (Approval Number: E-83045809).

Informed consent:

Signed informed consent was obtained from all study participants.

Data statement

The data used and analyzed during the current scoping review are available from the corresponding author upon reasonable request.

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The European Research Journal 2023;9(5):1142-1148 Original Article

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Comparison of L452R mutation variant diagnosis in SARS-COV-2 PCR positive samples with two different qPCR kits

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ABSTRACT

Objectives: Quantitative reverse transcription−polymerase chain reaction (qPCR) is used as the gold standard method to diagnose COVID-19 infection caused by SARS-CoV-2 which is the cause of the most important epidemic in world history. It was aimed to compare the results of two of the most commonly used commercial kits for the diagnosis of SARS-CoV-2 mutation in our laboratory during the pandemic.

Methods: Our study included 5000 SARS-CoV-2 PCR positive nasopharyngeal swab samples (2500 L452R mutation positive samples, 2500 L452R mutation negative samples). PCR positivity and negativity of the L452R mutation of the positive SARS-CoV-2 positive samples were identified with the Diagnovital® (DI-AGNO5plex NS SARS-CoV-2 Real Time PCR Kit [A1 Life Sciences Istanbul]) kit. The mentioned samples were also studied with a different commercial PCR kit, Bio-Speedy® (SARS-CoV-2 Emerging Plus Real Time PCR Kit [Bioeksen R&D Technologies Istanbul]).

Results: A total of 5000 samples included in the study were concluded as SARS-CoV-2 positive with both tests. One hundred and fifty of 2500 samples that were found positive for SARS-CoV-2 but negative for L452R mutations with the Diagnovital® kit were found positive with the Bio-Speedy® kit for SARS-CoV-2. The compatability between the two kits was found to be high (Kappa = 0.940). The mean Ct values of the samples found positive with the Diagnovital® kit and Bio-Speedy® kit were 24.15 ± 6.75 and 20.72 ± 7.17, respectively and the difference was statistically significant.

Conclusions: It was determined the two commercial kits included in the study were extremely compatible based on their analysis. Therefore both kits can be used safely for COVID-19 symptomatic patients.

Keywords: SARS-CoV-2, L452R mutation, COVID-19, qPCR, Laboratory diagnosis

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is the most important infectious agent that caused more than 6 million deaths, embarked an unprecedented burden upon the national and international health systems/agencies and the global economy with the COVID-19 pandemic. Its diagnostic procedures are carried out with quantitative reverse transcription−polymerase chain reaction (qPCR) which is still the gold standard method for the task [1, 2]. While commercial qPCR tests used in daily practice

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in the diagnosis of SARS-CoV-2 virus detect some gene regions of the virus, they can not accuretely respond to the issues such as prolonged positivity, disease stage, reinfection and clinical condition of the patient [3]. Diagnostic tests with the qPCR method developed for the SARS-CoV-2 infection detection is one of the most important mechanisms that helps limiting the spread of the virus along with the measures to be taken by monitoring it [4]. SARS-CoV-2 qPCR kits basically targets "hemagglutinin-esterase" HE, "open reading frame 1" ORF1, "envelope glycoproteins spike" S, "RNA-dependent RNA polymerase" RdRp, "helicase" Hel, "nucleocapsid protein targets" N, "envelope" E, and "transmembrane" M [5]. "Cycle threshold" of the PCR test "Ct" represents the number of cycles in which the signal resulting from target gene amplification reaches the positivity threshold level. The Ct value is considered to be inversely proportional to the viral load in the sample in which the lower the Ct value, the higher the viral RNA copy number in the sample. There are publications supporting that the Ct value may be useful in following the clinical course and prognosis of COVID-19 patients [6,7]. It has been reported that the viral load is higher and the Ct value is low in the first 12 days depending on the disease severity in cases with strong symptoms requiring intensive care admission, poor prognosis or immunosuppression [7, 8]. Initially the kits supplied by the Ministry of Health of the Republic of Turkey and then the commercial kits approved by the Turkish Medicines and Medical Devices Agency were used in laboratories authorized by the Ministry of Health to diagnose SARS-CoV-2 in our country. Turkish Medicines and Medical Devices Agency has given pre-authorization to many diagnostic products during the COVID-19 pandemic, and the effectiveness of these kits has been assesed by the General Directorate of Public Health [9]. However, many new variants of SARS-CoV-2 have emerged during this time period, posing a public health concern. Increased transmission and reinfection risks, immune response evasion, decreased vaccine effectiveness and worsening of the clinical picture are threats that may adversely affect the course of the pandemic along with the variants emerging during the pandemic. Therefore, detecting SARS-CoV-2 variants and tracking their mutations is critical in the fight against the COVID-19. Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) variants are classified as "variants of concern (VOCs)" by World Health Organization (WHO) [10]. The B.1.1.7 variant has a higher contagiousness due to its unusually high mutation rate and poses a global threat [11]. The E484K mutation significantly reduces antibody neutralization [12]. It has been reported that E484K has a high effect for Oxford-AstraZeneca, Novavax and Gamaleya, a high to minimal effect for Moderna and Pfizer and a moderate effect for Sinovac and Sinopharm in evading post-vaccine neutralizing antibodies [13]. The L452R mutation in one of the VOCs (B.1.617) has significantly increased SARS-CoV-2 transmission rate in countries such as America and India where it was detected.

It has also been reported that this mutation has the effect of avoiding neutralizing antibodies formed after vaccination [14]. The L452R mutation, which can be found in many other lineages, including B.1.1.7, should be tested regardless of lineage [13]. It has been difficult to provide quality assurance in diagnostic tests and laboratories from time to time with the increase in the number of samples studied with the spread of SARS-CoV-2 variants and use of a difficult method such as qPCR in the pandemic laboratories with inexperienced personnel in molecular methods [15]. There have been frequent changes in qPCR kit versions used in SARS-CoV-2 diagnostic laboratories and it has not been possible to obtain objective data about the performance of qPCR kits and eliminate uncertainities due to the vast amount of samples. Several researches were performed worldwide to compare different qPCR kits for SARS-CoV-2 identification [16-18].

It has been demonstrated that diagnostic kits give similar results in terms of accuracy and sensitivity even though different gene regions have been investigated in the studies. The aim of this study is to compare and independently evaluate the results of two of the most frequently used commercial kits in our laboratory for detecting L452R mutation in delta variants during the pandemic.

METHODS

Ethics committee approval of Yıldırım Beyazit University Yenimahalle Training and Research Hospital (Decision Number: 2022-47) was obtained for the study.

Specimen Collection

Our study was carried out at Ankara Provincial Health Directorate Public Health Molecular Diagnosis Laboratory, which is one of the pandemic laboratories with the highest sample working capacity in our country. Randomly selected 5000 nasopharyngeal swab samples (2500 SARS-CoV-2 L452 mutation positive, 2500 SARS-CoV-2 positive L452 mutation negative) were determined Sars-CoV-2 positive with Diagnovital® (DIAGNO5plex NS SARS-CoV-2 Real Time PCR Kit) kit and analyzed with a different commercial PCR kit, Bio-Speedy® (SARS-CoV-2 Emerging Plus Real Time PCR Kit) in the study.

The test outfits did not require any extra RNA extraction phase during the qPCR process since the samples were transfered directly into Viral Nucleic Acid Buffer (vNAT) with nucleic acid extraction. Extracting RNA of swap samples with vNAT solution required only the the vortex.

Molecular Assays

SARS-CoV-2 specific 'Orf1ab' and 'N' genes, mutation genes of Spike (S) E484K, Nucleocapsid (N) D3L and Spike (S) L452R are investigated with Diagnovital® kit. The sample-induced inhibition control and the kit reagent control include an internal control containing the RNase P gene. 7.5 µl reaction mixture and 2.5 µl sample were combined and reverse transcription (RT) was carried out at 52°C for 5 minutes, denaturation at 95°C for 20 seconds followed by 40 cycles of 1 second at 95°C and 60°C as suggested by the kit manufacturer. Bio-Speedy® kit, which we used as a comparison kit, is a multiplex-based quantitative real-time PCR kit using labeled oligonucleotides specific to target gene regions for SARS-CoV-2 found in nasopharyngeal swabs, oropharyngeal swabs, nasal swabs, nasopharyngeal aspirates, saliva and bronchoalveolar lavage samples. The Bio-Speedy® kit allows complementary DNA (cDNA) synthesis and qPCR reaction to be performed in the same tube. SARS-CoV-2 specific 'Orf1ab' and 'N' genes, mutations of Spike (S) E484K, Nucleocapsid (N) D3L, Spike (S) L452R along with Human RNase-P mRNA genes were targeted with this kit. Sample-induced inhibition control and kit reagent control were performed thanks to the RNaseP mRNA gene which was used as internal control.

RT at 52°C for 3 minutes and holding at 95°C for 10 seconds, followed by 1 second at 95°C, 12 seconds at 60°C, 5 cycles of reproduction at 85°C, 1 second, at 60°C, 35 cycles of denaturation, annealing, and extension, duplication and reading, respectively, were carried out for 1 second in compliance with the manufacturer's specifications. Bio-Rad CFX96 TouchTM device was used in our study. The test was applied once for each sample and Ct values were recorded.

Analysis of the Results

The interpretation of test results was carried out following the manufacturer's instructions for all two kits. For each sample, samples with a Ct value of 36 and below (Ct ≤ 36) in the internal control (Rnase P) in the HEX channel from the fluorescent reading channels when the reaction was completed were included in accordance with the manufacturer's recommendations in the Diagnovital® kit. The samples seen as sigmoidal curve and Ct ≤ 38 in both FAM and Cy5.5 channels are determined as positive for SARS-CoV-2, but a variant containing L452R mutation (Delta, Epsilon, Kappa) is positive while Alpha variant (B.1.1.7) and variants containing E484K (Beta, Gamma, Zeta, Eta, Theta, Iota) were considered negative when the reaction is completed according to manufacturer recommendations. Samples with a sigmoidal curve and Ct ≤ 38 only in the FAM channel were SARS-CoV-2 positive, but Alpha variant (B.1.1.7), variants carrying E484K (Beta, Gamma, Zeta, Eta, Theta, Iota) and variants carrying L452R (Delta, Epsilon, Kappa) were considered negative. Samples with non-sigmoidal curves were excluded from the study.

For each sample, in accordance with the manufacturer recommendations, samples with a Ct value of 32 and below (Ct ≤ 32) in the internal control (Human mRNA) in the HEX channel, one of the fluorescent reading channels, were included in the study when the reaction was completed with the Bio-Speedy® kit. In accordance with the manufacturer recommendations, when there is a sigmoidal curve in both FAM and Cy5.5 channels and Ct ≤ 33, the Ct differences between Cy5.5 and FAM and Cy5 are calculated and if [Ct_Cy5.5 – Ct_FAM] < 4 and [Ct_Cy5.5 – Ct_Cy5] < 0 then the result is found to be positive for SARS-CoV-2 and one of the variants containing L452R mutation (Delta, Epsilon, Kappa) was positive while Alpha variant (B.1.1.7) and variants containing E484K (Beta, Gamma, Zeta, Eta, Theta), iota) were consid-

ered negative. Samples with a sigmoidal curve and Ct ≤ 33 only in the FAM channel was SARS-CoV-2 positive, but Alpha variant (B.1.1.7), variants carrying E484K (Beta, Gamma, Zeta, Eta, Theta, Iota) and variants carrying L452R (Delta, Epsilon, Kappa) were considered negative. Samples with non-sigmoidal curves were excluded from the study.

Statistical Analysis

The results of both tests were recorded in SPSS Statistics (IBM, version 22) and evaluated with Kappa analysis and t test in dependent groups.

RESULTS

A total of 5000 samples included in the study were concluded as SARS-CoV-2 positive with both tests. One hundred and fifty of 2500 samples that were found positive for SARS-CoV-2 but negative for mutations with the Diagnovital® kit were found positive with the Bio-Speedy® kit for SARS-CoV-2 and one of the variants containing the L452R mutation (delta, epsilon, kappa). The compatability between the two kits was found to be high (Kappa = 0.940, p = 0.5) in Kappa analysis. The mean Ct values of the samples with positive delta mutation (n = 2650) with Bio-Speedy® kit were 20.72 ± 7.17, while the mean Ct values of samples with positive delta mutation with Diagnovital® (n = 2500) were 24.15 ± 6.75. The difference between the two groups was statistically significant ($p < 0.05$). Table 1 shows the positivity and negativity rates of both tests. The Ct value of 51.01% of the samples in the Bio-Speedy® kit is below 20. Table 2 shows the Ct value ranges of both kits.

DISCUSSION

Measures taken to reduce the spread of COVID-19 depend on rapid and accurate identification of the disease in SARS-CoV-2-infected individuals with the most sensitive and specific method available. qPCR is still a fast and accurate method for SARS-CoV-2 virus diagnosis in the world [19]. Although many diagnostic tests became available at the laboratories with rapid approval, many studies support that the kits are sensitive and reliable [20]. In this study, two locally manufactured and frequently used commercial kits that perform mutation analysis in samples deterimed to be positive for SARS-CoV-2 were compared and the compatability between them was found to be extremely high. False negative results are the main issue for qPCR in the early stages of infection, Incorrect application of reagents and incomplete extraction in qPCR cause false negative results [21]. Incorrect result qPCR problem caused issues during the delta variant intensive period. In addition, qPCR requires trained medical personnel, special tools and technical labor [22]. This sample constitutes only 3% of our group. The mean Ct values of the samples found positive with the Diagnovital® kit and Bio-Speedy® kit were 24.15 ± 6.75 and 20.72 ± 7.17 respectively and the difference was statistically significant ($p < 0.05$). The high compatability between the two kits with the positivity rates and average Ct values determined in this study shows that both kits can be used in the diagnosis of SARS-CoV-2 variants.

Van Kasteren *et al*. [20] also tested kits specific for common betacoronavirus and/or only SARS-CoV-2 virus and targeting very different gene regions at different dilutions with 13 samples with different viral

Table 1. Comparison of positivity and negativity of both kits

Method	Bio-Speedy® SARS-CoV-2 Emerging Plus Real Time PCR Kiti			
	Results	Positive	Negative	Total
Diagnovital® DIAGNO5plex NS SARS-CoV-2 Real Time PCR Kiti	Positive	2500	0	2500
	Negative	150	2350	2500
Total		2650	2350	5000

Table 2. Ct value ranges of mutation-positive samples with Diagnovital® DIAGNO5plex NS SARS-CoV-2 Real Time PCR Kit and Bio-Speedy® SARS-CoV-2 Emerging Plus Real Time PCR Kit

		Method			
		Diagnovital®		Bio-Speedy®	
		n	%	n	%
Ct value	< 20	410	16.4	1352	51.01
	20.00-24.99	1048	41.92	798	30.11
	25-29.99	945	37.8	400	15.09
	30-32.99	77	3.08	100	3.77
	33-37.99	20	0.8	0	0

Ct = Cycle threshold

loads and stated that all kits can be used in routine diagnosis in symptomatic patients. WHO recommends first performing qPCR testing targeting two different gene regions of the virus, or specific to the common gene region of the betacoronavirus family, and then confirming the test result with partial or whole genome sequencing specific to the SARS-CoV-2 virus in periods or places where the virus prevalence is low. On the other hand, an qPCR test targeting a single gene region specific to SARS-CoV-2 virus is sufficient in regions where SARS-CoV-2 is common [23].

Studies show that the performance of the qPCR test can be affected by many factors such as patient's viral load, disease stage, sample source (upper or lower respiratory tract), sample collection technique, sample handling conditions [24]. In the mutation analysis of the Bio-Speedy® kit, the Ct value differences between Cy5.5, FAM and Cy5 were calculated in accordance with manufacturer recommendations, and one of the variants containing the L452R mutation was evaluated as positive and the variants containing B.1.1.7 and E484K as negative. It has also been observed in our study that this proces complicates the work of doctors during the test evaluation phase and that it creates an extra burden on the result evaluation stress in a laboratory with a high daily case rate.

The Diagnovital® and Bio-Speedy® PCR kits were compared in another study which showed that both of the methods produce on par results for the negative and positive clinical specimens. However, it has been reported that the amplification graph of the Diagnovital® PCR kit was a more distinctive curve than the one produced by the Bio-Speedy® PCR kit. It was stated that the use of diverse kits targeting different genes in samples of unknown virus presence based on COVID-19 clinical symptoms could provide a more definitive SARS-CoV-2 diagnosis [25].

CONCLUSION

Different nucleic acid amplification tests were developed and used during the COVID-19 Pandemic. It has been understood that it is extremely important to determine the kits with low sensitivity and specificity so the health professionals can ward off false positive patients while they avert false negative patients from further spreading the infection and becoming infectious in the pandemic. In this study, there was no significant difference between the Diagnovital® kit and the Bio-Speedy® kit even though the test specificity was similar between the two kits. It is thought that both kits can be used safely in the diagnosis of COVID-19 infection and SARS-CoV-2 variants in patients with symptoms.

Authors' Contribution

Study Conception: BGG; Study Design: BGG, GGA; Supervision: GGA; Funding: BGG, GGA; Materials: BGG, GGA; Data Collection and/or Processing: BGG, GGA; Statistical Analysis and/or Data Interpretation: BGG, GGA; Literature Review: BGG, GGA; Manuscript Preparation: BGG and Critical Review: BGG, GGA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Effect of celecoxib on intra-abdominal sepsis-induced lung injury in rats

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ABSTRACT

Objectives: This experimental study investigated the preventive effects of Celecoxib, a selective COX-2 inhibitor, on lung injury induced by intra-abdominal sepsis in rats. The study assessed Celecoxib's potential to mitigate the harmful impacts of sepsis on lung tissue.

Methods: Thirty male Wistar albino rats, divided into three groups: a normal control group, a sepsis-induced group treated with saline, and a sepsis-induced group treated with Celecoxib. Sepsis was induced using fecal intraperitoneal injection (FIP), followed by a one-hour administration of Celecoxib at 50 mg/kg/day to the treatment group. Biochemical analysis of lung tissue measured oxidative stress markers (malondialdehyde [MDA]) and pro-inflammatory cytokines (Tumor Necrosis Faftor-α [TNF-α]). Histopathological examination evaluated lung tissue damage, encompassing alveolar congestion, hemorrhage, inflammatory cell aggregation, and edema. Arterial blood gas analysis quantified partial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) pressures.

Results: Celecoxib-treated rats exhibited reduced oxidative stress markers with lower MDA levels, indicating decreased oxidative damage in lung tissue. Moreover, TNF-α and other pro-inflammatory cytokines were significantly reduced in lung tissues of Celecoxib-treated rats, indicating its anti-inflammatory effects. Histopathological examination revealed reduced lung tissue damage in Celecoxib-treated rats, including alveolar congestion, hemorrhage, and inflammatory cell aggregation. Arterial blood gas analysis showed improved oxygenation (PaO_2) in the Celecoxib-treated group compared to untreated sepsis rats.

Conclusions: Celecoxib demonstrated preventive effects against sepsis-induced lung injury in rats by mitigating oxidative stress and inflammation, thereby preserving lung tissue integrity-further research, including clinical trials, to validate its effectiveness and safety in human sepsis management.

Keywords: Celecoxib, lung injury, sepsis, anti-inflammation

Sepsis is a significant clinical condition with global implications, particularly within critical care units. The occurrence of multiple organ dysfunction during the hyperactive immune response to infections is closely linked to a significant mortality rate ranging from 25% to 52% [1]. The lungs, kidneys, and liver

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are among the organs that are primarily impacted in the early stages of sepsis. Dysfunction in two or three of these factors strongly correlates with increased mortality rates in patients with sepsis [2]. The hyperactive immune response aimed at combating and containing an infection leads to a phenomenon known as a "cytokine storm" [3]. Cytokines play a significant role as pleiotropic regulators in modulating the immune response and are crucial in the intricate pathophysiology of sepsis. By exhibiting dual pro- and anti-inflammatory characteristics, they can modulate the immune response in the context of infection.

Prior studies have investigated the effects of inhibiting inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α). At the same time, other research has explored the outcomes of suppressing Cyclooxygenase 2 (COX-2) activity in sepsis. For instance, Ozer *et al*. [4] conducted a study where they investigated the effects of administering infliximab (IFX), an antibody that targets TNF-α, as a preventive measure prior to cecal ligation and puncture (CLP) surgery. The findings of their research demonstrated improved survival rates in septic animals. Another study investigated a reduced mortality rate when Celecoxib (CLX), a specific COX-2 inhibitor, was applied after CLP [5]. The survival rates of 57% and 43% were reported in the studies, indicating the potential to improve these outcomes by adjusting the treatment dosage and timing [6]. As substantiated by prior research, these factors are widely recognized as pivotal in efficiently managing sepsis [7-9].

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are pathological conditions characterized by the sudden onset of respiratory failure, resulting in substantial morbidity and mortality [10]. Empirical evidence suggests that individuals who successfully recover from acute lung injury (ALI) experience a detrimental effect on their long-term quality of life [11]. Significant progress has been made in comprehending the epidemiological aspects, pathogenic mechanisms, and therapeutic approaches to this ailment. Nevertheless, additional advancements are required in order to diminish further the rates of mortality and morbidity associated with Acute Lung Injury (ALI) and acute respiratory distress syndrome (ARDS) [12].

Celecoxib is a pharmaceutical agent from non-steroidal anti-inflammatory drugs. This substance is frequently employed to alleviate pain, inflammation, and swelling resulting from various conditions, including arthritis, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and menstrual cramps [6]. The mechanism of action of Celecoxib involves the inhibition of COX-2, an enzyme. The enzyme COX-2 is accountable for synthesizing prostaglandins, bioactive lipid mediators that contribute to inflammation and pain perception [13]. Celecoxib works as a medicine by stopping the cyclooxygenase-2 (COX-2) pathway. This is a mechanism that stops prostaglandin production from the beginning. This mechanism of action ultimately results in the attenuation of inflammatory responses and the alleviation of pain [13].

Celecoxib differs from other non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and naproxen because it is a selective COX-2 inhibitor. This means that it mainly targets COX-2 and leaves COX-1 alone. The enzyme COX-1 plays a crucial role in synthesizing prostaglandins, which are essential for maintaining the protective mucosal lining in the gastrointestinal tract, which encompasses both the stomach and intestines [14].

This experimental study aims to evaluate the preventive effect of Celecoxib in a rat sepsis model in the primarily affected organ, the lung.

METHODS

Animals

The present study employed a sample of 30 male Wistar albino mature rats, whose average weight was 200 to 250 g. The experiments carried out in this study adhered to the guidelines specified in the Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health in the United States. Following the acquisition of ethical approval from the Animal Ethics Committee (Ethical number: 2523075001) at Demiroğlu Bilim University, the laboratory rats utilized in the experiment were obtained from the Experimental Animal Laboratory at the same institution. The rats were granted unrestricted food availability and were housed in steel enclosures within a controlled environment, where the temperature was maintained at 22 ± 2 °C and a light/dark cycle of 12 hours was upheld.

Experimental Procedures

A research investigation was undertaken, encompassing a cohort of 30 rodents. A total of twenty rats were allocated randomly into three separate groups. The rats were subjected to the feces intraperitoneal-injection group (FIP) procedure to induce a sepsis model. A cohort of ten rats was partitioned into two distinct groups: one group received regular treatment, while the other group remained untreated and served as the control. The FIP rat model was established utilizing a methodology previously delineated by Karaali *et al*. [15]. The collection of fecal samples was followed by their suspension in a saline solution, creating a fecal saline solution. Subsequently, the subjects were administered intraperitoneal injections at 1 gram per kilogram of body weight. The formation of study groups was designed subsequently: Group 1 comprised a cohort of ten individuals designated as the study's control group. The subjects did not undergo any surgical interventions and were administered nutrition orally.

In contrast, Group 2 comprised ten individuals diagnosed with Feces Intraperitoneal Injection (FIP). The experimental cohort was administered an intraperitoneal placebo of 1 ml/kg/day of 0.9% NaCl saline. In Group 3, 10 subjects received an intraperitoneal administration of a combination of FIP and Celecoxib at 50 mg/kg/day. All interventions were administered after a one-hour Focused Intervention Protocol (FIP). The research inquiry was completed within a 24-hour duration. A total of six rats experienced mortality within the initial 24-hour period following the procedure, leading to their subsequent exclusion from the study. Four rats from the placebo group and two from the Celecoxib group were found to have expired.

Following the completion of the study, euthanasia was performed on all animals using cervical dislocation, utilizing an anesthesia protocol comprising Ketamine (100 mg/kg, Ketasol, Richterpharma AG Austria) and xylazine (50 mg/kg, Rompun, Bayer, Germany). Blood samples were collected through a cardiac puncture to conduct a biochemical analysis.

Determination of TNF-α in Plasma

The measurement of plasma TNF-α levels was conducted using enzyme-linked immunosorbent assay (ELISA) kits that were commercially available and obtained from Biosciences and Abcam. The measurements were performed following the manufacturer's provided guidelines. The plasma samples underwent dilution at a ratio of 1:2 by the manufacturer's guidelines. The quantification of TNF-α was conducted in duplicate.

Measurement of Lipid Peroxidation

Lipid peroxidation was measured in plasma samples by evaluating malondialdehyde (MDA) levels as a thiobarbituric acid reactive substance. The experimental procedure consisted of adding trichloroacetic acid and TBARS reagent to the plasma samples, followed by thorough mixing and subsequent incubation at 100 °C for 60 minutes. After the samples were cooled on ice, centrifugation was conducted at a speed of 3000 revolutions per minute for 20 minutes. Following this, absorbance was measured on the resultant supernatant at a specific wavelength of 535 nm.

Histopathological Examination of Lung

To perform histological analysis, anesthesia was administered to all animals using intraperitoneal injections of ketamine (40 mg/kg, Alfamine®, Alfasan International B.V., Holland) and xylazine (4 mg/kg, Alfazyne®, Alfasan International B.V., Holland). The subjects underwent perfusion with a 200 ml solution containing 4% formaldehyde in 0.1 M phosphate-buffered saline (PBS). The kidney sections, which were five µm thick and had been preserved in formalin, underwent staining using the hematoxylin and eosin (H&E) technique. The sections were obtained using an Olympus C-5050 digital camera securely attached to an Olympus BX51 microscope. The primary histopathological lung damage score was calculated using the methodology described in prior research investigations. To summarize, the assessment of histopathological lung injury encompassed the measurement of several parameters, namely alveolar congestion (A.C.), hemorrhage (H), leukocyte infiltration or aggregation in air spaces/vessel walls (A.L.), perivascular/interstitial edema (P.E.), and the thickness of the alveolar wall/hyaline membrane formation (T.A.). The severity of each item was evaluated utilizing a grading scale encompassing a range from 1 to 4. Each grade was associated with a distinct percentage range: Grade 1 denoted a severity level ranging from 0% to 25%, Grade 2 denoted a severity level ranging

from 25% to 50%, Grade 3 denoted a severity level ranging from 50% to 75%, and Grade 4 denoted a severity level ranging from 75% to 100% [15].

Arterial Blood Gas Analysis

Blood samples, measuring 0.2 mL, were obtained from the carotid artery of rats belonging to each experimental group precisely 24 hours after the surgical procedure. Subsequently, the gathered blood samples were subjected to analysis employing a blood gas analyzer in order to quantify the concentrations of PaO_2 and $PaCO_2$.

Statistical Analysis

The data are presented as mean values accompanied by the standard error of the mean (SEM). The data analyses were conducted using SPSS version 15.0 for Windows. The data underwent analysis using the non-parametric Mann-Whitney U test. Statistical significance was attributed to p-values that were equal to or less than 0.05

RESULTS

Malondialdehyde (MDA) (nM/mg protein)

Compared to the Normal Control group (11.2 ± 0.9 nM/mg protein), the FIP and Saline Group demonstrated a significant increase in MDA levels (43.2 ± 2.5 nM/mg protein, $p < 0.001$). However, the FIP and 50 mg/kg Celecoxib Group showed a partial attenuation of MDA levels (27.6 ± 1.9 nM/mg protein) compared to the FIP and Saline Group ($p < 0.01$) (Table 1).

Tumor Necrosis Factor-alpha (TNF-α) (pg/mL)

The FIP and Saline Group exhibited a significant elevation in TNF alpha levels (415.1 ± 13.9 pg/mL) compared to the Normal Control group (13.8 ± 2.3 pg/mL, $p < 0.001$). Conversely, the FIP and 50 mg/kg Celecoxib Group displayed a significant reduction in TNF alpha levels (151.3 ± 7.6 pg/mL) compared to the FIP and Saline Group ($p < 0.001$) (Table 1).

Alveolar Congestion (AC)

Compared to the Normal Control group (0.1 ± 0.1), the FIP and Saline Group demonstrated a significant increase in alveolar congestion (3.1 ± 0.1, p < 0.001). However, the FIP and 50 mg/kg Celecoxib Group showed a substantial reduction in alveolar congestion (0.6 ± 0.2) compared to the FIP and Saline Group ($p < 0.001$). (Table 2, Fig. 1).

Hemorrhage (H)

The FIP and Saline Group exhibited a significant increase in hemorrhage (2.5 ± 0.3) compared to the Normal Control group (0.2 ± 0.2, $p < 0.001$). Conversely, the FIP and 50 mg/kg Celecoxib Group displayed a partial reduction in hemorrhage (0.8 ± 0.2) compared to the FIP and Saline Group ($p < 0.001$). (Table 2, Fig. 1).

Aggregation in Air Spaces/Vessel Walls (A.L.)

In comparison to the Normal Control group (0.1 ± 0.1), the FIP and Saline Group showed a significant increase in aggregation in air spaces/vessel walls (2.2 ± 0.1, $pp < 0.001$). On the other hand, the FIP and 50 mg/kg Celecoxib Group demonstrated a partial reduction in aggregation (0.9 ± 0.1) compared to the FIP and Saline Group ($p < 0.001$). (Table 2, Fig. 1).

Perivascular/Interstitial Edema (P.E.)

The FIP and Saline Group exhibited a significant increase in perivascular/interstitial edema (2.6 ± 0.3) compared to the Normal Control group (0.2 ± 0.1, $p <$

Table 1. The results of the biochemical analysis in the three study groups: Normal control, FIP and saline group, and FIP and 50 mg/kg celecoxib group

	Normal control	FIP and saline	FIP and 50 mg/kg celecoxib
MDA (nM/mg protein)	11.2 ± 0.9	43.2 ± 2.5**	27.6 ± 1.9$^{\#}$
TNF-α (pg/ml)	13.8 ± 2.3	415.1 ± 13.9**	151.3 ± 7.6$^{\#\#}$

Results were presented as mean ± SEM. MDA = Malondialdehyde, TNF = Tumor Necrosis Factor, FIP = Fecal Intraperitoneal Statistical analyses were performed by one- way ANOVA. *$p < 0.05$, **$p < 0.001$ different from normal groups; #$p < 0.01$, ##$p < 0.001$ different from FIP and saline group.

Table 2. The results of the histopathological examination of the lung in the three study groups: Normal control, FIP and saline group, and FIP and 50 mg/kg celecoxib group

	Normal control	FIP and saline	FIP and 50 mg/kg celecoxib
AC (alveolar congestion)	0.1 ± 0.1	3.1 ± 0.1*	0.6 ± 0.2##
H (hemorrhage)	0.2 ± 0.2	2.5 ± 0.3**	0.8 ± 0.2##
AL (aggregation in air spaces/vessel walls)	0.1 ± 0.1	2.2 ± 0.1**	0.9 ± 0.1##
PE (perivascular/interstitial edema)	0.2 ± 0.1	2.6 ± 0.3**	1.4 ± 0.2#
TA (thickness of the alveolar wall)	0.1 ± 0.1	2.6 ± 0.4**	1.1 ± 0.1##

Results were presented as mean ± SEM. FIP = Fecal Intraperitoneal

Statistical analyses were performed by one- way ANOVA. *$p < 0.01$, ** $p < 0.001$ different from normal groups; # $p < 0.05$, ## $p < 0.001$ different from FIP and saline group

0.001). Conversely, the FIP and 50 mg/kg Celecoxib Group displayed a partial reduction in edema (1.4 ± 0.2) compared to the FIP and Saline Group ($p < 0.05$). (Table 2, Fig. 1).

Thickness of the Alveolar Wall (T.A.)

In contrast to the Normal Control group (0.1 ± 0.1), the FIP and Saline Group demonstrated a significant increase in the thickness of the alveolar wall (2.6 ± 0.4, $p < 0.001$). However, the FIP and 50 mg/kg Celecoxib Group showed a substantial reduction in alveolar wall thickness (1.1 ± 0.1) compared to the FIP and Saline Group ($p < 0.001$). (Table 2, Fig. 1).

Partial Pressure of Oxygen (PaO_2) in mmHg

Compared to the Normal Control group (104.2 ± 5.3 mmHg), the FIP and Saline Group demonstrated a significant reduction in PaO_2 (65.2 ± 8.1 mmHg, $p < 0.01$). However, the FIP and 50 mg/kg Celecoxib Group showed a partial improvement in PaO_2 (78.5 ± 6.3 mmHg) compared to the FIP and Saline Group ($p < 0.05$) (Table 3).

Partial Pressure of Carbon Dioxide ($PaCO_2$) in mmHg

In comparison to the Normal Control group (42.3 ± 3.5 mmHg), the FIP and Saline Group displayed a significant decrease in $PaCO_2$ (32.3 ± 2.5 mmHg, $p < 0.05$). There was no significant difference in PaCO2 between the FIP and 50 mg/kg Celecoxib Group (33.1 ± 4.9 mmHg) and the FIP and Saline Group (Table 3)

DISCUSSION

This study examines the potential protective effects of Celecoxib in mitigating lung damage induced by sep-

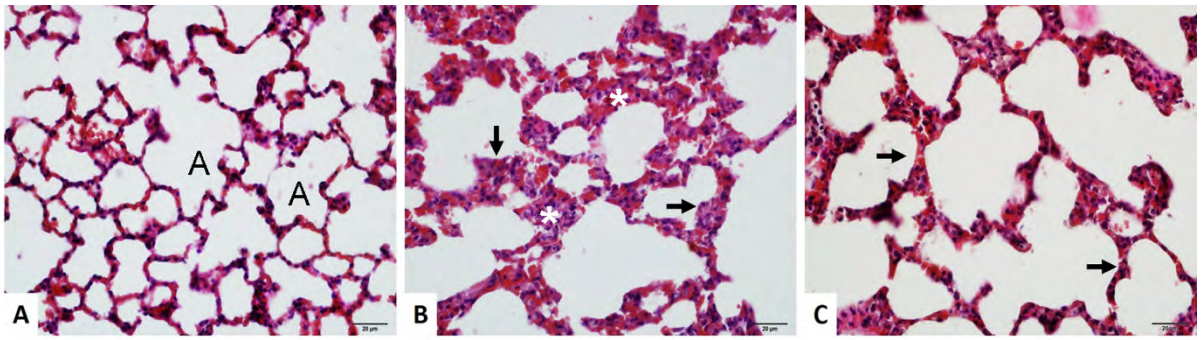

Fig. 1. Lung histopathology x40 magnification H&E staining. (A) Normal control group lung, (A = Alvelol), (B) FIP groups showed severe histopathologic alteration related to increased alveolar inflammation (*) and septal thickness (arrow), (C) FIP and 1 ml/kg % 0.9 NaCl saline (placebo) groups showed severe histopathologic alteration related to increased alveolar inflammation (*) and septal thickness (arrow), and (D) FIP and 50 mg/kg Celecoxib groups showed decreased inflammation and septal thickening (arrow)

Table 3. The results of the blood gas analysis in the three study groups: Normal control, FIP and saline group, and FIP and 50 mg/kg celecoxib group

	Normal control	FIP and saline	FIP and 50 mg/kg celecoxib
PaO_2 (mmHg)	104.2 ± 5.3	65.2 ± 8.1*	78.5 ± 6.3#
$PaCO_2$ (mmHg)	42.3 ± 3.5	32.3 ± 2.5*	33.1 ± 4.9

Blood gase analysis: results were presented as mean ± SEM. FIP = Fecal Intraperitoneal

Statistical analyses were performed by one- way ANOVA and post- hoc Bonferroni test. * $p < 0.05$, different from normal groups; #$p < 0.05$ different from FIP and saline group

sis. Sepsis is a critical medical condition distinguished by an aberrant immune response to infection, resulting in extensive inflammation and impaired organ function, such as ALI and ARDS. The lungs are particularly vulnerable to the detrimental effects of sepsis due to the massive release of pro-inflammatory cytokines and chemokines, leading to endothelial and epithelial cell injury, increased vascular permeability, and infiltration of inflammatory cells.

The results of this study demonstrate that Celecoxib administration exerts a protective role against sepsis-induced lung damage. Histopathological examination revealed that septic animals treated with Celecoxib exhibited reduced alveolar congestion, hemorrhage, aggregation in air spaces/vessel walls, and perivascular/interstitial edema compared to untreated septic animals [16]. These findings suggest that Celecoxib attenuates lung inflammation and edema, preserving the lung architecture. In a study by Liu [17], they describe similar results histologically in a model of lung injury induced by hyperoxia in rats.

Furthermore, the biochemical analysis showed a significant decrease in oxidative stress markers, such as MDA, in the lungs of septic animals treated with Celecoxib. Oxidative stress is a crucial contributor to the pathogenesis of sepsis-induced lung injury, and Celecoxib's antioxidant properties likely play a crucial role in mitigating oxidative damage in lung tissue. By reducing oxidative stress, Celecoxib may protect lung cells from oxidative injury and maintain their function. Mazhari *et al*. [18]'s study, observed a decreased MDA in a group that takes Celecoxib and prevents oxidative stress in varicocele.

Moreover, Celecoxib treatment decreased pro-inflammatory cytokines, such as TNF-alpha, in the lung tissue of septic animals. These cytokines are critical mediators of the inflammatory response in sepsis, and their excessive production contributes to tissue damage. Celecoxib's anti-inflammatory effects, mainly through inhibition of COX-2, may attenuate the inflammatory cascade, leading to reduced lung injury. COX-2 has a crucial role in inflammation) process, the significant effect of Celecoxib in the septic process is by inhibiting COX-2 [17]. The results of the biochemical analysis revealed that FIP induction led to a significant increase in oxidative stress marker MDA and pro-inflammatory cytokine TNF alpha levels. These findings align with previous studies that have shown the involvement of oxidative stress and inflammation in the pathogenesis of FIP [17-19]. Celecoxib administration at 50 mg/kg significantly reduced MDA and TNF alpha levels, indicating its potential to alleviate oxidative stress and inflammation in FIP. Celecoxib's anti-inflammatory and antioxidant properties likely contribute to these effects, as it inhibits the cyclooxygenase-2 enzyme and reduces the production of pro-inflammatory mediators [20, 21]. Similarly, Gurusamy *et al*. [19] study show the anti-inflammatory effect of Celecoxib in acute lung injury in mice with COX-2 inhibition.

Additionally, Celecoxib enhances the lung's barrier function by preserving the expression of tight junction proteins, such as occludin and claudin-5 [22]. The integrity of the lung's endothelial and epithelial barriers is critical for preventing fluid leakage and maintaining lung homeostasis. As shown in preventing gut barrier tight junction failure [23], Celecoxib's protective effects on tight junction proteins may attenuate lung edema and vascular leakage.

The overall findings of this study suggest that Celecoxib, through its anti-inflammatory, antioxidant, and barrier-protective properties, plays a beneficial role in mitigating sepsis-induced lung damage. By modulating the inflammatory response and oxidative

stress, Celecoxib may prevent excessive lung tissue injury and improve lung function in septic animals. These promising results warrant further investigations to explore the potential therapeutic use of Celecoxib as an adjunct treatment in clinical settings for sepsis-associated lung injury and ARDS.

Limitations

It is essential to acknowledge the limitations of this study, such as the animal model used, the dosing regimen of Celecoxib, and the specific mechanisms underlying its protective effects in sepsis-induced lung damage. Further studies, including clinical trials, are needed to validate these findings and determine the safety and efficacy of Celecoxib in human sepsis patients.

CONCLUSION

The present study provides evidence supporting the protective effects of Celecoxib against sepsis-induced lung damage. By targeting inflammation, oxidative stress, and barrier function, Celecoxib holds potential as a therapeutic agent to mitigate the devastating consequences of sepsis on the lungs. These findings contribute to the growing research on potential treatments for sepsis-induced organ dysfunction, particularly in acute lung injury. They may pave the way for developing novel therapeutic strategies to improve the clinical outcomes of septic patients.

Authors' Contribution

Study Conception: ESB, OE, CD; Study Design: EE, ESB; Supervision: OE, CD; Funding: N/A; Materials: OE; Data Collection and/or Processing: YU, OE; Statistical Analysis and/or Data Interpretation: OE, ESB; Literature Review: ESB; Manuscript Preparation: ESB, CD and Critical Review: OE, GY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Potential prognostic parameters and real-world data in patients with primary central nervous system lymphoma: a new brick on the old ones

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ABSTRACT

Objectives: We aimed to evaluate the potential prognostic factors of patients with primary central nervous system lymphoma (PCNSL).

Methods: Thirty-two patients with PCNSL were retrospectively analyzed.

Results: All the patients received high doses of methotrexate-based chemotherapy as the first-line treatment. Overall survival was 30.0 ± 7.2 months. Those with partial response and without response had a higher risk of mortality. The increased leukocyte and neutrophil levels were associated with high mortality. Besides, the SIIL as a product of the systemic immune inflammation (SII) and lactate dehydrogenase (LDH); the SIRIL as a product of systemic immune response index (SIRI) and LDH; and the NLL as a product of neutrophil-lymphocyte ratio and LDH were taken into consideration for the first time for the purposes of the present study. Elevated NLL, SIIL, and SIRIL indexes were associated with mortality. Elevated SIIL level, radiotherapy, and partial and no response were the independent predictors of mortality on the basis of the multivariable regression model including the risk factors associated with mortality.

Conclusions: SIIL, SIRIL and NLL are prognostic factors in PCNSL. Determining the prognostic factors and risk profile may predict the requirement for more intensive treatment, especially in young patients at high risk.

Keywords: Primary central nervous system lymphoma, prognostic score and parameters

Primary central nervous system lymphoma (PCNSL) accounts for approximately 3% of brain tumors and 4-6% of all the extranodal lymphomas. PCNSL is an aggressive form of non-Hodgkin lymphoma (NHL), which occurs in the brain, spinal cord, eye, or leptomeninx without systemic involvement. Its annual incidence is 0.47/100.000 [1]. High dose methotrexate-based regimens are used in treatment. Although scores such as International Extranodal Lymphoma Study Group (IELSG), The Nottingham / Barcelona (NB) score and Memorial Sloan-Kettering Cancer Center (MSKCC) score are used to predict prognosis, challenges are still encountered. [2-4]. Therefore, biomarkers that can better predict prognosis need to be developed to achieve more appropriate treatment.

It is known that inflammation increases tumor risk and has an effect on all stages. Many inflammatory

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markers such as C-reactive protein (CRP), neutrophil count-lymphocyte ratio (NLR) and platelet count-lymphocyte ratio (PLR) have been associated with poor prognosis in many malignancies [5-7]. However, systemic immune inflammation index (SII), which is formed by using absolute neutrophil, platelet and lymphocyte counts in peripheral blood, and systemic immune response index (SIRI), which is formed by using neutrophilia, monocyte and lymphocyte counts, have started to be used as inflammatory biomarkers in many cancers in recent years [8-11]. Prognocytic nutritional index (PNI) is a measurement calculated by using serum albumin and absolute lymphocyte value and reflects the inflammatory, nutritional and immune status of patients with cancer. There have been studies showing the prognostic importance of PNI in many cancer types [12-14].

In our study, we analyzed the prognostic impact of inflammatory markers and SIIL, SIRIL and NLL measurements obtained by multiplying SII, SIRI and NLR by serum LDH value in PCNSL patients.

METHODS

Patient Recruitment

The demographic and clinicopathological data of 32 patients with PCNSL followed-up at Bursa Uludag University Hematology Department between 2010 and 2021 were retrospectively reviewed. The criteria for inclusion included pathologically confirmed PCNSL diagnosis and age over 18 years.

Data Collection

Clinical data included gender, age, symptoms at diagnosis, examination findings, KPS, localization and number of lesions, biopsy type, pathological subtype, Ki-67(%), Memorial Sloan-Kettering Cancer Center (MSKCC) score, lactate dehydrogenase (LDH), β2 microglobulin (B2M), sedimentation (Sed), CRP, albumin, globulin, total bilirubin, ferritin level, complete blood count, NLR, PLR, platelet count × neutrophil count/ lymphocyte count (SII), neutrophil count × monocytes count/lymphocytes count (SIRI), lymphocyte count-to-monocyte ratio (LMR), albumin-globulin ratio (AGR), serum albumin (g/L) + 5 × lymphocytes count (×109/L) [prognostic nutritional index (PNI), LDH-lymphocyte ratio (LLR), WBC-lymphocyte ratio (WLR), Ferritin-LDH ratio (FLR), CRP-albumin ratio (CAR), PLR × LDH (PLL), SII × LDH (SIIL), SIRI × LDH (SIRIL), NLR × LDH (NLL) indexes and treatment regimens and responses. The results of routine blood tests performed within one week prior to the onset of the treatment, were retrospectively retrieved from medical records. The location, number, and size of the lesions in all the patients were evaluated by means of magnetic resonance imaging (MRI).

MSKCC Score

The MSKCC model is comprised of two variables, including age and KPS, and defines three prognostic classes: Class 1 (age < 50), Class 2 (age ≥ 50 and KPS ≥ 70) and Class 3 (age ≥ 50 and KPS < 70).

Response Evaluation

Responses were evaluated according to international working group recommendations as defined by Abrey *et al*. [15].

Statistical Analysis

IBM Statistical Package for the Social Sciences (SPSS) v.20 software (IBM Corp., Armonk, NY, USA) was used for the purposes of analyses in the scope of the study. The distribution of normality hypothesis was tested using the Kolmogorov-Smirnov test. Numerical variables with and without normal distribution were expressed as mean ± standard deviation and median (min-max), respectively. The categorical variables were expressed as numbers and percentages. Univariable Cox Regression analysis was used to identify the potential risk factors associated with mortality, and the statistically significant factors were included in the multivariable regression model. ANOVA test (post hoc: Bonferroni test) and Kruskal-Wallis H test (post hoc: Dunn's test) were used to compare the numerical variables by class groups depending upon the normality of distribution. Fisher's Exact and Chi-square tests were used to compare categorical variables. A p value of < 0.05 was considered statistically significant.

RESULTS

The Entire Population and Mortality Relation

The study population was comprised of 32 patients,

Table 1. Demographic and clinical characteristics of patients

Variables	Data (n = 32)
Gender, n (%)	
Female	18 (56.3)
Male	14 (43.8)
Age (years)	54.1 ± 14.3
Female	61.1 ± 8.1
Male	45.1 ± 15.6
Number of involvements, n (%)	
Solitary	18 (56.2)
Multiple	14 (43.8)
Involvement area, n (%)	
Cerebral hemisphere	15 (46.9)
Cerebellum	3 (9.4)
Periventricular	6 (18.8)
Hemisphere	1 (3.1)
Thalamus	5 (15.6)
Corpus callosum	1 (3.1)
Eye	1 (3.1)
Involvement of deeper brain structures, n (%)	
None	18 (53.3)
Yes	14 (43.8)
Pathology/cytology, n (%)	
DLBCL	29 (90.6)
Others	3 (9.4)
Biopsy, n (%)	
SBX	19 (59.4)
EBX	13 (40.6)
Ki-67(%)	80 (20-98)
Treatment, n (%)	
MATRIX	7 (21.9)
HD-MTX	2 (6.3)
HD-MTX+RTX	1 (3.1)
HD-MTX+RTX+VINC	8 (25.0)
HD-MTX+VINC	14 (43.8)
Radiotherapy, n (%)	
None	13 (40.6)
Yes	19 (59.4)
Radiotherapy duration (days)	25 (8-45)
Response, n (%)	
Complete response	13 (40.6)
Partial response	6 (18.8)
No response	2 (6.3)
Could not be assessed	11 (34.4)
ASCT, n (%)	
None	29 (90.6)
Yes	3 (9.4)
Follow-up duration (days)	7.2 (0.2-93.8)

DLBCL = diffuse large B cell lymphoma, SBX = stereotactic biopsy, EBX = excisional biopsy, Ki-67 = proliferation index, MATRIX = high dose methotrexate+cytosine arabinoside + thiotepa + rituximab, HD-MTX = high dose methotrexate, RTX = rituximab, VINC = vincristine, ASCT = autologous stem cell transplantation

including 18 female and 14 male patients (mean age: 54.1 ± 14.3 years). The patients most frequently complained about dysphasia (n = 7; 21.9%), headache (n = 6; 18.8%), and impaired balance (n = 6; 18.8%). Paresthesia was the most prevalent manifestation during the neurological examinations (31.3%). Eighteen patients had solitary and 14 had multiple lesions. The cerebral hemisphere was the most frequently affected area (n = 15; 46.9%). Twenty-nine (90.6%) cases involved diffuse large B-cell lymphoma (DLBCL) subtype 68.8% (n = 22) of the patients died. There was no association between the demographic characteristics and mortality. Demographic and clinical characteristics of patients are shown in Table 1. Relationship between clinical findings and mortality were included in detail in.The patients with partial or no response had a higher risk of mortality compared to those with complete response (HR: 12.93, p = 0,003; HR: 10.64, p = 0.025, respectively). There was no association with other clinical findings and mortality.

