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The Middle Black Sea Journal of Health Science, which is international journal, is published by Ordu University Institute of Health Sciences on behalf of the Middle Black Sea Universities Collaboration Platform

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The aim of the journal is to contribute to the international literature with clinical and experimental research articles, case reports, reviews and letters to the editor in the field of health sciences.

The target audience of the journal is all scientists working in the field of health, graduate students and researchers in this field.

Middle Black Sea Journal of Health Science is an open access, independent and impartial, international journal based on double-blind peer-reviewed principles.

The publication language of the journal is English. The journal is published every three months, in February, May, August and November, and four volumes are completed.

Middle Black Sea Journal of Health Science - adheres to the standards of publication ethics in health science research, Higher Education Council's Scientific Research and Publication Ethics Directive, Committee on Publication Ethics (COPE), Directory of Open Access Journals (DOAJ), Open Access Scholarly Publishers It also adopts the ethical publishing principles published by the Association (OASPA) and the World Association of Medical Editors (WAME).

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Middle Black Sea Journal of Health Science

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Ethics Committee Approval: Ethics committee approval was received for this study from ……. Clinical Research Ethics Committee of …… University (No…….).

Author Contributions: Concept: …….., Design: …….., Literature search:……..... Data Collection and Processing: ……..., Analysis and Interpretation:……, Writing - …….

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Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed.

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Levine WC, Pope V, Bhoomkar A, Tambe P, Lewis JS, Zaidi AA, et al. Increase in endocervical CD4 lymphocytes among women with nonulcerative sexually transmitted diseases. J Infect Dis. 1998;177(1):167–174.

Chapter in Edited Book: Hornbeck P. Assay for antibody production. In: Colign JE. Kruisbeek AM, Marguiles DH, editors. Current Protocols in Immunology. New York: Greene Publishing Associates; 1991. p. 105-32.

Book with a Single Author: Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York: John Wiley and Sons; 1981. p. 105-32.

Editor(s) as Author: Balows A. Mousier WJ, Herramaflfl KL, editors. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press. 1990. p. 105-32.

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Revisions: Authors should mark the changes they made on the main text in color while submitting their article revision files. The responses to the referees should be specified in a separate Word file. Revised articles should be sent to the journal within one month following the decision letter. If the revised version of the article is not uploaded within the specified time, the revision option may be canceled. If the authors need additional time for revision, they are required to submit their extension requests to the journal before the end of one month.

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a) Original research: Prospective, retrospective and all kinds of experimental studies

Structure

Title

Abstract should be structured with subheadings (Objective, Methods, Results, and Conclusion) (average 200-400 word)

Key words

Introduction

Methods

Results

Discussion

Conclusion

Acknowledgements

References (most 40)

Whole text should not exceed 4500 words except for resources and English summary.

b) Short papers: Prospective, retrospective and all kinds of experimental studies

Structure

Title

Abstract should be structured with subheadings (Objective, Methods, Results, and Conclusion) (average 200-400 word)

Key Words

Introduction

Methods

Results

Discussion

Conclusion

Acknowledgements

References (most 20)

Whole text should not exceed 2700 words except for resources and English summary.

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Structure

Title

Abstract (average 100-300 word)

Key words

Introduction

Case report

Discussion

Conclusion

Acknowledgements

References (most 20)

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d) Review articles

Structure

Title

Abstract (average 200-400 word)

Key words

Introduction

The compilation text also including appropriate sub-headings,

Conclusion

Acknowledgements

References (most 50)

Whole text should not exceed 6550 words except for resources and English summary.

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CONTENTS

EDITORIAL

In the Shadow of the Earthquake

While we expect 2023 to be a much better year in every way after the hard times we went through due to the Covid-19 epidemic, the date of February 6, 2023, was painfully etched in our memories due to the earthquake disaster we experienced in our country. We wish God's mercy on our citizens who lost their lives due to this great disaster, a speedy recovery to all injured people, and pray that God will give the patience to endure their suffering and the strength to accept what they have been through to all of the relatives and friends left behind.

This grim picture once again revealed that pure knowledge can only be useful to the extent that it is applicable, and how necessary it is to carry out scientific and academic studies that will lead to new developments in every field.