Distribution of laboratory findings and their relationship with mortality included in detail in Table 2. Elevated hemoglobin level (HR: 1.42; p = 0.017), elevated leukocyte level (HR: 1.14; p = 0.050), elevated neutrophil level (HR: 1.17; p = 0.023), elevated SIIL (HR: 1.02; p = 0.008), elevated SIRIL (HR: 1.04; p = 0.021), and elevated NLL index (HR: 1.28; p = 0.037) were associated with mortality. There was no relation between other laboratory findings and mortality.

Elevated SIIL level (HR: 1.03; p = 0.019) and partial and no response (HR: 17.6, p = 0.009; HR:11.6, p = 0.004, respectively) were the independent predictors of mortality on the basis of the multivariable regression model including the potential risk factors associated with mortality. Independent predictors of mortality are included in detail in Table 3.

The median neutrophil level and SII score were lower in Class 1 patients than in the others ($p < 0.05$). Those with Class III had a higher median SII score, median PLR, median NLR, median CRP, and lower median creatinine than others. Other laboratory findings did not differ significantly between the groups. Distribution of laboratory findings by prognostic score are included in detail in Table 4.

The predictive value of the SIIL index in prediction of mortality was > 377.4 × 103 with a sensitivity of 59.1% and specificity of 100% (AUC ± SE = 0.73 ± 0.08; 95% CI = 0.546-0.872; p = 0.008) (Fig. 1A).

Table 2. Distribution of laboratory findings and their relationship with mortality

Variables	Survival			Univariable Cox regression		
	Total (n = 32)	Alive (n = 10)	Dead (n = 22)	HR	95% CI	*p* value
Hemoglobin (g/dL)	12.9 ± 1.6	11.4 ± 1.5	13.5 ± 1.0	1.42	1.06-1.90	**0.017***
Leukocyte ($\times 10^3/\mu L$)	10.2 (5.1-18.8)	8.1 (6.3-13.4)	10.3 (5.1-18.8)	1.14	1.01-1.31	**0.050***
Neutrophils ($\times 10^3/\mu L$)	8.7 (3.0-16.8)	6.7 (3.0-10.8)	9.0 (3.4-16.8)	1.17	1.02-1.35	**0.023***
SII	1255.3 (252.6-6283.6)	1219.3 (252.6-1585.8)	1760 (330-6283.6)	1.00	0.99-1.02	0.094
SIIL	365.8 (59.1-1994.7)	238.3 (59.1-377.4)	475.5 (90.1-1994.7)	1.02	1.01-1.03	**0.008***
SIRIL	7.1 (1.3-53.7)	4.7 (1.3-15.1)	8.9 (1.9-53.7)	1.04	1.01-1.07	**0.021***
NLL	1.8 (0.3-6.0)	1.2 (0.02-3.4)	2.2 (0.4-6.0)	1.28	1.02-1.062	**0.037***
PLL	51.5 (18.2-175.0)	25.5 (19.7-68.0)	57.1 (18.2-174.9)	1.01	0.98-1.02	0.060

Categorical variables were expressed as numbers (%). Numerical variables with and without normal distribution were expressed as mean ± SD and median (min-max), respectively. SII = systemic immune inflammation index, SIIL = SII × LDH, SIRIL = SIRI × LDH, NLL = Neutrophil/Lymphocyte × LDH, PLL = Platelet/Lymphocyte × LDH, HR = Hazard ratio, CI = Confidence interval

SII × LDH levels are divided by 1000. SIRI × LDH levels are divided by 100.

*$p < 0.05$ is considered statistically significant.

The risk of mortality was 5.9 times higher in patients with a SIIL index of > 377.4 × 103 compared to patients with a SIIL index of ≤ 377.4 x103 (HR: 5.9; $p < 0.001$) (Fig. 1B).

MSKCC Prognostic Scoring Relationship

There was a lower rate of male patients as MSKCC prognostic score increased ($p = 0.031$). There was no relation between other demographic character-

Table 3. Independent predictors of mortality

Variables	Multivariable Cox regression		
	HR	95% CI	*p* value
SIIL	1.03	1.01-1.05	**0.019***
Radiotherapy			
None	ref		
Yes	0.15	0.02-0.34	**< 0.001***
Response			
Complete response	ref		
Partial response	12.8	1.6-103.8	**0.017***
No response	12.2	1.9-75.7	**0.007***
Could not be assessed	40.6	0.1-728.3	0.893

SIIL = SII × LDH, HR = Hazard ratio, CI = Confidence interval.

2 Log Likelihood: 82.2; $p < 0.001$

*$p < 0.05$ is considered statistically significant.

Table 4. Distribution of laboratory findings by prognostic score

Variables	MSKCC Class			*p* value
	1 (n = 7)	2 (n = 14)	3 (n = 11)	
Leukocyte (×10^3/µL)	7.6 (5.1-14.6)	10.2 (6.3-18.8)	11.5 (6.8-18.0)	0.061
Neutrophils (×10^3/µL)	4.9 (3.6-9.7)	8.7 (3-16.8)	9.3 (5.9-16.4)	**0.029***
CRP	0.3 (0-0.4)	0.3 (0.3-5.8)	0.9 (0.1-42)	**0.030***
SII	619.3 (330-1503.5)	1255.3 (252.6-6283.6)	1857.4 (946,7-4199.4)	**0.007***
PLR	117.1 (66.5-227.1)	133.2 (84.2-667.8)	191.5 (106.4-368.4)	**0.048***
NLR	3.3 (2-7.9)	5.6 (1.2-30.7)	11.2 (2.9-20.8)	**0.019***
LLR, %	3.9 (2.3-6.9)	2.4 (1.4-10.5)	2 (1.2-4.5)	0.064
WLR	0.5 (0.3-0.9)	0.7 (0.2-3.4)	1.2 (0.4-2.3)	**0.024***
CAR	0.1 (0-0.1)	0.1 (0.1-1.5)	0.3 (0-11.7)	**0.050***
LNR	0.7 (0.6-0.9)	0.7 (0.5-0.9)	0.9 (0.7-0.9)	**0.029***
SIIL	209.5 (90.1-401.5)	316.1 (59.1-1237.8)	780.1 (148.3-1994.7)	0.062
NLL	1.7 (0.5-2)	1.6 (0.3-6)	3.4 (0.4-5.5)	0.094

Categorical variables were expressed as numbers (%). Numerical variables with and without normal distribution were expressed as mean ± SD and median (min-max), respectively.

MSKCC = Memorial Sloan-Kettering Cancer Center score, SII = systemic immune inflammation index, SIRI = systemic immune response index, PLR = platelet-lymphocyte ratio, NLR = neutrophil-lymphocyte ratio, LLR = LDH/lymphocyte ratio, WLR = WBC/lymphocyte ratio, CAR = CRP/albumin ratio, LNR = Lymphocyte/neutrophil ratio, SIIL = SII × LDH, SIRIL = SIRI × LDH, NLL = Neutrophil/Lymphocyte × LDH, HR = Hazard ratio, CI = Confidence interval

*$p < 0.05$ is considered statistically significant.

istics and clinical findings and prognostic score. Patients classified under MSKCC class 1, had lower median neutrophil and median SII scores compared to the others ($p < 0.05$). Patients classified under Class 3 had higher median CRP level, median SII score, median PLR level, median NLR level, median WLR level, median CAR level, and median LNR level and lower median creatinine compared to others. There was no significant difference by other laboratory findings between the groups.

DISCUSSION

There is growing evidence that cancer-related inflammation (CRI) may promote malignant cell proliferation, invasion, and metastasis [16, 17]. Tumor-related macrophages play a leading role in CRI, the prognostic value of which has been demonstrated as regards a number of lymphoproliferative malignancies [18]. The prognostic value of other systemic inflammation response indicators represented by NLR and LMR has also been verified in several cancers [19-22]. Neutrophils as a part of the innate immune system may promote oncogenesis and suppress the function of lymphocytes that work for antitumor immunity [23]. Monocytes, which can be recruited by large B-cell lymphoma cells through CCL5 that adhere leukocytes, may promote the survival and proliferation of tumor cells [24].

In a study on 60 patients with PCNSL, the LMR (HR 6.195, $p = 0.093$), SII (HR 5.144, $p = 0.012$), and total bilirubin level (HR: 3.892, $p = 0.009$) were suggested as the independent risk factors for OS [25]. Nevertheless, NLR and LMR were not associated with mortality in the present study. Yet, elevated NLL index (HR: 1.28; $p = 0.037$), a product of NLR value and LDH, was associated with mortality.

In an in vitro experiment, platelets activated tumor cell invasion by increasing the secretion of metalloproteinase-9 (MMP-9) [26]. However, there was no correlation in the present study between platelet counts

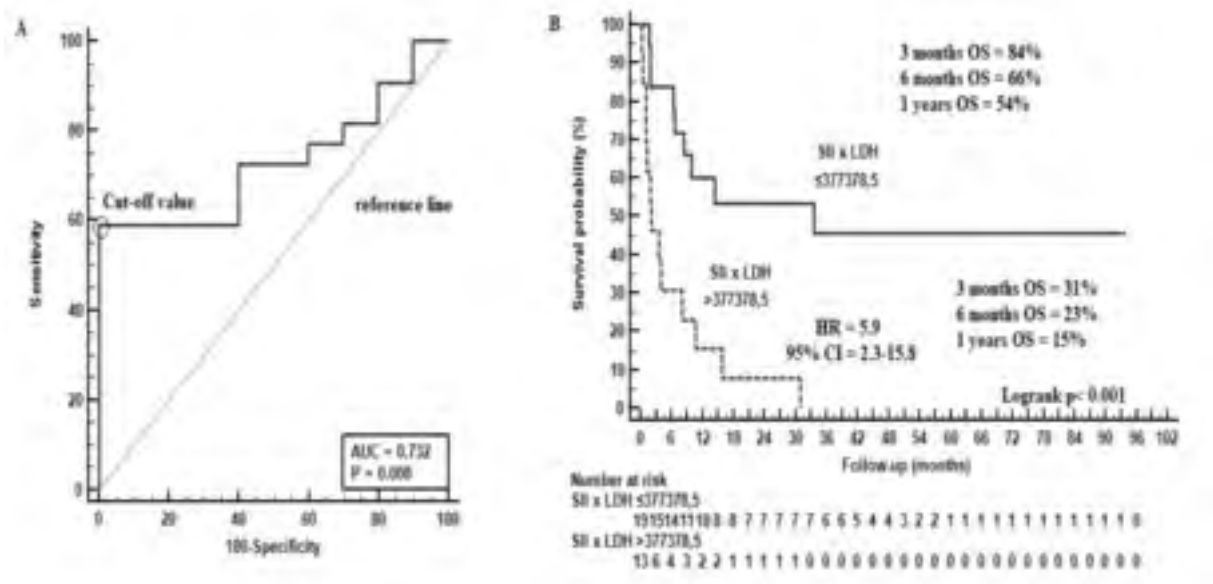

Fig. 1. **(A) SIIL diagnostic performance assessment and (B) survival risk based on the predictive value. SIIL = SII × LDH**

and PLR, and mortality.

In a study, which retrospectively investigated 73 patients with PCNSL, both age and MSKCC scores were correlated with lower progression-free survival (PFS) and OS ($p < 0.05$) rates and that elevated NLR, PLR, SII, and SIRI levels were suggested as significant predictors of shorter PFS and OS rates ($p < 0.05$) [27]. It was shown that upon the combination of neutrophil, lymphocyte, and platelet counts, the prognostic ability of the SII was higher compared to NLR, LMR, and PLR in lung cancer [28] and classic Hodgkin lymphoma [29]. Nevertheless, SII was not prognostic upon the single-variable analysis for the purposes of the present study. On the other hand, elevated SIIL (HR: 1.03; $p = 0.019$) was identified as an independent risk factor for mortality according to the multivariable regression model. The predictive value of the SIIL index in prediction of mortality was > 377.4 x 103 with a sensitivity of 59.1% and specificity of 100%. Patients with a SIIL level of > 377.4 × 103 were at 5.9 times higher mortality risk compared to patients with a SIIL level of ≤ 377.4 × 103.

PNI is an indicator of systemic inflammation and nutritional status, nevertheless, there was no association between PNI and survival in the present study.

Although the IELSG model was derived from a relatively large group of patients from multiple centers, there was no data on LDH level or the CSF protein in two-thirds of the samples. Information about the LDH level or CSF protein was not always available in clinical practice, which made IELSG difficult to apply and verify in many previous studies [4, 30-32].

In addition, CSF protein concentration among those parameters is not easily applicable. Routine lumbar puncture cannot be performed due to the high intracranial pressure in patients [2, 4, 30-32]. The Nottingham/Barcelona (NB) model was derived form a relatively smaller patient population, where the patients received legacy chemotherapy regimens. Therefore, its application for the PCNSL populations of the day is limited. A few recent studies suggested that there was no adequate correlation between the MSKCC score and survival [30, 33]. This raises doubts with regard to the reliability of the said two-parameter model.

The rather wide survival range in patients with PCNSL indicates the need to develop a reliable prognostic model that is able to predict disease outcomes and facilitate decision-making for further treatments. In addition, given the low incidence of PCNSL, there is a comparatively limited number of large randomized phase III studies with regard to optimal standard therapy, and thus consensus is mainly based on the comparative analysis of retrospective and phase II studies [34-36]. A number of studies in the relevant literature have investigated the prognostic factors for PCNSL. Age and performance status (PS) are the two factors

that are reported to have consistently associated with disease survival [2-4, 37, 38]. In the present study age limits of > 50, < 50 and of > 60, < 60 years were not associated with mortality. There was no association between the patients' KPS scores of > 70 and < 70 and mortality.

A recent study of 167 patients with PCNSL reported a median OS rate of 7 months (95% CI, 25-49) with a follow-up period of 25 months (1-152). The post-operative residual tumor, HD-MTX-free chemotherapy, and palliative therapy were identified as independent prognostic markers. Furthermore, the ECOG > 3, multifocal lesions, and palliative therapy were reported as negative independent prognostic markers for PFS [39].

While the role of MSKCC score is still controversial, it was not associated with mortality in patients treated with standard HD-MTX based therapy in the present study.

In PCNSL, there are various options for induction chemotherapy and consolidation therapy. Therefore, a better disease risk classification score can help with clinical decision-making and develop treatments tailored to the risk assessment. In PCNSL, the effect of surgical excision on survival has not been conclusively confirmed [40, 41]. In the present study, the rates of patients, who underwent excisional biopsy and stereotactic biopsy were 40.6% and 59.4%, respectively, where the was no difference in the two methods by survival.

The addition of rituximab as another important treatment for PCNSL is controversial [42, 43] and there was no significant difference in the present study. A previous study suggested that ASCT was better at consolidation treatment compared to WBRT [44], yet this was not confirmed in the present study, since probably only three patients were treated with ASCT, and one died of pneumonia subsequent to ASCT.

Therefore, there is a requirement for further studies to determine the better therapeutic options in PCNSL. However, the present study had several limitations. Firstly, the selection bias and information bias could not be avoided in the present study due to its retrospective and single-centered design. Secondly, the study population was relatively small. Despite these limitations, SIIL, SIRIL, NLL, PLL, FLR, CAR, and AGR prognostic factors were investigated for the first time in patients with PCNSL, who initially received standard HD-MTX-based chemotherapy.

PCNSL treatment has significantly improved in the last 20 years and long-term survival has been observed in approximately 15-20% of patients upon HD-MTX-based chemotherapy with or without radiotherapy. However, relapse is prevalent, and long-term survival rate is still not good enough. Although clinical prognostic scoring, including MSKCC and IELSG, is available in predicting prognosis and survival, it is still not adequate for today and there is a requirement for further prognostic parameters. Many studies [25, 27, 42] reported an association between alterations in laboratory parameters and outcomes in patients with PCNSL.

CONCLUSION

In brief, the likelihood of survival was higher in patients with PCNSL, who received RT, compared to RT-naive patients in this 6-year retrospective and single-centered study, which investigated the demographic and clinicopathological characteristics and possible prognostic factors. The risk of mortality was higher in patients with partial and no response compared patients with complete response. Elevated hemoglobin levels, elevated leukocyte levels, elevated neutrophil levels, elevated SIIL index, elevated SIRIL index, and elevated NLL index were associated with mortality. SIIL, SIRIL, and NLL indexes were investigated for the first time in the literature. Elevated SIIL index level and partial and no response were the independent predictors of mortality on the basis of the multivariable regression model including the potential risk factors associated with mortality. Other parameters (ALC, AMC, RDW, total bilirubin) and their respective ratios (LMR, PLR, NLR, PLL, FLR, AGR, WLR, CAR) were also not associated with OS. The serum LDH level was not associated with OS and there was a paradoxical association with anemia in the present study. The fact that a single-variable analysis was conducted, and the number of cases may account for the above. Elevated neutrophil levels, elevated leukocyte levels, and elevated SIIL, SIRIL, and NLL index levels are effective and promising blood markers as prognostic factors. Further studies and research are required for verification of these results and for the prognostic role of hematologic parameters in PCNSL.

Authors' Contribution

Study Conception: TE, FÖ; Study Design: TE; Supervision: TE, FÖ; Funding: TE; Materials: İEP, BO, CY, ÖC, RA; Data Collection and/or Processing: TGK, SÇ, TE; Statistical Analysis and/or Data Interpretation: TE; Literature Review: TE; Manuscript Preparation: TE, FÖ, VÖ and Critical Review: FÖ, VÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Ethics Committee Approval

Clinical Research Ethics Committee, Faculty of Medicine, Bursa Uludag University, 2021-8/3

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Exploring the impacts of a nuchal cord on perinatal outcomes in vaginal delivery

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ABSTRACT

Objectives: To investigate the frequency of cord entanglement and neonatal outcomes in vaginal deliveries.
Methods: A total of 24,623 patients who had vaginal delivery at at Kayseri City Hospital between July 2018 and January 2023 were included in the study. The incidence of nuchal cord was determined in the study group. The characteristics and perinatal outcomes of groups with and without nuchal cord were compared. Chi-square test was used for statistical evaluation. A p value less than 0.05 was considered significant in the evaluation.
Results: The rate of cord entanglement in the neck at birth was 15.7%. There was no statistically significant difference between the infant weights, genders, maternal ages, hospitalization rates in the neonatal intensive care unit, and apgar scores at the 1st and 5th minutes of the babies included in the study. We detected amniotic fluid with meconium in 506 (13.1%) patients with a nuchal cord and 270 (1.3%) without a nuchal cord, and the difference was found to be significant.
Conclusions: There is no significant relationship between vaginal deliveries with the nuchal cord and poor perinatal outcomes, except for meconium amniotic fluid. For this reason, pregnant women diagnosed with nuchal cord in the third trimester can deliver vaginally, but they should be carefully monitored in terms of meconium and related complications. However, neonates with nuchal cord do not have significantly longer neonatal hospital stays, and thus the adverse effects of nuchal cord may be transient.
Keywords: Nuchal cord, vaginal delivery, meconium, perinatal outcomes

The umbilical cord, an extension of the fetal cardiovascular system, is known to supply nutrients and oxygen to and removes wastes from the fetus. It consists of the outer layer of the amnion, Wharton's gel, two uterine arteries, and a vein. [1] Most umbilical cords are 40-70 cm long, and those < 30 cm and > 100 cm are known to be rare. It should be noted that extremely long cords increase the likelihood of cord entanglement. [2]. Not surprisingly, cord loops are frequently encountered when the umbilical cord gets wrapped around various fetal parts during fetal movements [2]. In this sense, a nuchal cord is a condition occurring when the umbilical cord wraps around the fetal neck. Nuchal cords can be encountered in any period of pregnancy, and 25-50% of them can resolve spontaneously before delivery [3, 4]. The incidence of nuchal cords at birth was previously reported to be 14.7%-33.7% [5-9]. On the other hand, the previous research identified various risk factors for a nuchal cord, such as monozygotic twinning, male fetus, long

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umbilical cord, previous birth with a nuchal cord, and posterior placentation. Nonetheless, the definitive etiology and pathogenesis have not been fully elucidated yet. Moreover, the clinical significance of nuchal cords remains controversial. A nuchal cord may cause cord compression during delivery, resulting in metabolic acidosis and decreased cardiac output and increased arterial resistance caused by fetal bradycardia. In the literature, some studies associated nuchal cords with variable fetal heart rate during delivery, increased prevalence of deceleration, low 1-minute Apgar score, and high incidence of meconium-stained amniotic fluid [10, 11]. Nevertheless, the literature also hosts a plethora of studies that could not conclude any relationship between nuchal cords and increased cesarean section rate, low 5-minute Apgar score, perinatal mortality, and neonatal intensive care unit admission [5, 6, 8, 12, 13]. Ultimately, the present study compared the perinatal outcomes of normal delivery patients with and without a nuchal cord.

METHODS

Research Design and Sample

After obtaining ethical approval from the Ethics Committee Kayseri City Hospital, we retrospectively analyzed the perinatal outcomes of 24,623 normal delivery patients in the Department of Gynecology and Obstetrics at the said hospital between July 2018 and January 2022. In the patient data, we discovered that gestational week was calculated according to the first day of the last menstrual period or the findings of ultrasound performed in the first trimester [14]. Accordingly, we considered the data of those with a calculated gestational week of 37 weeks and above. Yet, we excluded the patients with an intrauterine chromosomal anomaly or metabolic disease, neural tube defects, anencephaly (and other central nervous system anomalies), additional cardiovascular anomalies, no routine perinatology follow-ups, and intrauterine ex fetuses. Then, the patients were divided into two by the presence of a nuchal cord: 3,868 with a nuchal cord and 20,755 without a nuchal cord at normal delivery. We retrospectively reviewed the patients' files and compared their demographic characteristics and perinatal outcomes.

Statistical Analysis

In statistical analyses, we first resorted to the Kolmogorov-Smirnov test to check whether the data showed a normal distribution. Accordingly, while showing the normally distributed data as means (M) and standard deviations (SD), we present non-normally distributed as medians (min-max). Discrete variables are given as percentages. In group comparisons, we utilized independent samples t-test for normally-distributed variables. Moreover, we compared categorical variables using the Chi-square test. We performed all statistical analyses on the SPSS 17.0 program and accepted a p - value < 0.05 statistically significant.

RESULTS

Demographic characteristics of the patients in our study are shown in Table 1. The findings revealed the mean age of the patients to be 27.9 ± 4.1 years in the nuchal cord (+) group and 28.01 ± 4.2 years in the

Table 1. Patients' demographic characteristics

	NC (+) (n = 3,868)	NC (-) (n = 20,755)	*p* value
Maternal age (years)	27.9 ± 4.1	28.01 ± 4.2	0.216
Parity	1.2 ± 0.9	1.3 ± 1.1	0.324
BMI (kg/m^2)	28.9 ± 2.5	28.4 ± 2.3	0.241
Hemoglobin (g/dL)	11.6 ± 1.5	11.2 ± 1.3	0.189
Gestational week	40.1 ± 0.7	39.2 ± 0.6	**< 0.001**
Birth weight (g)	3,397 ± 440.6	3,407 ± 442.6	0.467

Data are given as mean ± standard deviation. NC = nuchal cord

nuchal cord (-) group, but there was no significant difference between the groups by age ($p = 0.216$). Nevertheless, we discovered a significant difference between the groups by the week of delivery. While the mean week of delivery was 40.1 ± 0.7 among the patients with a nuchal cord, it was 39.2 ± 0.6 among those without a nuchal cord ($p < 0.001$). On the other hand, while the mean parity of the patients with an NC was found to be 1.2 ± 0.9, it was 1.3 ± 1.1 among those without a nuchal cord. Yet the groups did not significantly differ by parity ($p = 0.324$).

In our study, we could not conclude a significant difference between the groups' mean body mass index (BMI) values, calculated to be 28.9 ± 2.5 kg/m^2 and 28.4 ± 2.3 kg/m^2, respectively ($p = 0.241$). It was also the case for hemoglobin values, discovered to be 11.6 ± 1.5 among the patient with a nuchal cord and 11.2 ± 1.3 among those without a nuchal cord ($p = 0.189$). Finally, despite no significant difference between the groups ($p = 0.467$), the mean birth weights of newborns with and without a nuchal cord were found to be 3,397 ± 440.6 g and 3,407 ± 442.6 g, respectively.

In this study, we retrospectively analyzed the data of 24,623 normal delivery patients. Among them, 3,868 (15.7%) had a nuchal cord at birth. We detected amniotic fluid with meconium in 506 (13.1%) patients with a nuchal cord and 270 (1.3%) without a nuchal cord, and the difference was found to be significant ($p < 0.001$). The 1- and 5-minute Apgar scores of newborns were similar in both groups. Although the number of male babies was higher in both groups, the difference was not statistically significant ($p = 0.337$). Moreover, the groups did not significantly differ by the number of newborns admitted to the neonatal intensive care unit (NICU) ($p = 0.561$). Accordingly, while 550 (14.2%) newborns were admitted to the NICU in the nuchal cord (+) group, this number was found to be 2,200 (10.6%) in the nuchal cord (-) group ($p = 0.561$). The groups' perinatal outcomes are presented in Table 2.

DISCUSSION

Our findings revealed the rate of a nuchal cord in the neck to be 15.7% in normal deliveries, overlapping with the previous results that showed the rate of cord entanglement in the neck between 14.7-33.7% [5-9].

Due to its sticky and viscous properties, meconium may cause mechanical obstruction in the airways, changes in the tracheobronchial mucociliary transport, and difficulties in gas exchange in the early period of pregnancy [15]. Later, it may lead to inflammation, chemical pneumonia, vasculitis, ischemia, mucosal necrosis, and a decrease in or inactivation of endogenous surfactant synthesis. In addition, meconine directly inhibits endogenous surfactant production and functions [16, 17]. Partial occlusion of small airways causes air accumulation in the alveoli and alveolar rupture, while complete occlusions lead to atelectasis [18]. Meconium and the bile salts in it also exert direct toxic effects on the lung tissue and blood vessels [15]. Therefore, the impacts of meconium lead to necrosis and ulceration in fetal membranes, placenta, and umbilical cord and vasoconstriction in placenta and fetal blood vessels [19]. An inflammatory response occurs in the lungs a few hours after aspiration, and there may be an increase in the release of cy-

Table 2. Patients' perinatal outcomes

	NC (+) (n = 3,868)	NC (-) (n = 20,755)	*p* value
Meconium, n (%)	506 (13.1)	270 (1.3)	< 0.001
1-min. Apgar	8 (4-9)	8 (7-9)	
5-min. Apgar	9 (5-10)	9 (8-10)	
Sex, n (%)			
Male	2050 (53)	10,585 (51)	0.337
Female	1818 (47)	10,170 (49)	
NICU admission, n (%)	550 (14.2)	2,200 (10.6)	0.561

Data are given as median (minimum-maximum) or n (%). NC = nuchal cord, NICU = neonatal intensive care unit

tokines involved in the inflammatory process. The released substances account for damage to the lung parenchyma, severe vasoconstriction of blood vessels, and pulmonary hypertension [19, 20]. When it comes to pregnancy, the probability of the fetus being born with meconium increases as the gestational age progresses ([21]. The previous research reported that about 10.5% of newborns with meconium bear meconium aspiration syndrome (MAS) and that the morbidity and mortality rate in newborns with severe MAS is 12% [20]. In this study, we concluded an increased rate of birth with meconium amniotic fluid among those with a nuchal cord ($p < 0.001$).

The previous research showed that the presence of a nuchal cord does not pose a risk of decreased 1-and 5-minute Apgar scores [5,9,22-27]. Similarly, we did not find a significant difference between the groups by Apgar scores. The groups did also not significantly differ by newborn birth weight. In their studies, Schaffer *et al*. [1] and Miser *et al*. [28] observed low birth weight in the nuchal cord (+) group but no significant difference between the groups by newborn birth weight. Yet, our findings showed a significant difference between the mean week of delivery among patients with a nuchal cord and that of those without a nuchal cord. Accordingly, the patients with an NC gave birth at later weeks than their counterparts ($p < 0.001$). Similarly, Uludağ *et al*. [29] found that the rate of delivery at the 41st week or later among cases with a nuchal cord was significantly higher than among those cases without a nuchal cord. Our findings showed no significant difference between the groups by age and parity, overlapping with the findings of Miser *et al*. [28] that discovered that maternal age and parity had no effect on the incidence of nuchal cords. Finally, there was no significant difference between the groups by the sex of newborns, although there seemed to be more male newborns in the group with an NC. Yet, Miser *et al*. [28] reported the incidence of a nuchal cord to be significantly more in male newborns.

Cord compression causes chronic, intermittent, or acute interruption of blood flow to the fetus, directs the blood flow to the extremities to the central circulation (heart, adrenal, and brain), and alters the fetus' body to take self-protective measures [11]. This situation, in turn, triggers the development of fetal hypoxia by allowing the release of catecholamine, cortisol, vasopressin, angiotensin, and other biochemicals by baroreceptors and chemoreceptors. While the relevant literature hosts research documenting that compression of the umbilical cord may cause poor newborn outcomes [10, 30-32], some other studies showed no significant connection between a nuchal cord and poor perinatal outcomes [9, 33, 34].

CONCLUSION

In conclusion, our findings revealed the presence of a nuchal cord not to be associated with adverse perinatal outcomes, except for meconium-containing amniotic fluid. Therefore, pregnant women diagnosed with an NC in the third trimester can deliver vaginally but may need to be carefully followed up in terms of meconium and related complications. Nuchal cord entanglement was associated with a higher risk of meconium; however, there was no additional risk for an adverse neonatal outcome. Most obstetricians experience anxiety in the presence of the nuchal cord, but our study did not detect any negative outcomes in newborns.

Authors' Contribution

Study Conception: MA, ŞDÇ; Study Design: MA, MBD; Supervision: MA, CRC; Funding: ŞDÇ, MBD; Materials: CDC, MBD; Data Collection and/or Processing: MA, CRC; Statistical Analysis and/or Data Interpretation: MA, CRC; Literature Review: MA, ŞDÇ, CRC; Manuscript Preparation: MBD, CRC, MA and Critical Review: MA, ŞDÇ, CRC, MBD.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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Relationship between uric acid/ albumin ratio and coronary slow flow

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ABSTRACT

Objectives: Although the pathophysiology of coronary slow flow is not fully understood, evidence suggesting endothelial dysfunction and subclinical widespread atherosclerosis in genesis has grown in recent years. Our aim in this study is to investigate the relationship between uric acid/ albumin ratio and coronary slow flow.

Methods: One hundred and five coronary slow flow patients (determined by the Thrombolysis in Myocardial Infarction-frame count method) and one-hundred patients with normal coronary low were included retrospectively. The uric acid/ albumin ratio was investigated in all patients participating.

Results: In the logistic regression analysis, it was revealed that high uric acid levels, uric acid/ albumin ratios, and male gender were independent predictors for coronary slow flow. Among these parameters, the uric acid/ albumin ratio was the best predictor of coronary slow flow. Based on the receiver operating characteristics (ROC) analysis, the cut-off value of uric acid/ albumin ratio ≥ 0.57 was found to predict coronary slow flow with 68.3% sensitivity and 68.7% specificity. In multivariate logistic regression analysis, high uric acid levels (OR: 2.22; 95% CI (1.551-3.200), $p < 0.001$), high serum uric acid/ albumin ratio (OR: 37.7 95% CI (8.176-234.387), $p < 0.001$), male gender (OR: 0.157; 95% CI (0.078-0.318), $p < 0.001$) were independent predictors of coronary slow flow.

Conclusions: High uric acid/ albumin ratio was detected as an independent predictor for coronary slow flow. Larger studies are needed to elucidate its role in the pathophysiology of coronary slow flow.

Keywords: Uric acid, uric acid/ albumin ratio, coronary slow flow

The coronary slow flow was distinguished by a noticeably reduced flow rate of contrast material in one or more coronary arteries during coronary angiography (CAG) despite the absence of coronary stenosis. Although individuals with coronary slow flow are identified as having "normal coronary arteries," it is reasonable to treat coronary slow flow as a separate disease entity. Tambe et colleagues reported "a normal coronary artery tree accompanied with a notably sluggish flow rate of angiographic dye from the main arteries" in individuals with chest discomfort in 1972 [1]. Since then, most studies have characterized coronary slow flow quantitatively by analyzing the Thrombolysis in Myocardial Infarction (TIMI) flow grade or corrected TIMI frame count (CTFC) [2]. The prevalence of coronary slow flow in CAG ranges from 1%

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to 5.5% [3]. Coronary slow flow has been linked to endothelial dysfunction. Endo-myocardial biopsies histological examinations have revealed signs of endothelial dysfunction and microvascular disease in the coronary slow flow scenario [3]. Endothelium-dependent flow-mediated vasodilation has been observed to be decreased in coronary slow- flow patients; however, nitroglycerin-induced endothelium-independent vasodilation was not altered in these people. In addition to arterial endothelial dysfunction, endothelium-dependent vasodilation is also weak in this group. In comparison to persons with normal coronary flow, these patients showed higher plasma levels of asymmetric dimethyl-arginine, endothelin-1, and homocysteine, and lower plasma levels of nitric oxide [4]. Furthermore, a link between microalbuminuria and endothelial dysfunction has been observed in the literature [5]. Albumin, which is a negative acute phase reactant, seems to decrease in this patient group due to renal excretion.

The end product of purine metabolism, serum uric acid, plays a significant role in the genesis and progression of coronary artery disease [6]. When comparing patients with myocardial infarction to healthy persons, the metabolism of amino acids, acyl-carnitines, and purines alters considerably [7]. Many epidemiologic studies have linked hyperuricemia to an increased risk of coronary heart disease, heart failure, and arrhythmias [8]. Elevated serum uric acid indicated the development of coronary artery calcification, and in patients with acute coronary syndrome, elevated serum uric acid was related to higher lipid content of coronary plaque [8]. Furthermore, several variables including age, gender, diet, and medical treatment might alter serum uric acid metabolism. Previous studies have found that having serum uric acid levels above 7 mg/dL increased cardiovascular mortality in patients aged 70 and more [9]. Previous research has found that serum uric acid levels were higher in individuals with systemic hypertension, diabetes, and acute myocardial infarction [10]. Additionally, serum uric acid was associated in earlier research with raised coronary artery calcium, enhanced platelet adhesiveness, and smooth muscle cell proliferation [11]. The fundamental process has not, however, been completely clarified. We evaluated the association between hyperuricemia, the serum uric acid/ albumin ratio, and coronary slow flow in the current study.

METHODS

Study Population

In our clinic, angiography was performed on 1984 patients between July 2019 and December 2022. The coronary slow flow was observed in 130 patients. After exclusion criteria, 105 patients with coronary slow flow who had previously been diagnosed with coronary ischemia by non-invasive methods were consecutively included in this study. 105 patients with slow coronary flow were included in group 1. In the control group, 100 patients were included in group 2. Patients with severe renal failure (creatinine > 2 mg/dL), active infection, acute coronary syndrome or patients with malignancy were excluded from the study. By examining our hospital's database, we were able to determine the fundamental clinical features of the patients. Throughout each patient's hospital stay, fasting blood samples were taken. Automatic equipment was used to measure biochemical values and do whole blood counts on the blood. Blood pressure over 140/90 mmHg or using an antihypertensive drug was considered to be hypertension. Fasting plasma glucose levels of 7.0 mmol/L (126 mg/dL), glycated hemoglobin A1c of 6.5%, or use of antidiabetic medications were considered to be indicators of diabetes mellitus. Being on lipid-lowering treatment or having a total cholesterol level over 220 mg/dL were considered to be symptoms of hyperlipidemia.

The study, which was conducted by the Helsinki Declaration, was approved by the local ethics committee (Date: 23.07.2023, Decision no: 2023.149.07.14).

Coronary Angiography

After receiving informed consent, a standard Judkins technique was used to perform coronary angiography through the right femoral artery with standard projections. Two independent cardiologists who were blinded to the patient information analyzed coronary angiograms. Significant stenosis was defined as 50% or more in at least one major coronary artery. Non-critical coronary artery stenosis was defined as less than 50%. Each patient's coronary frame count was determined using the Gibson *et al*. [12] described TIMI frame count computation. Since the left anterior descending coronary artery (LAD) length caused opacification to take a long time, the TFC value calculated for the LAD was divided by 1.7, and the CTFC was

calculated by multiplying the number of frames obtained for each vessel by 2, taking into account that angiographic recordings were taken in our clinic at a rate of 15 frames per second. The TIMI-3 flow threshold was established at 27 frame numbers. CTFC > 27 was seen as a sign of a malfunction of the microvascular perfusion, and CTFC < 27 was a sign of healthy microvascular perfusion. CTFC values of more than 27 are regarded as diagnostic for coronary slow flow [3].

Statistical Analysis

The statistical program SPSS 22.0 was used to conduct the statistical analysis (SPSS Inc, Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD) or median (minimum-maximum). Using chi-square or Fischer's exact tests, categorical variables were represented as percentages and compared. The Kolmogorov-Smirnov test was used to assess the normality of data distributions. For contin-

Table 1. Baseline characteristics of the groups

Variable	Group 1 (CSF (+)) (n = 105)	Group 2 (CSF (-)) (n = 100)	Total (n = 205)	*p value*
Age (years)	52 ± 9	50 ± 9	51.5 ± 9.5	0.24
Male	81 (77.1%)	78 (78%)	159 (77.5%)	0.82
Hyperlipidemia	45(42.9%)	35 (39.3%)	80 (41.2%)	0.61
Hypertension	39 (37.1%)	44 (45.8%)	83 (41.3%)	0.21
Smoking	37 (35.2%)	31 (32.3%)	68 (33.1%)	0.35
Diabetes mellitus	27 (25.7%)	35 (36.5%)	62 (30.8%)	0.09
Body mass index (kg/m^2)	27.4 ± 4.04	26.8 ± 4.85	26.4 ± 3.2	0.67
Pharmacological treatment				
Ca–channel blocker	12 (11.4%)	11 (11%)	23 (11.2%)	0.99
ACE-I	30 (28.6%)	24 (25.5%)	54 (27.1%)	0.63
Acetyl salicylic acid	38 (36.2%)	36(36%)	74 (36%)	0.96
Oral antidiabetic	9 (8.5%)	10 (10%)	19 (9.2%)	0.35
Statin	29 (27.6%)	27 (27%)	56 (27.3%)	0.95
Laboratory parameters				
Glucose (mg/dL)	115 ± 25	118.5 ± 30	131.7 ± 86.9	0.07
Creatinin (mg/dL)	0.86 ± 0.15	0.88 ± 0.59	0.87 ± 0.4	0.71
Uric acid (mg/dL)	5.44 (3.4-10.4)	2.28 (1.5-6.7)	2.88 (1-11)	**< 0.001**
Total cholesterol (mg/dL)	188.76 ± 40.2	195.63 ± 44.4	219.1 ± 30.6	0.44
Albumin (g/dL)	4.1 (3-5. 1)	4.9 (3.5-5.2)	4.3 (3-5. 2)	**0.02**
UAR	0.81 (0. 2-2.55)	0.49 (0.25-0.97)	0.67 (0. 2-2.55)	**< 0.001**
Angiographic measurements				
LAD TFC	40.3 ± 9. 9	19.6 ± 5. 8	30.4 ± 13. 2	**< 0.001**
LCX TFC	27.9 ± 6. 9	17.5 ± 4. 5	22.7 ± 8	**< 0.001**
RCA TFC	32.6 ± 9. 4	18.6 ± 6. 8	25.9 ± 10	**< 0.001**

CSF = Coronary slow flow; UAR = uric acid/ albumin ratio, TFC = Corrected TIMI frame count, LAD = Left anterior descending artery, LCX = Left circumflex artery, RCA = Right coronary artery

uously distributed data with a normal distribution, independent samples t-test was applied. The Mann-Whitney U test was used to examine non-normally distributed data. Receiver-operating characteristic analyses (ROC) were utilized to find the serum uric acid/ albumin ratio cut-off values for coronary slow flow prediction. Independent predictors of coronary slow flow were found using multivariate logistic regression analysis. The Spearman correlation test was used to analyze the relationship between the serum uric acid/albumin ratio and the coronary slow flow. Statistics were considered significant for p-values below 0.05.

RESULTS

The baseline characteristics and laboratory results of the patients are summarized in Table 1. The 205 patients' mean age was 51.5 ± 9.5 yrs, and 77.5% of them were male. There was no difference between the two groups in terms of medical treatment and baseline characteristics. The laboratory variables of the two groups were similar except for serum uric acid and the serum uric acid/ albumin ratio, which were higher in group 1 [serum uric acid 5.44 (3.4-10.4) and 2.28 (1.5-6.7), $p < 0.001$; serum uric acid/ albumin ratio 0.81 (0.2-2.55) and 0.49 (0.25-0.97), $p < 0.001$]. The result of the ROC analysis is as follows when the serum uric acid/albumin ratio cut-off value ≥ 0.57 is selected to estimate a coronary slow flow as follows: AUC 0.732; 95% confidence interval (CI) (0.660-0.805) with 68.3% sensitivity and 68.7% specificity (fig. 1). A moderately positive correlation between the serum uric acid/ albumin ratio and coronary slow flow was found using correlation analysis (r = 0.52, $p < 0.001$).

In multivariate logistic regression analysis, high uric acid levels, high serum uric acid/ albumin ratio, male gender were independent predictors of coronary slow flow (uric acid, odds ratio (OR): 2.22; 95% CI (1.551-3.200), $p < 0.001$; serum uric acid /albumin ratio OR: 37.7 95% CI (8.176-234.387), $p < 0.001$, male gender OR: 0.157; 95% CI (0.078-0.318), $p < 0.001$) (Table 2).

DISCUSSION

We investigated the relationship between the coronary slow flow and serum uric acid/ albumin ratio in this study. In our study, high uric acid levels, serum uric acid/ albumin ratio and male gender were independent predictors for coronary slow flow. We found high uric acid levels and serum uric acid/ albumin ratio in the

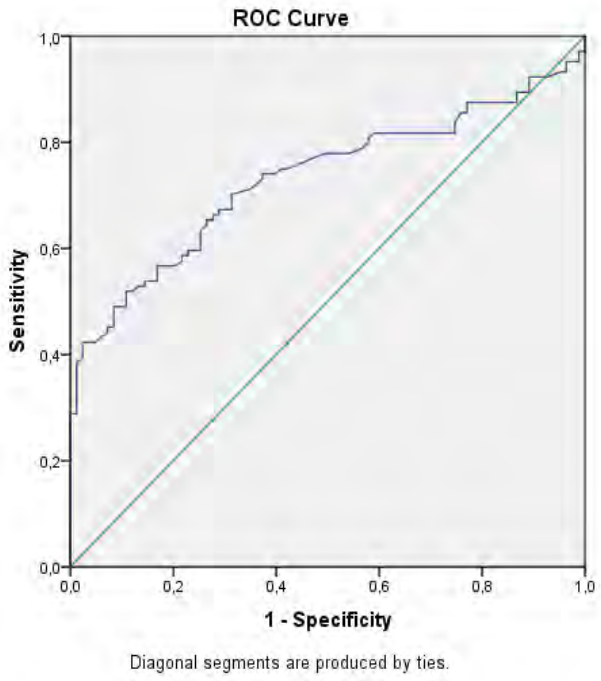

Fig. 1. ROC analysis of uric acid/albumin ratio for predicting coronary slow flow.

Table 2. Univariate and multivariate logistic regression analysis of the independent predictors of coronary slow flow

	Univariate analysis		Multivariate analysis	
Variables	**OR (95% CI)**	***p value***	**OR (95% CI)**	***p value***
UAR	41.4(9.745-176.348)	**< 0.001**	37.7(8.176-234.387)	**< 0.001**
Uric acid	2.451(1.752-3.427)	**< 0.001**	2.22 (1.551-3.200)	**< 0.001**
Age	1.017(0.988-1.048)	0.246	-	-
Gender(male)	0.148(0.079-0.276)	**< 0.001**	0.157(0.078-0.318)	**< 0.001**

UAR = uric acid/ albumin ratio

group with a coronary slow flow.

It has been noted in numerous earlier studies that people aged 70 years and older who have serum uric acid levels above 7 mg/dL have an increased risk of cardiovascular mortality [13]. When present in the acidic/ hydrophobic environment in the cytoplasm of cells or atherosclerotic plaques, serum uric acid transforms into a pro-oxidant substance and increases oxidative stress and through this mechanism, it causes cardiovascular and cerebrovascular diseases [14]. In previous studies, patients with systemic hypertension, diabetes mellitus, and acute myocardial infarction had elevated serum uric acid levels [15]. There was a gradual increase in the risk for acute myocardial infarction, stroke, or CHF over time in the Apolipoprotein MOrtality RISk (AMORIS) and the First National Health and Nutrition Examination Survey (NHANES I) studies [16, 17]. As uric acid permeates cell membranes, inflammation, and oxidation result. Additionally, it decreases the endothelium's production of nitric oxide and prevents cell division and migration. In addition, the expression of proinflammatory cytokines initiates the development of atherosclerosis as well as directly damaging vascular smooth muscle structures. Furthermore, high serum uric acid values and carotid intima-media thickness were found to be significantly correlated in a recent study with 15,843 (73.90% male) participants [18]. In earlier studies, serum uric acid was linked to elevated coronary artery calcium and ectasia, increased smooth muscle cell proliferation, and increased platelet adhesiveness [19]. High serum uric acid levels directly affect vascular smooth muscle, causing endothelial dysfunction and microvascular damage as well as an increase in reactive oxygen species and the activation of the renin-angiotensin system [20]. The underlying mechanism hasn't been fully explained, though. Uric acid is produced in the liver and is primarily eliminated by the kidneys. Kidney damage and cardiovascular disease have both been linked to hyperuricemia, according to studies [21]. An independent predictor of the development of microalbuminuria was baseline serum uric acid, according to a prospective cohort study with 1862 participants who did not have the condition [22]. Hyperuricemia can cause renal injury and microalbuminuria. Based on this, we decided to examine the serum uric acid/albumin ratio in patients with and without coronary slow flow, hoping that microalbuminuria may accompany as well as increased uric acid levels that may cause coronary slow flow. As a matter of fact, in our study, we found high uric acid levels and low albumin levels in the coronary slow flow group, which supports other studies.

Although the epicardial coronary arteries are open in patients with coronary slow flow, they apply to the emergency services due to recurrent chest pain. Although coronary slow flow is relatively benign, it impairs the quality of life. There are also life-threatening arrhythmias such as ventricular tachycardia, myocardial infarction, syncope, and sudden cardiac death in the literature due to coronary slow flow. Although this subgroup's reported mortality is less than 1%, certain individuals may have adverse long-term outcomes, such as myocardial infarction, significant perfusion abnormalities on scintigraphy, or prolonged QT interval on ECG [23]. Additionally, traditional antianginal medication based on guidelines is frequently ineffective for the long-term care of patients with coronary slow flow. Dipyridamole, which affects functional blockage in arteries with sizes under 200µm, was

demonstrated to normalize CTFC, however nitroglycerine, which dilates arteries with diameters more than 200µm, did not. Small coronary arteries under 400 µm have structural and functional abnormalities in the pathogenesis of coronary slow flow. Medial hypertrophy, endothelial swelling, and dysfunction are structural abnormalities, whereas increased resting coronary resistance response to vasodilator is a functional anomaly [24]. We demonstrated that elevated levels of uric acid and serum uric acid /albumin ratio may contribute to the pathogenesis of coronary slow flow in addition to earlier research.

Limitations

The fact that our study was done at a single facility with a small number of patients is its most significant drawback. More blood samples could be obtained from the patients included in our study. Another problem is that some patients were receiving diuretic-based antihypertensive therapy, which could have an impact on their serum uric acid levels. Urinalysis could be performed on patients for microalbuminuria. The results of this study need to be confirmed in larger, multi-center prospective studies.

CONCLUSION

According to our study results; we detected high serum uric acid and low albumin levels in patients with coronary slow flow, and higher serum uric acid/ albumin ratio may predict coronary slow flow.

Authors' Contribution

Study Conception: CA; Study Design: AD; Supervision: CA; Funding: AD; Materials: CA; Data Collection and/or Processing: AD; Statistical Analysis and/or Data Interpretation: CA; Literature Review: AD; Manuscript Preparation: CA and Critical Review: AD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Frequency of drug-associated hyperprolactinaemia: a single-center retrospective study

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ABSTRACT

Objectives: One of the causes of hyperprolactinaemia (HP) is drug-associated HP (DAHP). In this study, it was planned to investigate the frequency of DAHP.

Methods: In this study, a retrospective review of 296 individuals referred to the endocrinology outpatient clinic between June 2013 and March 2018 due to elevated prolactin (PRL) was performed.

Results: Of the 296 patients included in the study, 140 (47.3%) had HP (+), 80 (27.0%) had HP (-), 27 (9.1%) had DAHP and other causes (16.6%). The causes of DAHP were as follows; sulpiride in 7 (25.9%) patients, risperidone in 6 (22.2%), amisulpride in 4 (14.8%), domperidone in 3 (11.1%), haloperidol in 2 (7.4%), paliperidone, olanzapine, escitalopram, duloxetine and otilonium bromide in one patient each. PRL levels in the DAHP group were higher than in the HP (-) group (respectively; median 114.6 [interquartile range (IQR): 144.0], median 35 [IQR 37.3], $p < 0.001$). Patients with DAHP had an increased frequency of symptoms compared to the HP (-) group (oligomenorrhoea; 42.3%, 16.4%, $p = 0.007$, galactorrhoea; 53.8%, 30.1%, $p = 0.028$, respectively). PRL levels were higher and the frequency of clinical signs was higher in sulpiride than risperidone (PRL; median 195.0 [IQR 99.0], median 72.0 [IQR 57.9], $p = 0.022$, oligomenorrhoea; 100%, 20%, $p = 0.010$, respectively).

Conclusions: One of the 3 most common causes of patients referred for HP is DAHP and the most common cause of DAHP is anti-psychotic drugs. Sulpiride causes a higher rate of elevated PRL and frequency of clinical findings compared to other drugs.

Keywords: Hyperprolactinaemia, drug-associated hyperprolactinaemia, anti-psychotic drug

Prolactin (PRL) is a protein hormone produced from lactotroph cells in the pituitary (1). PRL release from lactotroph cells is under the control of negative and positive factors (1). The main factors that increase PRL production are suction, estrogen increase and stress (1). PRL production shows circadian production and is mainly under tonic control of inhibitory factors (1). The most important inhibitory factor is dopamine (DP) and it provides tonic control of PRL production via dopamine receptor 2 (DPR2) [1, 2]. The presence of sufficient amount of DP in the pituitary portal circulation suppresses production and pro-

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vides control of PRL synthesis (1, 2). This mechanism of action explains the effect of positive or negative dopaminergic effects of some drugs on PRL synthesis [3].

The most prominent drug group with anti-dopaminergic effects are anti-psychotic drugs (APD) and they constitute an important part of referrals due to hyperprolactinaemia (HP) [4-6]. Typical APD or 1st generation block DPR2 in all regions of the brain without any selectivity [7] and provide control of schizophrenic symptoms in this way [7]. On the other hand, 2nd generation or atypical APDs bind to serotonin 2 receptor (5HTR2) at a higher rate and DPR2 at a lower rate [7]. In addition, they show different effects in different regions of the brain (8). For example, 2nd generation APDs have a higher selectivity for the mesolimbic region than the striatal region [8]. Furthermore, while first generation neuroleptics have low affinity for DPR1, they show stronger antagonist effect against DPR2 [8]. All these mechanisms lead to the emergence of different effects of drugs on PRL.

On the other hand, increased PRL levels with APD cause drug-associated HP (DAHP). It is thought that increased PRL levels in DAHP disrupt the pulsatility of gonatotropins and lead to irregularities in menstrual cycles [9]. However, while this effect is observed in Caucasians, it is not observed in people of African descent [10], which leads to the questioning of the clinical significance of elevated PRL levels occurring in DAHP. The presence or absence of clinical findings in HP is one of the most important factors affecting the treatment approach [11, 12].

In this study, it was planned to determine the frequency of DAHP in patients referred with a prediagnosis of HP and to investigate whether there is an increase in the frequency of clinical findings in patients with DAHP.

METHODS

The study was conducted as a retrospective data analysis and individuals aged 18 years and over who were referred to the endocrinology, diabetes and metabolic diseases clinic of the Ministry of Health Çekirge State Hospital between June 2013 and March 2018 due to elevated PRL were included in the study.

The diagnosis and differential diagnosis of HP were made in accordance with the guidelines [11, 12].

Information about PRL, macro PRL (mPRL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr), thyroid stimulating hormone (TSH), age, oligomenorrhoea, galactorrhoea, hirsutism, headache, erectile dysfunction, pituitary magnetic resonance imaging (MRI) and drug use were obtained from patient files. PRL levels were analysed with the "Abbott ARCHITECT Prolactin Reagen B7K76T Kit system" between the specified dates, covering 90% of the expected normal range in women and the entire expected normal range in men; median 6.99 ng/ml, range 3.46-19.40 ng/ml in men, median 10.29 ng/mL, range 5.18-26.53 ng/ml in women. Those with prolactin levels above normal limits for gender were defined as HP (+) and those with prolactin levels within normal limits for gender were defined as HP (-).

The present study was approved by the ethics committee of the University of Health Sciences, Bursa Yüksek Ihtisas Training and Research Hospital (011-KAEK-25 2022/02-11). In light of the retrospective nature of the study, all procedures were performed as part of routine care. The researchers affirm that they adhered to the Declaration of Helsinki. If the manuscript is accepted, it will not be published elsewhere in the same form in English or any other language without your consent.

Statistical Analysis

IBM® Statistical Package for the Social Sciences (SPSS) statistics 20 (IBM® Corp., Armonk, NY, USA) was used to compare the data. After the normal distribution was determined, an independent samples t-test was applied to data with a normal distribution, and the Mann-Whitney U test was applied to compare the data that did not have a normal distribution. The Pearson's chi-squared test was used to compare ratios. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 296 patients (264 females and 32 males) were referred, the mean age of females was 37.0 ± 9.3 years and the mean age of males was 43.8 ± 13.6 years. Of the 296 patients included in the study, 140 (47.3%) had HP (+), 80 (27.0%) had HP (-), 27 (9.1%) had DAHP, 41 (13.9%) had mPRL-related HP and the remaining 8 (2. 7%) patients had HP due to other

Table 1. Demographic and laboratory characteristics of patients

	HP (+) (n = 140)	*p* value[a]	HP (-) (n = 80)	*p* value[b]	DAHP (+) (n = 27)	*p* value[c]
Age (year)	38.8 ± 10.6	**< 0.001**	34.4 ± 7.0	**< 0.001**	42.2 ± 9.6	0.08
Gender (F/M)	123/17	0.50	73/7	0.6	26/1	0.70
Cr (mg/dL)	0.73 ± 0.15	0.47	0.71 ± 0.15	0.41	0.76 ± 0.14	0.17
TSH (µIU/mL)	2.01 (IQR25-75:1.42)	0.16	2.10 (IQR25-75:1.00)	0.92	2.10 (IQR25-75:1.79)	0.35
AST (U/L)	19.9 (IQR 25-75:6.0)	0.6	17.7 (IQR25-75:7.8)	0.13	19.4 (IQR25-75:9.5)	0.98
ALT (U/L)	16.5 (IQR25-75:11.0)	**0.002**	16 (IQR25-75:7.8)	0.97	16.0 (IQR25-75:9.5)	0.78
rPRL(µg/L)	101.5 (IQR25-75:102.4)	**0.001**	50.6 (IQR25-75:25.4)	**< 0.001**	132.7 (IQR25-75:100.5)	**0.004**
PRL (µg/L)	133.2 (IQR25-75:91.5)	**0.001**	35 (IQR25-75:37.3)	**< 0.001**	114.6 (IQR25-75:144.0)	0.38

*Median and interquartile range (IQR) 25-75 as not normally distributed, HP = hyperprolactinemia, DAHP = drug-associated HP, F = female, M = male, TSH = thyroid stimulating hormone, AST = aspartate aminotransferase, ALT = alanine aminotransferase, PRL = prolactin, rPRL = referral PRL

[a]Comparison of HP (+) and HP (-) group,

[b]Comparison of HP (-) and DAHP (+) group,

[c]Comparison of HP (+) and DAHP (+) group

causes (acromegaly + prolactinoma in 1 patient, polycystic ovary syndrome (PCOS) in 2 patients, pregnancy-related HP in 2 patients, HP as a result of pituitary stalk incision due to pituitary operation in 2 patients and HP due to hypothyroidism in 1 patient).

Demographic and laboratory characteristics of the study patients are shown in Table 1. During the period when the study was designed, mPRL was not a routine test and was ordered in case of clinical inconsistency. Therefore, the number of patients with mPRL was 83,

Table 2. Distribution of symptoms and findings

	HP (+)	*p* value[a]	HP (-)	*p* value[b]	DAHP (+)	*p* value[c]
Female, n (%)						
Oligomenorrhea,	52/123 (42.3)	**< 0.001**	12/73 (16.4)	**0.007**	11/26 (42.3)	0.99
Galactorrhea	84/123 (68.3)	0.06	22/73 (30.1)	**0.028**	14/26 (53.8)	0.16
Hirsutism	25/123 (20.3)	**< 0.001**	19/73 (26.0)	**0.050**	2/26 (7.7)	0.13
Headache	6/123 (4.9)	0.36	0/70 (0)	0.019	2/26 (7.7)	0.57
Male, n (%)						
ED	13/16 (81.3)	**0.032**	2/6 (33.3)	0.69	0/1 (0)	0.63
Headache	1/16 (6.3)	0.53	1/7 (14.3)	0.50	0/6 (0)	0.80

HP = hyperprolactinemia, ED = erectile dysfunction, DAHP = drug-associated HP

[a]Comparison of HP (+) and HP (-) group,

[b]Comparison of HP (-) and DAHP group,

[c]Comparison of HP (+) and DAHP group

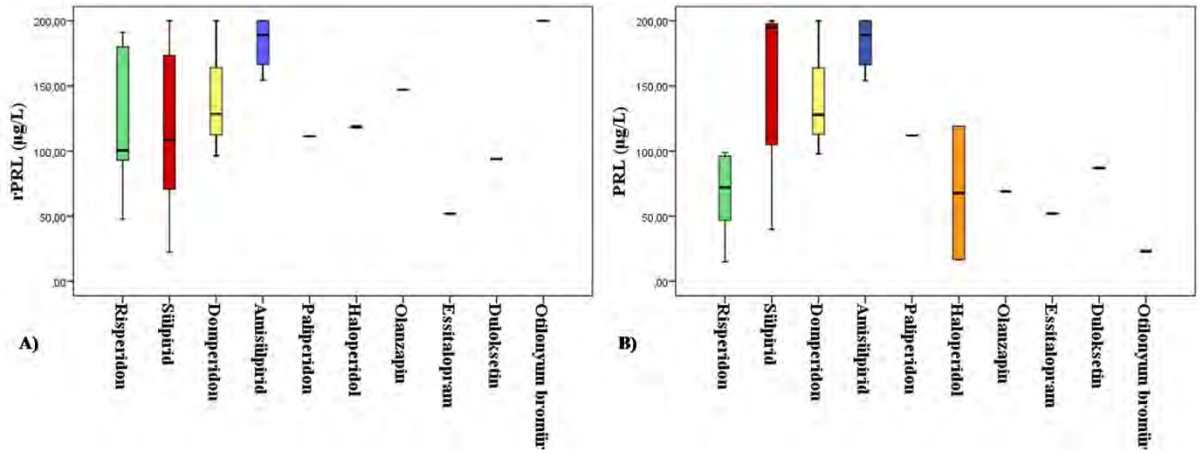

Fig. 1. rPRL (A) and admission PRL levels (B) according to etiological causes in drug-associated hyperprolactinemia. PRL = prolactin, rPRL = referral PRL.

the number of positive tests was 41, and the frequency of mPRL was 49.4% in ordered patiens group.

Clinical findings

The distribution of clinical findings according to the etiological causes of the patients in the study group is shown in Table 2. The frequency of symptoms increased in patients with DAHP compared to HP (-) group ($p = 0.028$).

Imaging data of 76 of 140 patients with HP (+) were available. Of the 76 patients whose imaging data were obtained, 65 were female and 11 were male. Of the 57 female patients with adenoma, 49 (75.4%) had microadenoma, 8 (12.3%) had macroadenoma, and 8 (12.3%)patients had no adenoma. Of the 11 male patients, 7 (63.6%) had microadenoma and 4 had macroadenoma (36.4%).

DAHP and Its Etiological Causes

The distribution of 27 patients with DAHP was as follows; sulpiride in 7 (25.9%), risperidone in 6 (22.2%), amisulpride in 4 (14.8%), domperidone in 3 (11.1%), haloperidol in 2 (7.4%), paliperidone, olanzapine, escitalopram, duloxetine and otilonium bromide in one patient each. 26 of 27 patients were female and 1 was male (Risperidone). The distribution of rPRL and PRL according to the etiological causes of DAHP is shown in the graph below (Fig. 1). While 21/27 (77.8%) of the patients had APD, the frequency of HP due to antidepressants was 7.4% (2/27) in the group with DAHP.

Age, rPRL and PRL levels of the three most common drugs are shown in Table 3. Amisulpiride, sulpiride, domperidone and haloperidol cause more severe HP compared to other agents.

Although all drugs increased PRL via dopamine in DAHP, the distribution of clinical findings was not the same (Table 4). Sulpiride causes more severe HP and the frequency of clinical findings is higher than risperidone ($p = 0.010$). Although more severe HP developed in domperidone users, the frequency of clinical findings was relatively less; two patients on haloperidol both had oligomenorrhoea and galactorrhoea (one of whom was postmenopausal), in dom-

Table 3. Characteristics of the three most common drugs causing DAH

	Age (years)	rPRL (µg/L)	PRL (µg/L)
Sülpirid (n = 7)	49.9 (IQR 25-75: 5.0)	108.6 (IQR 25-75: 160.0)	195.0 (IQR 25-75: 99.0)
Risperidon (n = 6)	38.0 (IQR 25-75: 12.8)	100.5 (IQR 25-75: 101.2)	72.0 (IQR 25-75: 57.9)
Amisülpirid (n = 4)	38.0 (IQR 25-75: 12.0)	189.3 (IQR 25-75: 39.4)	189.3 (IQR 25-75: 39.9)

Median and interquartile range (IQR) 25-75 as not normally distributed. DAHP = drug-associated hyperprolactinemia, PRL = prolactin, rPRL = referral PRL

Table 4. Distribution of clinical findings of the three most common drugs causing DAHP

	Oligomenorrhea	Galactorrhea
Sülpirid (n = 7)	7/7	4/7
Risperidon (n = 6)	1/5*	1/5*
Amisülpirid (n = 4)	2/4	3/4

DAHP = drug-associated hyperprolactinemia.
*In the risperidone group, there were 5 female patients and 1 male patient

peridone users; oligomenorrhoea was detected in 1/3 patients, that one patient with oligomenorrhoea was also postmenopausal, none of had galactorrhoea. Sulpiride, amisulpiride and haloperidol also cause severe HP and clinical findings are observed in most of the patients. Patients were premenopausal except for 2 patients using sulpiride (64 years) and domperidone (57 years).

DISCUSSION

It is observed that there is an increase in HP-related symptoms in DAHP compared to the HP-negative group. However, APDs causing DAHP have different mechanisms of action and clinical findings associated with HP at different frequencies.

In our study, DAHP constituted 9.1% of all admissions due to elevated PRL. In the PROLEARS study [13], which is one of the largest studies on the etiology of HP, the frequency of DAHP was found to be 45.9%. The study is a very large-scale investigation covering 20 years in Tayside, Scotland, where 400.000 inhabitants live and 1301 patients with HP were included [13]. The fact that it reflects community data is also an important aspect of the study. In the PROLEARS study, the frequency of DAHP was found to be considerably higher compared to our study. Possible reasons for this may be as follows; firstly, the frequency with which APDs are used may vary depending on the community concerned. and from region to region in the same community. In the literature review (PROSPERO) conducted by Junqueira *et al*. [14], in observational studies, APDs constituted a significant proportion of DAHP (90%), whereas antidepressants constituted 3%, prokinetic agents 5% and oral contraceptives 5%. In our study, APDs constituted 77.8% of DAHP, whereas antidepressants constituted 7.4%. As it is clearly seen in our study and previous studies, a significant proportion of DAHP are caused by APDs, whereas antidepressants constitute a very small proportion of DAHP (7.4%), although antidepressant use is very common in the in the community. In the study of Vilar *et al*. [15], the prevalence of DAHP was found to be 14.5% and the prevalence of prolactinoma was found to be 56.2%. In our study, the prevalence of DAHP was 7.4%, which is lower than that of Vilar *et al*. [15] and PROLEARS study [13]. The potential reason for this may be that the drugs were not questioned sufficiently. In our study, pituitary MRI imaging was requested from all patients who were thought to have HP, but MRI data of all patients could not be accessed. The prevalence of prolactinoma was 89.5%, and patients with HP constituted 47.3% of all presentations, so it can be assumed that the prevalence of prolactinoma was 42.3% in the whole study group. DAHP is one of the 3 most common causes of HP in clinical practice. Since mPRL was not requested from all patients, it is not possible to give a definite number in our study, but it is possible to say that it is more frequent than DAHP considering the positive rate in the requested patients.

DAHP consists of a very wide range of patient population including many different drug groups. Drugs in this group cause HP by different mechanisms. In general, APDs act by antagonising the effect of dopamine [16]. The anti-psychotic effect of APDs is by affecting DPR2 and DPR4 receptors in the mesolimbic area in the brain. Its effect on PRL secretion is again mediated by dopamine, blocking the effect of DPR2 receptors in the tuberoinfundubular area in the hypothalamus and removing the dopamine tonic inhibition on lactotrophs and thus PRL secretion increases [16]. DPR2 binding and selectivity of drugs show considerable variability [7, 17]. Old drugs, namely typical APD, phenothiazines (chlorpromazine, thioridazine, mesoridazine, trifluoperazine, fluphenazine, perphenazine), thioxanthenes (thiothixene), butyrophenones (haloperidol), and dibenzoxazepine (loxapine) are agents with very strong anti-psychotic effects [16]. Strong anti-psychotic effect also means strong anti-dopaminergic effect and strong HP-constructive effect [16, 17]. As their effects on DPR are different, DPR binding potentials in all regions of the

brain are not the same [16, 17]. New generation APDs such as clozapine, olanzapine, quetiapine, ziprasidone and aripiprazole act on DPR in different parts of the brain at different levels and lead to HP to a lesser extent [16-22]. In the study of Turrone *et al*. [20], it was found that olanzepine, an atypical APD, caused a slight increase in PRL levels, but this change was not significant. Since atypical APDs show anti-dopaminergic effect by transiently and mildly binding to DPR2, it is thought that the HP effect in these drugs is much milder and transient compared to typical APDs [16]. In a study conducted in individuals taking clozapine or olanzapine, PRL levels start to increase 2 to 4 hours after drug intake, the increase is generally limited to 1.5 to 2.5 times, and returns to baseline levels 8 hours after onset [20]. On the other hand, this effect starts at a similar time in Risperidone, but the effect of the drug lasts longer than 24 hours [209. However, this effect is not seen in 100% of patients, why does not PRL rise in all patients even though they are all taking the same drug? The answer to this question may be polymorphisms in the Taq1 A gene encoding DPR2 [23]. A decrease in DPR2 activity and number was found in individuals with A1 polymorphism, and APDs probably cause more HP in individuals with A1 polymorphism [23]. In general, HP caused by APDs returns to baseline levels 3-4 days after the drug is discontinued [16, 17]. In our study, PRL increase was higher in amisulpride, sulpiride and haloperidol compared to other drug groups, and mild HP was found in a patient using olanzepine. The findings were compatible with the general literature data.

As the HP-causing effects of APDs are different, there are also differences in the emergence of clinical findings [10]. In our study, oligomenorrhoea and galactorrhoea were found in 1 of 5 female patients using risperidone, whereas oligomenorrhoea was found in 7 of 7 patients and galactorrhoea was found in 4 of 7 patients using sülpirid, which increased PRL at similar levels. The number of patients in our study is not suitable for generalisation, the findings may be completely coincidental or the drugs may cause different frequencies of clinical findings at the same PRL levels. In the literature, oligomenorrhoea was found in 80% and galactorrhoea in 74% of the patients [14]. However, whether different drugs cause different clinical findings at the same PRL levels was not analysed in the studies [14]. Our study differs from the literature in this respect. This subject needs to be analysed and prospective and controlled studies in this field will contribute to a better understanding of the subject.

Limitations

Our study has some limitations. Firstly, since the study was conducted in retrospective design, there were problems in accessing some data. Again, it is likely that the same standard was not applied in the questioning of drugs, which are the most common causes of HP, and this attitude may have caused some unintentional errors to occur.

CONCLUSION

In our study, it was revealed that drugs were one of the three most common causes of HP and APDs was the most common cause of DAHP. It was observed that the frequency of clinical findings was higher in the DAHP group compared to the HP negative group. There is a strong suspicion that the effects of different drug groups on the frequency of clinical findings at similar PRL levels are also different, but further studies are needed in this regard.

Authors' Contribution

Study Conception: MG; Study Design: EG, MG; Supervision: MG; Funding: MG, EG; Materials: MG; Data Collection and/or Processing: EG, MG; Statistical Analysis and/or Data Interpretation: MG, EG; Literature Review: MG, EG; Manuscript Preparation: EG and Critical Review: MG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The effect of adenoidectomy on pulmonary function in children: prospective controlled study

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ABSTRACT

Objectives: Adenotonsillar hypertrophy (AH) is a prevalent condition in children that can cause significant complications if left untreated. In this study, we investigated the impact of adenoidectomy on pulmonary function tests (PFTs) and explored the relationship between spirometric parameters in affected children. By evaluating these factors, we can better understand the post-surgical outcomes and the potential benefits of surgical intervention.

Methods: The present study utilized a prospective controlled design to conduct a before and after clinical trial involving 23 children diagnosed with upper airway obstruction resulting from AH. Five specific spirometric parameters were selected to evaluate pulmonary function before and 1-3 months following the adenoidectomy procedure. Additionally, adenoid grade scores and gender differences were recorded for each patient to assess their effect on the lung.

Results: Peak expiratory flow (PEF) ($p = 0.002$), the first second of expiration (FEV1) ($p < 0.001$), and the ratio of FEV1/FVC ($p = 0.001$) significantly increased postoperatively. However, no significant correlations were found between the forced vital capacity (FVC) ($p = 0.39$) and mid-expiratory forced expiratory flow (FEF25-75) ($p = 0.2$). Rising of the FVC, PEF, FEV1, and FEV1/FVC was observed in AH grade III patients compared to AH grade IV patients following the surgical intervention, in comparison to the preoperative baseline, especially statistical significance was FEV ($p = 0.047$), indicating a noteworthy change in lung function.

Conclusions: These findings emphasize the beneficial effects of adenoidectomy on PFTs and highlight that adenoidectomy positively affects the upper and lower airways.

Keywords: Adenotonsillar hypertrophy, pulmonary function tests, adenoidectomy, spirometric parameters, upper airway obstruction

Adenoid hypertrophy (AH) is a common condition characterized by the enlargement of the adenoid tissues. This condition primarily affects children and can result in upper airway obstruction, leading to a range of clinical manifestations and potential systemic effects [1-5]. AH can obstruct the nasal passage and impede the smooth flow of air through the upper respiratory tract. Upper airway obstruction caused by AH

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can significantly impact pulmonary and cardiovascular function in children, leading to symptoms such as nasal congestion, mouth breathing, snoring, and disturbed sleep patterns [6, 7]. Impaired oxygen exchange and increased carbon dioxide retention may contribute to hypoxemia, hypercarbia, and respiratory acidosis [8-10].

The cardiovascular system can also be affected by AH-related upper airway obstruction. The compromised airflow and increased respiratory effort in affected children impose an additional workload on the heart. This increased cardiac demand can lead to heart rate, blood pressure, and cardiac output changes. Over time, these alterations may contribute to cardiovascular complications such as hypertension, right ventricular dysfunction, and cardiac arrhythmias [2, 11-15].

Understanding the complex interactions between AH, upper airway obstruction, and the various physiological systems involved is crucial for appropriate diagnosis and management. Timely recognition of AH allows for targeted interventions, such as adenoidectomy, to alleviate upper airway obstruction and improve respiratory function, cardiovascular health, and overall well-being in affected children [6, 16, 17].

Spirometry, a widely utilized technique, is a non-invasive method that assesses lung function by measuring volumes above the residual volume and flow volume rates. This study obtained spirometric values using a spirometer (P.K. Morgan Ltd., UK). Spirometry is a valuable tool for evaluating lung function and diagnosing various respiratory conditions in children [18, 19]. Spirometry is recommended in several clinical scenarios for children with symptoms suggestive of obstructive airway disease, such as recurrent wheezing, coughing, or shortness of breath. It aids in assessing airway obstruction and can assist in distinguishing between asthma, chronic bronchitis, or other respiratory disorders [20, 2].

Additionally, spirometry helps monitor disease progression and response to treatment in children with known respiratory conditions. Regular spirometric measurements can provide objective data on lung function over time, allowing healthcare professionals to assess the effectiveness of interventions and adjust treatment plans accordingly. Spirometry can be valuable in preoperative evaluations for children undergoing surgical procedures that may impact lung function, such as adenoidectomy or tonsillectomy. Baseline spirometric measurements provide a reference point for postoperative comparisons and can help identify any changes in pulmonary function following the procedure [18-21]. In this study, our primary objective is to investigate the effects of adenoidectomy on PFTs in children with AH.

METHODS

Patients

From October 2016 to January 2020, in a university hospital, we conducted a prospective trial enrolling a total of 23 children (8 boys and 15 girls) between the ages of 4 and 16. They were scheduled for adenoidectomy due to adenoid hypertrophy. The study received approval from the institutional review board (IRB), and written informed consent was obtained from the parents of the participating children, following the guidelines outlined in the Declaration of Helsinki.

All patients underwent a comprehensive evaluation, including detailed medical history, clinical examination, and laboratory tests. Patients with grade 3 (adenoid obstructions 51% to 75% of posterior choana) and grade 4 (adenoid obstructions 76% to 100% of posterior choana) were included in the study and scheduled for adenoidectomy. Patients with chronic adenoiditis and tonsillitis and who have cardiopulmonary diseases, neurological involvement, obesity (body mass index [BMI] > 30 kg/m^2), other causes of nasal obstruction such as polyps, nasal septal deviations, thoracic skeletal deformity, hypertrophic tonsils (grade 2 or 3), subglottic or secondary airway stenosis resulting from previous surgeries, cleft palate, and children unable to perform PFTs were excluded.

During the study, we recorded several parameters related to pulmonary function, including vital capacity (VC), forced vital capacity (FVC), forced expiratory volume during the first second of expiration (FEV1), the ratio of FEV1 to FVC (FEV1/FVC), and mid-expiratory forced expiratory flow (FEF25-75). PFTs were performed one week before the surgery and continued until 1-3 months after the procedure, allowing us to assess the changes in pulmonary function over the postoperative period.

Spirometry

Spirometry is a fundamental tool for diagnosing and monitoring lung function in children. It plays a critical role in assessing respiratory abnormalities, determining the severity of respiratory diseases, and guiding clinical decision-making. By conducting PFTs through Spirometry, we focused specifically on flow rates in accordance with the guidelines established by the American Thoracic Society (ATS) [19-22]. During the spirometry tests, the patients were comfortably seated, with their noses clipped, and there were no restrictions on chest expansion, such as tight clothing or orthodontic braces. To ensure accurate results, the patients were encouraged to perform the test up to five times, and the highest recorded value was considered for analysis. The spirometry values were then compared to age, gender, and height-specific reference values, expressed as a percentage ratio. A minimum of three adequate measurements were recorded to ensure accuracy, following the reference values provided by Knudson *et al*. All measurements were completed within a maximum 2-minute interval [23]. The same trained operator performed all the respiratory tests throughout the study to maintain consistency and reliability. The operator provided consistent support and encouragement to the children during the testing process, ensuring their comfort and cooperation.

Statistical Analysis

In this study, descriptive statistics were used for continuous variables, and frequency analysis was used for categorical variables. Whether the variables fit the normal distribution was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. The dependent sample t-test was applied for the variables obtained from the dependent sample groups with normal distribution, and the Wilcoxon test was applied for the variables obtained from the dependent sample groups without the normal distribution. Independent sample t-test and Mann-Whitney U test were used to determine the differences between independent groups for the normal and non-normally distributed variables. The significance level was accepted as $p < 0.05$. Statistical analyses were made in the IBM SPSS 26 program.

RESULTS

The study enrolled a total of 23 children, consisting of 15 females and 8 males, with an age range between 5 and 16 years and a mean age of 9,43 ± 3,01 years. All participants demonstrated nasopharyngeal obstruction, evidenced by an adenoid-nasopharyngeal (A/N) ratio exceeding 0.85. Seven participants (30.4%) had grade III enlarged tonsils, while the remaining 16 cases

Table 1. Pulmonary function measures of all patients

Descriptive Statistics						Test Statistics[a]	
		Mean	SD	Min.	Max.	Z	Asymp. Prob
FVC	pre-operation	1.549	0.490	0.690	2.470	-0.853[b]	0.393
	post-operation	1.729	0.627	0.770	3.040		
FEV_1	post-operation	1.263	0.521	0.340	1.920	-3.681[b]	**< 0.001**
	post-operation	1.636	0.551	0.760	2.580		
FEV_1/FVC	pre-operation	80.024	19.519	30.631	105.682	-3.406[b]	**0.001**
	post-operation	96.277	14.456	80.142	146.269		
PEF	pre-operation	1.873	0.835	0.340	3.180	-3.073[b]	**0.002**
	post-operation	2.602	1.122	1.310	5.520		
FEF_{25-75}	pre-operation	3.516	7.281	0.000	27.000	-1.278[b]	0.201
	post-operation	1.766	0.560	1.080	3.020		

FVC = forced vital capacity, FEV_1 = first second expiration, PEF = peak expiratory flow, FEF_{25-75} = mid-expiratory forced expiratory flow

[a]Wilcoxon Signed Ranks Test

[b]Based on negative ranks

(69.4%) had grade IV.

Table 1 illustrates the improvements observed in various pulmonary function measures, including FVC, PEF, FEV1, FEV1/FVC, and FEF25-75, during the period of 1-3 months following the surgery in comparison to the preoperative measurements. The Wilcoxon Signed Ranks tests indicated significant improvements in three parameters: PEF, FEV1, and FEV1/FVC ($p < 0.05$).

Significant spirometric improvements followed adenoidectomy. PEF increased from 1.87 ± 0.84 preoperatively to 2.60 ± 1.12 postoperatively ($p = 0.002$), FEV1 increased from 1.26 ± 0.52 to 1.63 ± 0.55 ($p < 0.001$), and FEV1/FVC increased from 80.02 ± 19.51 to 96.28 ± 14.46 ($p = 0.001$). However, no significant correlations were found between FVC, FEF25-75, both pre and post-op ($p > 0.05$). FVC increased from 1.55 ± 0.49% pre-op to 1.73 ± 0.63% post-op ($p = 0.39$), while FEF25-75 improved, decreasing from 3.52 ± 7.28% to 1.76 ± 0.55% ($p = 0.2$). Differences in post/pre-PFTs were more pronounced in women but not statistically significant (Table 2).

The study revealed that among the variables measured, including FVC, PEF, FEV1, and the FEV1/FVC,

Table 2. Statistical Analysis of Pulmonary Functional Tests Based on Gender Differences

Descriptive Statistics						**Test Statistics[a]**		
		Mean	**SD**	**Min.**	**Max.**	**Z**	**Asymp. Prob**	**Exact Sig. [2*(1-tailed Sig.)]**
Dif. FVC Post-Pre operation	F	0.309	0.067	-0.09	1.94	-1.56	0.120	0.131[b]
	M	-0.006	0.29	- 0.43	0.44			
Dif. FEV_1 Post-Pre operation	F	0.52	0.72	- 0.13	2.24	-2.13	**0.033**	**0.034[b]**
	M	0.097	0.12	- 0.15	0.24			
Dif. FEV_1/FVC Post-Pre operation	F	19.11	18.39	-8.99	53.96	*Independent Samples Test		
	M	10.89	17.12	-19.15	40.59			
Dif. PEF Post-Pre operation	F	0.92	1.76	-0.27	5.18	-0.097	0.92	0.93[b]
	M	0.37	0.55	-0.20	1.00			
Dif. FEF_{25-75} Post-Pre operation	F	-2.78	9.06	-25.92	3.00	-0.06	0.95	0.98[b]
	M	0.12	0.39	-0.49	0.57			
*Independent Samples Test								
					t-test for Equality of Means			
Dif.FEV_1/FVC		t	Df	Prob	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
Post-Pre operation (F, M) (Equal variances assumed)		1.05	21	0.308	8.23	7.87	Lower	Upper
							-8.14	24.59

FVC = forced vital capacity, FEV_1 = first second expiration, PEF = peak expiratory flow, FEF_{25-75} = mid-expiratory forced expiratory flow, F = female, M = male

[a]Grouping variable: gender

[b]Based on negative ranks

there was a more significant increase in patients with AH grade III compared to those with AH grade IV following the surgical intervention when compared to the preoperative baseline. However, the variable FEF25-75 did not show significant differences (Table 3).

In Fig. 1, the spirometry results illustrate the variation in lung capacity before (B) and after (C) surgery. The normal airflow during nasopharyngeal breathing is provided, indicating the typical breathing time of a child with adenoid hypertrophy (D) after undergoing surgery (E). Adenoid hypertrophy hinders or obstructs the airflow passage through the nasopharynx, leading to reduced or complete blockage (Fig. 1).

DISCUSSION

The relationship between AH grade and PFTs has been a subject of interest in understanding the impact of adenoid enlargement on respiratory function. AH is characterized by the excessive growth of adenoid tissues in the upper airway, leading to varying degrees of airway obstruction. This obstruction can disrupt normal airflow and potentially affect lung function parameters measured by PFTs [23-25]. In our study, we found that adenoidectomy has a positive effect on pulmonary functions, and after surgery, there were significant improvements in three spirometric parameters:

Table 3. Statistical Analysis of Pulmonary Functional Tests Parameters in Relation to Adenoid Hypertrophy Grades

		Descriptive Statistics				Test Statistics[a]		
		Mean	SD	Min.	Max.	Z	Asymp. Prob	Exact Sig. [2*(1-tailed Sig.)]
Dif. FVC Post-Pre operation	Grade III	0.32	0.72	-0.9	1.94	-0.91	0.37	0.376[b]
	Grade IV	0.12	0.53	-0.43	1.93			
Dif. FEV_1 Post-Pre operation	Grade III	0.60	0.74	0.7	2.24	-2.01	**0.05**	**0.047[b]**
	Grade IV	0.27	0.54	-0.15	2.23			
Dif. FEV_1/FVC Post-Pre operation	Grade III	25.06	14.12	9.81	53.96	*Independent Samples Test		
	Grade IV	12.40	18.56	-19.56	53.91			
Dif. PEF Post-Pre operation	Grade III	1.13	1.83	-0.14	5.18	-1.07	0.28	0.308[b]
	Grade IV	0.55	1.30	-0.27	5.18			
Dif. FEF_{25-75} Post-Pre operation	Grade III	-2.99	10.15	-25.92	2.75	-1.10	0.27	0.278[b]
	Grade IV	-1.21	6.13	-23.98	3.00			

*Independent Samples Test

	t-test for Equality of Means						
Dif.FEV_1/FVC Post-Pre operation	t	df	Prob	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
	1.60	21	0.12	12.66	7.89	Lower	Upper
						-3.74	29.06

FVC = forced vital capacity, FEV_1 = first second expiration, PEF = peak expiratory flow, FEF_{25-75} = mid-expiratory forced expiratory flow

[a]Grouping variable: AH grade

[b]Not corrected for ties

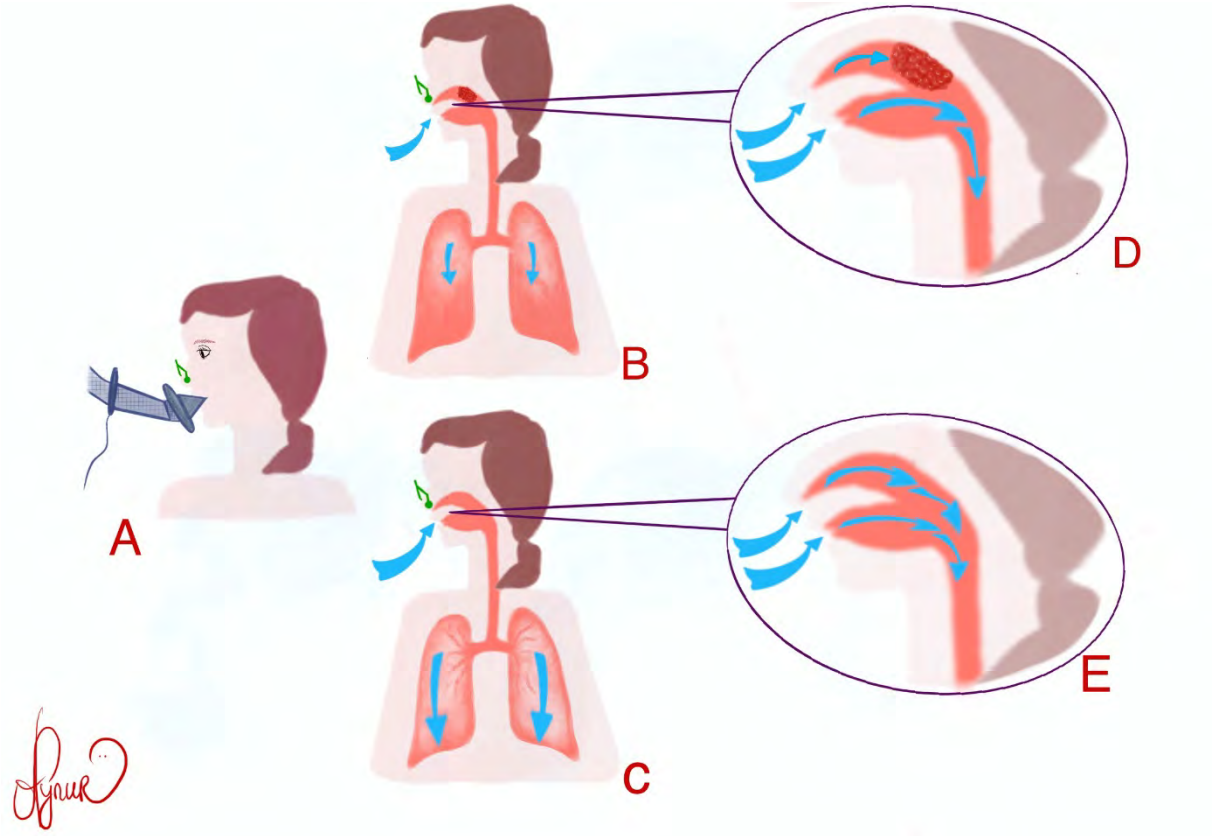

Fig. 1. Nasopharyngeal airflow in a child with adenoid hypertrophy and after adenoidectomy. A: The spirometry procedure in a child, B: Spirometry in a child with adenoid hypertrophy, C: Spirometry after the adenoidectomy, D: Airflow from the nasopharynx in a child with adenoid hypertrophy, and E: Airflow from the nasopharynx in a child after the adenoidectomy.

PEF, FEV1, and FEV1/FVC ($p < 0.05$).

AH notably impacts various systems, including the respiratory, cardiovascular, neurological, and other associated systems. Enlarging the adenoid tissue in the upper airway can give rise to patterns, leading to significant physiological disturbances and complications.

AH obstructs the nasal passages, causing airflow restriction during inhalation and exhalation. This obstruction results in mouth breathing as a compensatory mechanism, altering the normal breathing pattern. The restricted airflow can lead to increased respiratory effort, reduced lung volumes, and impaired gas exchange. Children with AH often experience symptoms such as snoring, sleep-disordered breathing, and recurrent respiratory infections. The compromised airflow can also disrupt sleepiness and reduce quality of life [3, 25-28].

Rogha *et al*. [28] found a significant increase in FVC after adenotonsillectomy. Removal of adenoids can alleviate upper airway obstruction, allowing for improved air movement in and out of the lungs and subsequently increasing FVC. Although the FVC parameter is not statistically significant ($p = 0.393$), our study observed a mathematical difference in the FVC parameters before and after the surgery. Initial findings hint at a potential positive link between adenoidectomy and FVC, but more data is needed for statistical significance.

FEV1 measures the air volume forcibly exhaled during forced expiration in one second. Adenoidectomy can contribute to an improvement in FEV1 by reducing airway resistance and facilitating smoother airflow. This can result in a greater volume of air expelled from the lungs within the first second of exhalation [1, 20, 23-26]. Our study observed a significant increase in FEV1, pre-op it was 1.26% ± 0.52%, post-op averaged 1.636% ± 0.551 ($p < 0.001$), showing adenoidectomy's impact on one-second forced exhalation.

FEV1/FVC ratio assesses airway function. A lower

ratio suggests obstruction or airflow restriction, higher in healthy cases. Our study also detected a significant increase in FEV1/FVC (Preoperative: 80.02 ± 19.52; postoperative: 96.28 ± 14.46) ($p = 0.001$).

PEF measures the maximum flow of air during forced expiration. Improvement in PEF signifies better expiratory flow and respiratory efficiency [10, 28]. PEF increased significantly from 1.87% ± 0.84% preoperation to 2.60% ± 1.12% post-operation ($p = 0.003$), aligning with literature. After adenoidectomy, the reduction in upper airway obstruction allows for more efficient airflow, leading to an increased PEF.

FEF25-75, a Spirometry measure of mid-exhalation airflow, reflects lung function in small and intermediate airways. In our study, FEF25-75 was 3.52% ± 7.28% preoperative and 1.76% ± 0.55% postoperative, without significant change ($p = 0.2$).

Gender-related PFT differences linked to adenoidectomy yield insights, but findings are inconclusive [25, 29, 30]. Our study found female's post-surgery PFTs increased compared to male's, lacking statistical significance (FVC; $p = 0.120$, FEV1; $p = 0.033$, FEV1/FVC; $p = 0.38$, PEF; $p = 0.92$, and FEF25-75; $p = 0.95$).

Our thorough spirometry provides reliable data, enhancing understanding of adenoidectomy's impact on pulmonary function in adenotonsillar hypertrophy children. Assessment of the presence and degree of reversibility of airflow obstruction is clinically significant in patients with asthma or chronic obstructive pulmonary disease. The measurement of PEF and FEV1 is a valuable method to assess the severity of obstruction and its degree of reversibility [31]. Our study showed that positive reversible changes can be observed in the lower respiratory tract after adenoidectomy.

Limitations

We only evaluated the short-term effects of adenoidectomy on pulmonary function tests. However, it will help provide valuable insights into the durability of the improvements and potential future complications that may arise over time. The study focused on five parameters of spirometry to evaluate pulmonary function. For future research, we recommended including other relevant parameters, such as lung volumes or airway resistance. Conducting multicenter studies involving a larger and more diverse population to improve the generalizability of the findings. Involving multiple centers would allow for a broader representation of different patient demographics, which could enhance the applicability of the results to a wider range of populations. Assessing parameters such as sleep quality, daytime functioning, academic performance, and behavioral outcomes can provide a more holistic understanding of the benefits of surgical intervention beyond the objective measurements of pulmonary function.

Highlights of this study

(1) Significant improvements in spirometric parameters: The study demonstrated significant improvements in spirometric parameters, including forced vital capacity (FVC), peak expiratory flow (PEF), forced expiratory volume during the first second of expiration (FEV1), and the ratio of FEV1 to FVC (FEV1/FVC), following surgical intervention for adenoid hypertrophy.

(2) Variation in improvement based on AH grade: The study observed a more substantial increase in spirometric parameters among patients with AH grade III compared to those with grade IV, indicating that the severity of AH may influence the extent of improvement in lung function following surgery.

(3) Limited impact on mid-expiratory forced expiratory flow (FEF25-75): The study found that the mid-expiratory forced expiratory flow (FEF25-75) did not show significant changes after the surgical intervention. This suggests that while other spirometric parameters improved, the FEF25-75 may be less influenced by adenoidectomy.

(4) Statistically significant improvement in FEV1: Among the spirometric variables, the FEV1 exhibited statistically significant improvement after surgery. This finding indicates a notable enhancement in the air volume forcefully exhaled during the first second of expiration, reflecting improved lung function.

(5) Clinical relevance and impact: The significant improvements observed in spirometric parameters following adenoidectomy highlight the clinical relevance of the surgical intervention in improving respiratory function. These findings can contribute to better respiratory care and enhanced overall respiratory health outcomes for patients with adenoid hypertrophy.

CONCLUSION

Compared with preoperative measurements, our study showed improvement in postoperative pulmonary function measurements (PEF, FEV1, FEV1/FVC). Gender differences were noted, more pronounced in females but not statistically significant. Grade III adenoid hypertrophy patients showed more remarkable postoperative improvement in lung function than grade IV patients, significantly in FEV1. FEV1 and PEF measurements are of clinical importance in guiding the reversibility of the lower airway. We observed positive reversible changes in the lower respiratory tract after adenoidectomy.

Authors' Contribution

Study Conception: OYA, AA; Study Design: OYA; Supervision: SK; Funding: N/A; Materials: N/A; Data Collection and Processing: OYA, AA; Statistical Analysis and Data Interpretation: OYA, AA; Literature Review: OYA; Manuscript Preparation: AA, OYA, and Critical Review: AA, OYA, SK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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Prognostic factors in atypical carcinoid tumors

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ABSTRACT

Objectives: Carcinoid tumors are rare neuroendocrine neoplasms of the lung. Although typical and atypical carcinoids have different clinical courses, most studies in the literature evaluate them together. Therefore, we aimed to investigate prognostic factors in patients with atypical carcinoids, excluding typical carcinoids.

Methods: We included 32 patients with atypical carcinoids according to WHO 2021 criteria admitted to Uludag University Hospital. We retrospectively extracted the clinicopathological characteristics from electronic medical records. The log-rank tests were used to determine the prognostic factors on survival.

Results: Median age was 57 (24-71) years. Pathological stages were as follows: stage I in 41%, II in 9%, III in 34%, and IV in 16%. Median Ki-67 index was 11% (1-50). Median follow-up time was 46.2 (0.7-184.2) months. 12-month and 48-month disease-free survival (DFS) rates were 92.3% and 79.2%, respectively. 12-month and 48-month overall survival (OS) rates were 93.8% and 86.2, respectively. Receiver operating characteristic curve analysis determined the Ki-67 cut-off as 12.5%. The log-rank test indicated that Ki-67 and stage were statistically significant prognostic factors for DFS and OS. The patients with a Ki-67 index lower than 12.5% had longer DFS and OS ($p = 0.007$ and $p = 0.020$, respectively).

Conclusions: The Ki-67 index and 8th TNM staging have prognostic value on DFS and OS in patients with atypical carcinoids. Large-scale studies are needed to define the optimal cut-off value of Ki-67.