In this issue of our journal, we tried to include the works of many valuable academicians and scientists, and articles with rich content in which they conveyed their observations and experiences. While we are trying to move our journal to a better level in the international scientific community with each new issue we publish, I would like to extend my sincere thanks to all our authors who participated with us in this process and to everyone who worked with us during the intensive preparation process until the magazine took its final form.

Although it will take time to heal the wounds of this heavy earthquake disaster, we have full faith that we will overcome this difficult process by working hand in hand with all our citizens. Hope to meet you in hopeful days and new issues, when discoveries that will make a difference in the life of humanity and leave a mark in the future, and mysteries that will shed light on science that have not yet been revealed...

PhD, Assoc. Prof. Ülkü KARAMAN

Editor

Middle Black Sea Journal of Health Science / Mid Blac Sea J Health Sci

February 2023; 9(1): 1-13

RESEARCH ARTICLE **DOI:** 10.19127/mbsjohs.1155632

The Relationship Between Coronary Slow Flow and Myocardial Ischaemia Evaluated with Timi Frame Count and Myocardial Perfusion Scintigraphy

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Abstract

Objective: Coronary slow flow (CSF) is known as a form or early stage of common atherosclerotic disease. Myocardial perfusion scintigraphy (MPS) is a valuable technique in the diagnosis of coronary artery disease and prediction of prognosis. The aim of this study was to investigate the relationship between the myocardial defect score and ischaemia in patients with CSF.

Methods: A total of 168 patients who applied with the complaint of angina pectoris and underwent SPECT as a non-invasive test followed by coronary angiography were included in this retrospective study. 9 patient was excluded from the study for various reasons. The study population comprised determined with CSF and no obstructive stricture in the coronary arteries and with normal flow. The mean age of the patients was 56±12 years. The scores obtained from Quantitative Perfusion SPECT (QPS) and Quantitative Gated SPECT (QGS) software were used in the myocardial perfusion evaluation. The TIMI frame counts were compared with the myocardial defect and ischaemia scores. The TIMI frame count method was used in the determination of CSF.

Results: In patients with slow flow in the circumflex (Cx) coronary artery, the stress total perfusion defect Cx (sTPD-Cx) was found to be 0.1 (range, 0.0-1.3), and in those with normal flow, it was 0.0 (range, 0.0-0.28) (p=0.002). The stress score Cx (sscore-Cx) was found to be 1.0 (range, 0.0-3.0) in patients with slow flow and 0.0 (range, 0.0-2.0) in those with normal flow (p=0.031). A linear correlation was determined between the Cx TIMI frame count and the sTPD-Cx and sscore-Cx values (r=0.207, p=0.009; r=0.159, p=0.045). No relationship was found between slow flow and the defect and ischemia scores in other myocardial regions.

Conclusion: In patients with slow flow in the Cx coronary artery, the sTPD-Cx and the sscore-Cx values were found to be significantly high. Although at a weak level, a linear correlation was found between the Cx TIMI frame count and the sTPD-Cx and the sscore-Cx values

Key words: Coronary slow flow, TIMI frame count, myocardial perfusion scintigraphy

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INTRODUCTION

It is a common clinical problem to explain the cause of chest pain in patients with anginal complaints suggestive of myocardial ischemia and with normal coronary arteries on angiography. Some patients with chest pain and myocardial ischemia detected by noninvasive testing but with normal coronary anatomy have been termed 'cardiac syndrome X' (1).

In 1972, despite normal coronary anatomy, it was noticed for the first time that the contrast medium progressed slowly in the coronary arteries, and this situation was named as coronary slow flow. Although many studies have been conducted on the etiological factors that may cause slow coronary flow, this issue has not yet been fully elucidated (2).

Although the pathophysiological cause of coronary slow flow (CSF) is not exactly known, previous studies have shown that microvascular dysfunction, endothelial and vasomotor dysfunction and occlusive disease could be underlying causes (3-6). Recent studies have determined intimal thickening in coronary arteries, widespread calcification and atheroma plaques not creating lumen irregularity in a significant proportion of CSF patients (7-9). Based on this information, it would be more accurate to evaluate CSF as another type of coronary artery disease (CAD) rather than the previous evaluation as a subgroup of cardiac syndrome X. (10).

The aim of this study was to investigate the relationship between coronary slow flow and myocardial ischaemia by retrospectively evaluating the TIMI (Thrombolysis In Myocardial Infarction) frame count and myocardial perfusion scintigraphy results.