Keywords: Lung carcinoid, atypical carcinoid, ki-67 index, stage, survival

Carcinoid tumors are a component of a heterogeneous group of pulmonary neuroendocrine neoplasms [1]. Although twenty percent of neuroendocrine tumors of all body sites are carcinoid and their incidence is reported to be increasing, carcinoid tumors constitute less than 2% of all lung malignancies [2, 3]. The World Health Organization has classified carcinoid tumors into two histological types according to histopathological criteria: typical (well-differentiated, low grade) and atypical carcinoid tumors (well-differentiated, intermediate grade) [4]. Atypical carcinoid tumors constitute approximately one-quarter of lung carcinoids [5].

Carcinoid tumors have better survival than other neuroendocrine neoplasms of the lung, small-cell lung cancer, and large-cell neuroendocrine carcinoma [6].

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Histological type is the most crucial factor in determining survival. In addendum to histological type, many factors affecting the survival of carcinoid tumors, such as stage, Ki-67 index, gender, tumor size, lymph node metastasis, and treatment modalities, were investigated [7-12]. In most of these studies, patients with typical and atypical carcinoid tumors were evaluated together. However, the survival, recurrence sites, and rates of typical and atypical carcinoid tumors were reported to differ [13-16]. Therefore, in the present study, we aimed to investigate the clinicopathological factors affecting the survival of patients with atypical.

METHODS

We included the patients admitted to Bursa Uludag University Hospital between January 2007 and December 2021 for atypical carcinoid tumors according to the criteria determined in the WHO 2021 classification of lung tumors [4]. We retrospectively reviewed the electronic medical records and extracted the demographic and clinical features of all the participants: age, gender, imaging modalities, tumor side, tumor localization, clinical manifestation, clinical stage, treatment modalities, site of recurrences, and areas of metastasis. We obtained histopathological features from the patients' pathology reports, including pathological T and N stage, tumor size, lymphovascular invasion, Ki-67 index, mitotic count, and necrosis. We excluded patients younger than 18 and those with a history of other sites' malignancy and incomplete clinicopathological data.

Ethical Statement

The study was per the 1964 Declaration of Helsinki and approved by the clinical research ethics committee of Bursa Uludag University Faculty of Medicine (Approval number: 2020-19/19).

Statistical Analysis

We defined overall survival (OS) as the time from diagnosis until death from any cause. Disease-free survival (DFS) was specified to the time from surgery until disease recurrence, confirmed by histological examination or imaging modalities, or death for any reason, whichever occurred first. The optimal cut-off points for the Ki-67 index and tumor size were deter-

Table 1. Clinicopathological characteristics of the patients

	Data (n = 32)
Age (years)	57.0 (24.6-71.3)
Gender	
Female	17 (53.1)
Male	15 (46.9)
Symptoms at diagnosis	
Cough	15 (46.9)
Dyspnea	7 (21.9)
Hemoptysis	3 (9.4)
Carcinoid Syndrome	2 (6.3)
Absent (asymptomatic)	5 (15.6)
Imaging at diagnosis	
Computed tomography	32 (100.0)
FDG PET/CT	21 (65.6)
Ga68-Dotatate PET/CT	9 (28.1)
Tumor side	
Right	18 (56.3)
Left	14 (43.7)
Localization	
Central	18 (56.3)
Peripheral	14 (43.7)
Surgery	
Lobectomy	21 (65.6)
Sublobar Resection	6 (18.8)
None	5 (15.6)
Stage at diagnosis	
I	13 (40.6)
II	3 (9.4)
III	11 (34.4)
IV	5 (15.6)
Tumor size (mm)	31 (5-75)
Ki-67 index (%)	11 (1-50)
Necrosis	
Absent	19 (59.4)
Present	13 (40.6)
Mitosis	
< 2	8 (25.0)
2-10	24 (75.0)
(Neo)Adjuvant Therapy	
Chemotherapy	11 (34.4)
Radiotherapy	6 (18.8)

Data are show as median (minimum-maximum or number (percent). FDG PET/CT = fluorodeoxyglucose positron emission tomography-computed tomography, Ga68-Dotatate PET/CT = Gallium-68 Dotatate positron emission tomography-computed tomography

mined using receiver operating characteristic (ROC) curve analysis, taking the DFS event as the endpoint of interest. Statistical analyses were performed using IBM SPSS version 28 software. Continuous and categorical variables were represented as median (minimum-maximum) and frequency, respectively. Kaplan–Meier analysis was operated for survival rates. The log-rank tests were used to compare the patient groups. Statistical significance was indicated by *p* - values less than 0.05.

RESULTS

Our study comprised thirty-two patients. Table 1 displays the clinicopathological characteristics of the patients included. The median age was 57.0 (24.6-71.3) years. Nearly half of the patients had a cough at presentation. Carcinoid syndrome was observed in only two patients. All patients had computed tomography (CT) scans during evaluation, and Ga68-Dotatate positron emission tomography-computed tomography (PET/CT) was performed in 28% of them at the initial diagnosis. Eighteen patients had centrally located tumors. Of the patients, 84% had nonmetastatic disease at presentation. Pathological stages were as follows: stage I in 41%, II in 9%, III in 34%, and IV in 16%. The median tumor size was 31 (5-75) mm, and the median Ki-67 index was 11% (1-50). Necrosis was present in 41% of the patients. (Neo)Adjuvant chemotherapy and radiotherapy were performed in 43% and 19%, respectively. All patients received a somatostatin analog and cytotoxic chemotherapy in the metastatic stage.

In ROC curve analysis, the cut-off values for the Ki-67 index and tumor size were determined as ≥ 12.5% (AUC:0.771, sensivity:100%, specificity: 75%), and ≥ 34.5 mm (AUC:0.604, sensivity:71%, specificity: 60%), respectively.

The median follow-up time was 46.2 (0.7-184.2) months. 6 (8.3%) patients had recurrences, 83% representing distant metastasis. 6 patients died, and five deaths were attributed to the disease. 12-month, and 48-month DFS rates were 92.3%, and 79.2%, respectively. 12-month and 48-month OS rates were 93.8%, and 86.2, respectively. Fig. 1 demonstrates the Kaplan Meier DFS (A) and OS (B) curves.

Table 2 represents the results of the log-rank test analyses set for DFS and OS. The log-rank test revealed that Ki-67 and stage were statistically significant prognostic factors for DFS and OS. The patients with a low Ki-67 index (≤ 12.5) had longer DFS and OS ($p = 0.007$ and $p = 0.020$, respectively). Figs. 2 and 3 show the Kaplan-Meier curves of DFS and OS according to the Ki-67 index (A) and the pathological stage (A).

DISCUSSION

In the current retrospective study, we analyzed the prognostic factors impacting survival in patients with atypical carcinoids, excluding typical carcinoids. We observed that the Ki-67 index and stage were statistically significant factors in both DFS and OS in this population.

Ki-67 is a non-histone DNA binding nucleolar protein first identified in 1993 [17]. Ki-67 protein is a component of the perichromosomal layer functioning as a platform during nucleolar assembly [18]. The lev-

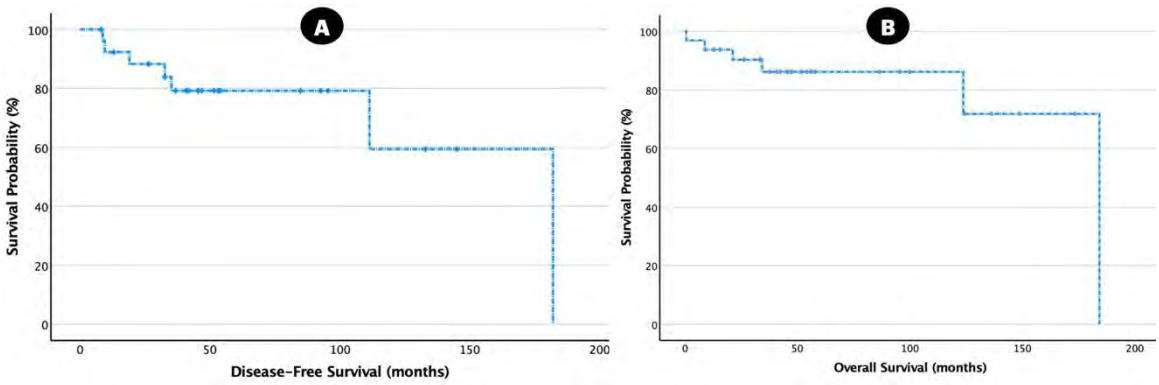

Fig. 1. Kaplan Meier curves of disease-free survival (A) and overall survival (B) of all patients.

Table 2. Results of the Log-rank test: factors affecting disease-free survival and overall survival

Factor	Disease-free survival			Overall survival		
	12-month (%)	48-month (%)	*p* value	12-month (%)	48-month (%)	*p* value
Age (years)						0.624
< 65	91.3	76.2	0.289	96.4	87.5	
≥ 65	100	100		75.0	75.0	
Gender						0.589
Female	86.7	86.7	0.897	94.1	87.8	
Male	90.9	72.7		93.3	84.8	
Tumor side						0.343
Right	93.8	79.1	0.533	94.4	81.7	
Left	90.0	80.0		92.9	92.9	
Tumor localization						0.951
Central	91.7	83.3	0.851	88.9	82.5	
Peripheral	92.9	73.1		100	92.3	
Surgery						0.289
Sublobar resection	100	80.0	0.822	100	95.0	
Lobectomy	90.0	79.3		100	100	
Tumor Size						0.529
< 34.5 mm	92.9	92.9	0.060	100	86.5	
≥ 34.5 mm	91.7	62.9		87.5	87.5	
Ki-67 index						**0.020**
< 12.5	100	100	0.007	100	100	
≥ 12.5	83.3	58.3		86.7	72.7	
Necrosis						0.089
Absent	100	87.5	0.266	100	83.0	
Present	86.7	72.2		89.5	77.1	
Mitosis						0.262
< 2	87.5	87.5	0.842	100	100	
2-10	94.7	77.9		91.7	82.2	
Pathological stage						**0.007**
I	100	90	0.034	100	100	
II	100	100		100	100	
III	80	58.3		100	90.0	
IV	-	-		60.0	30.0	
(Neo)Adjuvant treatment						0.819
No	100	82.5	0.233	100	100	
Yes	81.8	72.7		100	90.9	

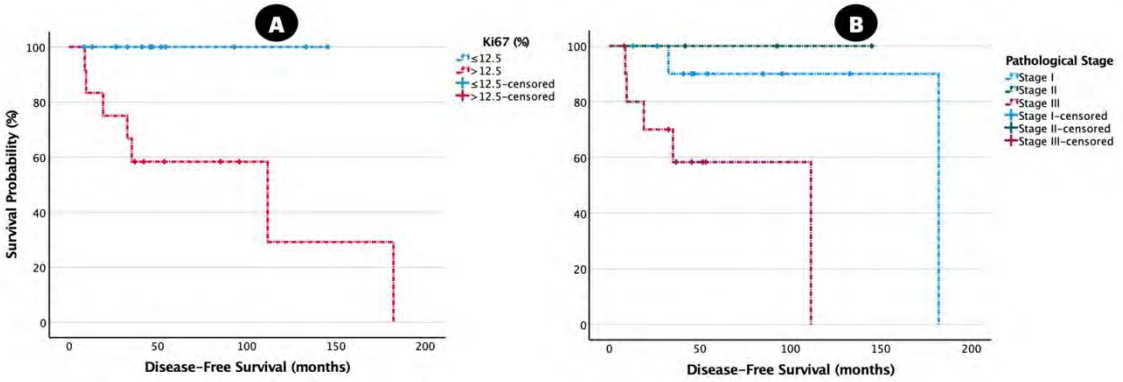

Fig. 2. Kaplan Meier curves of disease-free survival according to the Ki-67 index (A) and stage (B).

els of Ki-67 were reported to increase from the late G1 phase to the S phase and peak at mitosis [19]; therefore, it is widely used as a proliferative marker. The diagnostic, prognostic, and predictive values have been extensively studied in neuroendocrine neoplasms and other solid organ malignancies [14, 20-24].

Ki-67 is mandatory to grade gastroenteropancreatic neuroendocrine neoplasms [14, 25], but in lung carcinoid tumors, necrosis and mitotic count are recommended to grade rather than the Ki-67 index [4]. The Ki-67 index is recommended as a complementary tool to help to differentiate atypical carcinoids from lung neuroendocrine carcinomas (small cell carcinoma and large cell neuroendocrine carcinoma); a Ki-67 index below 30% is in favor of atypical carcinoids rather than high-grade neuroendocrine carcinomas [4, 10, 26-28]. In complement to this diagnostic value, studies on the prognostic value of Ki-67 level, particularly in lung carcinoid tumors, are increasing in the literature [11, 26, 29].

In 2018, Kasajima *et al*. [26] reported an evaluation of a multi-center retrospective study of 244 lung neuroendocrine neoplasms, of which 20 atypical carcinoids, and stated that patients with atypical carcinoids with a Ki-67 ≥ 20% had a worse prognosis compared to those with pulmonary carcinoid with a Ki-67 < %20, consistent with our findings. In 2020, Dermavan *et al*. [11] conducted the results of a retrospective cohort of 176 pulmonary carcinoids (11 atypical carcinoids). They found that Ki-67 is an independent prognostic factor in lung carcinoids, and integrating the Ki-67 index to histological grade and TNM staging is superior in predicting recurrence compared to TNM alone. Recently, Centonze *et al*. [29] reported that Ki-67 has a substantial predictive value in post-surgical recurrence in lung carcinoid tumors. Although the Ki-67 cut-off values in the studies mentioned above were reported to be lower than our study since atypical and typical carcinoids were assessed together, these studies also support our study's findings, indicating the prognostic value of the Ki-67 index.

Although scientific evidence concerning the diagnostic and prognostic value of the Ki-67 index in carcinoid tumors has expanded recently, there are some

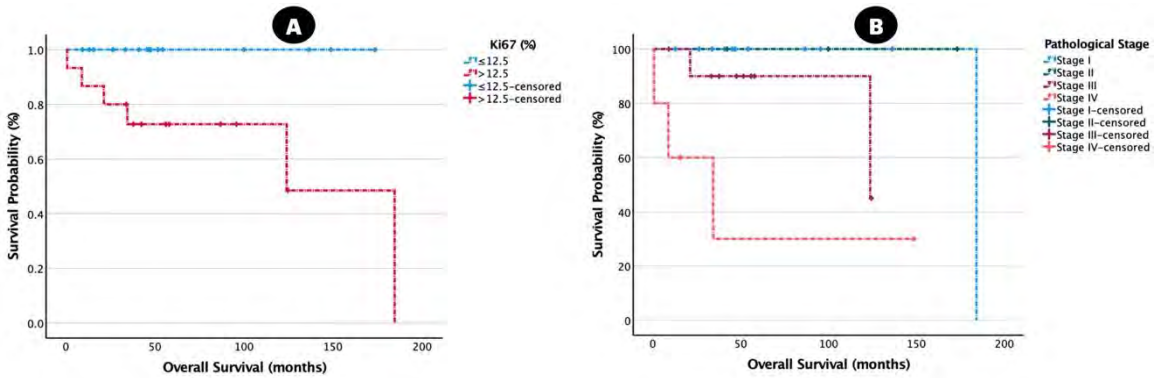

Fig. 3. Kaplan Meier curves of overall survival according to the Ki-67 index (A) and stage (B).

limitations regarding its clinical use: interobserver variability, intratumoral heterogeneity, and interlaboratory variability caused by various reasons such as fixation and tissue processing [30-32]. In addition, a substantial limitation is the lack of internationally accepted cut-off values in carcinoid tumors, in contrast to gastroenteropancreatic neuroendocrine neoplasms.

Several limitations to using TNM staging in lung carcinoids are reported [7, 13]. Although new staging recommendations have been proposed by incorporating prognostic parameters such as histological grade and ki-67, international guidelines recommend the 8th TNM edition [14, 16]. TNM staging is the most noteworthy prognostic parameter after histological grade [14]. Numerous reports support the prognostic value of TNM staging in the literature, consistent with our results [33, 34].

Limitations

Our study has several limitations, such as its retrospective design and limited number of patients due to the rarity of the disease. In addition, a multivariate analysis could not be performed due to the low number of cases and DFS events.

CONCLUSION

In conclusion, the Ki-67 index and 8th TNM staging have prognostic value on DFS and OS in patients with atypical carcinoids. Embodying Ki-67 into the TNM staging system may improve its prognostic value. Large-scale studies are needed to determine the optimal cut-off value.

Authors' Contribution

Study Conception: ABS, BO, EC; Study Design: TE, EC, ABS; Supervision: TE, EC, HM, ASB; Funding: N/A; Materials: N/A; Data Collection and/or Processing: BE, BC, BO, HM, EUA; Statistical Analysis and/or Data Interpretation: ABS, ASB, AD; Literature Review: BE, BC, BO, HM, EUA; Manuscript Preparation: ABS, BE, BC, AD and Critical Review: ASB, TE, EUA, AD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The role of intraoperative superior hypogastric plexus blocks in pain management for total abdominal hysterectomy: a comparative study

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ABSTRACT

Objectives: This study aimed to investigate the efficacy of intraoperative Superior Hypogastric Plexus Blocks (SHPBs) in managing postoperative pain following total abdominal hysterectomy, comparing pain scores and analgesic requirements between patients who received SHPBs and those who did not.

Methods: A prospective, randomized, controlled trial was conducted on 70 female patients undergoing elective total abdominal hysterectomy. Patients were randomly assigned to either the SHPB group or the non-SHPB group. In the SHPB group, intraoperative SHPBs were administered after uterine removal. Postoperative pain scores were assessed using the Visual Analogue Scale (VAS) at various time points. Analgesic consumption and adverse effects were also recorded.

Results: Patients in the SHPB group consistently exhibited lower pain scores compared to the non-SHPB group at various postoperative time intervals ($p < 0.05$). Initial analgesic requirements were significantly higher in the non-SHPB group, as was total analgesic consumption during the hospital stay ($p < 0.05$). No significant complications related to SHPB administration were observed.

Conclusions: Intraoperative Superior Hypogastric Plexus Blocks demonstrated a potential benefit in reducing postoperative pain scores and analgesic consumption in patients undergoing total abdominal hysterectomy. These findings highlight the potential of SHPBs as an effective approach to enhance pain management in this surgical population, warranting further investigation and refinement of administration protocols.

Keywords: Pain management, gynecological surgical procedures, analgesia, laparotomy, hysterectomy, elective surgical procedures

Postoperative pain management following total abdominal hysterectomy is a crucial aspect of patient care, as it influences recovery, psychological well-being, and overall surgical outcomes. Current strategies, such as epidural blocks, offer effective pain relief but can involve invasive procedures and potential complications. In light of these challenges, exploring alternative pain management approaches becomes paramount [1].

The pelvic region and its neural innervation play a pivotal role in pain perception, encompassing sympathetic, parasympathetic, and somatic nerves. Anatomically, spinal nerves originating from thoracolumbar and sacral segments contribute to this intri-

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cate network. Among the potential strategies, the use of superior hypogastric block (SHPB) emerges as an intriguing possibility [2]. Superior hypogastric plexus (SHP), originating from L3-L4 sympathetic ganglia, contains sympathetic, sacral parasympathetic, and somatic afferent fibers [3]. Its division into the right and left hypogastric nerves culminates in the formation of the inferior hypogastric plexus, which serves as a central hub for neuronal integration within the pelvis.

Superior hypogastric block, initially described by Plancarte, has gained attention as a method for managing pelvic pain [3]. Performed through percutaneous techniques guided by ultrasound, fluoroscopy, or computed tomography, SHP blocks offer a promising avenue for pain relief [4]. However, their proximity to major vascular structures and sensitive anatomical components poses inherent challenges, leading to potential complications. In the context of total abdominal hysterectomy, the opportunity to explore intraabdominal anatomy presents a unique advantage, potentially minimizing complications associated with percutaneous SHP blocks [5].

This study takes inspiration from previous research involving SHP blocks and extends its application to the specific context of total abdominal hysterectomy [6]. While previous studies have focused on diverse patient groups, our investigation centers on patients undergoing total abdominal hysterectomy. By implementing the SHP block technique, we aim to assess its efficacy in postoperative pain reduction and analgesic consumption, thereby contributing to the optimization of pain management strategies in this surgical population.

In this article, we present the results of our study, which aims to compare postoperative pain scores and analgesic requirements between patients who received intraoperative SHP block and those who did not. By shedding light on the potential benefits and challenges associated with this novel approach, we hope to offer valuable insights into improving postoperative pain management for individuals undergoing total abdominal hysterectomy.

METHODS

Study Design and Patient Selection

This prospective, randomized, and controlled trial aimed to investigate the efficacy of superior hypogastric plexus block (SHPB) in reducing postoperative analgesic requirements and pain scores following total abdominal hysterectomy. The study was conducted in accordance with the ethical standards of the institutional review board (Istanbul Kartal Dr. Lütfi Kırdar City Hospital) and complied with the principles outlined in the Helsinki Declaration (Ethical approval Number: 2022/514/236/28). Prior to participation, patients were fully informed about the study's purpose, procedures, and potential risks. Written informed consent was obtained from all participants.

A total of 70 female patients, aged 18 years and above, with American Society of Anesthesiologists (ASA) physical status I or II, who underwent elective total abdominal hysterectomy or total abdominal hysterectomy with salpingo-oophorectomy for benign conditions such as uterine fibroids, ovarian cysts, dysfunctional uterine bleeding, between 01 November 2022 and 01 January 2023, were included in the study. Patients who had a history of continuous analgesic drug usage for reasons other than the surgical indication, patients under 18 years of age, and those scheduled for surgery due to malignancy were excluded from the study. Patients meeting the inclusion criteria were assigned randomly to either the study group (SHPB group) or the control group (non-SHPB group) in a 1:1 ratio. The randomization process was carried out using a closed-envelope method.

Anesthesia

All patients underwent standardized general anesthesia induction, which included the administration of propofol, fentanyl, and rocuronium. Anesthesia was maintained using inhaled sevoflurane, an oxygen and air mixture, and intravenous remifentanil. Dexketoprofen trometamol and tramadol were routinely administered intraoperatively ~30 minutes before skin closure. Bladder catheterization was performed for all patients and was removed the day after surgery.

Surgery and Superior Hypogastric Plexus Block

Total abdominal hysterectomy procedures were performed through a Pfannenstiel incision. The duration of surgery was recorded from the time of the initial incision to skin closure. Subsequent to uterine removal and closure of the vaginal cuff, administration of SHP blocks was initiated. Identification of the

promontorium and aortic bifurcation was accomplished. The posterior peritoneum covering the promontorium was gently lifted using toothless tissue forceps, creating a tent-like elevation. A needle was then introduced at the apex of the tent and advanced approximately 1 cm inward, ensuring minimal contact with bony tissue. Following a negative aspiration, a retroperitoneal injection of 30 mL of 0.25% bupivacaine was administered.

Postoperative Pain Assessment

Patients were evaluated in the post-anesthesia care unit (PACU) for about 1 hour after surgery, and then transferred to the gynecology ward when their modified Aldrete scores reached ≥9. Postoperative pain was evaluated using a 10 cm Visual Analogue Scale (VAS) at predefined time points: 0, 15, 30, and 45 minutes, and 1, 2, 6, 12, and 24 hours (VAS0, VAS15, VAS30, VAS45, VAS-1, VAS-2, VAS-6, VAS-12, VAS-24) after surgery. Patients with VAS scores of 4 or higher received diclofenac sodium 75 mg/3mL intramuscularly as the first-line analgesic. Possible adverse effects of SHPB, such as bradycardia and hypotension, were monitored in the ward for 2 hours. The total number of analgesic doses administered during the postoperative period was recorded.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22.0. The demographic characteristics, VAS scores, initial analgesic requirement, and total analgesic intakes were assessed by scrutinizing the median, minimum, and maximum values. Non-parametric data were analyzed using the Mann-Whitney U test. A p-value of < 0.05 was considered statistically significant. Primary outcome measures included the comparison of VAS scores and analgesic requirements between the SHPB and No-SHPB groups. Secondary outcomes included the duration of the first analgesic effect, demographic characteristics, and surgical indication. We utilized the G*Power software to conduct a power analysis and determine the optimal sample size for our research investigating the effect of intraoperative superior hypogastric plexus blocks on categorical outcomes. Extending insights from a previous study that assessed the impact of SHPB on pain intensity in cesarean section patients, we initially calculated a total sample size of 54, with 27 patients allocated to each group [7]. While employing 27 participants per group would have resulted in a power of 0.95, our objective was to enhance the study's statistical robustness while accounting for potential data variations. As a result, we included 35 individuals in each group. Subsequent post hoc analysis unveiled an elevated study power of 0.98.

RESULTS

In this study, we conducted a comprehensive analysis to investigate the impact of administering Superior Hypogastric Blocks (SHPB) on postoperative pain and the requirement for analgesics following total abdominal hysterectomy. A total of 70 patients who underwent elective total abdominal hysterectomy for benign indications were included in the study. Detailed patient exclusion criteria were applied, resulting in a final evaluation of 70 patients, with 35 patients in each group (SHPB and No-SHPB).

Table 1. Demographic characteristics

	SHPB group (n = 35)	No-SHPB group (n = 35)
Gravida	3 (1-8)	3 (0-12)
Age (years)	48 (43-69]	50 (40-78)
BMI (kg/m^2)	28.37 (19.03-36.45)	30.04 (18.67-46.75)
Lenght of hospital stay (days)	2 (1-7)	2 (1-5)
Duration of surgery (min)	80 (40-130)	90 (30-180)

Data are shown as median (minimum-maximum). SHBP = Superior Hypogastric Plexus Block

Demographic attributes, surgical durations, and hospital lengths of stay were comparable between the two groups (Table 1), ensuring a balanced baseline for subsequent analyses. The study revealed significant differences in analgesic consumption and pain scores between the two groups.

Table 2 offers a detailed comparison of Visual Analog Scale (VAS) scores across different groups, along with pertinent statistical data. VAS scores, serving as a quantifiable measure of pain intensity on a scale of 0 to 10, were juxtaposed between the SHPB and No-SHPB groups. The table illustrates VAS scores at various postoperative time intervals: 0, 15, 30, and 45 minutes, 1 hour, 2 hours, 6 hours, 12 hours, and 24 hours. Additionally, we examined the time of initial analgesic administration and the cumulative analgesic intake over a 24-hour period.

Consistently, VAS scores favored the SHPB group across different time points, including VAS0, VAS15, VAS30, VAS45, VAS1, VAS2, VAS6, and VAS24 ($p < 0.05$) (Table 2). It's noteworthy that a p-value of 0.053 for VAS at 12 hours indicates a potential difference between the groups, even though statistical significance wasn't firmly established.

Initial analgesic requirements were notably higher in the No-SHPB group ($p < 0.05$), implying a potential benefit of SHPB administration in reducing analgesic consumption. Furthermore, total analgesic usage dur-

Table 2. Comparison of VAS scores among SHPB groups

		Median (minimum-maximum)	U	*p*value
VAS 0 minute	SHPB group	3 (0-7)	138,00	**0.001***
	No-SHPB group	6 (0-10)		
VAS 15 minute	SHPB group	3 (0-7)	138.000	**0.001***
	No-SHPB group	6 (0-10)		
VAS 30 minute	SHPB group	3 (0-7)	138.000	**0.001***
	No-SHPB group	6 (0-10)		
VAS 45 minute	SHPB group	3 (0-7)	136.000	**0.001***
	No-SHPB group	6 (0-10)		
VAS 1 hour	SHPB group	3 (0-8)	151.000	**0.001***
	No-SHPB group	7 (3-10)		
VAS 2 hour	SHPB group	3 (0-7)	84.000	**0.001***
	No-SHPB group	7 (4-10)		
VAS 6 hour	SHPB group	3 (0-8)	266.000	**0.001***
	No-SHPB group	5 (2-8)		
VAS 12 hour	SHPB group	3 (0-7)	454.000	0.053
	No-SHPB group	4 (2-8)		
VAS 24 hour	SHPB group	2 (0-7)	349.500	**0.002***
	No-SHPB group	3 (2-8)		
Initial analgesic requirements	SHPB group	2 (0-30)	360.000	**0.001***
	No-SHPB group	1 (1-2)		
Total analgesic intakes	SHPB group	2 (0-5)	127.000	**0.001***
	No-SHPB group	4 (2-5)		

VAS = Visual Analogue Scale, SHPB = Superior Hypogastric Block

ing the hospital stay was also significantly higher in the No-SHPB group ($p < 0.05$) (Table 2). These findings underscore the potential analgesic efficacy of SHPB administration in managing postoperative pain. Importantly, we observed no complications related to SHPB blocks, indicating the safety and feasibility of this intervention in the context of total abdominal hysterectomy.

DISCUSSION

In this study, we examine the effectiveness of intraoperative Superior Hypogastric Plexus Blocks (SHPB) as a promising approach for managing pain after surgery. In this context, our study embarks upon an exhaustive exploration into the integral role played by intraoperative Superior Hypogastric Blocks (SHPBs), with a particular focus on the dynamics surrounding total abdominal hysterectomy. Our aim is to underscore the potential benefits of SHPB in terms of reducing analgesic consumption and alleviating pain scores, ultimately enhancing the overall postoperative recovery experience for patients.

Aligned with the outcomes elucidated in the study of Aytuluk *et al*. [8], our study accentuates the pivotal role of SHPBs in promoting opioid-sparing effects and effective multimodal analgesia. The noticeable reduction in both analgesic consumption and pain scores corroborates the observations detailed in the study of Rapp *et al*. [9], thereby providing additional support for the effectiveness of this intervention.

Considering the intricate realm of sympathetic innervation, as discussed in the study of Aytuluk *et al*. [8], we gain insight into the complex landscape of visceral pain management, characterized by the interplay of multifaceted nociceptive mechanisms. While plexus blocks, such as the celiac plexus blocks have found utility in the realm of chronic pain management, their potential application in postoperative pain relief warrants further exploration [10].

The multifaceted nature of acute postoperative pain encompasses a diverse array of nociceptive structures, neuroendocrine pathways, and autonomic nervous systems [11]. The administration of SHPBs, leading to the blockade of excitatory sympathetic activation and nociception relayed through the superior hypogastric plexus, provides compelling evidence for the reduction in pain levels and analgesic utilization, thereby potentially mitigating the risk of chronic pain development [12].

The versatility of SHPBs is underscored by their applicability across various surgical scenarios. The convergence of our findings with those presented in the study of McDonell *et al*. [12] and Dooley *et al*. [13] underscores the promising potential of SHPBs as a valuable adjunct for both intraoperative anesthesia and postoperative pain relief.

The paramount consideration of safety in any therapeutic intervention remains paramount. The administration of intraoperative SHPBs attests to their safety profile, characterized by minimal complications such as vascular or bowel puncture. While concerns may arise regarding potential adverse effects such as bradycardia and hypotension, our empirical data indicates an overall hemodynamically stable trajectory post-SHPB administration.

Additionally, the theoretical concern of bladder dysfunction arising from SHPBs lacks substantial empirical support [14]. Our study signifies a notable absence of early-presenting bladder dysfunction among patients who underwent SHPBs.

Limitations

The inherent limitations of our study warrant acknowledgment. The delayed onset of SHPB effects underscores the importance of meticulous temporal planning to maximize pain relief. Moreover, the applicability of SHPB efficacy in surgical procedures involving retroperitoneal intervention necessitates further exploration.

CONCLUSION

In conclusion, our study sheds light on the potential advantages of intraoperative SHPBs in enhancing postoperative pain management. Future research endeavors should delve into refining administration protocols and exploring potential synergies with existing pain management modalities to optimize patient outcomes.

Authors' Contribution

Study Conception: ECG, TGY; Study Design: ECG, TGY; Supervision: ECG; Funding: ECG; Materials: ECG; Data Collection and Processing: ECG,

TGY; Statistical Analysis and Data Interpretation: ECG; Literature Review: ECG, TGY; Manuscript Preparation: ECG, TGY and Critical Review: ECG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Predictive role of ABCD2, ABCD3I, C-reactive protein, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and systemic immune-inflammation index in 90-day and long-term stroke after transient ischemic attack

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ABSTRACT

Objectives: Transient ischemic attack (TIA) is a neurological emergency and a precursor of ischemic stroke. ABCD2 and ABCD3I scores predict stroke after TIA, and clinicians use preclinical, clinical, and radiological parameters for calculating these scores. Our study aimed to investigate the efficacy of peripheral blood markers in predicting 90-day and long-term stroke risk after TIA.

Methods: This retrospective study was conducted in Kastamonu Training and Research Hospital between January 2015 and November 2022. The demographic data of 99 patients who applied with the diagnosis of TIA and peripheral blood markers at the time of first admission to the hospital were used in the study. These parameters was evaluated in 90-day and long-term (> 12 months) stroke after TIAs.

Results: Of the 99 patients in our study, 59% (n = 58) were male. The mean age of the patients was 70 ± 13 years. ABCD2 (age, blood pressure, clinical features, duration of symptoms, and presence of diabetes mellitus) and ABCD3I (age, blood pressure, clinical features, duration of symptoms, presence of diabetes mellitus, dual TIA, and ipsilatheral carotis stenosis) scores and C-reactive protein (CRP) were statistically significant in predicting 90-day stroke. ABCD2 and ABCD3I were not effective in predicting long-term stroke. In addition, CRP, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) parameters were statistically significant in long-term stroke. CRP (AUC=793, sensitivity = 82%, and specificity = 81%) values were higher than ABCD2 (AUC = 779, sensitivity = 73%, and specificity = 76%) and ABCD3I (AUC = 755, sensitivity = 82%, and specificity = 70%) scores in predicting 90-day stroke.

Conclusions: Our study showed that ABCD2, ABCD3I, and CRP effectively predict 90-day stroke after TIA. Furthermore, CRP was more effective than ABCD2 and ABCD3I scores in predicting 90-day stroke after TIA. CRP, NLR, PLR, and SII also effectively predicted long-term stroke after TIA.

Keywords: Transient ischemic attack, ABCD2, ABCD3I, C-reactive protein

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Transient ischemic attack (TIA) is a neurological emergency characterized by a focal neurological deficit lasting less than 24 hours. Clinically, it most commonly presents as dysarthria, aphasia, hemiparesis, hemihypoesthesia, and amaurosis fugax. Atrial fibrillation (AF), hypertension (HT), and diabetes mellitus (DM) are the most common etiologies. TIA is a critical clinical condition as it is a precursor of ischemic stroke that may develop in the future. Studies reported the risk of stroke following TIA between 1.7% and 20.6% at day 90 [1]. A reasonable etiological investigation and identification of risk factors can minimize the risk of ischemic stroke. Several scales were developed to estimate risk of stroke after TIA. ABCD2 (age, blood pressure, clinical features, duration of symptoms, and presence of diabetes mellitus) and ABCD3I (age, blood pressure, clinical features, duration of symptoms, presence of diabetes mellitus, dual TIA, and ipsilatheral carotis stenosis) scores are the most commonly used scores that predict 90-day stroke after TIA [2-4]. However, markers that are calculated and easily predicted based on laboratory data are still needed. In addition, it is important to differentiate patients with a poor prognosis with peripheral inflammatory markers, especially in cases where magnetic resonance imaging (MRI) is not accessible or takes time to be obtained in the emergency department, to start more effective treatments quickly. C-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), monocyte-lymphocyte ratio (MLR), and systemic immune-inflammation index (SII) parameters are used to predict stroke severity [5, 6]. However, few studies have investigated the role of these inflammatory parameters in predicting ischemic stroke after TIA. Moreover, as far as we know, there is no study investigating the efficacy of these parameters in predicting long-term stroke after TIA [7, 8].

In this study, we aimed to investigate the efficacy of peripheral inflammatory markers in predicting 90-day and long-term stroke after TIA.

METHODS

This retrospective study was conducted in Kastamonu Training and Research Hospital between January 2015 and November 2022. The study was started after the approval of the Kastamonu University Faculty of Medicine Clinical Research Ethics Committee with decision number 2023- KAEK - 12 and dated 30.01.2023. The Demographic data of 99 patients who applied with the diagnosis of TIA and hematological and biochemistry data at the time of first admission to the hospital were used in the study. ABCD2 and ABCD3I scores were calculated according to all patients' demographic, clinical, and radiological data. ABCD2 score was calculated as follows: A = age, 1 point for > 60 years; B = blood pressure, 1 point for > 140/90 mmHg; C = clinical feature, 2 points for unilateral weakness or 1 point for speech impairment without liability; D1 = duration, 2 points for > 60 minutes, 1 point for < 60 minutes; D2 = 1 point for the presence of diabetes mellitus). According to this scoring system, 1-3 points indicate low, 4-5 points indicate moderate, and 6-7 points indicate high stroke risk. ABCD3I score was calculated by adding dual TIA and vascular findings to ABCD2 [2, 3].

Patients under 18 years old, pregnant women, trauma patients, patients with malignancy, liver and kidney failure, and patients without hemogram and biochemistry data were excluded from the study. Hemogram and CRP tests were performed on Sysmex XN-1000 (Sysmex, Kobe, Japan) hematology and Beckman Coulter AU 5800 (Beckman Coulter, Brea, CA, USA) clinical chemistry auto analyzers, respectively. In addition, the D-dimer test was performed on CS 2500 (Sysmex, Kobe, Japan). The NLR, MLR, PLR, SII, and SIRI were calculated as follows:

NLR = Neutrophil count ($\times 10^9$/L)/ Lymphocyte count ($\times 10^9$/L)

MLR = Monocyte count ($\times 10^9$/L)/ Lymphocyte count ($\times 10^9$/L)

PLR = Platelet count ($\times 10^9$/L)/ Lymphocyte count ($\times 10^9$/L)

SII = Platelet count ($\times 10^9$/L) × Neutrophil count ($\times 10^9$/L)/ Lymphocyte count ($\times 10^9$/L)

SIRI = Neutrophil count ($\times 10^9$/L) × Monocyte count ($\times 10^9$/L)/ Lymphocyte count ($\times 10^9$/L).

Statistical Analysis

The "Statistical Package for Social Sciences 18.0 for Windows" (SPSS Inc., Chicago, USA) program was used for statistical analysis of the data. Descriptive statistics of the data obtained were given as numbers and percentages for categorical variables and as median (25, 75 Percentiles) for numerical variables.

Since the groups showed a nonparametric distribution, the Mann-Whitney U test was used to test the significance between the groups. A chi-square test was performed to see if there was a significant difference between the groups regarding gender, DM, HT, and HL. The receiver operating characteristic (ROC) analysis was performed, and Youden's index was used to determine the area under the curve (AUC), sensitivity, specificity, and optimal cut-off values. A p - value of < 0.05 was considered statistically significant.

RESULTS

Of the 99 patients in our study, 59% (n = 58) were male. The mean age of the patients was 70 ± 13 years (Table 1). ABCD2 and ABCD3I scores and CRP were statistically significant in predicting 90-day stroke (Table 2). ABCD2 and ABCD3I were not effective in predicting long-term stroke (Table 3). CRP (AUC = 793, sensitivity = 82%, and specificity = 81%) values were higher than ABCD2 (AUC = 779, sensitivity = 73%, and specificity = 76%) and ABCD3I (AUC = 755, sensitivity = 82, and specificity = 70) scores in predicting 90-day stroke (Table 4) (Fig. 1). Notably, CRP AUC, sensitivity, and specificity values were higher than ABCD2 and ABCD3I scores in predicting 90-day stroke.

Table 1. Demographic and clinical data of TIA patients

	Data
Age (years)	70 ± 13
Gender (male), n (%)	58 (59)
AF, n (%)	20 (20)
HVD, n (%)	37 (37)
DM, n (%)	56 (56)
HT, n (%)	56 (56)
CRP (mg/L)	6.76 ± 11
D-DIMER (ng/dL)	1.1 ± 5.5
NLR	4.6 ± 5.4
PLR	150 ± 105
MLR	0.44 ± 0.75
SII	1081 ± 1368
SIRI	3 ± 4.4

Data are shown as mean±standard deviation or n (%). AF = atrial fibrillation, HVD = heart valve disease, DM = diabetes mellitus, HT = hypertension, CRP = C-reactive protein, NLR = neutrophil-lymphocyte ratio, PLR = platelet-lymphocyte ratio, MLR = monocyte-lymphocyte ratio, SII = systemic immune-inflammation index (neutrophil × platelet/ lenfosit), SIRI = systemic inflammation response index (neutrophil × monocyte/ lenfosit)

In addition, CRP, NLR, PLR, and SII parameters were statistically significant in long-term stroke (Table 3). In the ROC analysis performed in patients with long-term stroke (12 months and above), SII (cut off: 505, AUC: 0.742), NLR (cut off: 3.7, AUC: 0.726), CRP (cut off: 4.8, AUC: 0.722) moderate-high predictive properties were detected in the tests (Fig 1).

In predicting 90-day stroke, ABCD2 and ABCD3I obtained from patients' data at admission to the hospital were statistically significantly different, as expected. However, CRP was also significantly different (Table 2).

ABCD2 and ABCD3I scores were not effective in predicting long-term stroke. However, CRP was again statistically significant in predicting long-term stroke. In addition, NLR, PLR, and SII parameters were significantly different (Table 3).

DISCUSSION

The ABCD2 score was developed for primary care and emergency physicians to predict TIA patients at high risk of stroke and significantly differentiate patients for hospitalization. Nevertheless, in some studies, it was thought to help identify patients with an increased risk of stroke after TIA, whereas this result could not be demonstrated in some studies [9]. These inconsistent results were attributed to methodological differences in the studies. Some of these studies were population-based, while others included only hospitalized patients. In addition, some studies were conducted by stroke specialists, while others were executed by primary care physicians. In our study, in which we examined patients evaluated in the emergency department and hospitalized in the neurology department, we found a significant association between high ABCD2 scores and 90-day stroke.

The clinicians developed the ABCD3I score for more sensitive scoring stroke risk after TIA. However, its disadvantage is that it is not suitable for use by pri-

Table 2. Comparison of hematological and biochemical parameters in predicting 90-day stroke after TIA

	Stroke Group (n = 11)	Non-stroke Group (n = 88)	*p* value
Age (years)	80 (55;83)	73 (58;81)	0.407
Male Gender, n (%)	5 (45.5)	53 (60.2)	0.348
ABCD2	5 (4;6)	4 (3;4)	**0.002**
ABCD3I	5 (5;6)	4 (3;5)	**0.005**
CRP (mg/L)	7.8 (5.26;20.4)	3.25 (1.22;5)	**0.002**
D-DIMER (ng/dL)	0.9 (0.3;1.21)	0.40 (0.26;0.53)	0.067
NLR	2.75 (1.90;1.20)	2.81 (1.86;4.82)	0.551
PLR	115 (101;158)	120 (91;164)	0.920
MLR	0.24 (0.20;0.45)	0.28 (0.19;042)	0.928
SII	608 (471;786)	629 (417;1090)	0.640
SIRI	1.61 (0.60;2.05)	1.62 (0.89;2.64)	0.577

Data are shown as median (25;75 percentiles) or numbers and percentages. ABCD2 scoring system (A = age, 1 point for > 60 years; B = blood pressure, 1 point for >140/90 mmHg; C = clinical feature, 2 points for unilateral weakness or 1 point for speech impairment without liability; D1 = duration, 2 points for > 60 minutes, 1 point for < 60 minutes; D2 = 1 point for the presence of diabetes mellitus), ABCD3I scoring system (A = age, 1 point for > 60 years; B = blood pressure, 1 point for >140/90 mmHg; C = clinical feature, 2 points for unilateral weakness or 1 point for speech impairment without liability; D1 = duration, 2 points for > 60 minutes, 1 point for < 60 minutes; D2 = 1 point for the presence of diabetes mellitus; D3 = dual TIA; I = ipsilatheral carotis stenosis), AF = atrial fibrillation, HVD = heart valve disease, DM, =diabetes mellitus, HT = hypertension, CRP = C-reactive protein, NLR = neutrophil-lymphocyte ratio, PLR = platelet-lymphocyte ratio, MLR = monocyte-lymphocyte ratio, SII = systemic immune-inflammation index (neutrophil × platelet/ lenfosit), SIRI = systemic inflammation response index (neutrophil × monocyte/ lenfosit)

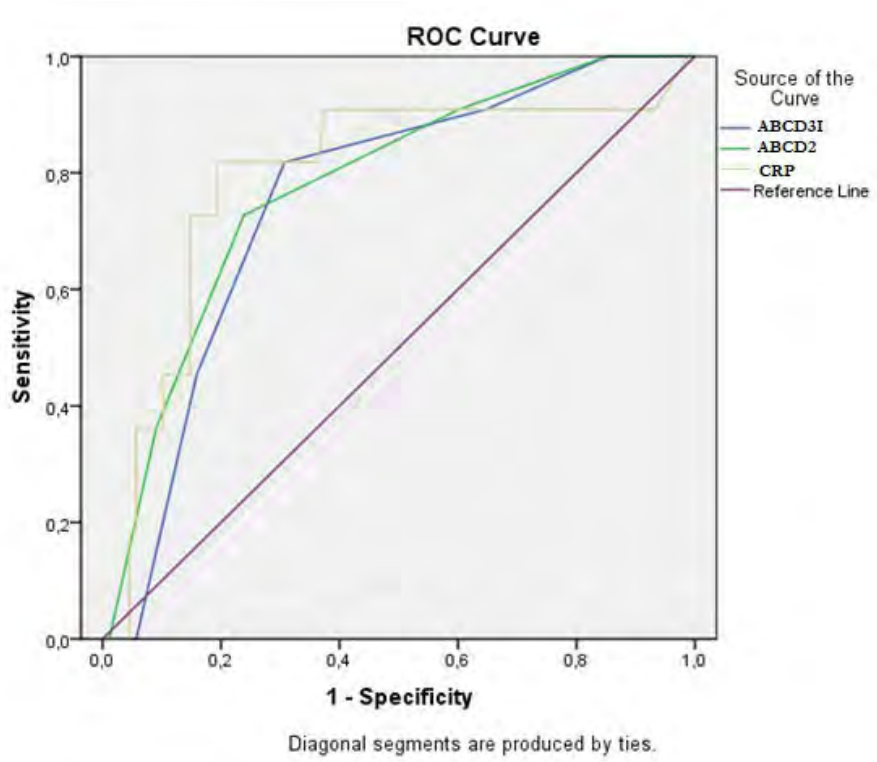

Fig. 1. ROC curve analysis of ABCD2, ABCD3I, and CRP data in TIA patients.

Tablo 3. Comparison of hematological and biochemical parameters in predicting long-term stroke after TIA

	Stroke Group (n = 9)	Non-Stroke Group (n = 90)	*p* value
Age (years)	76 (69;85)	73 (56;81)	0.315
Male Gender, n (%)	5 (55.6)	53 (58.9)	0.848
ABCD2	5 (4;5)	4 (3;5)	0.070
ABCD3I	5 (4;5)	4 (3;5)	0.143
CRP	5 (4;35)	3.4 (1.2;5.2)	**0.029**
D-DIMER	0.46 (0.29;0.50)	0.40 (0.26;0.60)	0.817
NLR	2.75 (1.90;1.20)	2.74 (1.81;4.39)	**0.026**
PLR	180 (119;347)	117 (91;157)	**0.041**
MLR	0.24 (0.20;0.45)	0.27 (0.19;0.39)	0.061
SII	1042 (614;4214)	600 (407;1007)	**0.017**
SIRI	2.47 (1.22;8.27)	1.48 (0.86;2.54)	0.070

Data are shown as median (25; 75 percentiles) or numbers and percentages. ABCD2 scoring system (A = age, 1 point for > 60 years; B = blood pressure, 1 point for >140/90 mmHg; C = clinical feature, 2 points for unilateral weakness or 1 point for speech impairment without liability; D1 = duration, 2 points for > 60 minutes, 1 point for < 60 minutes; D2 = 1 point for the presence of diabetes mellitus), ABCD3I scoring system (A = age, 1 point for > 60 years; B = blood pressure, 1 point for > 140/90 mmHg; C = clinical feature, 2 points for unilateral weakness or 1 point for speech impairment without liability; D1 = duration, 2 points for > 60 minutes, 1 point for < 60 minutes; D2 = 1 point for the presence of diabetes mellitus; D3 = dual TIA; I = ipsilatheral carotis stenosis), AF = atrial fibrillation, HVD = heart valve disease, DM, =diabetes mellitus, HT = hypertension, CRP = C-reactive protein, NLR = neutrophil-lymphocyte ratio, PLR = platelet-lymphocyte ratio, MLR = monocyte-lymphocyte ratio, SII = systemic immune-inflammation index (neutrophil × platelet/ lenfosit), SIRI = systemic inflammation response index (neutrophil × monocyte/ lenfosit)

mary care physicians as carotid artery imaging is required for complete scoring. An emergency department study revealed the ABCD3I score is to be helpful in long-term stroke prevention [10]. Studies showed that the ABCD3I score is superior to the ABCD2 score in predicting stroke. Interestingly, the ABCD2 score was more predictively than the ABCD3I score in our study.