METHODS

In this retrospective study, the TIMI frame counts were calculated for patients who presented with anginal complaints and were applied with SPECT and then coronary angiography. The TIMI frame counts, calculated separately for LAD, Cx, and RCA, were determined using the scintigraphic method for the perfusion defects and ischaemia scores corresponding to these vascular beds. A total of 159 patients were included in the study, comprising determined with CSF on angiography and normal coronary arteries, and with normal flow. Patients were excluded from the study if they had hypertension, left ventricle hypertrophy, diabetes mellitus, connective tissue disease, arrhythmia, sick sinus syndrome, or congenital or acquired valve disease.

Access to the coronary arteries was obtained with 7F sheath cannulation of the right femoral artery with the Judkins technique, and selective coronary angiography was performed with a G2100 device (*make and model**). The TIMI frame count (TFC) method was used to determine a CSF pattern (9). The normal frame counts corrected for

coronary artery length required for filling the coronary arteries were accepted as 36.2±2.6 for LAD, 22.2±4.1 for Cx, and 20.4±3.0 for RCA and values above these were evaluated as CSF.

ECG Gated Myocardial Perfusion SPECT Imaging was applied as stress-rest imaging with a myocardial perfusion scintigraphy single day protocol. The images were taken 30 mins after completing the Efor test and at 45-60 mins after finishing the infusion in the pharmacological stress test. The images were obtained with a single-head gamma camera (Siemens E. CAM) synchronised with ECG. The Gated Single Positron Emission Computed Tomography (SPECT) images were taken using a general purpose parallel hole collimator with a 64 x 64 imaging matrix, 180° circular orbit and 6-angle sampling. Processing of the images was performed on a Siemens e. soft computer system using QGS software (Cedar's Sinai, ENTEGRA View Workstation Version 2: Siemens Medical System). Following reconstruction with the filtered back projection method by the software, short axis, vertical, and long axis cross-sectional myocardial perfusion images and functional gated images were formed (Figure 1).

The ischaemia and defect scores were obtained separately by totalling the scores of the segments corresponding to the vascular beds of LAD, Cx, and RCA (Table 1). The scores in the segments shown with 1-2-3-7-8-13-14-19-20 for LAD, the scores in the segments 5-6-11-12-17-18 for Cx, and the scores in the segments 4-9-10-15-16 for RCA were totalled and the stress scores (sscore-LAD, sscore-Cx, sscore-RCA) and rest scores (rscore-LAD, rscore-Cx, rscore-RCA) were formed.

The ischaemia score was calculated by subtracting the rest score from the stress score, and a score of ≥2 was accepted as significant in respect of myocardial ischaemia.

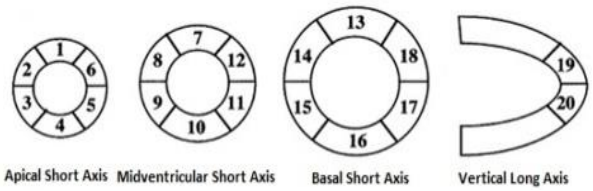

Figure 1. The scores in the segments and axial images

The total perfusion defect (TPD) was automatically calculated in the Cedars-Sinai QPS software using the following formula:

$$TPD = 100 \times \frac{\sum_{a=0}^{a<A} \sum_{p=0}^{p<P} score(a,p)}{4AP}$$

a, p; Radial coordinates on the polar map
A, P; Maximum sample count in each dimension
Score (a, p); Pixel score on the polar map (11).

Statistical Analysis

Statistical analyses were conducted with SPSS for Windows version 19.0 software (IBM SPSS Inc, Chicago, IL, USA). The Shapiro Wilk test was employed to assess the normal distribution of data. Numerical variables with normal distribution were presented as mean ± standard deviation, while

those without normal distribution were expressed as median (interquartile range) values. Categorical variables were stated as number (n) and percentage (%). Comparison of two-sample numerical variables was conducted using the Mann–Whitney U test. The relation between the variables was analyzed through Spearman's rank correlation analysis. The Pearson Chi-square test was also used to compare categorical variables. A two-tailed p value of <0.05 was considered statistically significant.

RESULTS

Evaluation was made of a total of 168 patients, comprising 113 (67.3%) females and 55 (32.7%) males, with a mean age of 56±12 years.