Inflammatory mechanisms in ischemic stroke have been investigated in studies. Proinflammatory factors are activated in the damaged brain region in the first minutes of a stroke. After ischemic stroke, migration of immune system mediators and release of cytokines occur, leading to impaired permeability of the blood-brain barrier, resulting in brain edema, increased infarct volume, and neuronal damage [11, 12]. In the

Table 4. ROC curve analysis of ABCD2, ABCD3I, and CRP data in TIA patients

	Cut-off	AUC	95%CI	*p* value	Sensitivity (%)	Specificity (%)
CRP	5.23	0.793	0.63-0.95	**0.002**	82	81
ABCD2	4.5	0.779	0.64-0.92	**0.003**	73	76
ABCD3I	4.5	0.755	0.62-0.89	**0.006**	82	70

ABCD2 scoring system (A = age, 1 point for > 60 years; B = blood pressure, 1 point for > 140/90 mmHg; C = clinical feature, 2 points for unilateral weakness or 1 point for speech impairment without liability; D1 = duration, 2 points for > 60 minutes, 1 point for < 60 minutes; D2 = 1 point for the presence of diabetes mellitus), ABCD3I scoring system (A = age, 1 point for > 60 years; B = blood pressure, 1 point for > 140/90 mmHg; C = clinical feature, 2 points for unilateral weakness or 1 point for speech impairment without liability; D1 = duration, 2 points for > 60 minutes, 1 point for < 60 minutes; D2 = 1 point for the presence of diabetes mellitus; D3 = dual TIA; I = ipsilatheral carotis stenosis), CRP = C-reactive protein.

early phase of stroke, neutrophils are the first inflammatory mediators to migrate to the damaged brain region. Clinical studies show that the severity of the inflammatory response is important in acute and chronic prognosis and the extent of brain damage [13]. Available data suggest that inflammation increases atherosclerosis, and the ongoing thrombotic process aggravates inflammation. Continued obstruction leads to inflammation and migration of other inflammatory mediators to the site [14].

Hematologic markers such as NLR are used in the diagnosis and prognosis monitoring of many diseases. In addition, these markers have recently been used in the prognosis prediction of many neurological disorders because they are easily accessible and inexpensive. However, there are conflicting results among studies. For example, Ross *et al*. [15] found that neutrophil counts were higher in patients with TIA and acute ischemic stroke (AIS) compared to control groups. Also, Gokhan *et al*. [16] showed that the NLR rate was significantly higher in AIS patients than in TIA patients. Cavrak *et al*. [17] found no significant difference in NLR value between these groups, including TIA, mild ischemic stroke, and stroke mimics. In addition, another study conducted on young patients showed that elevated NLR was a marker of poor prognosis in ischemic stroke and TIA [18]. In our research, NLR and PLR didn't predict 90-day stroke after TIA but predicted long-term stroke after TIA.

Effect of SII and SIRI on prognosis prediction in ischemic stroke was investigated in various studies. Hou *et al*. [19] found that SII was associated with ischemic stroke severity independently of all parameters. Furthermore, Lii *et al*. [20] showed that SII may be a prognostic marker in patients undergoing mechanical thrombectomy (MT) for large artery occlusion. However, another study found that higher SII was associated with greater stroke severity after AIS [21]. Besides, a study revealed that SII and SIRI is closely related to the short- and long-term prognosis of patients with AIS [22]. Moreover, similar to previous studies, Zhou *et al*. [23] showed that SII could predict the risk of adverse outcomes in patients with AIS with an accuracy of 80.2%). However, a prospective study showed that although SII showed significant differences during the first two weeks following stroke, the discrimination capacity of these changes was limited [24]. In our study, SII and SIRI did not predict 90-day stroke after TIA but SII predicted long-term stroke.

C-reaktive protein (CRP) is an important marker used in treatment follow-up in inflammatory diseases. In addition, it is an important prognostic marker in cardiovascular diseases due to the inflammatory mechanisms of these diseases. It is effective in prognosis prediction, especially in cardiovascular diseases, and associated with poor prognosis in ischemic stroke. (It is effective in prognosis prediction, especially in cardiovascular diseases, and associated with poor prognosis in ischemic stroke.) In one study, highly sensitive CRP predicted stroke after TIA [25]. Moreover, in a recent study, H-sensitive CRP (hs-CRP) was an independent indicator of recurrent cerebrovascular events [26]. In our study, CRP predicted 90-day and long-term stroke after TIA and was more effective than ABCD2 and ABCD3I in predicting a 90-day stroke after TIA. This was one of the most striking finding of our research.

The relationship between D-dimer levels and stroke is not consistent across studies. In contrast, some studies have found high D-dimer levels to be significantly associated with stroke [27, 28]. Zakai *et al*. [29] found a weak association between D-dimer levels and stroke. In addition, D-dimer levels were grouped according to different criteria between studies, which may explain the inconsistency in the results of the studies. However, a definitive association between D-dimer levels and stroke has not been found based on the limited available data. Increased D-dimer levels may reflect ongoing thrombosis in cerebral blood vessels [30]. D-dimer is reported to activate the inflammatory process, which may include the activation of monocytes and the release of proinflammatory cytokines such as interleukin-6 (IL-6) [30]. However, the D-dimer levels were ineffective in predicting 90-day and long-term stroke after TIA in our study.

Limitations

Our study had some limitations: It is a single-center and retrospective analysis. We didn't perform serial hemogram measurements. Another limitation is the absence of tumor necrosis factor and interleukin-6 levels, which we could not test in the emergency department.

CONCLUSION

Our study showed that ABCD2, ABCD3I, and C-reactive protein (CRP) effectively predict 90-day stroke after TIA. Furthermore, CRP was more effective than ABCD2 and ABCD3I in predicting 90-day stroke after TIA. CRP, NLR, PLR, and SII also effectively predicted long-term stroke after TIA.

Authors' Contribution

Study Conception: İK, SG; Study Design: İK, SG; Supervision: İK, SG; Funding: İK, SG; Materials: İK, SG; Data Collection and/or Processing: İK, SG; Statistical Analysis and/or Data Interpretation: SG; Literature Review: İK, SG; Manuscript Preparation: İK, SG and Critical Review: İK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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Effects of embryo characteristics in frozen-thawed single euploid blastocyst transfers on pregnancy outcomes: a retrospective cohort study

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ABSTRACT

Objectives: Our study examined the effects of the trophectoderm biopsy (TB) day and the presence of necrotic foci (NF) or separate blastomeres (SB) within euploid embryos on in vitro fertilization (IVF) pregnancy outcomes.

Methods: This retrospective cohort study was conducted from January 2017 to September 2021 at Memorial Sisli Hospital, Istanbul, Turkey. The study comprised a total of 2758 frozen-thawed euploid embryo transfer cycles. After thawing, blastocysts were graded using Gardner's classification Top-Quality (TQ), Good-Quality (GQ), Moderate-Quality (MQ), Poor-Quality (PQ) and further divided into groups according to the presence of NF and/or SB and evaluated for pregnancy outcomes.

Results: There were significant correlations between pregnancy outcomes and the degree of blastocoele expansion, as well as the presence of NF or SB in the euploid embryo. Ongoing pregnancy rates were lower in the group with NF in the inner cell mass (ICM) or trophectoderm (TE) than in the group without NF. The presence of SB decreased the rates of ongoing pregnancy and increased the rates of miscarriage. Embryos with expansion grades ≤ 3 had lower rates of ongoing pregnancy and higher rates of miscarriage compared to embryos with expansion grades > 3. TQ and GQ embryos had a higher rate of ongoing pregnancy and a lower rate of miscarriage than MQ and PQ embryos.

Conclusions: When selecting the embryo to be transferred to a patient, careful consideration should be given to the morphological grade of the embryo as well as whether or not it contains NF and SB.

Keywords: Necrotic foci, separate blastomeres, blastocyst morphology, pregnancy, miscarriage

Embryo aneuploidy is one of the most important reasons, causing in vitro fertilisation (IVF) failure [1]. Selecting chromosomally healthy embryos using preimplantation genetic testing for aneuploidies (PGT-A) programmes is very important for a healthy pregnancy [2-4]. However, choosing the best single euploid embryo to transfer is still challenging if the patient has more than one. On the other hand, selecting an embryo depending on ploidy alone does not guarantee a live birth. Therefore, embryo implantation fail-

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ure and pregnancy losses may still occur, although a euploid embryo was selected for transfer and other risk factors were eliminated [5-7].

The effect of blastocyst morphology on pregnancy outcomes is recognized, yet additional research is required to explore the influence of necrotic foci (NF) and/or separate blastomeres (SB) within the embryo on these outcomes. During embryo development, some of the blastomeres are observed to be excluded from the embryo. These separate blastomeres cannot integrate into either the trophectoderm (TE) or inner cell mass (ICM) as the embryo reaches the blastocyst stage, and they remain within the perivitelline space or blastocoel cavity. These blastomeres that are not included display disorganized or reduced expression of gap junction protein. It has been suggested that embryos employ apoptotic pathways to autonomously eliminate abnormal cells or fragments autonomously, thereby initiating a self-correction mechanism [8]. Lagalla *et al*. [9] in 2020, based on the results of chromosome analysis from biopsied embryos in the trophectoderm and separate blastomeres of the same embryo, suggested that separate blastomeres are a possible self-correction mechanism aimed at removing aneuploid cells from mosaic embryos and thus reducing or eliminating aneuploid load [9]. There have been limited studies investigating the effect of separate blastomere (SB) existence in blastocysts on IVF outcomes in the literature [9-11]. Blastocoele expansion degree has been studied in previous studies and was found to be related to higher implantation, ongoing pregnancy and live birth rates in both fresh and frozen embryo transfer cycles [12-14].

This study aimed to investigate whether the presence of necrotic foci (NF) or the SB, the blastocoele expansion degree, and the day of trophectoderm biopsy (TB) affect the pregnancy outcomes of euploid, frozen-thawed embryo transfer (FET) cycles.

METHODS

Patient Selection

A total of 2758 euploid FET cycles were included into this single-centered, retrospective cohort study that was conducted at Memorial Şişli Hospital, Istanbul, Turkey, between January 2017 and September 2021. Patients with ovarian, endometrial, or uterine abnormalities (Müllerian anomalies, severe endometriosis/adenomyosis, Asherman's syndrome, thin endometrium (< 7 mm) were excluded from the study.

Controlled Ovarian Stimulation (COS) Protocol

Gonadotropin-releasing hormone (GnRH) antagonist protocols were used in most of the patients (n = 2642, 95.8%). Others were administered long-stop protocol (GnRH agonist + antagonist) (n = 59), letrozole (n = 50), clomiphene citrate (n = 4), and in a few of the patients (n = 3) natural or semi-natural protocols. To stimulate the ovaries, recombinant follicle-stimulating hormone (rFSH) (Gonalf, Merck, Switzerland) or a combination of rFSH and recombinant luteinizing hormone (Pergoveris, Merck, Switzerland) or human menopausal gonadotropin (Menogon, Ferring, Germany) were used. The starting dosages were determined depending on the individual features of each patient. The oocyte pickup (OPU) was performed 36 hours following the administration of 250 mcg of recombinant human chorionic gonadotropin (rhCG) (Ovitrelle; Merck, Switzerland) or a GnRH analog (Lucrin; Abbott Laboratories, USA), by transvaginal ultrasound guidance.

Embryo Culture and Morphology Assessment

Embryos were cultured in single-step IVF medium (LifeGlobal, Cooper Surgical, Brussels, Belgium) for 5-6 days at 6% CO2, 5% O2, 37 ˚C, with pH 7.26-7.30 until embryo biopsy. On day 3, the culture medium was refreshed. Prior to vitrification and subsequent thawing, blastocysts were evaluated based on Gardner's classification and categorized into distinct groups based on their quality as follows; Top-Quality (TQ): Hatched AA, 6AA, 5AA, 4AA; Good-Quality (GQ): Hatched AB/BA/BB, 5AB/BA/BB, 4AB/BA/BB, 3AA; ModerateQuality (MQ): 3AB/BA, 2AA; and Poor-Quality (PQ): the rest of the embryos.

Embryo Biopsy and Genetic Analysis

A diode laser (RI Saturn 3, England) was utilized on the third day of embryo culture to create an artificial opening in the zona pellucida. This procedure aimed to promote the protrusion of trophectoderm cells following blastulation. A total of five and eight trophectoderm cells were extracted by the flicking technique, involving the use of a pipette with a 30 mm inner diameter (Origio, Denmark), which facilitated a mechanical incision. The ReproSeq kit (Ther-

moFisher, USA) was utilized for Next Generation Sequencing (NGS) in accordance with the manufacturer's guidelines. Initially, the PGM (Ion Personal Genome Machine, ThermoFisher, USA) was employed, followed by the S5 (ThermoFisher, USA) at a later stage. The genetic studies were conducted utilizing the Ion Reporter software package versions 5.2 and 5.6 (ThermoFisher, USA).

Embryo Freezing and Thawing Protocols

After the biopsy, the vitrification cryotops® procedure was performed with the Kitazato vitrification medium. Blastocysts were thawed in accordance with the manufacturer's instructions using Kitazato warming medium. 30 minutes and 2 hours after thawing, embryos were examined for viability. Each embryo was assessed for re-expansion and the presence of necrotic foci and separated blastomeres. Then, blastocysts that are eligible with at least 80% re-expansion and > 90% vitality were transferred.

Endometrial Preparation for Frozen-Thawed Euploid Embryo Transfer Cycles

All patients were evaluated by transvaginal ultrasonography on the second day of the menstrual cycle to check for ovarian, uterine and endometrial abnormalities. And FET cycles were started in cases where no pathology was detected. Patients who started endometrial preparation in mNC-FET cycle were checked 7-9 days after the first examination to determine the dominant follicle that developed spontaneously. When the dominant follicle reached 16-20 mm and LH levels were > 1 5 IU/L, r-hCG (Ovitrelle, Merck-Serono, Switzerland) was given. 2 days after hCG triggering, vaginal progesterone gel 1x1 (Crinone® Merck Serono, Switzerland) or vaginal progesterone tablets 2x1 (Lutinus® Ferring, Germany) were given as luteal phase support. The thawed embryo was transferred 5-6 days after the trigger. In the ERT-FET cycle, a 2 mg Estradiol tablet (Estrofem®, Novo Nordisk, Denmark) or a 3.9 mg Estradiol patch (Climara®, Bayer Turk, Turkey) was used for endometrial preparation. Luteal phase support was started after at least 12 days of Estradiol use and when the endometrium was > 8 mm. For this support, vaginal progesterone gel 2x1 (Crinone® Merck Serono, Switzerland) or vaginal progesterone tablets 2x2 (Lutinus® Ferring, Germany) were given. 9 days after embryo transfer, serum β-hCG was tested. Luteal phase support was given until the 10th week of gestation. At 7 weeks, patients were scheduled for ultrasonography and fetal heart rate was checked.

Ethical Approval

This stady was appoved by Istanbul Memorial Şişli Hospital Ethics Committee (approval number: 003, Date: 03.03.2023).

Statistical Analysis

The demographics and clinical characteristics of patients were evaluated using the t-test. The chi-square test was used for the analysis of frequencies. The logistic regression analysis was performed for the risk assessment by selecting dependent variables such as ongoing pregnancy and total miscarriage.

RESULTS

The demographic and clinical characteristics of patients were presented in Table 1. Semen samples were used for ICSI (93.5%) and most of embryos were biopsied, vitrified, and transferred on Day 5 (92.8%). The morphology of transferred euploid embryos was TQ 59.8%, GQ 33.4%, MQ 4.9%, PQ 1.8%, respectively (Table 1). After thawing, all viable euploid blastocysts were categorized into groups according to the presence of necrotic foci (Fig. 1), the presence of separate blastomeres (Fig. 2), the absence of necrotic foci or separate blastomeres (Fig. 3) and the degree of re-expansion (Fig. 4), and pregnancy outcomes were evaluated.

In the logistic regression analysis, significant correlations were found between pregnancy outcomes and blastocyst morphology, blastocoele expansion degree, the presence of NF or SB and day of biopsy in the euploid embryos (Table 2). TQ and GQ embryo grades showed a higher ongoing pregnancy rate and lower miscarriage rate than MQ and PQ embryos (ongoing pregnancy rates; TQ: 64.3%, GQ: 55.1%, MQ: 36.6%, PQ: 33.3%, total miscarriage rates; TQ: 18.3%, GQ: 21.5%, MQ: 32.9%, PQ: 36.6%). When pregnancy outcomes were compared according to blastocele expansion grade, ongoing pregnancy rates increased in embryo transfers with larger expansion grade (6: 61.4%, 5: 59.8%, 4: 59.3%, 3:38.3%, 2:

Table 1. Patient demographics and characteristics

n = 2758	Mean ± standard deviation	Minimum-maximum
Age (year)	36.85 ± 4.12	26.0-45.0
BMI (kg/m^2)	24.36 ± 4.34	13.8-42.3
AMH (ng/mL)	2.88 ± 2.40	0.01-22.0
Basal FSH	8.12-2.81	0.10-18.0
Infertility duration (year)	4.68 ± 3.95	2-25
Previous IVF cycles	4.84-2.81	2.0-26.0
Duration of stimulation (day)	9.07 ± 1.64	4.0-18.0
Daily gonadotropin doses	247.41 ± 70.95	87.50-725.0
Total gonadotropin doses	2246.61 ± 852.94	150.0-9450.0
Total oocyte	12.78 ± 8.14	1.0-58.0
MII oocyte (maturation%)	11.19 ± 7.03 (87.5%)	1.0-54.0
2PN (fertization %)	9.26 ± 6.02 (82.75%)	1.0-46.0
Sperm count (mill/mL)	14.18 ± 14.69	0.001-110.0
Sperm source for ICSI (%)		
Semen	2581 (93.5%)	
TESA	65 (2.4%)	
TESE	110 (4%)	
Embryo biopsy/ transfer day (%)		
Day 5	2561 (92.8%)	
Day 6	197 (7.1%)	
Embryo quality (%):		
TQ	1650 (59.8%)	
GQ	923 (33.4)	
MQ	134 (4.9%)	
PQ	51 (1.8%)	

Data are shown as mean ± standard deviation or number (percentage) or minimum-maximum. BMI = body mass index, AMH = anti-mullerian hormone, FSH = folliclestimulating hormone, MII = metaphase II, PN = pronucleus, TESA = testicular sperm aspiration, TESE = testicular sperm extraction, TQ = top-quality, GQ = good-quality, MQ = moderate-quality, PQ = poor-quality

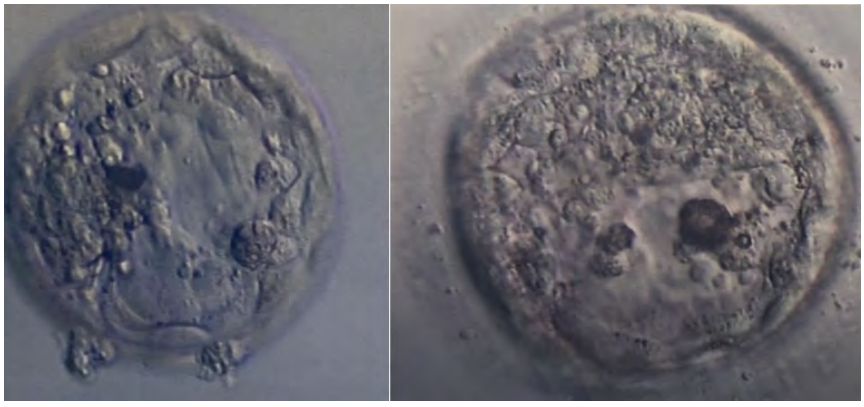

Fig. 1. Embryo containing necrotic foci.

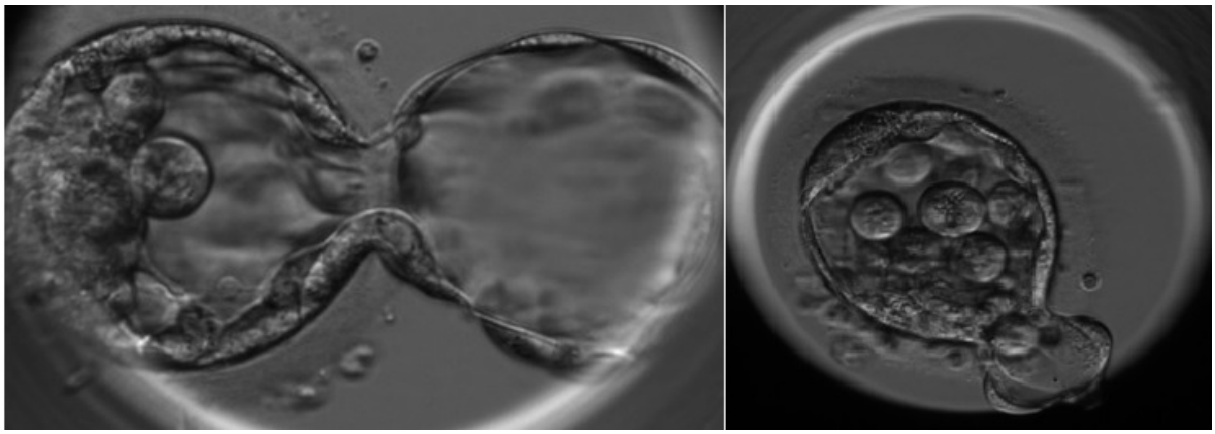

Fig. 2. **Embryo containing separated blastomeres.**

39.5%), while miscarriage rates increased in smaller expansion grades (6: 18.4%, 5: 21.2%, 4:21.0%, 3: 33.3%, 2: 35.0%). Embryos with expansion grades of ≤ 3 have lower ongoing pregnancy rates and higher miscarriage rates than embryos with expansion grades > 3.

The euploid embryos with the NF in the ICM or TE showed lower ongoing pregnancy rates than embryos without NF (negative vs. positive: 60.0% vs 41.0%, 59.9% vs 34.0%, respectively). The presence of SB in blastocysts were found to be associated with decreased ongoing pregnancy rates and increased miscarriage rates (negative vs. positive: 61.8% vs 50.3%, 18.8% vs 25.3%). Furthermore, a correlation was observed between the biopsy day and the rates of ongoing pregnancy and miscarriage. Consequently, euploid embryos that underwent biopsy and vitrification on Day 5 showed ongoing pregnancy rates and reduced miscarriage rates compared to those that underwent biopsy and vitrification on Day 6 (ongoing pregnancy rates for Day 5 vs. 6; 60.7% vs. 43.4%, miscarriage rates for Day 5 vs. 6; 19.6% vs. 25.9%) (Table 2).

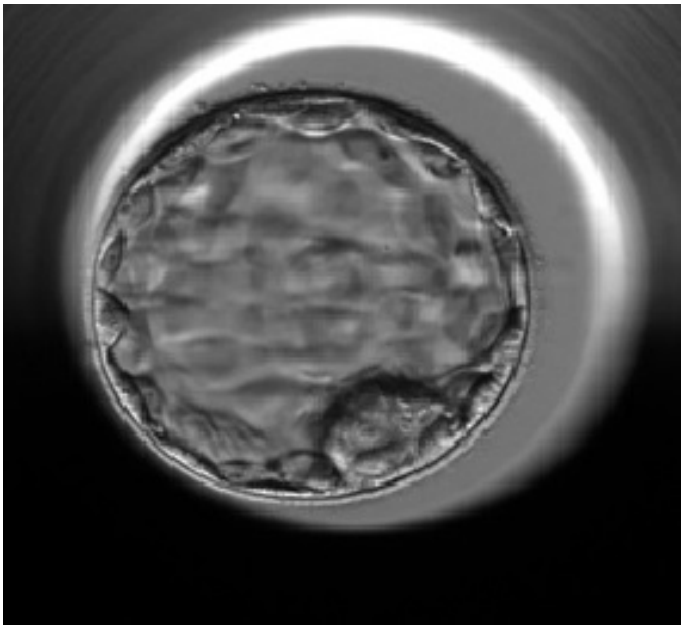

Fig. 3. **Embryo without necrotic foci or separate blastomeres.**

DISCUSSION

Even when a euploid embryo is chosen for transfer and other risk factors are eliminated, there can still be occurrences of implantation failure and pregnancy losses. In our study, we found that in addition to embryo morphology, the day of TB and the presence of separate blastomeres or necrotic foci in the embryo were effective on pregnancy outcomes. We have shown that embryos undergoing TB on day 6 have a lower continuing pregnancy rate and a higher abortion rate than embryos undergoing TB on day 5. Only a few publications in the literature claim the opposite of our study [15, 16]. Capalbo *et al*. [15] revealed that implantation rates did not differ between the group biopsied on day 5 and the group biopsied on day 6 (51.2% on day 5 vs. 48.8% on day 6). Similarly, in a smaller sample, Gonzalez *et al*. [16] showed no difference between the implantation rates of embryos undergoing TB on day 5 and day 6.

Numerous studies in the literature indicate similar results to our study on the effect of biopsy day on pregnancy rates [5, 17-20]. In a recently published

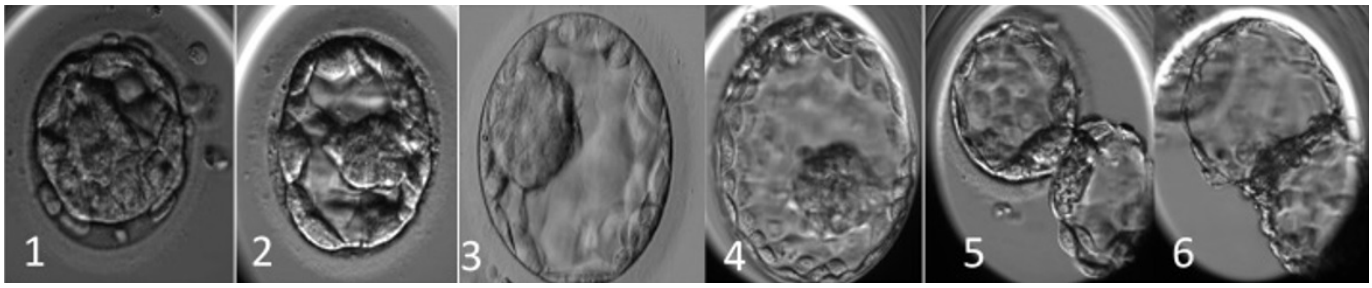

Fig. 4. **Embryos by grade of expansion.**

Table 2. **Logistic regression analysis of risk assessment of ongoing pregnancy and total miscarriage rates in euploid blastocyst transfer cycles**

Morphological features of blastocysts	No. of cycles (n = 2758)	Ongoing Pregnancy (%)	*p* value	Total Miscarriage (%)	*p* value
Blastocyst morphology					
TQ	1650	64.3%	**< 0.001**	18.3%	Ref.
GQ	923	55.1%	**< 0.001**	21.5%	0.10
MQ	134	39.6%	0.43	32.9%	**0.02**
PQ	51	33.3%	Ref.	36.1%	**0.01**
Day of biopsy					
Day 5	2561	60.7%	**< 0.001**	19.6%	Ref.
Day 6	197	43.4 %	Ref.	25.9 %	**0.004**
Presence of NF in ICM					
negative	2680	60.0%	**0.001**	19.8%	Ref.
positive	78	41.0%	Ref.	27.3%	**0.05**
Presence of NF in TE					
negative	2711	59.9%	**0.001**	19.7%	Ref.
positive	47	34%	Ref.	38.5%	**0.02**
Presence of SB					
negative	2201	61.8%	**< 0.001**	18.8%	Ref.
positive	557	50.3%	Ref.	25.3%	**0.004**
Degree of blastocoele expansion					
6	1415	61.4%	**0.005**	18.4%	Ref.
5	520	59.8%	**0.01**	21.2%	0.50
4	696	59.3%	**0.01**	21.0%	0.57
3	94	38.3%	0.89	33.3%	**0.01**
2	43	39.5%	Ref.	35.0%	**0.01**

TQ = top-quality, GQ = good-quality, MQ = moderate-quality, PQ = poor-quality, ICM = inner-cell mass, TE = trophectoderm, NF = necrotic foci, SB = separate blastomere

study, Li *et al*. [19] demonstrated that the live birth rate in embryo transfer cycles with biopsy on day 5 was considerably higher than in embryo transfer cycles with biopsy on day 6 (57.75% vs. 41.67%). Irani *et al*. [17] also showed that the live birth rate was 44.8% in embryo transfer cycles in which the biopsy was performed on day 6 and 60.4% in embryo transfer cycles in which the biopsy was performed on day 5. It is believed that aberrant gene expression in embryos or inappropriate oocyte cytoplasmic maturation contributes to the lower live birth rates of day 6 embryo transfers compared to day 5 embryos, which reach the blastocyst stage more slowly [21]. According to the findings of these studies and our own, if a patient has more than one euploid embryo, we recommend transferring the same-quality embryo biopsied on day 5.

Numerous studies in the academic literature investigate the correlation between embryo morphology and pregnancy outcomes. We have shown that TQ and GQ embryo transfers have higher ongoing pregnancy rates than MQ and PQ embryo transfers. Contrary to our findings, Capolbo *et al*. [15] concluded in a study involving 956 blastocysts that morphology did not influence pregnancy outcomes in euploid embryo transfer cycles. However, the study was limited by only including 13 embryo transfers of poor quality [15]. In a subsequent study with a larger study group comprising 103 PQ embryo transfers, they demonstrated that TQ and GQ blastocyst transfers had a twofold higher pregnancy rate and a twenty-fivefold lower miscarriage rate compared to PQ euploid embryo transfers and underscored the effectiveness of ICM in predicting pregnancy outcomes [17]. Similarly, in the study by Zhang *et al*. [22], it was reported that pregnancy rates were low, especially in the group with ICM/C. In another study involving 914 single euploid embryo transfers, it was shown that embryo morphology affected pregnancy outcomes, but ICM and TB morphology had similar effects. [23]. In contrast to these studies, in the study examining the results of 660 FET cycles with RPL history, it was shown that ICM in C did not affect the live birth and abortion rates, but, especially, TE C negatively affected the live birth rates.

The results of our study demonstrated that euploid embryos containing separate blastomeres exhibit lower ongoing pregnancy rates and higher abortion rates than those without separate blastomeres. A study by Shenoy *et al*. [24] showed that embryos with separate blastomeres at early or late stages of embryo development had higher aneuploidy rates (68% vs. 53%; $p < 0.001$. Coticchio *et al*. [10] showed that forming separate blastomeres before or after the morula stage in embryos without preimplantation genetic testing (PGT) negatively affected IVF success rates. In particular, they showed that an increase in the number of separate blastomeres leads to loss of embryo material, affecting the number of cells in the TE and ICM and ultimately affecting embryo viability and adversely affecting pregnancy outcomes. They also suggested that these findings should be considered when the embryos without PGT are selected using time-lapse culture systems [10]. Lagalla *et al*. [9] analyzed the results of 1271 PGT/A embryos, showing that separate blastomeres in embryos in the full and partial compaction stages did not affect aneuploidy and implantation rates. They stated that it is associated with abnormal division and represents a self-correction mechanism to eliminate aneuploid cells [9]. The hypothesis suggesting that separate blastomeres act as a self-correction mechanism to diminish or remove the impact of mosaicism presents potential opportunities for future research.

To the best of our knowledge, our study is the first examination of the influence of necrotic foci on pregnancy outcomes in euploid embryos. Necrosis and apoptosis are two distinct cell death processes with distinct morphological characteristics and biological significance. Following irreversible injury, necrosis involves swelling of cells and membrane rupture. The presence of necrotic foci in a blastocyst can be a concerning sign, indicating that some regions of the developing embryo have not received sufficient oxygen and nutrients, leading to cell damage. Few studies in the literature have examined the impact of necrotic foci on pregnancy outcomes in embryo transfer without PGT. Kovacic *et al*. [25] reported that necrotic foci negatively affect pregnancy outcomes, showing that the live birth rate was 45% in good-quality blastocysts without PGT and 32.8% in embryos with necrotic foci on ICM or TB. In contrast to this study, Ebner *et al*. [26] showed that the presence of necrotic foci had no effect on pregnancy outcomes in a small study group of 129 blastocysts without PGT. Our study differs from these studies in that it included only euploid embryos. In our study, we observed a decreased ongoing pregnancy rate (59% vs. 34%) and an increased mis-

carriage rate, especially in necrotic foci in trophectoderm cells. A systematic review by Wang *et al*. [27] supports our results, showing that the number and growth of viable blastomeres in freeze-thawed embryos are important indicators for the potential development of embryos and for predicting clinical outcomes related to implantation.

Limitations

The limitation of this study is its retrospective nature. We believe that our study will lead to further research and novel suggestions on this subject and more detailed studies on this subject should be conducted.

CONCLUSION

These results may provide important guidance on optimizing euploid embryo selection and transfer procedures and identifying better candidates to improve pregnancy success. When selecting the optimal embryo to be transferred in cases, it is important to consider both the embryo's morphological grading and the presence of NF and SB. Our findings also suggest that TB and cryopreservation on Day 5 are important for higher pregnancy outcomes.

Authors' Contribution

Study Conception: GÖzer; Study Design: GÖzer; Supervision: GÖzer; Funding: N/A; Materials: GÖzer; Data Collection and Processing: GÖzer; Statistical Analysis and Data Interpretation: GÖzer; Literature Review: GÖzer; Manuscript Preparation: GÖzer, GÖzkara and Critical Review: GÖzer, GÖzkara.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(5):1224-1231 Original Article

DOI: 10.18621/eurj.1342084 *Pediatric Intensive Care*

The severity of hyponatremia worsens the outcome in pediatric intensive care patients

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ABSTRACT

Objectives: Hyponatremia is known to increase mortality and morbidity in adult patients. However, the significance of hyponatremia in critically ill pediatric patients is unknown, unlike in adults. We tried to determine the prevalance of hyponatremia in critically ill children and whether the severity of hyponatremia contributes to hospital stay and mortality.

Methods: The results of 190 patients who met the inclusion criteria and were admitted to the pediatric intensive care unit between April 2014 and April 2017 were analyzed.

Results: Eighty-six (45.3%) patients developed hyponatremia at the time of hospitalization, and Hospital−Acquired Hyponatremia (HAH) developed in 46 (24.2%) patients during the hospitalization. Fifty-eight (30.5%) patients were normonatremic. The patients in the HAH group were significantly more septic ($p = 0.015$). The duration of intensive care hospitalization was significantly longer in the HAH group ($p < 0.001$) and significantly less in the normonatremic group ($p = 0.008$). Total mortality was 41% (n = 78). There was no difference between the groups regarding mortality ($p = 0.4$). However, the degree of hyponatremia was associated with mortality. Mortality was 24.1% in mildly hyponatremic patients, 45.6% in moderate patients, and 58.8% in severe patients (OR: 2.636, 95% CI: 1.189-5.842; OR: 4.490, 95% CI:1.439-14.008, $p = 0.01$). We discovered that as hyponatremia severity increased, so did the length of stay in the intensive care unit, the need for invasive ventilation, and the need for vasoactive drugs ($p = 0.009$, $p = 0.018$, and $p = 0.006$, respectively).

Conclusions: Unlike adults, the prognostic value of hyponatremia in terms of mortality has not been determined in critically ill children. However, as the severity of hyponatremia increased, it was seen that the length of stay in the intensive care unit and mortality increased.

Keywords: Children, critically ill patients, hospital−acquired hyponatremia, hyponatremia, mortality, severity of hyponatremia

The most common electrolyte imbalance in hospitalized patients is sodium-related electrolyte imbalance [1]. Hospital-acquired hyponatremia was a cause of morbidity and mortality for decades, with the use of hypotonic fluids recommended by Holiday and Segar [1] in 1957 as intravenous maintenance fluid,

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considering the sodium content in breast milk. In accordance with the scientific data of the time, patients were given 77 mEq/L sodium-containing fluid as maintenance fluid during the study [2, 3]. However, we found that hyponatremia is a common problem in hospitalized patients. As a result of dozens of controlled studies, the UK's NICE guideline published in 2017 [4] and the American Academy of Pediatrics Clinical Practice Guidelines published in 2018 recommend isotonic intravenous fluids for maintenance fluid therapy [5].

Hyponatremia is a serum sodium (Na) level below 135 mEq/L. According to the published data, the incidence of hyponatremia in pediatric patients can increase up to 60% at admission to the hospital [6, 7]. The incidence of Hospital−acquired Hyponatremia (HAH) in children is 19.6% [6]. Symptoms of hyponatremia can range from non-specific findings such as restlessness, agitation, headache, and weakness that may be overlooked to serious neurological symptoms such as seizures, confusion, and coma [7]. The symptoms of hyponatremia are determined by the severity and duration of hyponatremia.

In adult patients, hyponatremia is thought to be an independent predictor of mortality [8-10]. In the literature, studies report that hyponatremia increases mortality, but mortality decreases as the severity of hyponatremia worsens [11, 12]. In previous studies, hyponatremia has been evaluated either at hospital admission or in hospital-acquired patient groups. It is a study conducted to eliminate an important information gap in the pediatric patient group. We also aimed to investigate the effects of severity of hyponatremia on the length of stay in the intensive care unit and mortality.

METHODS

The study was initiated after the approval of the Gaziantep University clinical research ethics committee (Decision No: 166). The files of all patients admitted to Gaziantep University Faculty of Medicine's 7-bed tertiary pediatric intensive care unit between April 2014 and April 2017 were reviewed retrospectively. During the current period, 247 patients were hospitalized in our unit, and 34 patients were excluded because they were hypernatremic (Na > 145 mEq/L) at the time of admission. Nine hyperlipidemic and 5 hyperglycemic patients were considered pseudohyponatremic and excluded from the study. The first admission was accepted for patients with recurrent hospitalizations during the study, thus 9 more patients were excluded from the study. The study was initiated with 190 patients. The patients were divided into three groups: Hyponatremia group (patients with hyponatremia at admission hospitalization), HAH group (patients without hyponatremia at admission but with hyponatremia during hospitalization), normonatremia group (patients without hyponatremia during admission and hospitalization).

Plasma sodium levels below 135 mEq/L were defined as hyponatremia. Patients were also grouped according to the severity of hyponatremia as mild (130-134 mEq/L), moderate (125-129 mEq/L) and severe (below 125 mEq/L). The underlying major diagnostic categories were grouped as: Respiratory, cardiac, neurologic, hematological, nephrological, hepatic, trauma, endocrinological and intoxication. As a mortality indicator, the pediatric risk of mortality III (PRISM III) score was used, which was calculated within the first 24 hours of intensive care admission [13].

The medical records of all patients were reviewed, and age, sex, PRISM III scores, cause of ICU hospitalization, need for mechanical ventilation, need for vasoactive medication, presence of sepsis, presence of hyponatremia during the ICU hospitalization, the severity of hyponatremia, and outcome of the patients were noted.

Statistical Analysis

Data were analyzed using SPSS version 22.0. Data were expressed as the means ±standard deviation for normally distributed variables and as the median with interquartile range for non-normally distributed data. Categorical data were expressed as numbers and percentages. Patients with and without hyponatremia during admission to the intensive care unit were compared using the chi-square test for categorical variables. Student's t−test or ANOVA was used to compare the association between groups for continuous data following normal distribution; otherwise, Mann–Whitney U−test was used. Kruskal-Wallis was used as a non-parametric test in multi-group comparison. $P < 0.05$ was considered statistically significant.

RESULTS

The study included 190 patients who met the inclusion criteria among the 247 patients hospitalized between April 2014 and April 2017. At admission, 86 (45.3%) patients had hyponatremia (serum Na = 130 mEq/L [range: 126-132 130 mEq/L]). When patients with hyponatremia at admission and patients who were normonatremic on admission were compared, no statistically significant difference was found between the two groups in terms of respiratory failure (p = 0.11). The hyponatremic group at admission was associated with significantly less mechanical ventilator need compared to the normonatremic group on admission (OR: 0.377, 95% CI: 0.195-0.731; p = 0.005,) (Table 1). The mean age of the hyponatremic group and the normonatremic group at admission was 46 months (12.7- 120) and 20.5 (8-84) months. Although no statistically significant difference was found in age between the groups, the mean age of the normonatremic group was significantly lower (p = 0.08). The need for mechanical ventilation in normonatremic patients was thought to increase due to a decrease in tolerance to respiratory failure due to anatomical and physiological reasons in the respiratory tract as age decreased. Although there was no statistically significant difference between the groups in terms of PRISM scores, the PRISM values of the hyponatremic group were lower than the normothermic group (p = 0.056) (Table 1).

Acquired hyponatremia developed in 46 (24.2%) patients during the course of ICU hospitalization (HAH group). Patients in the HAH group were significantly more septic (OR: 2.571, 95% CI: 1.185-5.581; p = 0.015,). Hyponatremia never seen in 58 (30.5%) patients, and this patient group was defined as normonatremia. There was no statistical difference between the groups in terms of age and sex. On the other hand, there was no significant difference in the septic picture in the normonatremia and hyponatremia groups (p = 0.25 and p = 0.36, respectively). While the length of ICU stay was significantly longer in the HAH group and significantly shorter in normonatremic patients, no significant difference was observed in hyponatremic patients at admission (p = 0.000, p = 0.008 and p = 0.524, respectively). Total mortality was 41% (n =78). There was no difference between the groups regarding mortality (p = 0.4) (Table 2).

Table 1. Baseline characteristics of the patients at admissions

	Hyponatremia at admission (n = 86)	Normonatremia at admission (n = 104)	*p value*
Age (month)	46 (12.7-120)	20.5 (8-84)	0.08
Gender, n (%)			0.25
Male	45 (52.3)	63 (60.6)	
Female	41 (47.7)	41 (39.4)	
Serum sodium level (mmol/L)	130 (126-132)	140 (136-141)	-
Respiratory failure, n (%)	66 (76.7)	89 (85.6)	0.11
Need for MV, n (%)	54 (62.8)	85 (81.7)	**0.005**
Need for inotropes, n (%)	47 (54.6)	58 (55.8)	0.88
CRRT, n (%)	21 (24.4)	18 (17.3)	0.27
PRISM III	15 (10-20.2)	18 (12-24)	0.056
Length of PICU stay (day)	9 (5-17)	10 (5-17.7)	0.52
Diagnosis of sepsis, n (%)	51 (59.3)	69 (66.3)	0.36 [a]
Mortality, n (%)	33 (38.4)	45 (43.3)	0.49

Data are shown as median with interquartile range (25-75) or number (percent). CRRT = Continuous renal replacement therapy MV = Mechanical ventilation, PICU = pediatric intensive care unit, PRISM = pediatric risk of mortality

Table 2. Comparison of etiological characteristics and outcome in study groups

	Hyponatremia at admission (n = 86)	Hospital-acquired hyponatremia (n = 46)	Normonatremia (n = 58)	*p value*
Age (month)	46 (12.75-120)	18 (6.75-93)	24 (8-87)	0.22
Age group, n (%)				0.19
1-12 month	21 (24.4)	19 (41.3)	24 (41.4)	
12-60 month	27 (31.4)	12 (26.1)	14 (24.1)	
> 60 months	38 (44.2)	15 (32.6)	20 (34.5)	
Gender, n (%)				0.49
Male	45 (52.3)	27 (58.7)	36 (62.1)	
Female	41 (47.7)	19 (41.3)	22 (37.9)	
Respiratory failure, n (%)	66 (76.7)	39 (84.8)	50 (86.2)	0.29
Need for MV, n (%)	54 (62.8)	37 (80.4)	48 (82.7)	**0.013**
Need for vasoactive drugs, n (%)	47 (54.6)	28 (60.9)	30 (51.7)	0.64
Length of hospital stay (day)	9 (5-17)	17 (9-29.2)	8 (4-14.2)	**0.001**
Sepsis n (%)	51 (59.3)	36 (78,3)	33 (56.9)	**0.049**
Major diagnostic category, n (%)				0.17
Respiratory	10 (11.6)	7 (15.2)	7 (12.1)	
Cardiac	15 (17.4)	10 (21.7)	14 (24.1)	
Neurological	14 (16.3)	10 (21.7)	13 (22.4)	
Hematological	19 (22.1)	3 (6.5)	5 (8.6)	
Nephrological	21 (24.4)	9 (19.6)	6 (10.3)	
Hepatic	2 (2.3)	1 (2.2)	1 (1.7)	
Trauma	1 (1.2)	1 (2.2)	3 (5.8)	
Endocrinological	4 (4.6)	3 (6.5)	5 (8.6)	
Intoxication	0 (0.0)	2 (4.3)	4 (6.9)	
Mortality, n (%)	33 (38.4)	17 (36.9)	28 (48.3)	0.4

Data are shown as median with interquartile range (25-75) or number (percent). MV = Mechanical ventilation, PICU = pediatric intensive care unit

However, the severity of hyponatremia was associated with mortality. Mortality was 24.1% in mildly hyponatremic patients, 45.6% in moderate patients, and 58.8% in severe patients (OR: 2.636, 95% CI:1.189-5.842; OR: 4.490, 95% CI:1.439-14.008, $p = 0.01$). We found that as the severity of hyponatremia increased, the length of stay in the intensive care unit, the need for invasive ventilation, and the need for vasoactive medications increased ($p = 0.009$, $p = 0.018$ and $p = 0.006$, respectively) (Table 3).

Sepsis, need for invasive mechanical ventilation, need for vasopressor, and need for CRRT were significantly higher in the deceased group than in the survived group ($p = 0.001$, $p = 0.001$, $p = 0.01$ and $p = 0.029$, respectively). However, there was no difference in terms of mortality between the groups. We found that as the severity of hyponatremia increased, the length of stay in the intensive care unit, the need for invasive ventilation, vasoactive drugs, and even mortality increased.

Table 3. Association of hyponatremia severity with management and outcomes of children in PICU

	Severity of hyponatremia			
	130-135 (mmol/L) (n = 58)	**125-130 (mmol/L) (n = 57)**	**< 125 (mmol/L) (n = 17)**	***p value***
Gender, n (%)				0.11
Male	27 (45.6)	37 (64.9)	8 (47.1)	
Female	31 (53.4)	20 (35.1)	9 (52.9)	
Age category, n (%)				0.81
1-12 months	17 (29.3)	16 (28.1)	7 (23.5)	
12-60 months	18 (31)	16 (28.1)	5 (29.4)	
> 60 months	23 (39.6)	25 (43.8)	5 (29.4)	
Length of PICU stay (day)	8.5 (5-14.7)	14 (7-25.5)	13 (8-32)	**0.009**
Need for MV n (%)	33 (56.9)	43 (75.4)	15 (88.2)	**0.018**
Need for vasoactive drugs, n (%)	24 (41.4)	39 (68.4)	12 (70.6)	**0.006**
Major diagnostic category, n (%)				0.47
Respiratory	8 813.8)	7 (12.3)	2 (11.8)	
Cardiac	7 (12.1)	16 (28.1)	2 (11.8)	
Neurological	11 (19)	9 (15.8)	4 (23.5)	
Hematological	8 (13.8)	12 (21.1)	2 (11.8)	
Nephrological	14 (24.1)	11 (19.3)	5 (29.4)	
Hepatic	2 (3.4)	1 (1.7)	0 (0.0)	
Trauma	2 (3.4)	0 (0.0)	0 (0.0)	
Endocrinological	4 (6.9)	1 (1.7)	2 (11.8)	
Intoxication	2 (3.4)	0 (0.0)	0 (0.0)	
Mortality n (%)	14 (24.1)	26 (45.6)	10 (58.8)	**0.01**

Data are shown as median with interquartile range (25-75) or number (percent). MV = Mechanical ventilation, PICU = pediatric intensive care unit

DISCUSSION

Holliday and Segar [1] proposed a theoretical approach to the maintenance fluid's content and calculation 1957. They stated that hypotonic fluids approaching the sodium and potassium concentrations of breast or cow's milk could meet acutely ill children's water and electrolyte needs. In 1992, a seminal study was published in the British Medical Journal in which 15 of 16 previously healthy pediatric patients were treated with hypotonic fluids post-operatively; 15 died, and the remaining patient suffered permanent brain damage caused by hyponatremia [14]. It was determined that increased ADH due to non-osmotic reasons such as postoperative condition, pain, anxiety, stress, pneumonia, and meningitis in hospitalized patients led to inappropriate ADH release syndrome (SIADH) [15, 16]. Hyponatremia, which developed especially in hospitalized patients, was most often associated with the use of hypotonic fluids [17]. In accordance with the scientific data of the time, patients were given 77 mEq/L sodium-containing fluid as maintenance fluid during the study [2, 3]. Despite this, we discovered that hyponatremia is a common problem in hospitalized patients in our clinic.

Eighty-six of our patients (45.3%) were hypona-

tremic at admission. Forty-six (24.2%) patients who were not hyponatremic at the beginning became hyponatremic during the course. One of the very few studies conducted on pediatric intensive care patients is the study of Bibi *et al*. [18] in Pakistan. In their study, 865 pediatric intensive care patients were evaluated in a tertiary university hospital; 405 (46.8%) patients had hyponatremia at admission, while hospital-acquired hyponatremia was observed in 240 (27.7%) patients [18]. It is compatible with our cohort. When patients with hyponatremia at admission and patients who were normonatremic on admission were compared, no statistically significant difference was found between the two groups in terms of respiratory failure (p = 0.11). In a study comparing hyponatremic and normonatremic patients with bronchiolitis who needed PICU admission, it was found that there was no difference between the two groups in terms of the need for intubation [19]. In our study, interestingly, the hyponatremic group had significantly less need for mechanical ventilators at admission than the normonatremic group.

Unsurprisingly, untreated, rapidly developing severe hyponatremia results in death. The publications about mild-moderate hyponatremia increasing mortality in adult patients, which was previously thought to be almost harmless, are increasing daily [20]. In a seven-year study of over 53,000 hospitalized adult patients, aggravated hyponatremia in the hospital was independently associated with increased mortality [20]. Although there are studies specific to disease subgroups in the adult age group, we realized there were not enough studies on pediatric patients that we treated with hypotonic maintenance fluids for decades. We investigated the impact of hyponatremia on mortality in our clinic, where we could only accept critically ill patients with high PRISM scores due to bed constraints.

Gill *et al*. [21], who evaluated adult patients with serum sodium levels below 125 mEq/L as hyponatremic and compared them with normonatremic patients, found that mortality increased with the deepening of hyponatremia. However, although the threshold value for hyponatremia was accepted as < 125 mEq/L, like Gill *et al*. [21], Chawla *et al*. [11] found the highest mortality rate in patients with sodium values between 120-124 mEq/L, they reported a lower mortality rate of hyponatremia level was less or more severe. They showed it with a parabolic radiograph [11]. They reported that the disparity was due to the nature of the deceased patients' underlying diseases.

Studies on hyponatremia reported that children generally depend on the hypotonic maintenance fluids used. Price *et al*. [22] found the frequency of HAH to be 75% in their study of children with heart failure. All 13 patients with severe hyponatremia either died or required mechanical circulatory support. Wald *et al*. [20] published a study reporting that even mild hyponatremia (1 mEq/L decrease in serum Na) increased in-hospital mortality by 2.3%. They noticed that the relationship between Na level and mortality graph has a U shape, with the lowest death rate in the study being at the 140 mEq/L serum Na level [20]. According to this study, the more severe the hyponatremia, the higher the mortality rate [20]. In this retrospective study on the patients admitted to our pediatric intensive care unit, we discovered that mortality increased as the hyponatremia severity increased. Mortality was 24% in mild hyponatremia, 45.6% in moderate hyponatremia, and 58.8% in severe hyponatremia.

Most studies predict a longer hospital stay in hyponatremic patients [6, 20, 23]. Consistent with the literature, the length of hospital stay of normonatremic patients was significantly lower in our study compared to the other two groups ($p = 0.008$).

In the study conducted on 168 hospitalized severely hyponatremic (< 115 mEq/L) patients, sepsis was highlighted as one of the independent predictors for mortality in multivariate analysis [24]. Shimoyama *et al*. [25] discovered hyponatremia was associated with 28-day mortality in their study of 83 hyponatremic intensive care patients. However, hyponatremia was not associated with mortality in critically ill patients with sepsis [25]. Our study found that the frequency of sepsis increased in the HAH group, the need for longer intensive care unit stay was longer, and we found no increase in mortality. These data are consistent with the existing literature.

Despite many studies emphasizing that hyponatremia is associated with an increased risk of death in adult patients in different disease groups, whether hyponatremia is an indicator of the severity of the underlying comorbidities is still a matter of debate [8, 26, 27]. Luu *et al*. [19] reported that pediatric patients with hyponatremic bronchiolitis admitted to the PICU had worse outcomes and significantly increased mortality

compared to normonatremic patients. However, when the study was reviewed in detail, it was seen that 3 (13%) patients who died had comorbidities (metastatic rhabdoid tumor in the first patient, heart failure and severe respiratory failure in the second patient, chronic lung disease and multi-organ failure in the third patient) that caused a high risk of death [19]. In our opinion, comparing mortality with the patient group with only bronchiolitis was not fair. The cause of the significant increase in mortality in the hyponatremia group was thought to be related to the severity of the patients' underlying diseases. In a study by Sachdev *et al*. [6] in India, in which the HAH group and isonatremic control group in PICU were evaluated, no difference was found in terms of mortality in HAH cases, despite the need for prolonged PICU hospitalization and more mechanical ventilation.

Limitations

The limitations of our study are as follows. Our study is a single-centered retrospective study. In accordance with the scientific data of the time, patients were given 77 mEq/L sodium-containing fluid as maintenance fluid during the study. It does not include the patient group in which isotonic fluids are used after the 2018 AAP guidelines recommendations. Furthermore, due to the study's retrospective nature, the amount of fluid intake and sodium content of the patients prior to ICU admission were unknown.

CONCLUSION

In conclusion, the main finding of this study is that mortality increases with the severity of hyponatremia. However, hyponatremia alone is not associated with increased mortality in critically ill pediatric patients. ospital-acquired hyponatremia is associated with a longer intensive care stay and is more common in patients with sepsis. More prospective studies are needed to separate the underlying disease groups one by one and evaluate each factor that may contribute to mortality separately before it can be said that hyponatremia increases mortality in pediatric patients.

Authors' Contribution

Study Conception: MB, AO; Study Design: AO, MB; Supervision: SE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: AO; Statistical Analysis and/or Data Interpretation: AO, SE, MB; Literature Review: SE; Manuscript Preparation: AO and Critical Review: AO, MB, SE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The effect of achieving guideline-based target low-density lipoprotein cholesterol levels on mortality in transcatheter aortic valve implantation patients with coronary artery disease

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ABSTRACT

Objectives: The aim of this retrospective study was to evaluate the effects of bringing low-density lipoprotein cholesterol (LDL-C) values to levels in line with guideline recommendations on long-term mortality in patients with a known history of coronary artery disease (CAD), undergoing transcatheter aortic valve implantation (TAVI), and long-term pre-treatment with statins.

Methods: This is a retrospective and observational study of patients undergoing TAVI at a tertiary heart center with a history of CAD and long-term statin therapy. Ninety-nine patients were included in the study. The relationship between LDL-C levels in accordance with the guidelines and 5-year mortality was determined by regression analysis.

Results: When the study population was divided into 2 groups with and without 5-year mortality, LDL-C values were found to be significantly higher in the mortality group (120 mg/dL vs. 93.9 mg/dL, $p < 0.001$). Parameters associated with the development of 5-year mortality were evaluated with univariate and multivariate logistic regression analysis. LDL-C ≥ 100 mg/dL (OR: 6.59, 95% CI: 2.17-20.01) and LDL-C ≥ 70 mg/dL (OR:3.88, 95% CI: 1.16-12.93) parameters were determined as independent predictors of mortality independent of other parameters.

Conclusions: The most important result obtained in this study is that achieving the LDL-C level targets specified in the guidelines significantly reduces the in-hospital and 5-year mortality rates in patients with a previous history of CAD and statin use and undergoing TAVI. Although all patients included in the study used statins, the mortality rate was significantly higher in patients who did not reach the target LDL-C value.

Keywords: Transcatheter aortic valve implantation, low-density lipoprotein cholesterol, statin, coronary artery disease

Severe aortic stenosis (AS) may result from rheumatic heart disease or, more commonly, from calcification of the congenital bicuspid or three-leaf aortic valve [1]. The prevalence of AS increases with age, while it is around 0.2% at younger ages, it rises to 9.8% after the age of 80 [1]. AS is the most common

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indication for surgical heart valve replacement in many countries. In patients who are not suitable for surgery, transcatheter aortic valve implantation (TAVI) is now an established and safe treatment option that continues to evolve [2, 3].

Despite its minimally invasive nature, TAVI is always associated with numerous complications that may affect outcomes in elderly patients. It is very important to identify patients likely to benefit from TAVI. Although procedural outcomes have improved significantly thanks to increased operator experience and progressive improvements in TAVI devices, to date there is no satisfactory TAVI risk score that can determine individual prognosis in patients undergoing TAVI (post-TAVI patients). Studies are continuing to develop new risk score systems specific to TAVI in order to predict and reduce complications. To date, the prognostic significance of various clinical and laboratory parameters such as chronic pulmonary obstructive disease, chronic kidney disease, frailty, pulmonary hypertension, serum albumin levels, and red cell distribution width (RDW) have been demonstrated in patients undergoing TAVI [4-6].

The coexistence of AS and coronary artery disease (CAD) is frequently observed [7]. The coexistence of these two diseases can be attributed to similar risk factors and pathophysiology of the diseases [8-10]. The prevalence of CAD in severe AS ranges from 30% to 50% and increases with age [11, 12].

Statins are widely used for primary and secondary prevention of atherosclerotic cardiovascular diseases and coronary artery disease and have been shown to be associated with lower mortality rates. In addition to their lipid-lowering properties, statins have been shown to have pleotropic effects such as improving endothelial function and anti-inflammatory effects [13]. In a previous multicenter study, statin therapy was shown to reduce mortality in TAVI patients. It was found to be more effective on mortality, especially in patients with a history of coronary artery disease (CAD) and receiving treatment [14].

It has been shown in many previous randomized studies that mortality is reduced by lowering low-density lipoprotein cholesterol (LDL-C) values with statin therapy in patients with CAD [15]. In this retrospective study, we aimed to investigate the effect of bringing LDL-C values to the recommended levels in the guidelines on long-term mortality in patients with a history of CAD who underwent TAVI for severe AS and long-term use of statins.

METHODS

Study Population

This is a retrospective and observational study of patients undergoing TAVI at a tertiary heart center with a history of CAD and long-term statin therapy. All patients who met the criteria from January 2014 to January 2018 were consecutively included in the study.

The inclusion criteria used to enroll patients in the study are: (1) patients are between 20 and 90 years old, (2) having been on statin therapy for at least 6 months, (3) patients who underwent TAVI for severe aortic stenosis (4) patients who had undergone coronary artery bypass graft or percutaneous coronary intervention for severe coronary lesion before TAVI.The exclusion criteria used in the study were: (1) evidence of acute or chronic infection, (2) systemic inflammatory or autoimmune disease, (3) any history of liver disease (more than three times the upper limit of normal for liver function tests), (4) clinically any endocrine, hematological, or metabolic disease found to be significant, (5) malignancy, (6) missing clinical data for LDL-C values. 99 patients who met the current criteria were included in the study. All patients had been on statin therapy for at least 6 months and had a known history of CAD. Clinical and laboratory data of all patients were obtained from the electronic database of our hospital. The patients were scanned from the hospital database and divided into two groups according to their 5-year mortality data (mortality [+] and mortality [-] groups). Demographic characteristics, comorbidities, and laboratory characteristics of the patients were compared between those two groups. In addition, the procedural conditions of the patients were compared according to their LDL-C values (LDL-C < 100 mg/dL and LDL-C ≥ 100 mg/dL) (preprocedural, procedural, and procedural complications).

Informed consent was obtained for all cases prior to the TAVI procedure. The selection of patients with severe symptomatic AS was based on expected perioperative or short-term mortality estimated from the risk model of the European system for the cardiac operative risk assessment II (euroSCORE II) algorithm. All patients were evaluated by the multidisciplinary

heart team before the TAVI operation. Patients with severe AS were considered eligible for TAVI after they were determined to be at high or very high risk for cardiac surgery. Valve Academic Research Consortium 2 (VARC-2) criteria were used to define procedural complications [16].

Laboratory Analysis

In this study, blood samples showing hemoglobin values, white blood cell values, platelet values, and blood glucose levels were measured at admission in all patients included in the study. Low-density lipoprotein cholesterol levels were determined after 8 to 12 hours of night fasting. eGFR was calculated according to the Modification of Diet in Renal Disease formula (eGFR [mL/min/1.73 m2] = 186 × [creatinine/88.4]-1.154 × [age]- 0.203 × [0.742 female, 1.210 black]) [17].

Definitions

The term in-hospital mortality was used for deaths occurring after the procedure until the patients were discharged. The hospital's electronic database was used for 5-year mortality data. In our study, the definition of CAD included patients who had undergone previous percutaneous coronary intervention or coronary artery bypass grafting due to severe and ischemia-causing coronary artery disease. Fasting blood glucose monitoring meeting the criteria of the American Diabetes Association or using oral antidiabetic or insulin were accepted as diagnostic criteria for diabetes mellitus [18]. In the definition of stroke, transient ischemic attack (TIA) was defined if symptoms included neurologic deficit for < 24 hours. If neurological symptoms lasting longer than 24 hours were present, it was considered a stroke.

Ethics Committee Approval

All the procedures in this study including human participants were applied in compliance with the ethical standards of the institutional research committee and the 1964 Helsinki Declaration and subsequent revisions or comparable ethical standards. No animals were used in this study. Approval for the study was granted by the Local Ethics Committee (Bursa Yüksek İhtisas Training and Research Clinical Research Ethics Committee, Decision no = 2011-KAEK-25 2022/11-15, Date = 02.11.2022).

Statistical Analysis

Statistical analysis of the data in this study was performed with the Statistical Package for the Social Sciences (SPSS) version 24.0 software program (IBM Corp., Armonk, NY, USA). Continuous variables were given as mean ± standard deviation (if normal distribution) and medians (interquartile ranges (IQR)) (if not normal distribution). Whether the distribution of continuous variables was close to normal was investigated with the Kolmogorov Smirnov test and the homogeneity of the variances was investigated with the Levene test. Analysis of baseline characteristics according to survival status was compared with Student's t-test or Mann-Whitney U tests. Categorical variables were analyzed with Pearson's Chi-Square test. Descriptive statistics were shown as median and interquartile range for continuous variables, and number of cases and (%) for categorical variables. Parameters that may have an effect on 5-year mortality were investigated by binary logistic regression analysis. As a result of univariate statistical analyses, the combined effects of risk factors (diabetes, contrast material amount, LDL-C level, LDL-C ≥ 70 mm/dL, and LDL-C ≥ 100 mg/dL) on mortality were evaluated. Three different models were created in which multivariate regression analysis was analyzed by adding the LDL-C parameter to the diabetes parameter as a continuous and nominal variable. As nominal variables, cut-off values were defined for the recommended values of 70 and 100 mg/dL in the guidelines. These models were evaluated separately by multivariate regression analysis. The odds ratio and 95% confidence intervals for each variable were calculated. For $p < 0.05$, the results were considered statistically significant.

RESULTS

A total of 99 patients with a previous history of CAD, using statins, and undergoing TAVI were included in the study (Median [IQR] age, 78.1 [74-84] years; 47 patients [47.5%] female). The mean follow-up period of the patients was 49.9 ± 20.1 months. In-hospital mortality was observed in 5 of the patients (5.1%) during the follow-up period. In the follow-up of the remaining 94 patients, mortality was detected in 11 patients (11.7%) after 2 years. In total, mortality was detected in 25 patients during the 5-year follow-up.

Table 1. Basic characteristics and laboratory investigations of TAVI patients by 5-year mortality status

	All patients (n = 99)	5 years mortality (-) (n = 74)	5 years mortality (+) (n = 25)	*p* value
Demographic features				
Age (years)	78.1 (74-84)	77.2 (73-83)	80,9 (76-84)	0.064
Male gender, n (%)	52 (52.5)	36 (48.6)	16 (64)	0.184
BMI	26.4 (24.3-28.1)	26,2 (24,4-27)	26.9 (24.2-29.1)	0.224
Follow-up, months	49.9 (52-62)	60.6 (60-62)	18.3 (3-29)	**< 0.001**
Comorbidities				
Hypertension, n (%)	84 (84.8)	61 (82.4)	23 (92)	0.249
Diabetes mellitus, n (%)	45 (45.5)	39 (52.7)	6 (24)	**0.013**
COPD, n (%)	16 (16.2)	10 (13.5)	6 (24)	0.218
CRF history, n (%)	26 (26.3)	20 (27)	6 (24)	0.766
Heart failure, n (%)	31 (31.3)	22 (29.7)	9 (36)	0.559
CVA history, n (%)	8 (8.1)	6 (8.1)	2 (8)	0.986
Medications				
Acetylsalicylic acid, n (%)	59 (59.6)	47 (63.5)	12 (48)	0.172
Beta blocers, n (%)	70 (70.7)	52 (70.3)	18 (72)	0.869
RAS blockers, n (%)	59 (59.6)	45 (60.8)	14 (56)	0.672
Diuretic, n (%)	40 (40.4)	31 (41.9)	9 (36)	0.604
Laboratory Values				
Hemoglobin (g/dL)	11.5 (10.4-12.7)	11.5 (10.4-12.5)	11.4 (10.3-12.8)	0.522
WBC ($\times 10^3$/mL)	8.36 (6.2-9.3)	8.54 (6.3-9.5)	7.84 (6-8.3)	0.288
Creatinine, mg/dL	1.26 (0.89-1.4)	1.21 (0.85-1.43)	1.39 (0.92-1.35)	0.869
eGFR (ml/min/1.73 m^2)	57.98 (42.2-74.3)	58,38 (42.9-74.3)	56.8 (42.2-68.7)	0.831
CRP (mg/L)	4.98 (1.6-5.7)	4.96 (1.6-5)	5.02 (1.8-6.8)	0.646
LDL-C (mg/dL)	100.5 (67-124)	93.9 (66-113)	120 (110-129)	**< 0.001**
LDL-C ≥ 70 mm/dL	66 (66.7)	45 (60.8)	21 (84)	**0.033**
LDL-C ≥ 100 mg/dL	47 (47.5)	27 (36.5)	20 (80)	**< 0.001**

Continuous variables are presented as mean ± SD or median (IQR), and nominal variables were presented as frequency (%). BMI = body mass index, COPD = chronic obstructive pulmonary disease, CRF = chronic renal failure, CVA = cerebrovascular accident, eGFR = estimated glomerular filtration rate; LDL-C = low-density lipoprotein cholesterol, RAS = renin-angiotensin system

The median LDL-C values of the patient population were calculated as 100.5 mg/dL (67-124).

When the causes of death of the patients were examined, 2 of those who developed in-hospital mortality were due to in-hospital pneumonia. Out of 25 deaths in total, 1 patient died due to malignancy and 2 patients died due to cerebrovascular events. Of the deaths that occurred, the remaining 20 patients died from cardiovascular events (80%).

Demographic, clinical, and laboratory characteristics of patients with and without mortality in the 5-year follow-up were compared in Table 1. When comorbidities were compared between the 2 groups, the rate of diabetes was found to be significantly lower

in the group with 5-year mortality ($p = 0.013$). There was no significant difference between the 2 groups in terms of demographic characteristics, other comorbidities, and drugs used. LDL-C cholesterol values were found to be significantly higher in the mortality group (120 mg/dL vs. 93.9 mg/dL, $p < 0.001$). No significant difference was observed between other laboratory parameters. When the groups were compared in terms of duration of statin use, it was found that there was no significant difference between the duration of use in the 2 groups ($p = 0.123$).

In Table 2, the patients were divided into 2 groups with LDL-C values ≥ 100 mg/dL and < 100 mg/dL, and the preprocedural and procedural characteristics of the patients and their post-procedural complication status were compared. The amount of contrast material used during the procedure was found to be significantly higher in the group with LDL-C < 100 mg/dL ($p = 0.024$). There was no significant difference between other preprocedural and procedural characteristics. When the groups were compared in terms of complication development, in-hospital mortality ($p = 0.021$) and 5-year mortality rates ($p < 0.001$) were found to be significantly higher in the group with LDL-C ≥ 100 mg/dL. There was no significant difference between the groups in terms of other complications. In addition, when the groups were classified as LDL-C < 70 mg/dL and ≥ 70 mg/dL, 5-year mortality was found to be significantly lower in the group with LDL-C < 70 mg/dL ($p = 0.033$).

Parameters associated with the development of 5-year mortality were evaluated with univariate and multivariate logistic regression analysis. Multivariate analysis was performed in the form of 3 different models in which the LDL-C parameter was analyzed as a continuous and nominal variable for the diabetes presence parameter, which was determined to be significant by univariate analysis. Both the LDL-C ≥ 100 mg/dL predictive value (OR:6.59, 95% CI: 2.17-20.01) and the LDL-C ≥ 70 mg/dL predictive value

Tablo 2. Preprocedural and procedural characteristic and complications of all cases according to the LDL

	All patients (n = 99)	LDL-C < 100 (n = 52)	LDL-C ≥ 100 (n = 47)	*p* value
Preprocedural and procedural features				
Aortic valve area (cm^2)	0.66 ± 0.09	0.66 ± 0.1	0.66 ± 0.09	0.989
LV ejection fraction (%)	43.6 ± 13	42.3 ± 13.1	45.1 ± 12.9	0.322
EuroSCORE II, median (IOR)	30.9 (20-40.8)	30.3 (19.9-40.1)	31.7 (21-41.9)	0.385
Predilatation, n (%)	18 (18.2)	12 (23.1)	6 (12.8)	0.184
Postdilatation, n (%)	19 (19.2)	10 (19.2)	9 (19.1)	0.992
Amount of contrast agent (mL)	140.3 ± 19.6	144.1 ± 21.7	136.1 ± 16.1	**0.024**
Type of valve, n (%)				
Balloon-expandable	15 (15.2)	9 (17.3)	6 (12.8)	0.529
Self-expandable	84 (84.8)	43 (82.7)	41 (87.2)	
Complications				
Major vascular complications, n (%)	13 (13.1)	6 (11.5)	7 (14.9)	0.622
Permanent pacemaker, n (%)	14 (14.1)	6 (11.5)	8 (17)	0.434
Postprocedural IS or TIA, n (%)	4 (4)	3 (5.8)	1 (2.1)	0.358
In-hospital mortality, n (%)	5 (5.1)	0	5 (10.6)	**0.021**
5-year mortality, n (%)	25 (25.3)	5 (9.6)	20 (42.6)	**< 0.001**

Continuous variables are presented as mean ± SD or median (IQR), and nominal variables were presented as frequency (%).
IS = ischemic stroke, TIA = transit ischemic attack, LV = left ventricle

Tablo 3. Univariate and multivariate regression analysis models for determining the predictors of 5-year mortality

	Univariate odds ratio (95% CI)	*p* value	Multivariate odds ratio (95% CI)	*p* value
DM	0.28 (0.10-0.79)	**0.016**	0.31 (0.10-0.94)	**0.039**
Amount of contrast agent	1.00 (0.98-1.02)	0.937		
***LDL-C**	1.01 (1.00-1.02)	**0.021**	1.01 (1.00-1.02)	**0.044**
***LDL-C ≥ 70 mg/dL**	3.38 (1.05-10.86)	**0.041**	3.88 (1.16-12.93)	**0.027**
***LDL-C ≥ 100 mg/dL**	6.96 (2.34-20.67)	**< 0.001**	6.59 (2.17-20.01)	**0.001**

DM = diabetes mellitus, LDL-C = low-density lipoprotein cholesterol

* Tree different multivariance regression analysis models were performed on mortality in which LDL-C parameter was analyzed as a continuous and nominal variable

(OR:3.88, 95% CI: 1.16-12.93) were found to be independent predictors of 5-year mortality in multivariate analyses. When LDL-C values were evaluated in the multivariate analysis without determining the lower limit, LDL-C elevation was found to be an independent predictor of 5-year mortality (OR:1.01, 95% CI:1.00-1.02). In addition, when multivariate analysis was performed for the presence of diabetes, it was observed that the significance remained independent of other parameters (OR: 0.31, 95% CI: 0.10-0.94).

DISCUSSION

The most important result of our study is that reaching the LDL-C targets specified in the guidelines significantly reduces the risk of in-hospital and 5-year mortality in patients with a previous diagnosis of CAD, long-term use of statins, and undergoing TAVI. We found that reaching the LDL-C < 100 mg/dL targets significantly reduced the 5-year mortality rates, even after adjusting for confounding factors. To the best of our knowledge, our study is the first in the literature to show that there is a significant relationship between reaching the target LDL-C levels specified in the guideline and the 5-year mortality rate in patients with a diagnosis of CAD and undergoing TAVI.

Some previous studies have evaluated the effect of statin therapy on mortality after TAVI. Peri-Okonny *et al*. [19], using the PARTNER II and Sapien 3 clinical trials or associated registries, showed that those receiving statin therapy were associated with a reduction in 2-year all-cause, cardiovascular and non-cardiovascular mortality compared with those not receiving statin therapy. Merdler *et al*. [20] showed that high-intensity statin therapy is associated with a reduction in mortality after TAVI, using data from 1238 cases from a single-center registry. Huded *et al*. [21] also showed that high-intensity statin therapy was associated with a reduction in all-cause mortality based on 294 cases.

The underlying mechanism of statin therapy's reduction in all-cause and cardiovascular mortality risks is thought to be related to the reduction in ischemic events. [19-22]. In this study, we investigated the relationship between the effect of statin therapy, which has been shown to reduce mortality in previous studies, and LDL-C levels. The entire patient population was patients on long-term statin therapy. When we compared the patients according to the LDL-C levels recommended in the international guidelines, we found that mortality was significantly lower in patients who remained below the recommended LDL-C levels. In our study, it was observed that the 5-year mortality rate was significantly lower in patients (< 70 mg/dL) who met the LDL targets recommended for high-risk patients in the latest ESC guideline [23] ($p = 0.033$). In addition, in-hospital ($p = 0.021$) and 5-year mortality ($p < 0.001$) were found to be significantly lower in patients (< 100 mg/dL) who achieved the LDL targets recommended for intermediate-risk patients. Thus, in this study, we showed that it is necessary to closely monitor the LDL-C value in order to reduce mortality

in patients using statins, and the importance of reducing the LDL-C value to the values recommended in the guidelines.

When subgroup analyzes were performed in our study, in-hospital mortality was observed in 5 patients. When considering the 2-year mortality rate, mortality was observed in 16 patients (16.2%) at 2-year follow-up. When the causes of mortality of the patients were examined, it was observed that mortality developed from cardiovascular causes in 20 patients (%80).

In our study, mortality rates were found to be lower in patients with diabetes ($p = 0.013$). When we look at the literature on this subject, it was found that previous studies had similar results to ours. In a study by Van Nieuwkerk *et al*. [24], patients with diabetes who underwent TAVI had lower mortality rates than those without diabetes. They attributed this to the earlier development of aortic stenosis in patients with diabetes and to the application of TAVI at an earlier age. The findings of our study were found to be consistent with the literature. In addition, the follow-up period was found to be significantly lower in the group with 5-year mortality ($p < 0.001$). It is an expected result that the follow-up times are short since the patients with mortality are left to be followed up due to the mortality observed over time.

Together with these results, the effect of lowering LDL-C levels to the recommended levels in the guidelines on mortality in patients with CAD diagnosis, TAVI procedure, and chronic statin use has been demonstrated. It has been shown that lowering LDL-C levels to the levels recommended in the guidelines significantly reduces mortality. However, due to the design of our current study, prospective studies with a higher number of patients are needed to confirm our findings and study results.

Limitations

Our study had some limitations. First, the study had a retrospective and observational design, which could be accepted as the major limitation of the study. Second, this study had a small sample size. Third, the mortality data of the patients in our study were limited. We could not obtain detailed data on the causes of mortality. Finally, our findings warrant prospective and multicenter studies with larger sample sizes to elucidate the association between LDL-C levels and long-term mortality following TAVI in AS patients with CAD.

CONCLUSION

The most important result obtained in this study is that achieving the LDL-C level targets specified in the guidelines significantly reduces the in-hospital and 5-year mortality rates in patients with a previous history of CAD and statin use and undergoing TAVI. Although all patients included in the study used statins, the mortality rate was significantly higher in patients who did not reach the target LDL-C value. We found that achieving the LDL-C < 100 mg/dL targets significantly reduced the risk of in-hospital and 5-year mortality, even after adjusting for influencing factors. With these results, we showed that it is not enough to only give statin therapy to patients and that it is important to reach the guideline targets in LDL-C values in patient follow-ups.

Authors' Contribution

Study Conception: ÖFD; Study Design: ÖFD; Supervision: ÖFD; Funding: FL; Materials: FL; Data Collection and/or Processing: ÖFD; Statistical Analysis and/or Data Interpretation: ÖFD; Literature Review: ÖFD; Manuscript Preparation: ÖFD and Critical Review: FL.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Histopathological diagnoses revealed by indication-based renal allograft biopsies: a retrospective analysis

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ABSTRACT

Objectives: Although there have been several advances in post-solid organ transplantation immunosuppression medications over the last two decades, the long-term survival of renal allografts did not significantly improve. Renal allograft biopsy is a helpful tool for determining the cause of graft dysfunction and adjusting patient management.

Methods: Patients who received kidney transplantation and underwent allograft biopsy in Istinye University Hospital between January 2017 and January 2023 constituted the target population of this study. Demographic parameters, clinical data and biopsy indications, and histopathological assessment results of the patients were retrospectively analyzed.

Results: Overall, 74 patients were included. The histopathology results included acute T-Cell mediated rejection (TCMR) (n = 15, 20%), tubular atrophy/chronic allograft nephropathy (IFTA) (n = 11, 15%), calcineurin inhibitor (CNI) toxicity (n = 2, 3%), chronic antibody-mediated rejection (ABMR) (n = 2, 3%), borderline pathology (n = 10, 13.5%), normal histology (n = 5, 6.5%), transplant glomerulopathy (TG) (n = 5, 6.5%), acute ABMR (n = 4, 5%), acute tubular necrosis (n = 7, 9%), polyomavirus nephropathy (n = 3, 4%) and non-specific changes (n = 10, 13.5%). The C4d was positive in 12% (n = 9) of the graft biopsies. In 73% (n = 54) of cases, the treatment strategy was changed based on biopsy results. Among all patients, 19 (25.6%) lost their grafts during follow-up.

Conclusions: According to the histopathological analysis results, acute TCMR, IFTA, and borderline pathology were the most common causes of renal graft dysfunction. Renal allograft biopsy led to a remarkable change in treatment strategies in a significant number of cases.

Keywords: Renal, allograft, biopsy, histopathology, diagnosis

Renal transplantation (RT) is the optimal treatment for end-stage kidney disease (ESRD) [1]. Although the advances in surgical techniques and immunosuppression (IS) protocols led to a significant increase in graft survival rates during the last two decades, renal allograft biopsy still has a significant

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role in patient management, particularly in the setting of graft dysfunction [2].

It is known that a broad spectrum of clinical entities can lead to graft damage [3]. In clinical practice, indication biopsies are performed after every attempt is made to diagnose or exclude all potential causes of renal allograft dysfunction such as hypovolemia, drug interactions leading to an increase in the trough calcineurin levels, vascular or urological problems including arterial stenosis, ureteral stricture, lower urinary tract obstruction or systemic infections or recurrence of the primary kidney disease [4]. On the one hand, it is known that renal allograft biopsy is not exempt from potential complications such as bleeding, hematoma or urinoma formation, infections, and graft loss; thus, it is considered the last resort in the diagnostic management of RT patients [5]. On the other hand, delaying or ignoring renal allograft biopsy can lead to irreversible outcomes [5]. In order to prevent permanent damage to the graft, the treatment strategies can be tailored according to the biopsy result, and the longevity of the graft function can be saved. Therefore, transplant practitioners need to know the impact of their "indication biopsy" strategy on their approaches regarding patient treatment.

This study was performed to analyze the renal allograft biopsy results and determine the impact of indication biopsies on treatment strategies at our transplant center.

METHODS

This study was designed as a retrospective single-center study. After obtaining approval from the Ethical Review Committee of Istinye University Hospital (Date:12.04.2023, Decision No.: 23/102), data of the patients who received kidney transplantation and underwent a conclusive renal allograft biopsy at our center between January 2017 and January 2023 were reviewed. All patients consented to the use of their medical data for research purposes.

The initial retrospective review revealed 84 patients. Among those, 6 were excluded since they underwent renal transplantation at another center, while 2 were not included due to patients with incomplete follow-up data. In addition, 2 patients with inconclusive biopsies were omitted. Thus, 74 patients were included in this study. The inconclusive biopsies repeated for obtaining a conclusive biopsy specimen according to the Banff criteria were also omitted [6]. All renal allograft biopsies were performed on a specific indication. No protocol biopsies were performed.

Data including demographic parameters (i.e., age and gender), donor age, the primary reason for ESRD, type of donor (i.e., deceased donor, live-related or live-unrelated), the time between RT and renal allograft biopsy, indication for biopsy, major histocompatibility complex antibody (anti-MHC) and C4d status, histopathological diagnosis, shift in treatment strategy after obtaining the biopsy result and duration of follow-up after renal allograft biopsy were retrieved from electronic patient folders. All renal allograft biopsy specimens were analyzed by the same pathologist experienced in this field. The pathologist based the assessments on the Banff 2007 classification [6]. The retrieved data were transferred to an electronic database for statistical analysis.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS v23, IBM, Armonk, NY, US) software. The data were analyzed using descriptive statistics. Frequencies, percentages, and means were presented where appropriate.

RESULTS

The histopathological diagnoses revealed by 74 renal allograft biopsies were included in the study. Three

Table 1. Primary diseases of the patients

Primary disease	n (%)
Diabetes mellitus	16 (21.5)
Hypertension	13 (17.5)
IgA nephropathy	3 (4)
Focal sclerosing glomerulonephritis	3 (4)
Reflux nephropathy	2 (2.5)
Nephronophthisis	2 (2.5)
Obstructive uropathy	2 (2.5)
Unknown	9 (12)
Other	24 (33.5)

Table 2. Renal allograft biopsy indications

Indication	n (%)
Increased serum creatinine level	60 (81)
Proteinuria	7 (9)
Increased serum creatinine level and proteinuria	2 (3)
Delayed graft function	5 (7)

(4%) of the biopsy specimens were obtained by repeat biopsies since the initial intervention failed to provide an "adequate" specimen for a satisfactory histopathological assessment. Patients were aged between 3 and 67, and the mean patient age was 3.4 years. Among these patients, 58 (78.4%) were male, and 16 (21.6%) were female. Live-related, live-unrelated, and deceased donors were the donor types in 58 (78.4%), 10 (13.5%), and 6 (8.1%), respectively. The mean donor age was 44.5 years (range: 21-68 years). The mean duration between RT and renal allograft biopsy was 217 day (range: 8-1680 days). The primary diseases leading to end-stage renal disease are listed in Table 1.

The renal graft biopsy indications are displayed in Table 2. An increase in serum creatinine level was the most common indication (81%) for biopsy. The histopathological diagnoses are listed in Table 3. Analysis of the histopathological assessment reports revealed that acute T cell-mediated rejection (TCMR) was the most frequent (20%) diagnosis. The shifts in the treatment strategies based on renal allograft biopsy results are displayed in Table 4.

This analysis revealed that biopsy results led to a significant change in treatment in 73% (n = 54) of the patients. The mean duration of follow-up after renal allograft biopsy was 30.5 [1-60] months. None of the patients died during the study period; however, 25.5% (n = 19) of grafts failed. Among these 19 grafts, 8 were diagnosed with acute rejection, 3 had transplant glomerulopathy (TG), and 2 had interstitial fibrosis and tubular atrophy (IFTA).

Table 3. Histopathological diagnoses of the patients

Diagnosis	n (%)
Acute T-cell mediated rejection	15 (20)
Acute antibody-mediated rejection	4 (5)
Interstitial fibrosis and tubular atrophy	11 (15)
Chronic antibody-mediated rejection	2 (3)
Calcineurin inhibitor toxicity	2 (3)
Borderline pathology	10 (13.5)
Acute tubular necrosis	7 (9)
BK virus nephropathy	3 (4)
Non-specific changes	10 (13.5)
Normal histology	5 (7)
Transplant glomerulopathy	5 (7)

DISCUSSION

Since renal allograft biopsy is an invasive intervention with potential complications endangering the survival of the graft or the recipient, it is challenging for clinicians to proceed with this procedure during the follow-up of kidney transplant recipients [7, 8]. On the other hand, while some centers, like ours, perform on-indication biopsies only, others perform protocol biopsies and, therefore, have a heterogeneous renal allograft biopsy specimen pool. We conducted our study with the belief that a retrospective review of our on-indication biopsy renal allograft biopsy data and analyzing their positive impact on patent management could encourage transplant practitioners during the decision-making processes for on-indication biopsies.

It is known that renal allograft biopsy is the technique of choice for diagnosing rejection and other potential causes of graft dysfunction [7]. Although some centers perform periodic protocol biopsies, most kidney transplant centers suggest allograft biopsies on

Table 4. Changes in treatment strategies based on graft biopsy results

Shift in treatment strategy	n (%)
Plasmapheresis	4 (5.5)
Pulse steroid treatment	30 (40.5)
Follow-up	20 (27)
Intravenous hydration	7 (9.5)
Reduction in immunosuppression	3 (4)
Other	10 (13.5)

specific indications [8]. The main reason for this approach is that rejection may lead to adverse outcomes regarding the survival of the graft, especially if it remains undiagnosed or diagnosed late [9]. In our study, most renal transplant biopsies were performed due to increased serum creatinine levels, and the biopsies led to a significant shift in patient management in 73% of the cases. This finding highlights the importance of performing renal allograft biopsy for early diagnosis and tailoring the patient management protocols.

McDonald *et al*. [10] worked on the impact of acute TCMR and renal transplant outcomes. These authors analyzed the data of 4325 renal transplant recipients and concluded that acute rejection increased the risk of graft loss [10]. In our study, 25% of the cases were diagnosed with acute rejection. While the patients with acute TCMR were managed with pulse steroids and increasing the dose of maintenance IS, those with acute antibody-mediated rejection (ABMR) were mainly treated by plasmapheresis.

In consistency with acute rejection, chronic rejection was also reported to be related to graft failure. It is known that chronic ABMR is characterized by C4d deposits in peritubular capillaries, transplant glomerulopathy, peritubular capillary basement membrane layering, and intimal fibrous thickening [10]. Based on these criteria, chronic ABMR was diagnosed in 3% of the cases in our study.

Parajuli *et al*. [11] analyzed the histopathological characteristics of the renal allograft biopsy specimens and the reasons for renal graft failure in a cohort including 329 patients with graft failure. These authors noted that the three most common causes of graft failure were acute rejection (40%), TG (17%), and IFTA (13%)- the distribution of the reasons for graft failure in our cohort aligned with these data.

In 2023, Afrakoti *et al*. [12] reported the histopathological findings of their patients with renal allograft dysfunction. They worked on the data from 300 renal allograft biopsies and concluded that acute TCMR, IFTA, and calcineurin inhibitor (CNI) toxicity were the most common causes of allograft dysfunction in their cohort. In line with these data, the most frequent histopathological diagnosis was acute TCMR in our cohort. Afrakoti *et al*. [12] stated that indication biopsies were beneficial in selecting the optimal treatment plans for preventing permanent graft failure. Our results led to a similar conclusion.

Our review revealed that 3 (4%) patients were diagnosed with polyomavirus (i.e., BK virus) infection. Although this rate is relatively low, it is known that BK virus infection might affect allograft survival. Hogan *et al*. [13] noted that the BK virus had a significant role in allograft failure. In line with this finding, Sharma *et al*. [14] reported that early detection of BK-related nephropathy was beneficial for preventing early graft loss. In our cohort, timely diagnosis of BK virus nephropathy led to the timely modification of the IS regimens and prevention of graft loss in all cases with BK virus infection.

In compliance with BK nephropathy, timely diagnosis and treatment of rejection is also crucial in preventing graft loss [15]. Since acute rejection necessitates initiating anti-rejection treatments with potential adverse effects, including increased risk of opportunistic infections and development of malignant disorders, correct diagnosis of this clinical entity is critical [16]. According to the Banff criteria, the pathologist should evaluate the specimen adequacy as the first step of the histopathological assessment of the renal allograft biopsy specimens [6]. Cimen *et al*. [17] worked on the impact of specimen adequacy in the histopathological interpretation of the renal allograft biopsy specimens and the interobserver variations between the pathologists. These authors stated that the interobserver variation significantly increased in the setting of unsatisfactory biopsy specimens. In line with this conclusion, the "minimal" or "unsatisfactory" specimens were not used for histopathological diagnosis in our cohort, and these biopsies were repeated.

Limitations

Our study has some limitations that must be considered while evaluating its findings. First, it is a single-center, retrospective study with a small sample. Second, the follow-up duration is short. Finally, all biopsy specimens were evaluated by a single pathologist, and there was no chance to analyze the validity of the data by assessing the interobserver variation.

CONCLUSION

We conclude that a timely-performed, conclusive indication biopsy is valuable in diagnosing the reasons for renal allograft dysfunction. This approach can sig-

nificantly change patient management and contribute to all endeavors to prevent graft loss.

Authors' Contribution

Study Conception: EE, MT; Study Design: AA, TŞ; Supervision: AD; Funding: N/A; Materials: N/A; Data Collection and/or Processing: HBU, SA; Statistical Analysis and/or Data Interpretation: EE, MT; Literature Review: EE, AD; Manuscript Preparation: EE, TŞ and Critical Review: HBU, AD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Evaluation of malignant breast masses with abbreviated breast magnetic resonance imaging

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ABSTRACT

Objectives: Our study aimed to investigate the sensitivity of the abbreviated magnetic resonance imaging (MRI) in the detection of tumors in breast cancer patients.

Methods: Patients who underwent breast MRI between March 2018 and October 2021 were reviewed retrospectively. Patients with a histologic diagnosis of breast malignancy were included in the study. Patients who underwent a biopsy or an interventional procedure before the MRI examination and who received neoadjuvant chemotherapy were excluded from the study. Abbreviated MR protocol included a pre-contrast T1-weighted, 1st minute contrast-enhanced T1-weighted, and 1st minute subtracted series. Additionally, 2nd minute post-contrast series were evaluated.

Results: A total of 83 lesions with a histologic diagnosis of breast cancer were evaluated in 81 patients. The mean age of the patients included in the study was 51.08 years (range: 27-79 years). Seventy-four of the 83 breast lesions showed contrast enhancement in the 1st-minute contrast-enhanced images and subtraction images (sensitivity 89.1%). When missed cases were re-evaluated all of them were visible in the second-minute contrast-enhanced series.

Conclusions: In this study, malignant lesions could be detected with high-sensitivity abbreviated MRI protocol and the addition of second-minute contrast-enhanced series to the protocol significantly improve lesion detection. We believe that MRI with the abbreviated MRI protocol can be used for screening purposes in high-risk women with dense breasts.

Keywords: Breast cancer, magnetic resonance imaging, abbreviated MRI, mammography, breast cancer screening

Magnetic resonance imaging (MRI) of the breast is the method with the highest sensitivity in the diagnosis of breast cancer. Cases in the high-risk group (with BRCA gene mutation, lifetime breast cancer risk over 20-25% with statistical models based on family history, radiotherapy history at the age of 10-30, Li Fraumeni syndrome, Cowden, Bannayan-Riley -Those with Ruvalcaba syndrome or their first-degree relatives) annual breast MRI scan is performed. In recent years, annual screening indications for breast MRI have been increasing [1].

Mammography is the basic imaging method in breast cancer screening, although the risk of breast cancer in dense breasts is 4-6 times higher than in fatty breasts, the sensitivity of mammography decreases below 50% [2, 3]. In addition, mammography sensi-

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tivity is low (40%-30%) in fast-growing tumors that do not cause spiculation and distortion (often triple-negative and human epidermal growth factor receptor 2 positive type tumors) [4, 5]. In many studies conducted in recent years, the rate of interval cancer has significantly decreased in patients with dense breasts with the addition of MRI [1, 6-8]. In line with these data, in the declaration published by EUSOBI (European Breast Imaging Society) in 2022, it is recommended that a breast MRI be performed every 2-4 years for women aged 50-70 years with extremely dense breasts [9].

Breast MRI as been increasingly used to study breast cancer for screening. However, a method to be used for screening should be simple, short-term, and easy to evaluate, as in mammography. Breast MRI is not a cost-effective imaging method with a long acquisition and evaluation time. Abreviated breast MRI, or an abbreviated protocol for breast MRI, was first proposed by Cristiane Kuhl *et al*. [10] in 2014. It was designed to be used for breast cancer screening and only needed the acquisition of two sequences: T1-weighted images taken before and right after the injection of gadolinium. The abbreviated MRI protocol includes differences in various studies [10-12].

In this study, we aimed to evaluate the cases with malignant masses in the breast with the abbreviated MRI protocol retrospectively and to determine the detection rate of the lesions.

METHODS

Our study is a single-center retrospective study. Between March 2018 and October 2021, patients with a histologic diagnosis of breast cancer diagnosed by biopsy and MRI of the breast were included in the study. Patients who underwent a biopsy procedure recently performed before MRI, and who received neoadjuvant chemotherapy were excluded from the study.

Ethics committee approval (2023-09/303) and informed patient consent was waived. Our study was conducted in accordance with the Declaration of Helsinki.

MRI Technique

All MRI scans were performed on a 1.5 Tesla (T) MRI device with an 18-channel superficial breast coil in the prone position (Aera; Siemens Healthcare, Erlangen, Germany). If the patient is premenopausal, the study is performed between the 5th and 15th days of the menstrual cycle.

Multiparametric MR sequences (fat-suppressed T2-weighted sequence, diffusion-weighted sequence (DWI), pre-contrast non-fat-suppressed T1-weighted sequence, and post-contrast T1-weighted sequences with dynamic contrast in 5 series) were obtained in all patients. For series with dynamic contrast, 0.1 mmol of contrast agent per kilogram (Gadovist; Bayer Healthcare Pharmaceutical, Berlin, Germany) was injected, and approximately 20 cc of saline was administered after the infusion of the contrast agent to ensure homogeneous distribution of the contrast agent. Post-contrast enhanced images were obtained from 30 s after contrast injection and the acquisition time for one scanning was about 60 s. Subtraction images were created automatically by the device.

Table 1. Comparison of routine Breast MRI protocol and abbreviated MRI protocols

MRI Protocol		Dynamic Series				
		1st min	2nd min	3rd min	4th min	5th min
		80. s	143. s	206. s	269. s	332. s
Conventional Routine Scanning Protocol	Fat saturated T2WI, DWI, T1WI	✓	✓	✓	✓	✓
Abbreviated MRI Scanning Protocol	T1WI	✓				

MRI = magnetic resonance imaging, DWI = diffusion-weighted imaging, T_1WI = T_1-weighted imaging, T_2WI =T_2-weighted imaging

Evaluation of Images

Images were retrieved from the archive system (PACS). Pre-contrast T1-weighted images, 1st-minute post-contrast T1-weighted images, and 1st-minute subtraction images were included in this protocol (Table 1). A breast radiologist (EY) with approximately 10 years of experience in breast imaging evaluated the images with the abbreviated MRI protocol. The cases had been reported by a different breast radiologist. The radiologist (EY) was informed that all patients are diagnosed with a malignant mass, but she was not informed about the localization of the masses, previous examinations of the patient, or the patient's history. The radiologist reviewed the images and noted any pathologic contrast enhancement. The findings were recorded as present or absent in the Excel file. Results were checked later whether the contrast enhancement matched the original tumor.

Statistical Analysis

Descriptive statistics were used to describe continuous variables (mean, standard deviation, minimum, median, maximum).