Table 1. Baseline Characteristics of Patients with determined with CSF and no obstructive stricture in the coronary arteries and with normal flow

Characteristics	n (%)
Age (years)	56 ± 12
Cender (Female/Male)	113/55 (67.3%)
Weight (Kg)	86.2 ± 9.3
Height (cm)	174.3 ± 11.2
BMI (kg/m2)	28.37 ± 7.6

In the LAD, normal flow was determined in 81% (129) and CSF in 19% (30), in the Cx normal flow was determined in 42% (68) and CSF in 58% (91), and in the RCA normal flow was determined in 49% (78) and CSF in 51% (Figure 2).

No significant correlation was found between the LAD frame count and the sTPD-LAD, sscore-LAD, iscore-LAD (p=0.249). A weak positive linear correlation was found between the Cx TIMI frame count and the sTPD-Cx and sscore-Cx (r= 0.207 p= 0.009; r= 0.159 p= 0.045, respectively). No significant correlation was determined between the Cx TIMI frame count and the iscore-Cx (p=0.505). No significant correlation was determined between the RCA frame count and the sTPD-RCA, sscore-RCA, iscore-RCA (p>0.05 for all). A weak positive correlation was determined between sESV and RCA-TFC (r=0.204, p=0.028) (Table1).

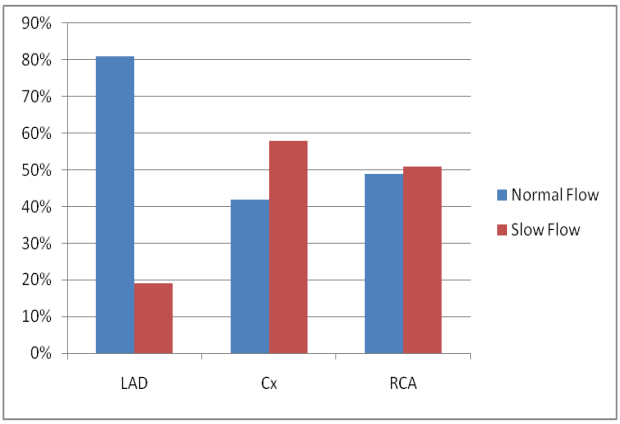

Figure 2. Graphic representation of the distribution of normal flow and slow flow of the coronary arteries.

The stress score of ≥4 was considered significant for myocardial perfusion defect and in the comparison of the normal flow groups with the slow flow groups for each vessel (LAD, Cx, RCA), no statistically significant difference was determined (p>0.05 for all) (Table 2).

The ischaemia score of ≥2 was considered significant for ischaemia and in the comparison of the normal flow groups with the slow flow groups for each vessel (LAD, Cx, RCA), no statistically significant difference was found (p>0.05 for all) (Table 3).

Table 2. Correlations between the parameters related to LAD, Cx and RCA TIMI Frame Counts

LAD-TFC			Cx-TFC			RCA-TFC		
	r	**p**		**r**	**p**		**r**	**p**
sTPD-LAD	0.093	0.249	sTPD-Cx	**0.207**	**0.009**	sTPD-RCA	0.041	0.605
fTPD-LAD	0.056	0.488	fTPD-Cx	0.015	0.850	fTPD-RCA	0.002	0.981
sscore-LAD	0.026	0.744	sscore-Cx	**0.159**	**0.045**	sscore-RCA	0.08	0.316
iscore-LAD	-0.039	0.631	iscore-Cx	0.053	0.505	iscore-RCA	0.073	0.358
~~s~~EF	-0.011	0.911	~~s~~EF	-0.055	0.559	~~s~~EF	-0.156	0.094
~~s~~ESV	0.037	0.693	~~s~~ESV	0.041	0.665	~~s~~ESV	**0.204**	**0.028**
~~s~~EDV	0.111	0.242	~~s~~EDV	0.04	0.671	~~s~~EDV	0.179	0.055

Cx, circumflex coronary artery; Cx-TFC, Cx TIMI frame count; sEF, post exercise left ventricle ejection fraction; sEDV, stress left ventricle diastolic end volume; sESV, stress left ventricle systolic end volume, fTPD- Cx, difference between total perfusion defect and Cx; fTPD- LAD, difference between total perfusion defect and LAD; fTPD-RCA, difference between total perfusion defect and RCA; iscore-Cx, ischaemia score Cx; iscore-LAD; ischaemia score LAD; iscore-RCA, ischaemia score RCA; LAD, left anterior descending coronary artery; LAD-TFC, LAD TIMI frame count; RCA, right coronary artery; RCA-TFC, RCA TIMI frame count; sscore-Cx, stress score Cx; sscore-LAD, stress score LAD; sscore-RCA, stress score RCA; sTPD-Cx, stress total perfusion defect-Cx; sTPD-LAD, stress total perfusion defect-LAD; sTPD-RCA, stress total perfusion defect-RCA; TFC, TIMI frame count; TIMI, thrombolysis in myocardial infarction.

Table 3. Comparison of the stress scores according to the flow characteristics of LAD, Cx and RCA

			LAD		***p***
			Normal Flow	Slow Flow	
sscore-LAD	<4	Count	82 (65%)	17 (56%)	
	≥4	Count	45 (35%)	13 (44%)	
Total		Count	127 (100%)	30 (100%)	p= 0.42
			Cx		
			Normal Flow	Slow Flow	
sscore-Cx	<4	Count	63 (92.6%)	77 (84.6%)	
	≥4	Count	5 (7.4%)	14 (15.4%)	
Total		Count	68 (100%)	91 (100%)	P= 0.122
			RCA		
			Normal Flow	Slow Flow	
sscore-RCA	<4	Count	72 (92.3%)	73 (90.1%)	
	≥4	Count	6 (7.7%)	8 (9.9%)	
Total		Count	78 (100,0%)	81 (100.0%)	P= 0.627

Cx, circumflex coronary artery; LAD, left anterior descending coronary artery; RCA, right coronary artery; sscore-Cx, stress score Cx; sscore-LAD, stress score LAD; sscore-RCA, stress score RCA.

Table 4. Comparğiğşison of the ischaemia scores according to the flow characteristics of LAD, Cx, and RCA

			LAD		***p***
			Normal Flow	Slow Flow	
iscore-LAD	<2	Count	49 (38%)	19 (63%)	
	≥2	Count	78 (62%)	11 (37%)	
Total		Count	127 (100%)	30 (100%)	P= 0.014
			Cx		
			Normal Flow	Slow Flow	
iscore-Cx	<2	Count	61 (89.7%)	75 (82.4%)	
	≥2	Count	7 (10.3%)	16 (17.6%)	
Total		Count	68 (100,0%)	91 (100.0%)	P= 0.196
			RCA		
			Normal Flow	Slow Flow	
iscore-RCA	<2	Count	69 (88.5%)	68 (84.0%)	
	≥2	Count	9 (11.5%)	13 (16.0%)	
Total		Count	78 (100.0%)	81 (100.0%)	P= 0.410

Cx, circumflex coronary artery; iscore-Cx, ischaemia score Cx; iscore-LAD, ischaemia score LAD; iscore-RCA, ischaemia score RCA; LAD, left anterior descending coronary artery; RCA, right coronary artery.

Table 5. Comparison of the parameters of the groups with normal flow and slow flow in LAD

	LAD-Normal Flow (N=127)	LAD-Slow Flow (N=30)	*p*
sTPD-LAD	3.0 (1.6-5.0)	2.85 (1.220-5.95)	0.909
fTPD-LAD	1.9 (0.3-3.4)	1.15 (0.0-4.55)	0.688
Sscore-LAD	3.0 (1.0-4.0)	3.0 (0.0-5.0)	0.751
iscore-LAD	2.0 (0.0-3.0)	1.0 (0.0-3.25)	0.225
sEF	67.0 (57.0-78.0)	62.0 (52.75-74.25)	0.255
sESV	21.5 (13.0-34.75)	27.0 (16.5-40.75)	0.167
sEDV	66.0 (53.25-80.75)	79.0 (61.75-96.5)	0.066

fTPD- LAD, difference between total perfusion defect and LAD; iscore-LAD, ischaemia score LAD; sEF, post exercise left ventricle ejection fraction; sEDV, stress left ventricle diastolic end volume; sESV, stress left ventricle systolic end volume; sscore-LAD, stress score LAD; sTPD-LAD, stress total perfusion defect-LAD.