RESULTS

The mean age of the patients included in the study was 51.08 years (27-79 years). A total of 83 histologically proven malignant breast lesions were evaluated in 81 patients. Of these lesions, 91.5% were invasive breast carcinoma (73 invasive ductal carcinomas, 2 invasive lobular carcinomas, and 1 metaplastic carcinoma), and 8.4% were ductal carcinoma in situ (7 patients). The mean diameter of the lesions was 27.8 mm (range: 3-100 mm).

Seventy-four of the 83 malignant breast lesions showed contrast enhancement in the 1st minute contrast-enhanced series and were visible in subtracted images (sensitivity 89.1%) (Fig. 1). Nine lesions were not detected (Fig. 2). The contrast-enhanced T1-weighted and subtracted series (Fig. 2) acquired in the second minute showed all nine of the lesions that the abbreviated protocol had failed to detect. The mean and median diameter of these nine missed cancers were 20.7 and 16.2 mm respectively. The size and histologic type of the missed cancers are given in Table 2.

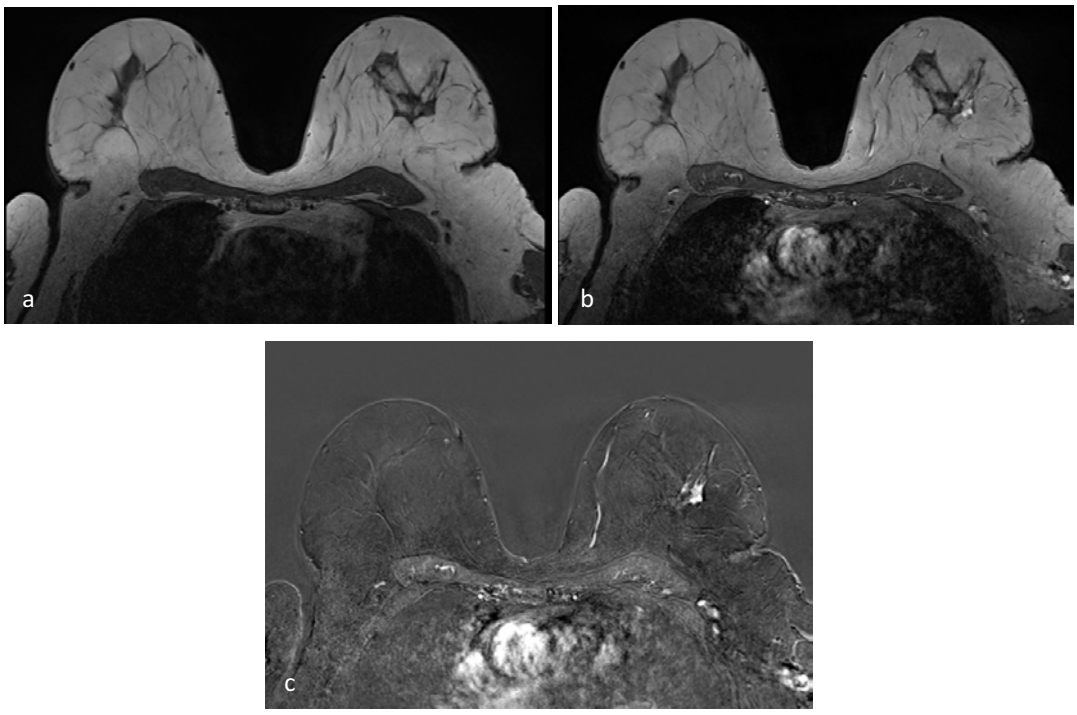

Fig. 1. Screening mammography of the 54-year-old female patient revealed a spiculated mass in the left breast. MRI was performed to evaluate the contralateral breast preoperatively. (a) T1-weighted image without contrast, (b) 1st-minute T1-weighted image with contrast, and (c) 1st-minute subtraction image; An enhancing irregular mass is observed in the outer quadrant of the left breast. The biopsy result was reported as luminal b invasive ductal carcinoma.

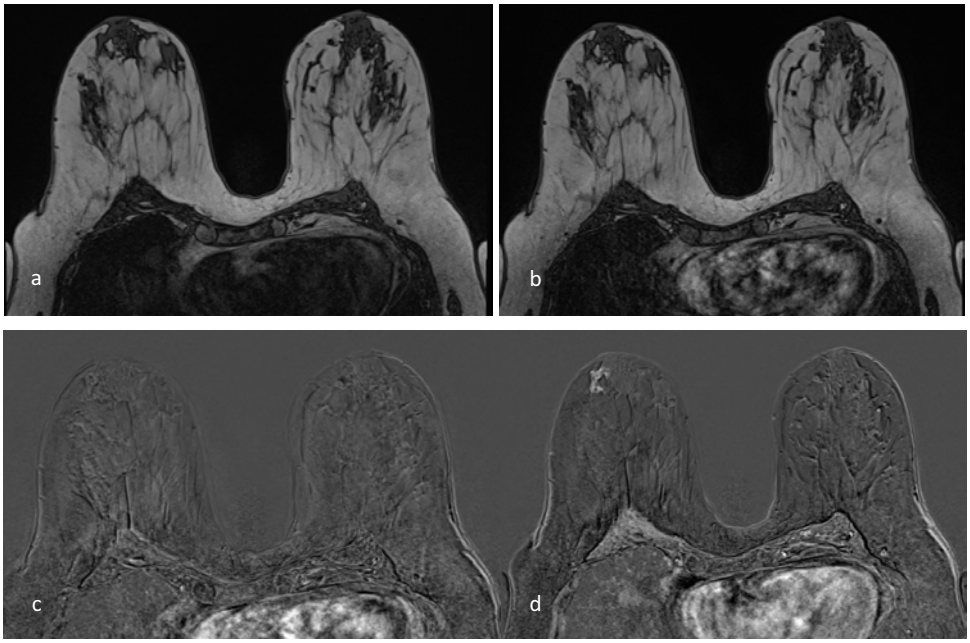

Fig. 2. A 51-year-old patient who applied to our clinic with palpable stiffness. On mammography, an irregular high-density mass was detected in the periareolar area of the right breast. Preoperative MRI images are obtained. (a) T1-weighted image without contrast, (b) 1st-minute T1-weighted image with contrast, (c) 1st-minute subtraction and (d) 2nd-minute subtraction image; An irregular enhanced mass is observed in the periareolar area of the right breast. The mass was not observed in the 1st-minute subtraction image. It became visible in the second-minute series. The biopsy result was reported as luminal b invasive ductal carcinoma.

DISCUSSION

Magnetic resonance imaging (MRI) is a multiplanar imaging method with high soft tissue resolution. Unlike mammography and ultrasonography (US), dynamic evaluation is performed in addition to morphological evaluation with MRI. The injection of a contrast agent during MRI examination is aimed to detect the pathological early contrast enhancement due to tumor neoangiogenesis. In many studies conducted in recent years, it has been shown that MRI has a higher cancer detection rate than mammography and US, and reduces the interval cancer rate [1].

The indications of breast MRI have increased in

Table 2. Masses undetected by the abbreviated MRI protocol

	Patient's Age (year)	Lesion size (mm)	Histologic type
1	74	6.1	Luminal type b invasive ductal carcinoma
2	73	10.4	Luminal type b invasive ductal carcinoma
3	63	19.2	Luminal type b invasive ductal carcinoma
4	72	40.5	Luminal type b invasive ductal carcinoma
5	66	9.7	Metaplastic carcinoma
6	39	14.1	Luminal type b invasive ductal carcinoma
7	51	22	Luminal type b invasive ductal carcinoma
8	58	16.2	Luminal type a invasive ductal carcinoma
9	37	50	Ductal carcinoma in situ

MRI = magnetic resonance imaging

recent years. Kuhl *et al*. [10] discussed whether it can be used for screening purposes or not. However, for an imaging method to be used for screening purposes, it must be easily accessible, fast, and easy to evaluate. For this purpose, an abbreviated MRI protocol has been developed [10].

Many different abbreviated MRI protocols have been used in the literature. While the first-minute T1-weighted sequence, first-minute subtraction image, and maximum intensity projection image are frequently used in studies [7, 10, 11] as the abbreviated breast MRI protocol, much other research also includes T2-weighted sequences and diffusion-weighted sequences to the protocol [13-16]. Due to the high vascular permeability secondary to neoangiogenesis, breast tumors tend to have early contrast enhancement. Sequences acquired later minutes are often obtained to characterize the lesion rather than detect it. In our study, similar to Kuhl *et al*.'s study [10] we determined the 1st minute T1-weighted sequence and the 1st-minute subtraction image as the abbreviated MRI protocol. In our clinic, the routine full diagnostic breast MRI protocol, which includes a fat-sat STIR T2W sequence, a precontrast DWI sequence, and dynamic contrast-enhanced T1W sequences, takes 25 minutes, and the abbreviated MRI protocol is com p l e t e d in 2.5 minutes.

In their study, Mango *et al*. [11] reported 96% mean sensitivity for the abbreviated MRI protocol. In the study by Panigrahi *et al*. [17], the sensitivity was reported as 81.2%, however, the sensitivity is claimed as 100% when compared to the standard MRI technique. All malignant tumors found with standard MRI were also detected with abbreviated MRI. According to Chen *et al*.'s study [7], the sensitivity was reported to be 93.8% and just one case could not be detected with abbreviated MRI.

In our single-center retrospective study, we aimed to determine the efficacy of abbreviated breast MRI in the detection of malignant lesions. In our study group, the patients were scanned with a full complete breast MRI protocol. But only the series included in the abbreviated MRI protocol were evaluated. Study results showed a high rate of invasive breast cancer detection. Nine lesions among 83 malignant masses were missed by abbreviated MRI. The sensitivity of abbreviated MRI was found to be 89.1%. When these cases were re-evaluated, all the missed lesions could be seen in the second-minute contrast-enhanced series. In the literature, there is no detailed information about the evaluation of the cases with the 2nd-minute contrast-enhanced series. We saw that some of the cancers may show delayed enhancement in our study. We thought that adding the series acquired at the second minute would allow us to detect late-enhancing tumors. This may raise the question of increased of false positivity and decreased specificity. However, in the literature, there are studies that reported high specificity of abbreviated MRI protocols including second-minute contrast-enhanced sequences. Park *et al*. [18] included 2nd minute contrast-enhanced series in their abbreviated MRI protocol and they noted a higher specificity of abbreviated MRI than full diagnostic MRI. In a different study, Kwon *et al*. [19] used first- and second-minute contrast-enhanced series and achieved a specificity of 98.2%.

Limitations

There are several limitations in our study that should be noted. First of all, it was a single center, single observer, and retrospective study. Secondly, the study group included only patients with malignant masses. False positivity, specificity, and false negativity levels could not be evaluated because the patient group did not include benign lesions. In addition, only index tumors were evaluated in malignant masses, and no evaluation was made in terms of multifocality or multicentricity. Another limitation is that the evaluation time was not noted.

CONCLUSION

Abbreviated breast MRI, which shortens MRI acquisition and evaluation time, is a highly sensitive imaging method. In this study, we found that adding a 2-minute contrast-enhanced series will increase the sensitivity of abbreviated MRI significantly. But larger studies are needed to evaluate this finding. We believe that in screening high-risk women with dense breasts, an abbreviated breast MRI may be a good alternative to a full diagnostic examination.

Authors' Contribution

Study Conception: EY, NG; Study Design: EY; Supervision: EY, NG; Funding: N/A; Materials: N/A;

Data Collection and Processing: EY; Statistical Analysis and Data Interpretation: EY, NG; Literature Review: EY; Manuscript Preparation: EY, NG and Critical Review: EY, NG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Cardiac biomarkers comparison between acute myocarditis/ myopericarditis and multisystem inflammatory syndrome in children

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ABSTRACT

Objectives: Acute myocarditis/myopericarditis is a heterogeneous disorder of unknown origin, the viral etiology leading the first row. There could be also myocardial involvement in multisystem inflammatory syndrome in children (MIS-C). In this study, we aimed to investigate cardiac biomarkers of acute myocarditis/myopericarditis and MIS-C and to compare these parameters between the two diseases.

Methods: Patients who are diagnosed with MIS-C, isolated viral myocarditis/myopericarditis at a university hospital from October 2021 to March 2023 are included in this study.

Results: There were 38 MIS-C patients and 53 patients with myocarditis/myopericarditis. The mean age was 141.2 ± 38.2 months (4 to 18 years old) in MISC, and 145.8 ± 52.1 months (7 to 18 years old) in myocarditis/myopericarditis. Median troponin I level was 145 ng/L in MIS-C patients and it was 901 ng/L in myocarditis/myopericarditis patients. Creatinine kinase-myocardial band (CK-MB) median was 2.25 ng/mL (0.6-6.3) versus 6.7 ng/mL in MIS-C and myocarditis/myopericarditis, respectively. Pro Brain natriuretic peptide (Pro-BNP) median level was 2714.5 pg/mL (< 300) in MIS-C, and it was 294 in patients with myocarditis/myopericarditis. Troponin I, CK-MB was significantly higher in myocarditis/myopericarditis, while Pro-BNP was significantly higher in MIS-C patients ($p < 0.05$). The separating power of CK-MB, troponin I, and Pro-BNP level was significantly higher in the differential diagnosis of these two group patients ($p < 0.001$). MIS-C patients with high pro-BNP levels had more prolonged hospitalization and left ventricular function impairment according to myocarditis/myopericarditis.

Conclusions: Cardiac biomarkers (CK-MB, troponin I, and Pro-BNP) could be good markers to estimate the course of the diseases.

Keywords: Cardiac biomarkers, myocarditis/myopericarditis, troponin, pro-BNP, multisystem inflammatory syndrome

Isolated myocarditis/myopericarditis is an inflammatory disease of the myocardium and pericardium with many various reasons, most of which are viral infections. In some other systemic diseases, hypoxia could also affect the myocardial tissue and start the inflammatory process. The clinical spectrum could be mild to lethal and varies individually [1, 2].

A novel coronavirus came about in late 2019 [3].

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It was called COVID-19, meaning coronavirus 2019, by The World Health Organization (WHO). The virus was officially designated as "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2). Although the acute illness was usually mild in children, there could be a systemic inflammatory response with a severe shock-like illness in children with features of incomplete Kawasaki disease (KD) or toxic shock syndrome in rare cases after the acute phase [4]. This manifestation is called multisystem inflammatory syndrome in children (MIS-C). Whilst the 2020 Centers for Disease Control and Prevention (CDC) MIS-C case definition did not entitle Kawasaki disease (KD) as a different diagnosis, the Council of State and Territorial Epidemiologists (CTSE)/CDC MIS-C case definition does specify KD as an alternative diagnosis that should activate telling the CDC KD passive surveillance organization [5, 6]. It will be more cumbersome to characterize patients with incident KD who have seroconverted from past SARS Co-V2 infections from patients with MIS-C who supply KD criteria. Thus, it is crucial to accentuate treatment if KD high-risk criteria exist.

Myocardial involvement may be present in MIS-C and Kawasaki-like patients thought to be an inflammatory vasculitis response [3]. However, distinguishing myocardial involvement in this and other similar systemic diseases from isolated viral myocarditis/myopericarditis seems important in the treatment plan and subsequent follow-up [7]. Because the cases are intertwined, it may be useful to use cardiac-specific markers outside the clinic [8]. While anti-inflammatory agents, including intravenous immunoglobulin (IVIG), are frequently used in the treatment plan in patients with MIS-C and Kawasaki-like syndrome with the new definition, supportive treatment is usually given in mild myocarditis/myopericarditis [3, 9].

Cardiac biomarkers such as creatinine kinase-myocardial band (CK-MB), troponin I, and pro Brain natriuretic peptide (pro-BNP) can be used in both diagnosis, follow-up, and treatment to show myocardial involvement and to determine the severity and prognosis of the clinic [10, 11]. Cut-off values for these markers may not be as clear in children as in adults [12]. Studies on the exact importance of these markers continue [13].

In this study, we aimed to investigate the clinical, laboratory, and imaging characteristics of acute myocarditis/myopericarditis and MIS-C (Kawasaki-like disease), and to compare the differences between them. It is also aimed to check out the usefulness of these characteristics and cardiac biomarkers on the course of diseases.

METHODS

Patients diagnosed with MIS-C, isolated viral myocarditis/myopericarditis, at a university hospital from October 2021 to March 2023 were included in this study. The local ethics committee (Mersin University Clinic Research Ethics Committee, Decision no.: 608, Date 08.09.2021) approved the protocol. The Helsinki Declaration was taken into consideration. The data was collected retrospectively.

MIS-C is diagnosed according to the CDC and the World Health Organization (WHO) criteria (5, 14). Myocarditis/myopericarditis diagnosis is made according to the algorithm of the American Heart Association and European Society of Cardiology [1, 2]. It includes history, clinical, laboratory, electrocardiography (ECG), and echocardiography findings with the addition of cardiac magnetic resonance imaging (CMRI) confirming the diagnosis [15, 16]. All patients had chest pain and elevation of cardiac biomarkers (troponin I and CK-MB). There was a viral prodrome within 1-4 weeks in all patients with myocarditis/myopericarditis. Electrocardiography showed ST segment changes/ T wave inversion.

Complete blood count, acute phase reactants [C-reactive protein (CRP)], creatinine kinase (CK), cardiac biomarkers (troponin I and CK-MB), Pro-BNP were also evaluated in MIS-C and myocarditis/myopericarditis.

Inclusion criteria for patients were to be diagnosed and followed up in this single-center clinic, and that they could have no other cardiologic diseases including structural heart diseases and arrhythmia syndromes. The exclusion criteria for patients were that they have other systemic diseases. Therefore, children with chronic illnesses and those taking daily medications were excluded from the study.

Standard 12-lead ECG was performed for the patients at a paper speed of 25 mm/second under similar conditions. A Nihon Kohden ECG 1250 Cardio fax S

(2009, Tokyo, Japan) device was used at standard velocity and amplitude. Transthoracic echocardiography, performed via Vivid E9 Pro Ultrasound System (GE Medical Systems, Canada) by using 3 and 6 MHz transducers as 2D, M-mode and colored Doppler, conventional continuous-wave (CW) and pulse wave (PW) Doppler visualizing methods. Two experienced pediatric cardiologists performed all studies.

Statistical Analysis

Categorical data were summarized through numbers and percentages. Normal distribution control of continuous data was done with Shapiro-Wilk's test. The continuous data were summarized as mean (± standard deviation) or median and quartiles according to normality assumption. Group comparisons were made with an independent t-test or Mann-Whitney U test. In addition, diagnostic performances of Troponin I and CK-MB parameters were evaluated using Receiver Operating Curve analysis. The sensitivity and specificity values for the obtained cut-off values were summarized. The statistical significance value was taken as $p < 0.05$. Statistical analyses were performed with the STATISTICA 13.0 package program. Power analysis is done to determine the number of patients and control participants.

RESULTS

There were 38 patients with MIS-C and 53 patients with myocarditis/myopericarditis. The mean age was 141.2 ± 38.2 months (4 to 18 years old) in MISC, and 145.8 ± 52.1 months (7 to 18 years old) in peri/myocarditis. Whilst 11 of the MIS-C patients suffered from hypotension requiring follow-up in the intensive care unit, the clinical progress was better in myocarditis/myopericarditis, and hypotensive value was observed in only one patient.

Imaging and Other Examination Results

Left ventricular systolic function slightly decreased (EF: 45-53%), in 11 patients with MIS-C; while it was in 5 patients with myocarditis/myopericarditis. The median ventricular ejection fraction was significantly lower in the MISC group (62.1% vs 69.6%; $p = 0.023$) and the median and z score of left ventricular end-diastolic dimension (LVEDD) was normal in all patients. All patients had recovery of cardiac function at discharge.

Considering the duration of hospitalization, the median hospitalization day was 16 days (5- 67 days) in patients with MIS-C, the median stay was 5 days (2-21 days) in patients with isolated viral myocarditis/myopericarditis. It was significantly longer in patients with MIS-C ($p = 0.034$).

Systolic and diastolic blood pressures were lower in MIS-C patients than in the others. The baseline characteristics of all participants are summarized in Table 1.

Laboratory Biomarkers

Complete blood count and biochemistry markers are all evaluated in both groups. Acute phase reactants

Table 1. Demographic features and physical examination results of the groups

	MIS-C	Isolated PERI/ myocarditis	*p* value
Age (months)	141.2 ± 38.2	145.8 ± 52.1	**0.192**
Gender, n (%)			
Male	23 (60.5)	29 (54.7)	**0.092**
Female	15 (39.4)	24 (45.2)	
Systolic blood pressure (mmHg)	102.45 ± 21.52	117.85 ± 11.24	**0.045**
Diastolic blood pressure (mmHg)	68.23 ± 15.33	76.21 ± 12.68	**0.038**
Height (cm)	138.13 ± 26.54	144.53 ± 38.21	**0.321**
Weight (kg)	37.21 ± 16.35	45.76 ± 23.72	**0.233**

Data are shown as mean ± standard deviation or n (%). MIS-C = Multi-system inflammatory disease in children.

Table 2. The comparison of cardiac biomarkers between MIS-C and isolated myocarditis/myopericarditis

Groups	MIS-C (n = 38)			Myocarditis/ Myopericarditis (n = 53)			*p* - value
	Median	1st Quarter (Q1)	3rd Quarter (Q3)	Median	1st Quarter (Q1)	3rd Quarter (Q3)	
Troponin I*	145.00	114.98	207.50	901.00	234.00	2802.00	**< 0.001**
CK[ɜ]	125.00	118.00	189.00	226.00	101.25	456.00	**0.035**
CK-MB[Ψ]	2.25	0.88	3.30	6.70	2.75	19.20	**< 0.001**
Pro-BNP[Φ]	2714.50	136.00	19219.50	294.00	92.25	3744.25	0.225

MIS-C = Multi-system inflammatory disease in children.

*Troponin I normal range: 12-20 ng/L

[ɜ]Creatinin kinase (CK) normal range: < 170 U/L

[Ψ]Creatinin kinase-myocardial band (CK-MB) normal range: 0.6-6.3 ng/mL

[Φ]pro Brain natriuretic peptide (ProBNP) normal range: < 300 pg/mL.

were higher in all patients with MIS-C. CRP normal range was below 5mg/L, and it was significantly higher in MIS-C patients than in isolated myocarditis/myopericarditis [median 121.6 mg/L (36.5-221) versus 12.6 mg/L (4.2- 90), respectively].

Troponin I level was high in all of the patients (Normal range is 12-20 ng/L). The median troponin I level was 145 ng/L (95-1220) in MIS-C patients, and it was 901 ng/L (196- >20.000) in myocarditis/myopericarditis. Normal values for CK-MB were between 0.6 to 6.3 ng/mL. CK-MB median was 2.25 ng/mL versus 6.7 ng/mL in MIS-C and myocarditis/myopericarditis, respectively. Pro-BNP level should be below 300 pg/mL, and it was 2714.5 pg/mL in MIS-C, and 294 pg/mL in patients with myocarditis/myopericarditis. Troponin I, CK-MB was significantly higher in myocarditis/myopericarditis, while Pro-BNP was significantly higher in MIS-C patients ($p < 0.05$) (Table 2).

The discriminating power of troponin I and CK-MB parameters on patients with MIS-C and myocarditis/myopericarditis was evaluated. The success of the parameters in classification was found to be statistically significant ($p < 0.001$). The area under the curve was Receiver Operating Curve (ROC) = 0.908 [0.82 – 0.96] and ROC = 0.800 [0.68 – 0.88], respectively. According to this model, individuals with a Troponin l parameter value below 100 were classified as MIS-C, while individuals with a CK-MB parameter value below 4.30 were classified as MIS-C (Table 3).

DISCUSSION

This study highlights the important differences between isolated myocarditis/myopericarditis and MIS−C myocarditis. Compared with isolated viral myocarditis/myopericarditis, those with MIS−C had

Table 3. The differential diagnosis power of cardiac biomarkers in isolated myocarditis/myopericarditis and MIS-C

Parameter	ROC [CI]	*p* value	Cut off	Sensitivity	95%CI	Specificity	95% CI
Troponin l	0.908 [0.82-0.96]	**< 0.001**	≤ 100	76.67	57.7-90.1	93.02	80.9-98.5
CK-MB	0.800[0.68-0.88]	**< 0.001**	≤ 4.3	90.00	73.5-97.9	64.29	48.0-78.4

CK-MB = Creatinin kinase-myocardial band, MIS-C = Multiystem innflammatory disease in children, ROC = Receiver Operating Curve

more significant elevation in pro-BNP value, and worse inflammation at presentation, but had lower troponin values with a much longer hospitalization stay. Cut-off values were calculated by this study for each entity with myocardial involvement.

It seems challenging to diagnose isolated myocarditis/myopericarditis in the pediatric population, especially in the era of other systemic diseases such as MIS-C. The clinical course and treatment could vary between these diseases and optimal clinical care and prognosis would be predictable due to laboratory markers. This study showed that laboratory markers could be utilized to make a differential diagnosis between the MIS-C and isolated myocarditis/myopericarditis. Although other clinical findings and history could help clinicians distinguish them, in some controversial situations, it is better to use a reliable marker to be sure of the diagnosis and treatment. Also, this comparison could give us the point that elevation of cardiac enzymes like troponin I doesn't correlate with the worse clinical condition all the time in the means of myocardial involvement. To our knowledge, this is the first study to analyze the cardiac biomarkers between these two groups.

There have been some previous studies comparing myocarditis and MIS-C in terms of clinical findings and prognosis [7, 17]. Cardiac involvement is not always present in MIS-C, it may occur secondary to systemic involvement [3]. In some cases, these two clinical conditions could be confused with each other. The course and severity of these two diseases and the use of cardiac biomarkers in the diagnosis and differential diagnosis were evaluated in this study.

The importance of cardiac biomarkers in the diagnosis of isolated myocarditis/myopericarditis is known. Elevated troponin I and pro-BNP are found to be associated with presentation in shock and LV dysfunction in some studies [18]. Although some studies have shown that the disease progresses more severely in cases with COVID-19-related myocarditis with high cardiac biomarkers [19], it has been reported that the very high troponin value in isolated viral myocarditis/myopericarditis doesn't mean severe myocardial damage [20]. Although troponin is more effective in demonstrating cardiac damage than CK-MB, it still has a low specificity. In this study, while elevated cardiac enzymes were higher in patients with isolated myocarditis/myopericarditis, troponin I was lower in patients with systemic involvement, such as MIS-C. However, clinical deterioration and worsening of cardiac functions were not evident in this disease group with myocarditis/myopericarditis. We attributed this to the predominance of pericardial involvement rather than myocardial damage in the isolated myocarditis/myopericarditis group. Pericardial involvement could also lead to higher troponin I levels [21].

Another cardiac biomarker is Pro-BNP. Elevated pro-BNP was also found to be associated with bad prognosis in diseases with myocardial involvement [22]. Higher pro-BNP values in MIS-C are another important parameter that may benefit clinicians in terms of both differential diagnosis and clinical course. The increase in pro-BNP value was found to be more pronounced in MIS-C patients than troponin in another study, and pro-BNP elevation was correlated with worse left ventricular function and a higher risk of cardiogenic shock [22, 23]. In fact, MIS-C patients also had higher troponin levels, but troponin was lower according to the myocarditis/myopericarditis patients in this study. MIS-C could be defined as a kind of vasculitis and systemic effects due to cytokines may affect cardiac functions [24]. Especially, pro-BNP may be more effective in demonstrating cardiac involvement in this kind of patient with multisystemic involvement.

In MIS-C patients, high pro-BNP levels were associated with prolonged length of stay in this study. The decrease in EF values was more pronounced in some MIS-C patients. ROC analyses were also performed, and limit values specific to cardiac involvement seen in MIS-C were found with cardiac biomarkers. Cut-off values that distinguish MIS-C and myocarditis and that may be useful in the diagnosis were determined. ROC analyses have been evaluated in other systemic diseases in the literature and the results supported the clinical use of these cut-off values [25].

Acute phase reactants were also higher in the MIS-C group, which is also consistent with widespread systemic inflammation and intensive care unit needs. In another study, acute phase reactants increased significantly in MIS-C and adversely affected the prognosis [17].

The management of acute myocarditis/myopericarditis is mainly conducive [2]. The use of IVIG and

steroids which have antiviral, anti-inflammatory, and immunomodulatory effects, remains controversial; IVIG has been shown to provide meaningful benefit in some pediatric patients, though not definitively [2]. On the contrary, in MIS-C, there is some evidence supporting that combination therapy with IVIG and steroids is related to good prognosis with reduced intensive care unit hospitalization [18]. Management of MIS-C and myocarditis/myopericarditis could differ among institutional protocols. When we evaluate the effects of cardiac biomarkers on treatment, as the higher troponin I didn't correlate with bad clinical course in this study, it seems higher pro-BNP correlates more with longer hospital stay and EF decrease. Thus, compatible with the literature, it seems that IVIG and steroid therapy are not essential in patients with myocarditis/myopericarditis associated with higher troponin I and lower pro-BNP.

Limitations

This is a single-center study and these diseases are so scarce, especially MIS-C. Finally, further large sample works should be accompanied to analyze the clinical usage of cardiac biomarkers in different clinical situations that comprise myocardial involvement and differential diagnosis.

CONCLUSION

In conclusion, both MIS-C and isolated viral myocarditis/myopericarditis could cause pro-BNP elevation, in which MIS-C is most prominent. Cardiac enzymes could rise in both diseases but most significantly in myocarditis/myopericarditis. These values will be useful in determining the diagnosis and risk of intensive care unit need for cardiac, and other systemic effects. Randomized controlled studies with a larger number of patients should be done in this manner.

Authors' Contribution

Study Conception: DD, DK; Study Design: DD, DK; Supervision: DD, DK; Funding: N/A; Materials: DD, DK; Data Collection and Processing: DD, DK; Statistical Analysis and Data Interpretation: DD, DK; Literature Review: DD, DK; Manuscript Preparation: DD and Critical Review: DD, DK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The effect of aromatherapy on labor pain, duration of labor, anxiety and Apgar score outcome: a systematic review and meta-analysis

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ABSTRACT

Objectives: This study was conducted to conduct a systematic review and meta-analysis of studies examining the effect of aromatherapy intervention on labor pain, duration of labor, anxiety and apgar scores in primiparous women.

Methods: The literature search was conducted in PubMed, CINAHL, Scopus and Science Citation Index (Web of Science) until February 2023. This study is based on the recommendations of the Cochrane guidelines. The data were analyzed using the Review Manager computer program (Version 5.4).

Results: The analysis was completed with 10 studies including 950 primiparous pregnant women. The average pooled results of the studies showed that there was a significant difference in the effect of aromatherapy on labor pain (SMD: -0.68 95% CI: -0.76 to -0.60, Z = 16.32, $p < 0.01$) and duration (SMD: -0.36 95% CI: -0.47 to -0.25, Z = 6.40, $p < 0.00001$) in the latent, active, and transition phase. When the mean results of anxiety scores were examined, it was determined that the difference between the groups was significant (SMD: -15.89 95% CI: -16.78 to -14.99, Z = 34.79, $p < 0.00001$).

Conclusions: While the aromatherapy application used in childbirth reduced the duration and pain of the latent transition and active phase of birth, it was found that it reduced the anxiety of the pregnants in the active and transition phase.

Keywords: Aromatherapy, labor pain, labour, anxiety, Apgar score, meta-analysis

Aromatherapy is one of the main complementary and alternative medicine methods widely used in the world and is a branch of phytotherapy. Aromatherapy is used to ensure the physical and psychological well-being of the individual by using the high concentration of essential oil and smell obtained by distillation of the plant to use the therapeutic properties of the plants [1, 2]. Aromatherapy can reach the cerebral cortex directly through connections extending to the limbic system and hypothalamus through smell. The smell reaching the cortex creates spiritual, physical, and behavioral effects on the individual [3]. The pleasant smell that essential oils and other fragrances give to the environment is used as an encouragement to the

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woman during childbirth. Among the most common types of aromatherapies during childbirth include massage, bathing, and inhalation [1, 2].

Although pregnancy and childbirth are a physiological event, pain due to uterine contractions in the process of action is defined among the strongest pains and is among the important factors that cause many women to fear labor. Therefore, control of labor pain is one of the main goals of the care given to women who give birth [4, 5]. Although scientific studies have not shown its full effectiveness, it is believed that aromatherapy can act as a drug on the brain and nervous system. Aromatherapy is thought to be beneficial in reducing pain and causing relaxation by increasing neurotransmitters and reducing the amount of epinephrine and norepinephrine in the blood [2, 6, 7]. The results of the studies included in a systematic review showed that the aromatherapy applied increased the quality of sleep, increased the pain tolerance during the applied procedures, and reduced the level of pain and anxiety felt. In addition, it has been reported that the application of aromatherapy positively affects vital signs and increases the level of comfort and satisfaction [3]. Although aromatherapy applications are used in various areas of women's health in the literature, the evidence for the effectiveness of the applications is limited. The aim of this analysis is to systematically review and meta-analyze studies examining the effect of aromatherapy intervention on labor pain, duration of labor, anxiety and Apgar score in primiparous women.

For this purpose, analysis questions; (a) What is the effect of the aromatherapy intervention given to primiparous women on labor pain? (b) What is the effect of aromatherapy on the duration of labor? (c) What is the effect of aromatherapy on anxiety at birth? (d) What is the effect of aromatherapy on Apgar score at birth?

METHODS

This study was conducted to conduct a systematic review and meta-analysis of studies examining the effect of aromatreapy intervention on labor pain, duration of labor, anxiety and apgar score in primiparous women. In the preparation of the systematic review and meta-analysis, the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement) directive was followed [8]. During the study, literature review, article selection, data extraction and quality evaluation of the included articles were independently performed by two researchers to keep the risk of bias under control. In case of disagreement on any issue, all the researchers came together for a discussion and a final consensus. During the study, there was no situation that would require a deviation from the protocol and the study was concluded in accordance with the protocol entered in the PROSPERO database.

Eligibility Criteria

The following criteria (PICOS) were considered in the selection of the studies to be included in the study:

Participant (P): Primiparous pregnant women

Intervention (I): Aromatherapy

Comparison (C); Pregnant women who are not allowed to aromatherapy during labor.

Results (O); labor pain, duration of labor, anxiety, Apgar score

Study design (S); Randomized controlled experimental studies published in English and Turkish between 2013 and 2023.

Studies examining antenatal or postnatal fear, studies reflecting pregnant women with psychological illnesses, as well as articles using measurement tools that have no validity, and traditional and systematic reviews were excluded.

In addition, reviews, quasi-experimental studies, case reports, qualitative studies, unpublished theses, congress papers and descriptive studies formed the exclusion criteria of the study.

Search Strategy

The literature review for this systematic review was conducted between February and March 2023 using four electronic databases (PubMed, CINAHL, Scopus and WOS). Primipar pregnant women were screened for tocophobia using medical topics or keywords. The keywords were: "birth," OR "childbirth,"OR "labor," AND "pain"OR "labor pain" AND "aromatherapy," OR "essential oils". The search strategy was changed according to the characteristics of each database. In addition, reviews on articles included in systematic reference lists and other previous systematic reviews were checked to reach further studies.

Selection of Studies and Data Extraction

After removing duplicate articles from different databases, two researchers (A.Y.K. and F.Ş.B.) independently conducted a literature review, article selection, data extraction and quality evaluation of the included articles to control the risk of bias during the study. The two independent reviewers first scanned the titles and abstracts to determine which studies met the inclusion and exclusion criteria. Those that met the inclusion criteria or could not be identified from the title/abstract scan were examined for full text, and when consensus could not be reached, the researchers considered the study in common. A data extraction tool developed by the researchers was used to obtain the research data. Two reviewers (A.Y.K. and F.Ş.B.) obtain data on the location and year of the study, year of publication, research design, sample size, inclusion, and exclusion criteria, aromotherapy intervention and delivery pain and duration with this data extraction tool (Table 1).

Statistical Analysis

Meta-analysis was performed using Review Manager 5.4 (The Nordic Cochrane Center, Copenhagen, Denmark) for data analysis. The heterogeneity between the studies was evaluated using Cochran's Q test and Higgins' I^2, and it was accepted that I^2 greater than 50% showed significant heterogeneity. Accordingly, random effect results were considered when I^2 was greater than 50%, and fixed effect results were considered if it was less than the value. Odds ratio (OR) for categorical variables, mean difference (MD) and standardized mean difference (SMD) for continuous variables were calculated. MD or SMD, along with the corresponding 95% confidence interval (CI), is appropriately pooled for continuous variables based on whether the results are measured on the same scales. All tests were calculated from two-pronged tests, and a p value of less than 0.05 was considered statistically significant.

Risk of Bias

The quality of the articles in randomized controlled trials and the Version 2 of the Cochrane Risk-of-Bias tool (RoB-2) were used for randomized trials. All selected articles were independently conducted by an author (A.Y.K.) using the Cochrane tool to assess the risk of nepotism. The criteria outlined in the Cochrane Handbook for Systematic Investigations of Interventions are; were classified into six areas: ((random sequence generation (selection bias), allocation obfuscation (selection bias), blinding of participants and staff (performance bias), blinding of outcome evaluation (detection bias), handling of missing outcome data (attrition bias), selective outcome reporting (notification bias), and other potential sources of bias (conflict of interest and funding sources)). The risk of bias for each area is classified as "low risk", "high risk" or "uncertain risk" according to the decision criteria in the "bias risk" assessment tool.

RESULTS

Through electronic database research and manual search, 4932 articles were found. After repeated registrations, 4900 articles were evaluated. Titles and abstracts were read to identify relevant articles, review articles, protocols, duplications, different populations, and 4856 articles were removed because they did not meet the inclusion criteria. The remaining 71 full texts were evaluated for eligibility. 10 RCT articles were included in the analysis because they met the desired criteria (Fig. 1).

Study Characteristics

The characteristics of the studies are summarized in Table 1. This systematic review and meta-analysis included 10 studies conducted in five countries, involving a total of 950 primiparous pregnant women for the effect of aromatherapy on labor pain. Study data were found in five different countries in Egypt [9], Indian [10], Thailand [11], Turkey [12], and Iran [13-18]. All studies included in this systematic review and meta-analysis included primipar pregnant women in the study. The design of all his works included in the meta-analysis is RCT. In all articles included in the study, aromatherapy was evaluated in the latent, active, and transitional phase for relieving labor pain [9-18]. While the pregnant woman in the control group was left blank in most of the articles, plesabo in one study [16], normal salline in one study [14], distilled water in two studies [13, 17], massage alone versus aromatherapy massage in three studies [9, 10, 18] in one study Entonox gas [15] was given. While all the groups in the study were completed with two groups,

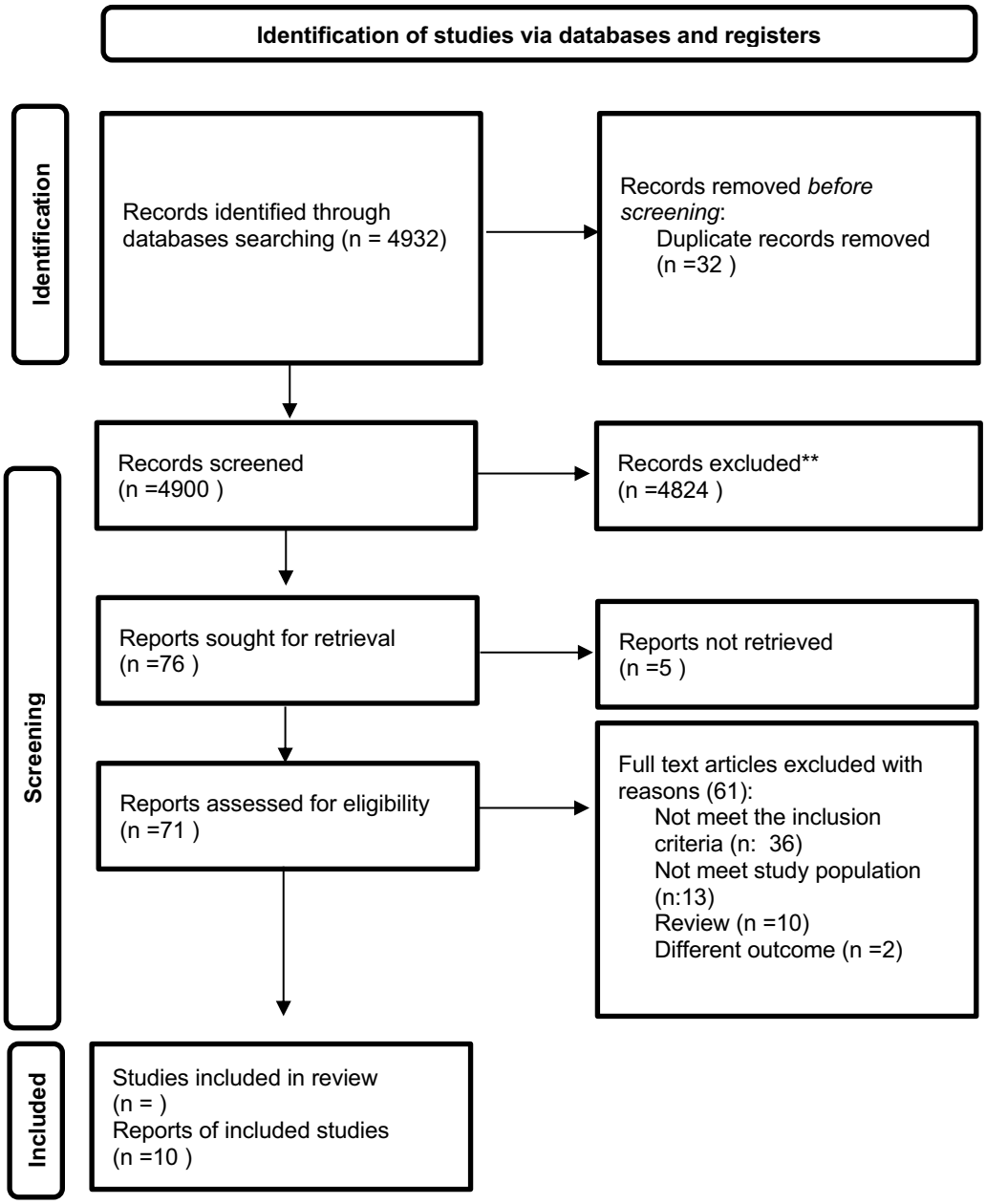

Fig. 1. PRISMA flow diagram. PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

not intervention and control groups, in two studies [12, 17] conducted a study with three groups. Pregnant women in the intervention group applied the following aromatherapy oils for labor pain; Lavander in six studies [9, 10, 12, 13, 15, 18], rosa in one study [14], essential oil in one study [169, Salvia and Jassimine in a study, and lavender, geranium rose, citrus and jasmine oils in one study were presented depending on participant preference [11]. The entire pregnant population included females (primiparous) who would give birth for the first time. In all studies, labor pain scores were considered as the primary outcome. In six of the studies, birth time [9-12, 17, 18] maternal anxiety in two [9, 14], five reported apgar scores [11, 13, 14, 16, 17].

Primary Outcomes

In Fig. 2, the results of the meta-analysis of the effect of aromatherapy on labor pain were presented as Forest Pilot. In four of the studies, labor pain was associated with the Numeric Rating Scale (NRS) [11, 13, 14, 16] with VAS in six [9, 10, 12, 15, 17, 18].

Table 1. Study characteristics

References, Country	Study Design	Study Period	Data Collection Tools	Age	Sample Size/ Parite	Aromatherapy Type	Aromatherapy Time and Method	Stage of intervention	Labor Pain (VAS Score)	Labor duration (min+hours)	Anxiety, Apgar scores
Lamadah [9], Egypt	RCT	June - September 2013	VAS	**N\A**	**Aromatherapy:** 30 **Control**: 30 Primiparous	Researchers' selections (lavender oil dissolved)	40 minutes back massage	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 7.0 ± 0.11 Active Phase: 6.4 ± 0.20 Transitional Phase: 7.7 ± 0.17 **Control** Latent Phase: 8.1 ± 0.14 Active Phase: 8.9 ± 0.19 Transitional Phase: 9.6 ± .50	**Aromatherapy:** Latent Phase (hours): 2.73 ± 1.05 Active Phase (min): 23.60 ± 15.35 Transitional Phase (min): 36.63 ± 8.05 **Control:** Latent Phase (hours): 3.17 ± 1.23 Active Phase (min): 27.23 ± 2.21 Transitional Phase (min): 24.93 ± 8.37	**Aromatherapy** Latent Phase: 55.47 ± 9.91 Active Phase: 38.40 ± 6.53 Transitional Phase: 36.63 ± 8.05 **Control** Latent Phase: 50.40 ± 5.75 Active Phase: 45.13 ± 9.10 Transitional Phase: 44.07 ± 9.95
Raju[10], Indian	RCT	December 2012-March 2013	VAS	**N\A**	**Aromatherapy:** 30 Control: 30 Primiparous	Researchers' selections (lavender oil dissolved)	N\A Back massage	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 6.2 ± 0.13 Active Phase: 7.5 ± 0.21 Transitional Phase: 8.3 ± 0.47 **Control** Latent Phase: 8.6 ± 0.5 Active Phase: 9.0 ± 0.34 Transitional Phase: 9.6 ± .21	**Aromatherapy**: Latent Phase (hours): 11.55 ± 2.4 Active Phase (hours): 1.5 ± 0.33 Transitional Phase (hours): 0.28 ± 0.02 **Control:** Latent Phase (hours): 14.58 ± 2.9 Active Phase (hours): 1.58 ± 0.37 Transitional Phase (hours): 0.30 ± 0.05	**N\A**
Tanvisut [11], Tayland	RCT	December 2015-December 2016	NPS	**Aromatherapy:** 26.54 ± 4.69 **Control**: 24.92 ± 4.31	**Aromatherapy:** 53 **Control:** 53 Primiparous	Researchers' selections (Aroma oil)	Diffused water	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 1.88 ± 2.24 Active Phase: 3.82 ± 2.45 Transitional Phase: 5.45 ± 2.28 **Control** Latent Phase: 2.60 ± 2.21 Active Phase: 4.39 ± 2.10 Transitional Phase: 5.62 ± 2.10	**Aromatherapy:** Latent Phase (min) 775.6 ± 334.9 Active Phase (min): 30.2 ± 28.1 Transitional Phase (min): 36.63 ± 8.05 **Control:** Latent Phase(min): 669.7 ± 463.7 Active Phase(min): 23.6 ± 20.4	**N\A**
Karatopuk[12], Turkey	RCT	February 2021-April 2021	VAS	**Aromatherapy massage**: 21.32 ± 2.73 Aromatherapy inhalasion: 20.61 ± 2.38 **Control:** 20.92 ± 3.02	**Aromatherapy massage**: 37 **Aramoterapy inhalasion:** 44 **Control:** 40 Primiparous	Researchers' selections (Lavender oil)	15 minutes massage 3 minutes inhalasion	Latent Phase Active Phase Transitional Phase	**Aromatherapy massage** Latent Phase: 1.54 ± 0.65 Active Phase: 2.81 ± 0.88 Transitional Phase: 4.46 ± 1.41 **Aromatherapy inhalasyon** Latent Phase :1.89 ± 0.92 Active Phase: 4.0 ± 1.45 Transitional Phase: 6.34 ± 1.60 **Control** Latent Phase :3.20 ± 0.99 Active Phase: 6.68 ± 1.07 Transitional Phase: 9.08 ± 0.73	**Aromatherapy massage** Latent Phase: 8.51 ± 1.95 Active Phase: 2.68 ± 0.58 Transitional Phase: 1.92 ± 0.83 **Aromatherapy inhalasyon** Latent Phase: 8.45 ± 1.78 Active Phase: 2.84 ± 0.71 Transitional Phase: 2.11 ± 0.84 **Control** Latent Phase: 9.43 ± 1.72 Active Phase: 2.73 ± 0.45 Transitional Phase: 2.13 ± 0.76	**N\A**
Yazdkhasti [13], Iran	RCT	September 2011-January 2012	NPS	**Aromatherapy:** 18.26 ± 2.83 **Control:** 19.13 ± 2.56	**Aromatherapy:**60 **Control:** 60 Primiparous	Researchers' selections (Lavender oil)	3 minutes massage and inhalasion	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 6.1 ± 2.3 Active Phase: 6.7 ± 2.0 Transitional Phase: 7.93 ± 2.1 **Control** Latent Phase: 7.7 ± 2.1 Active Phase: 8.6 ± 1.6 Transitional Phase: 9.4 ± 1.1	**Aromatherapy:** Active Phase (min): 170.2 ± 91.08 Transitional Phase (min): 59.4 ± 35.4 **Control:** Active Phase (min): 181.5 ± 93.6 Transitional Phase (min): 48.66 ± 23.5	**Aromatherapy** 1st min Apgar score: 8.8 ± 0.37 5th min apgar score: 9.9 ± 0.35 **Control** 1st min Apgar score: 8.7 ± 1.01 5th min apgar score: 9.7 ± 1.01

Table 1 Continued. **Study characteristics**

References, Country	Study Design	Study Period	Data Collection Tools	Age	Sample Size/ Parite	Aromatherapy Type	Aromatherapy Time and Method	Stage of intervention	Labor Pain (VAS Score)	Labor duration (min+hours)	Anxiety, Apgar scores
Hamdamian [14], Iran	RCT	N\A	NPS	**Aromatherapy:** 25.87 ± 5.17 **Control**: 26.24 ± 5.15	**Aromatherapy:** 58 **Control:** 58 Primiparous	Researchers' selections (R. damascene oil)	3 minutes inhalasion	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 3,25 ± 1,02 Active Phase: 5,11 ± 0,71 Transitional Phase: 6,69 ± 0,47 **Control** Latent Phase: 6,36 ± 1,02 Active Phase: 8,42 ± 0,50 Transitional Phase: 9,78 ± 0,42	**Aromatherapy:** Latent Phase (min): 37,18 ± 4,38 Active Phase (min): 47,27 ± 3,16 Transitional Phase (min): 55,82 ± 3,82 **Control:** Latent Phase (min): 38,91 ± 4,78 Active Phase (min): 47,09 ± 3,93 Transitional Phase (min): 54,54 ± 3,76	**Aromatherapy** Latent Phase: 35,14 ± 5,95 Active Phase: 46,12 ± 4,23 Transitional Phase: 55,14 ± 3,42 **Control** Latent Phase: 39,5 ± 8,28 Active Phase: 65,33 ± 4,78 Transitional Phase: 75,51 ± 3,55
Azizi Salimeh [15], Iran	RCT	June to September 2015	VAS	**Aromatherapy:** 26.7 ± 5.5 **Control:** 25.8 ± 5.5	Aromatherapy: 63 Control: 63 Primiparous	Mothers' selections (Lavander oil)	3 minutes inhalasion	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 4.7 ± 1.8 Active Phase: 5.1 ± 1.9 Transitional Phase: 5.9 ± 1.9 **Control** Latent Phase: 6.02 ± 2.04 Active Phase: 6.5 ± 2.1 Transitional Phase: 6.9 ± 2.2	N\A	**N\A**
Saeieh [16], Iran	RCT	March 2007-June 2008	NRS	**N\A**	**Aromatherapy:** 63 **Control:**63 Primiparous	Researchers' selections (essential oil)	Every 30 minutes inhalasion	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 4.98 ± 0.93 Active Phase: 5.79 ± 1.13 Transitional Phase: 6.35 ± 1.63 **Control** Latent Phase: 6.68 ± 1.28 Active Phase: 7.23 ± 1.54 Transitional Phase:7.71 ± 1.38	**N\A**	**Aromatherapy** 1st min Apgar score: 8.77 ± 0.37 5th min apgar score: 9.97 ± 0.17 **Control** 1st min Apgar score: 8.67 ± 0.56 5th min apgar score: 9.95 ± 0.21
Kaviani [17], Iran	RCT	October 2009-March 2010	VAS	**N\A**	**Salvia:** 52 **Aromatherapy:** 52 **Control:** 52 Primiparous	Researchers' selections (Jasmine oil)	15 minutes inhaliasion	30 min after the intervention 60 min after the intervention	**Salvia:** 30 min after: 3.19 ± 1.1 60 min after: 4.31 ± 0.9 **Aromatherapy** 30 min after: 2.46 ± 0.87 60 min after: 4.0 ± 0.84 **Control** 30 min after: 3.17 ± 1.2 60 min after: 3.98 ± 1.27	**Salvia:** First stage: 493.6 ± 59.8 Second stage: 46 ± 7.2 **Aromatherapy** First stage: 460.3 ± 65.5 Second stage: 44.3 ± 7.6 **Control** First stage: 509.3 ± 60.1 Second stage:49.83 ± 8.6	Salvia: 1st min Apgar score: 8.9 ± 0.44 5th min apgar score: 9.98 ± 0.15 Aromatherapy 1st min Apgar score: 8.88 ± 0.53 5th min apgar score: 9.96 ± 0.2 Control 1st min Apgar score: 8.98 ± 0.15 5th min apgar score: 10.0 ± 0.0
Zahra [18], Iran	RCT	March 2007-June 2008	VAS	**Aromatherapy:** 22.63 ± 3.48 **Control**: 26.66 ± 3.67	**Aromatherapy:** 30 **Control:** 30 Primiparous	Researchers' selections (a Lavander oil)	60 min back massage	Latent Phase Active Phase Transitional Phase	**Aromatherapy** Latent Phase: 3.20 ± 1.2 Active Phase: 5 ± 0.78 Transitional Phase: 6.16 ± 0.54 **Control** Latent Phase: 4.2 ± 1,02 Active Phase: 6.7 ± 0,50 Transitional Phase: 7.53 ± 0,42	**Aromatherapy** Latent Phase: 4.05 ± 1.95 Active Phase: 29± 10.46 Transitional Phase: 5.66 ± 2.66 **Control** Latent Phase: 5.21 ± 2.52 Active Phase: 42.36 ± 13.86 Transitional Phase: 5.86 ± 2.52	N\A

PC = Placebo group, AG = Aromaterapy group, CG = Control Group, RCT = Randomized Control Trial, PW = Primiparous Woman, MG = Massage Group, VAS = Visual Analogue Pain İntensity Scale, NRS = Numeric Pain Rating Scales

The Effect of Aromatherapy on Labor Pain

In all the studies examined, the authors reported results on the effect of aromatherapy on labor pain. The average pooled results of the studies showed that the effect of aromatherapy on labor pain was significant difference in the latent phase (SMD: -0.53 95% CI: -0.67 to -0.36, Z = 7.58, $p < 0.0001$) in the active phase (SMD: -0.84 95% CI: - 0.98 to -0.69, Z = 11.29, $p < 0.00001$) and in the transition phase (SMD: -0.69 95% CI: -0.83 to -0.55, Z = 9.66, $p < 0.00001$) (Fig. 2). When the mean results of the pain assessment were examined, there was a significant difference between the groups (SMD: -0.68 95% CI: -0.76 to -0.60, Z = 16.32, $p < 0.01$).

Second Outcomes

In Fig. 3, the results of the meta-analysis of the effect of aromatherapy on the duration of birth were presented as Forest Pilot. Six of the studies reported results on the duration of birth in the active, latent, and transitional phase of labor [9-12, 17, 18]. No scales were used for the duration of birth. In Fig. 4, the effect of aromatherapy on maternal anxiety is presented in a forest chart of the meta-analysis. In two of the studies, information was provided about the outcomes of maternal anxiety in the active, latent, and transitional phase of delivery [9, 14]. Spielberger state-trait anxiety questionnaire was not used to assess maternal anxiety.

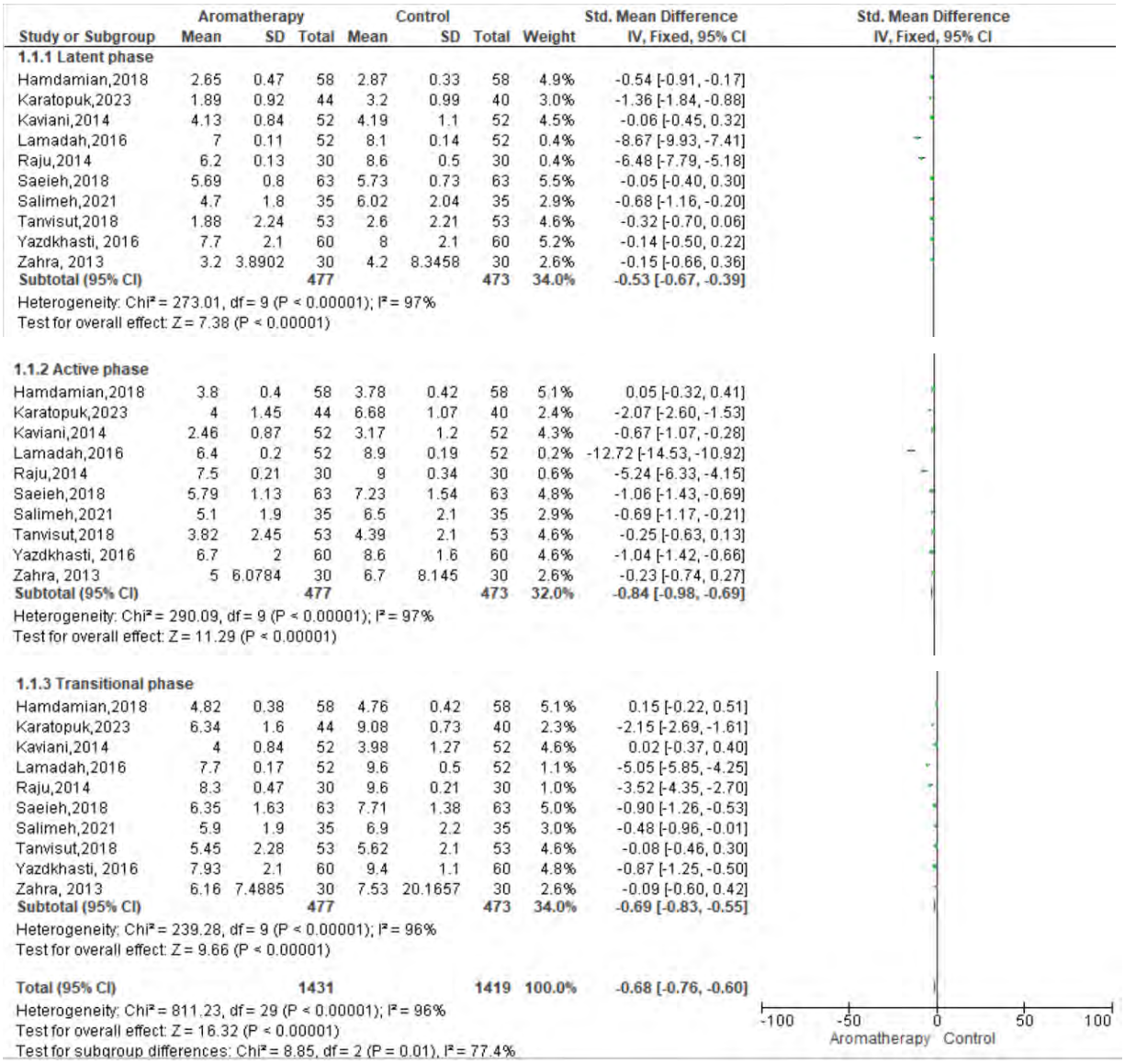

Fig. 2. Meta-analysis results on the effect of aromatherepy on the pain of labor: (1.1.1) latent phases (1.1.2.) active phases, (1.1.3) transtional phases

The Effect of Aromatherapy on the Duration of Labor

In the seven studies examined, the authors reported results on the effect of aromatherapy on the duration of labor. The average pooled results of the studies showed that the effect of aromatherapy on duration of labor was a significant difference in the latent phase (SMD: -0.48 95% CI: -0.69 to -0.26, Z = 4.36, $p < 0.0001$) in the active phase (SMD: -0.20 95% CI: - 0.38 to -0.02, Z=2.14, $p = 0.03$) and transition phase (SMD: -0.44 95% CI: -0.62 to -0.26, Z = 4.74, $p < 0.00001$) (Fig. 3). When the mean results of the duration evaluation were examined, it was determined that the difference between the groups was significant (SMD: -0.36 95% CI: -0.47 to -0.25, Z = 6.40, $p < 0.00001$). The studies had high heterogeneity.

The Effect of Aromatherapy on Anxiety

In the two studies examined, the authors reported results on the effect of aromatherapy on anxiety. The average pooled results of the studies showed that the effect of aromatherapy on anxiety was not significant in the latent phase (SMD: -1.52 95% CI: -3.77 to -0.74, Z = 1.32, $p < 0.19$), while there was a significant difference in the active phase (SMD: -17.33 95% CI: -18.89 to -15.78, Z = 21.85, $p < 0.00001$) and in the transition phase (SMD: -19.40 95% CI: -20.66 to -18.15, Z = 30.25, $p < 0.00001$). When the mean results of anxiety scores were examined, it was determined that the difference between the groups was significant (SMD: -15.89 95% CI: -16.78 to -14.99, Z = 34.79, $p < 0.00001$). There was high heterogeneity among the studies included in the study (Fig. 4).

The Effect of Aromatherapy on Apgar Score After Newborn

In the five studies examined, the authors reported

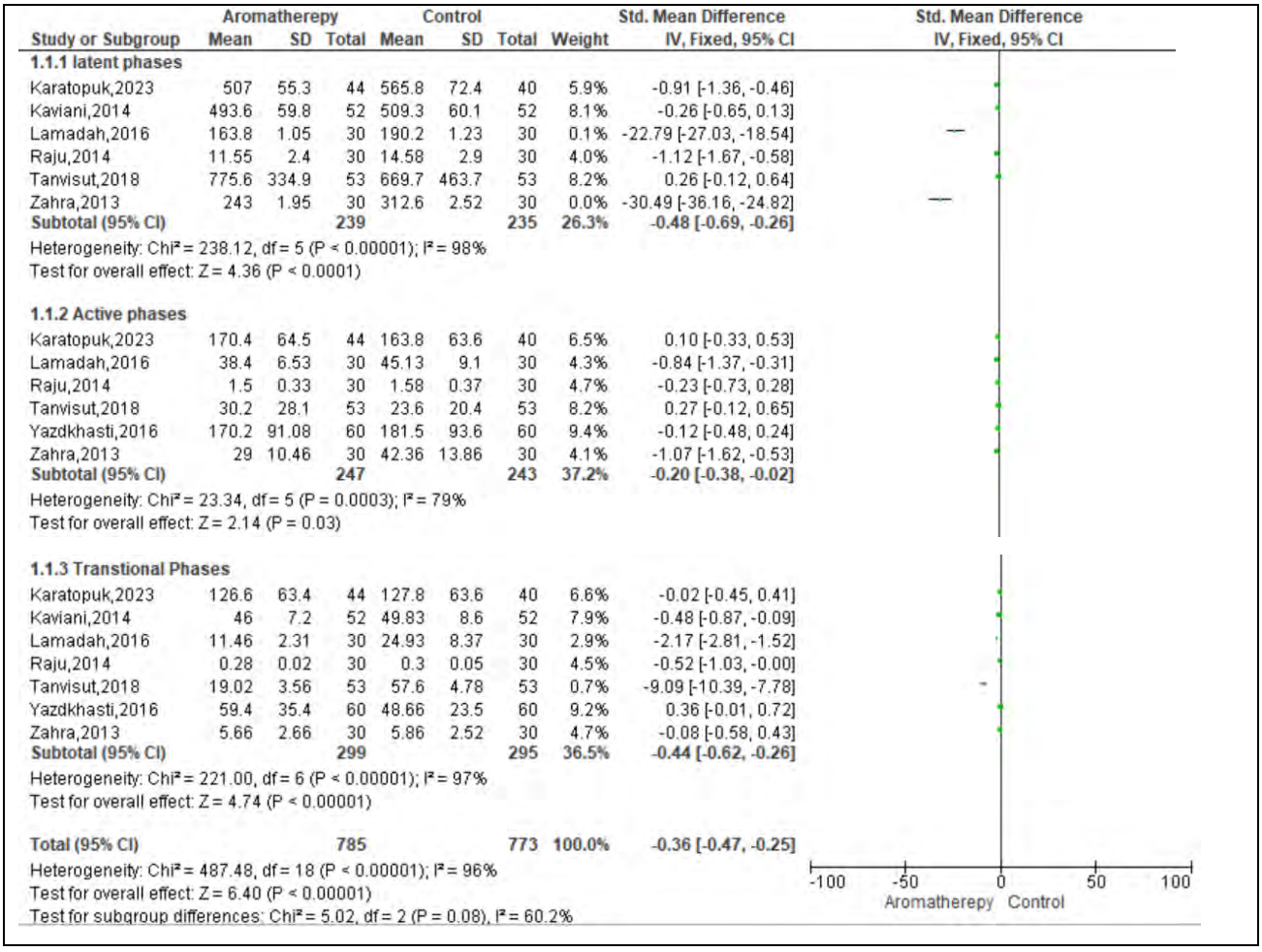

Fig. 3. Meta-analysis results on the effect of aromatherepy on the duration of labor: (1.1.1) latent phases (1.1.2.) active phases, (1.1.3) transtional phases

results on the effect of aromatherapy on the Apgar score in the postpartum period. The mean pooled results of the studies showed that the effect of aromatherapy on apgar score was significant in the first minute (SMD: -0.20 95% CI: -0.36 to -0.03, Z = 2.27, *p* p = 0.02) and that there was no significant difference in the fifth minute (SMD: -0.12 95% CI: -0.29 to -0.05, Z = 1.42, *p* = 0.16). When we looked at the average results of the Apgar scores, it was determined that the difference between the groups was not significant (SMD: -0.16 95% CI: -0.28 to -0.04, Z = 2.61, *p* = 0.54). There was high heterogeneity among the studies included in the study (Fig. 5).

Risk of Bias Assessment

All studies have identified an adequate method for randomly assigning participants to aromatherapy groups [9-18]. For this reason, we evaluated these studies in this area as a low risk of nepotism. Three studies reported adequate allocation confidentiality using sequentially numbered and sealed opaque envelopes and evaluated them at risk of under-favoritism error [11, 13, 18]. One study was judged to be at high risk [9] and other studies to be at risk of uncertainty bias due to insufficient information or lack of mention of factors [10, 12, 15-17]. In the three studies included in the meta-analysis, participants and researchers who participated in the experiment were assessed at low risk of bias in this area while providing information about their blindness to the study [13-15]. The other seven studies were not possible for the participants and researchers who participated in the experiment to be blind to the study, so all studies were evaluated at risk of bias in blinding participants and employees, and this was considered when interpreting the findings [9-12, 16-18]. Two studies are at low risk of blinding outcome evaluation [15, 16]. Other studies have also evaluated the outcome evaluation without blinding it and as having a high risk of favoritism error [9-14, 17, 18]. In all studies [9-18] was found to have a low risk of attrition because the work stoppages were balanced between the intervention and control groups or because there were few releases that would not affect the study. In all studies [9-18] included in the meta-analysis, they were assessed at risk of reporting low bias because they discussed the reported significant results, including negative outcomes, and matched those reported in their records. For each study included, we described important concerns regarding other possible sources of bias that had not previously been addressed in the above categories. Specifically, we looked for a declaration of conflict of interest and a source of funding. Six of the included studies reported no other risk of bias [10-13, 17, 18].

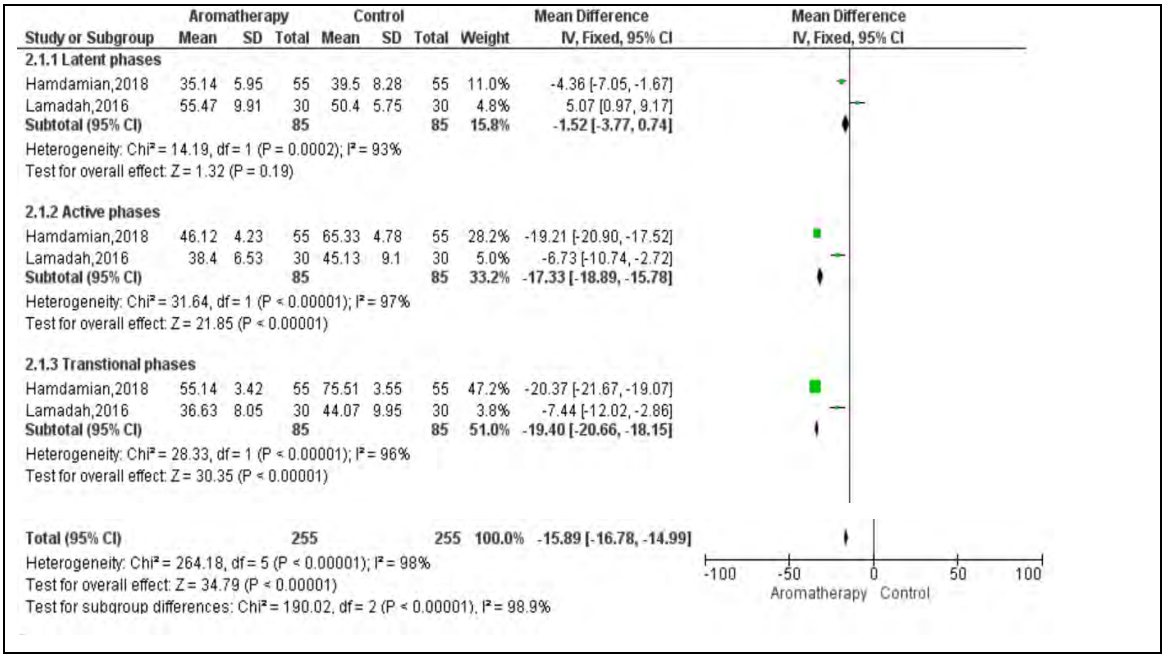

Fig. 4. Meta-analysis results on the effect of aromatherepy on the anxiety score: (2.1.1) latent phases (2.1.2.) active phases, (2.1.3) transtional phases

DISCUSSION

This study was conducted to conduct a systematic review and meta-analysis of studies examining the effect of aromatherapy intervention on labor pain, delivery time, anxiety and apgar score in primiparous women. As a result of this analysis, it was found that the aromatherapy application used in childbirth reduced the duration and pain in the latent transition and active phase of birth, while reducing the anxiety of pregnant women in the active and transition phase and increasing the apgar first minute score.

Labor pain is one of the most severe pains and is considered a complex physiological phenomenon that includes psychological, emotional, spiritual, and physical dimensions [19]. In this study, it was found that aromatherapy intervention reduced labor pain in the latent, transition and active phase. A methalytic study similarly reported to reduce labor pain [20]. In a systematic review, it was found that aromatherapy reduced labor pain [21]. Also, of the studies included in this study, Lavander in six studies [9, 10, 12, 13, 15, 18], rosa in one study [14], BC essential oil in one study [16], Salvia and Jassimine in a study, and lavender, geranium rose, citrus and jasmine oils in one study were presented depending on participant preference [11]. Rose essential oil has long been known to be effective in the reproductive system in women and has been shown to be effective in reducing pain and anxiety during childbirth [14]. Yazdkhasti *et al*. [13] reported that lavender essential oil aromatherapy may be an effective and beneficial treatment option for pain management among pregnant women. In the review published by Tabatabaeichehr and Mortazavi [22] it was reported that the most mentioned essential oil in the studies is lavender, which can be used as a single essential oil or in combination with other essential oils. The literature and study findings are in line with the literature and lavender oil was found to be the most used oil in the management of labor pain. However, the lack of a standard for applications suggests the need for more randomized controlled methodological studies.

Although the birth process is seen as a natural process, the woman who gives birth is faced with serious physiological changes, as well as discomfort, tension and sensory changes that will force her. Due to these changes, women state that they want to use more non-pharmacological methods [2, 11]. As a result of this analysis, it was found that the application of aromatherapy during childbirth reduced anxiety in women during the transition and active phase of labor.

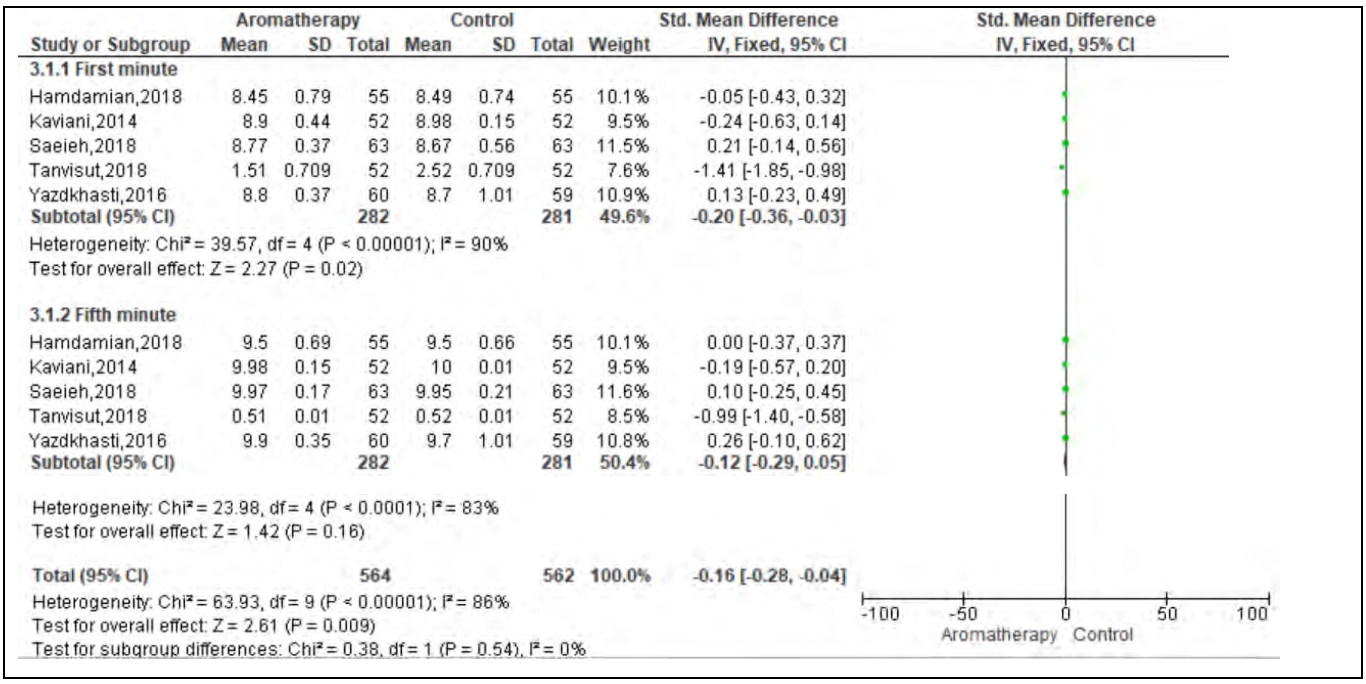

Fig. 5. Meta-analysis results on the effect of aromatherepy on the apgar score: (3.1.1) first minute (3.1.2.) Fifth minute

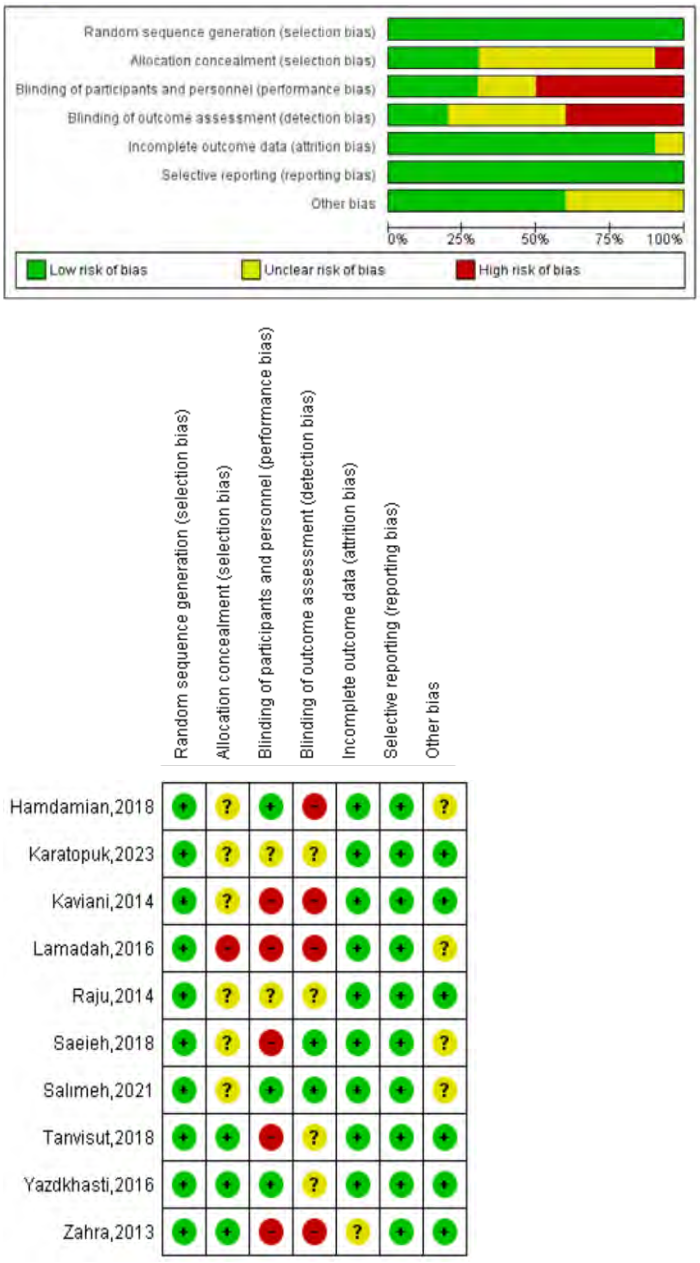

Fig. 6. Risk of bias domains: ROB-2, ROB-2: Risk-of-Bias tool for randomized trials.

In a systematic review study, it was shown that chamomile essential oil during labor significantly reduced anxiety in women during the first stage of labor [23]. As a result of this analysis, it was found that the first minute apgar score increased and shortened the duration of delivery in the group that underwent aromatherapy. This suggests that the positive effect on the duration of labor is reflected in the apgary. In a methalytic study, it was reported that aromatherapy applied at birth did not affect the apgar score [20]. In one study, it was reported that there was no difference between the two groups between the mean duration of the active phase and the second phase of birth [13]. The results of aromatherapy on anxiety, duration of delivery and newborn health at birth are very limited. More work is needed to establish evidence.

When the temporal evolution of publications is evaluated, it is an increasing trend, especially in the last seven years. This is because aromatherapy is increasingly sought by people for complementary me-

dicinal treatments that are low-cost, sustainable, and proven effective. In addition, WHO promotes the addition of complementary medicine to the health services of member states due to the important role of this practice. In addition, due to the side effects and cost-benefit associated with drugs, people are looking for natural treatments [21, 24, 25].

CONCLUSION

As a result of this analysis, it was found that aromatrapy application as the primary output reduced the pain of labour. As a secondary output, it was found to shorten the duration of labor, reduce anxiety in the active and transitional phase, and increase the first minute apgar scores of the newborn. The most preferred essential oil was lavender and rose oil. However, studies need to improve methodological quality to provide high-quality evidence. Although this meta-analysis study showed positive results regarding the use of aromatherapy for labor pain relief, most comparisons of the subgroups showed high heterogeneity, as the interventions were performed by different methods. This reinforces the need for standardization of experimental procedures performed in studies to provide safe evidence for the application of aromatherapy in pain management. In addition, more studies based on standardized methodology for duration, anxiety, comfort, and cardiovascular health outcomes other than labor pain alone are recommended.

Authors' Contribution

Study Conception: AYK, FŞB; Study Design: AYK; Supervision: AYK; Funding: N/A; Materials: AYK; Data Collection and/or Processing: AYK; Statistical Analysis and/or Data Interpretation: AYK, FŞB; Literature Review: AYK, FŞB; Manuscript Preparation: AYK, FŞB and Critical Review: AYK, FŞB.

Conflict of interest

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Pharmacobiology of topical Ankaferd hemostat in neoplastic disorders

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ABSTRACT

Ankaferd Hemostat (ABS) is a hemostatic agent of plant-extract acting on red blood cells used for achieving hemostasis. ABS has anti-inflammatory, anti-microbial, anti-fungal, anti-oxidative and anti-neoplastic effects. Cancer treatment is a challenging clinical condition that can lead to numerous clinical complications of different severity. Antineoplastic features of ABS had been depicted in many solid and hematological tumors. Supportive treatment of cancer is very important to decrease the mortality and morbidity of the cancer patients. ABS prevents and treats chemotherapy associated mucositis with its unique effects on the blood cells, endothelium, angiogenesis, cellular regeneration, wound healing and vascular dynamics. Those features of ABS bring it to be also beneficial for necrotizing enterocolitis as well. Besides its supportive and preventative roles in the cancer patients, ABS can also be potentially utilized as a chemoembolization agent within intratumoral treatment modality. The aim of this review is to summarize current pharmacobiology of topical ABS in neoplastic disorders.

Keywords: Ankaferd hemostat, neoplastic disorders, pharmacobiology

Ankaferd Hemostat (ABS) is a plant-based hemostatic agent which is mainly used for achieving hemostasis. ABS has critical effects on angiogenesis, endothelium, hematopoietic cells, cellular reproduction, wound healing and vascular dynamics [1]. Moreover, ABS also has anti-inflammatory, anti-microbial, anti-fungal, anti-oxidative and anti-neoplastic roles [2-5]. Chemotherapy could lead to many complications, one of which is oral mucositis leading to morbidity [6]. Oral mucositis is defined as the ulcerative lesions in the mucosa of the patients who were given chemotherapy; mucositis is frequently encountered as a complication of anti-cancer chemoradiotherapy. Oral mucositis is seen in 40-80% of patients who are given chemotherapy [7]. ABS is a useful wound-healing agent in the treatment of chemotherapy associated severe oral mucositis in patients with hematological malignancies [8]. ABS had been administered to the patients with severe mucositis and with a median healing time was 6.6 days. Thus, ABS is quite effective in the treatment of cancer-related mucositis [8]. Gastrointestinal tract cancer bleedings are another major problem in cancer patients. The mortality rate is approximately 5-10% for peptic ulcer hemorrhage and 15-20% for variceal bleedings [9]. Endoscopic treatment decreases the rates of re-bleeding, surgery, and mortality in active bleeding; however early recurrence is still approximately 20% even with active early he-

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mostatic measures. ABS is also effective in the controlling of the gastrointestinal tracts bleedings in the cancer patients. The endoscopic use of ABS for bleeding gastrointestinal tumors is associated with immediate hemostatic response, easy application and minimum side effects [10]. ABS can be used as a treatment agent in peptic ulcer disease, radiation colitis, sphincterotomy bleeding, Mallory-Weiss syndrome, post-polypectomy bleeding, Dieulafoy's lesion, solitary rectal ulcer and other neoplastic gastrointestinal bleedings [9]. The aim of this review is to summarize the pharmacobiology of topical ankaferd hemostat in neoplastic disorders.

MOLECULAR BASIS OF TOPICAL ANKAFERD HEMOSTAT

The antineoplastic features of topical ABS depend on the unique transcriptomics, proteomics, metabolomics features of ABS. ABS stimulates the cellular factors that have important role in the regulate the cell cycle machinery, pro-apoptotic pathways, angiogenesis, signal transduction and other metabolic pathways. Some of those factors are nuclear factor-1 (NF-1), interferon-(IFN-) stimulated response element (ISRE), protein-2, androgen receptor, cyclic AMP response element binding protein, SMAD2/3,cyclic AMP response element or stimulating transcription factor-1, Myc-Max, E2F1-5, peroxisome proliferator-activated receptor, E2F6, EGR, protein 53, and Yin-Yang (YY1) [11] . ABS may prevent oxidative damage on DNA. The effect of ABS on superoxide dismutase, 8-hydroxy-2′-deoxyguanosine, myeloperoxidase levels over pleural adhesions in rabbits with pulmonary parenchymal damage was demonstrated [12]. The preventive effect of ABS on oxidative DNA damage was confirmed by the study.

ABS has also antioxidant and antimutagenic effects. Those effects of ABS had been tested with two different methods [13]. β-carotene-linoleic acid tests and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging were used to analyze the antioxidant features of ABS. The Ames Salmonella/microsome mutagenicity test with the bacterial mutant strains Salmonella typhimurium TA98 and TA100 was used to analyze the antimutagenic features of ABS. The antioxidant and antimutagenic effects of ABS was demonstrated with these test in a previous study [13].

The main mechanism behind the antineoplastic effects of ABS is apoptosis. Protease-activated receptor 1 (PAR1) is a part of proteinase-activated receptor (PARs) family which is found in seven transmembrane G-protein-coupled receptors group [14]. Increased PAR1 alters intra-cellular signaling by coupling G proteins. PAR1 and EPCR expression in K-562 and Jurkat cells is controlled by ABS. The effect of PAR1 and p21 in pro-apoptotic pathways was demonstrated in Jurkat cells. ABS controls PAR1- and p53-independent p21 involvement in pro-apoptosis in leukemia cells [5].

ABS has also various pleiotropic features, like antineoplastic and antimicrobial roles and tissue supportive features. ABS increases the expression of CREBZF leading to activation of the antineoplastic protein p53 [15]. Moreover, HNF-4a is a component of ABS and has antineoplastic features [16]. ME-1 is also a component of ABS which has significant effects of cancer metabolism [17]. ABS increases the UCHL1 and RPL5 which are tumor suppressor proteins [18]. The transcription factors of TRE/AP1, E2F6, AP2, AR, CREB, CREATF1, E2F1-5, EGR, ISRE, HNF1, MycMax, NF1, NF-κB, p53, PPAR, GATA, SMAD2/3, SP1 and YY1 which were involved in different biological mechanisms, such as hemostasis, infection, cellular growth, and inflammation; were stimulated by ABS [19]. Likewise, ABS increases several transcription factors that controls cell growth scuh as AP2, AR, SMAD2/3, CRE-ATF1, CREB, E2F1-5, ISRE, E2F6, EGR, Myc-Max, NF1, NF-κB, TRE/AP1, p53, PPAR, SP1, and YY1 [19].

ANKAFERD HEMOSTAT IN THE CANCER MANAGEMENT

ABS has anti-neoplastic and anti-proliferative effects on solid tumors as well as hematological tumors (Table 1). ABS has antineoplastic features on the lymphoid cells [20]. ABS showed anti-proliferative effects of chronic lymphoid leukemia cell lines at higher doses (> 0.5 µg/mL); whereas ABS had been found to increase of cellular differentiation at lower doses (< 0.5 µg/mL) (Fig. 1) [20]. The anti-proliferative effects

of ABS on myeloma cell lines were also demonstrated [21]. ABS exerts anti-cancer effects of melanoma cells via reactive oxygen generating (ROS), genotoxic, cytotoxic, pro-apoptotic mechanisms [22]. Anti-tumoral features of ABS on SaO_2 osteosarcoma cell lines were shown by previous studies [23]. Human CaCo-2 colon cancer cells lost cellular proliferative features with the administration of ABS [24]. ABS exert its antineoplastic effects on bladder cancer cells. A decrease in the viability of bladder cancer cells decrease was detected with ABS [25]. ABS induces necroptosis in breast cell cultures [26]. HEPG2 hepatocellular carcinoma cells were inhibited when the cells were exposed to ABS [27]. ABS may have a role in the treatment of solid and hematological cancer cells. Future human studies are needed to clarify the clinical efficacy of ABS in the cancer treatment.

ANKAFERD HEMOSTAT IN CANCER SUPPORTIVE TREATMENT

Cancer treatment could lead to many complications. The conventional cytotoxic chemotherapy agents could exert damage to normal tissue cells. ABS may be helpful in reducing the cancer related complication by its chemopreventive, antioxidant, and supportive features. Oral mucositis is a major chemotherapy associated problem in cancer patients. It affects 40-80% of cancer patients. The role of ABS in the treatment of

Table 1. Anti-neoplastic features of Ankaferd Hemostat

Authors	Cancer Type	Study Summary
Akalın *et al.* [20]	Lymphoid neoplastic cells	ABS administrated chronic lymphocytic leukemia cells stopped proliferation. Transformation of lymphocytic leukemia cells to the blastic aggressive lymphoid forms was inhibited by ABS.
Avcu *et al.* [21]	Multiple myeloma cells	ABS exerts anti-neoplastic effect on myeloma cells which was detected by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-dye reduction assay in Balb/c mice
Turk *et al.* [42]	Melanoma cells	A decrease in cell viability was observed after ABS administration to SK-MEL-10 (CVCL_6020), SK-MEL-9 (CVCL_ U934), A2058 (ATCC® CRL-11147™), and MeWo (ATCC HTB-65™) melanoma cell lines.
Kocyigit *et al.* [22]	Melanoma cells	ABS stimulate DNA injury, apoptosis, and ROS levels in melanoma cells.
Goker *et al.* [23]	Osteosarcoma cells	Following the ABS administration, a dose-dependent reduction was observed in cell proliferation and survival of Saos-2 cells.
Goker *et al.* [24]	Colon cancer cells	ABS exerts inhibitory effects on cellular reproduction of CaCo-2 cells and CaCo-2 cells lose their viabilities after ABS exposure.
Nenni *et al.* [43]	Colon cancer cells	ABS effects the glucose, fatty acids, and protein metabolism and cell cycle machinery. ABS increases cancer suppressor proteins such as carboxyl-terminal hydrolase 1, 60S ribosomal protein L5, Tumor protein D52-like2, karyopherin alpha 2, and protein deglycase DJ-1 in Caco-2 cells.
Sarı *et al.* [25]	Bladder cancer cells	ABS induces apoptosis and decreases cell viability of bladder cancer cells. Necroptosis was observed following ABS administration to the bladder cancer cells.
Fidan *et al.* [26]	Breast cancer cells	ABS decreases the cell viability ratio in breast cancer cells. Necroptosis and apoptosis were detected in breast cell cultures. The cytotoxic role of ABS on breast cancer cells were observed.
Nenni *et al.* [27]	Hepatocellular carcinoma	ABS inhibited cell viability of HEPG2 hepatocellular carcinoma cells

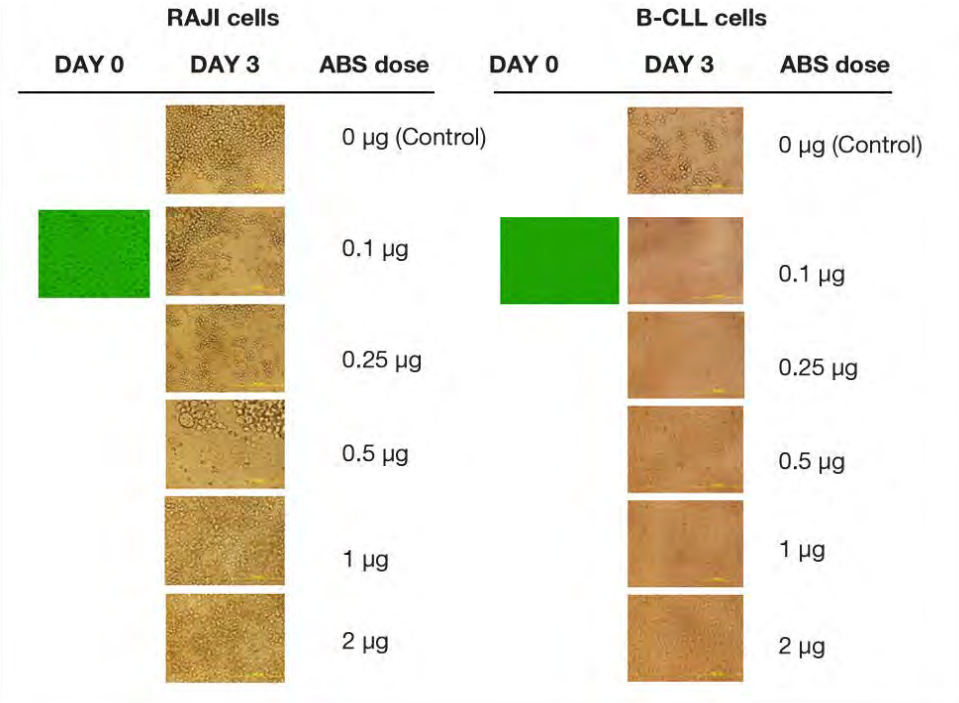

Fig. 1. RAJI (Human Burkitt's lymphoma cell line) and BCLL (B-chronic lymphoid leukemia) cells. Ankaferd treated B-CLL cells (at doses 0.5, 1 and 2 µg/mL) ceased to inflate and more than 50% of tumor cells were died compared to 0.1 and 0.25 µg/mL doses. ABS exerts anti-neoplastic effects at higher doses (> 0.5 µg/mL) whereas it stimulates the cellular differentiation at lower doses (< 0.5 µg/mL).
Reproduced from: Akalın, Ibrahim, et al. Acute in vitro effects of ABS (Ankaferd Hemostat) on the lymphoid neoplastic cells (B-CLL and RAJI tumor cell lines). Int J Hematol Oncol 2014;24:253-9, doi: 10.4999/uhod.13026.

chemotherapy-associated oral mucositis in the patients with hematological malignancies was clarified previously. ABS is efficient in the treatment of oral mucositis due to anti-cancer agents in childhood cancers [29]. Moreover, ABS is beneficial in the chemotherapy-associated oral mucositis in adult patients also [8]. ABS may decrease epithelial dysplasia. ABS has been shown to reduce the 7,12-dimethylbenz[a]anthracene associated oral epithelial dysplasia [28]. Cancer patients may also suffer from necrotizing enterocolitis which increases morbidity and mortality of the cancer patients. Inflammation, prematurity, oxidative stress may induce the development of necrotizing enterocolitis. ABS has preventive effect on intestinal damage in necrotizing enterocolitis with its anti-inflammatory, antioxidant, and antiapoptotic features on intestinal tissue cells [30]. On the other hand, hepatocyte cell is also positively affected with the antioxidative and hepatoprotective features of ABS since it has high levels of vitamin E and other trace elements such as magnesium, vitamin B12, vitamin D, vitamin B9, vitamin A, calcium [31-35].

ANKAFERD HEMOSTAT IN THE INTRATUMORAL TREATMENT

Intratumoral treatment of cancers is preferred in selected patients because of the minimal systemic toxicity with this method. Transarterial chemoembolization (TACE) aims to localize chemotherapeutic agents specially to the cancer site [36]. Conventionally, ethanol and lipiodol embolization is the preferred method for TACE in order to ablate the tumor. Although TACE is safe method that directs the tumor site, it has also several complications [37]. Local complications in TACE method is generally expected but also systemic complications such as tumor lysis syndrome or metabolic problems may develop [38]. ABS has a potential in intratumoral treatment. The antineoplastic features of ABS on myeloma cell line were analyzed by intraperitoneal preterm injection in vitro in Balb/c mice [21]. Moreover, ABS was given as an embolizer in splenic and renal arteries for medical nephrectomy and splenectomy in experimental animal models [39, 40]. In a previous study, ABS depicted success in the he-

patic embolization when compared to the alcohol [41]. According to those data, ABS may be potentially useful for intratumoral treatment since it has unique antineoplastic features on several cancers. ABS may be a superior chemoembolization agent than ethanol and lipiodol which are used frequently in TACE method. There is a need for future clinical studies that will clarify the role of ABS in intratumoral treatment as an embolizing agent.

CONCLUSION AND PERSPECTIVES

Cancer treatment is a challenging clinical condition which can lead to several serious clinical complications. ABS is shown to possess antineoplastic features along with other anti-inflammatory, anti-microbial, anti-fungal, anti-oxidative effects. Anti-neoplastic features of ABS have been proven in many solid and hematological tumors. Supportive treatment in cancer is very important to minimize the morbidity and mortality of patients. ABS prevents and treats chemotherapy associated mucositis with its unique effects on endothelium, blood cells, angiogenesis, cellular reproduction, vascular dynamics and wound healing. These features of ABS bring it to be also beneficial for necrotizing enterocolitis. Besides its supportive and preventative roles in cancer patients, ABS can also be utilized as a chemoembolization agent in intratumoral treatment modality.

Authors' Contribution

Study Conception: ÜYM, İCH; Study Design: ÜYM, İCH; Supervision: ÜYM, İCH; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ÜYM; Statistical Analysis and/or Data Interpretation: ÜYM, İCH; Literature Review: ÜYM; Manuscript Preparation: ÜYM, İCH and Critical Review: İCH.

Conflict of interest

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