Table 6. Comparison of the parameters of the groups with normal flow and slow flow in RCA

	RCA-Normal Flow (N=78)	RCA-Slow Flow (N=81)	*p*
sTPD-RCA	0.1(0.0-0.85)	0.0(0.0-1.4)	0.901
fTPD-RCA	0.0(0.0-0.5)	0.0(0.0-0.5)	0.960
sscore-RCA	0.0(0.0-2.0)	0.0(0.0-3.0)	0.965
iscore-RCA	0.0(0.0-1.0	0.0(0.0-2.0)	0.657
stEF	70.0(57.5-79.0)	64.0(54.0-77.0)	0.109
stESV	20.0(11.5-28.5)	25.0(14.0-40.0)	0.058
stEDV	66.0(52.5-79.0)	70.0(57.0-89.0)	0.106

fTPD-RCA, difference between total perfusion defect and RCA; iscore-RCA, ischaemia score RCA; sEF, post exercise left ventricle ejection fraction; sEDV, stress left ventricle diastolic end volume; sESV, stress left ventricle systolic end volume; sscore-RCA, stress score RCA; sTPD-RCA, stress total perfusion defect-RCA.

Table 7. Comparison of the parameters of the groups with normal flow and slow flow in Cx

	RCA-Normal Flow (N=78)	RCA-Slow Flow (N=81)	*p*
sTPD-Cx	0.0(0.0-0.28)	0.1(0.0-1.3)	**0.002***
fTPD-Cx	0.0(0.0-0.1)	0.0(0.0-0.4)	0.186
sscore-Cx	0.0 (0.0-2.0)	1.0(0.0-3.0)	**0.031***
iscore-Cx	0.0(0.0-1.0)	0.0(0.0-2.0)	0.177
stEF	67.0(55.5-78.0)	66.0(56.0-77.0)	0.780
stESV	22.0(13.5-34.5)	23.0(13.0-38.0)	0.984
stEDV	0.0(0.0-0.28)	0.1(0.0-1.3)	**0.002***

Cx, circumflex coronary artery; sEF, post exercise left ventricle ejection fraction; sEDV, stress left ventricle diastolic end volume; sESV, stress left ventricle systolic end volume; fTPD- Cx, difference between total perfusion defect and Cx;

No statistically significant difference was found between the LAD and RCA normal flow and slow flow groups in respect of the median values of the sTPD, sscore and iscore (Tables 4, 5). The sTPD and sscore median values of the patients with slow flow in Cx were found to be significantly higher than those of the patients with normal flow in Cx (p=0.002, p=0.031). No statistically significant difference was found between the Cx normal flow and slow flow groups in respect of the median iscore value (p=0.177) (Table 6,7).

DISCUSSION

Recent studies have shown that CSF is a type of coronary artery disease and could be associated with increased mortality (14-16). Although the etiopathogenesis of CSF has not been fully determined, the clinical and pathological characteristics have been defined (17). Clinical presentation may sometimes be

in the form of angina occurring with effort but is more often in the form of angina at rest. Patients may sometimes present with clinical acute coronary syndrome requiring admission to the coronary intensive care unit (18, 19). In patients with stable angina, coronary ischaemia can be shown with the exercise stress test or myocardial perfusion scintigraphy (20). There is no visible atherosclerotic lesion on coronary angiography, but invasive studies have shown that resting coronary artery resistance is abnormally high in these patients, consistent with delayed opaque transition (8). IVUS studies in recent years have shown that the coronary arteries are not normal in these patients, and there are widespread atherosclerotic changes and widespread calcifications (7, 8).

In a study by Yaymaci et al, (21) in which cardiac ischaemia was created with atrial pacing in patients with CSF, using as measures the lactate production and the difference in coronary arteriovenous oxygen content, which are measures of metabolic ischaemia, it was reported that although anginal complaints were seen in the majority of patients with atrial pacing, metabolic ischaemia was determined in only 17%. In 83.4% (n:5) of the patients determined with metabolic ischaemia, a perfusion defect was found on myocardial perfusion scintigraphy (MPS), anatomically consistent with the vessel determined with CSF. Consequently, it was emphasized that anginal complaints in patients determined with CSF did not originate from myocardial ischaemia. However, as the number of patients determined with ischaemia on MPS was extremely low in that study, the relationship between myocardial ischaemia or defect and CSF was not directly examined. In the current study with a larger patient population, the myocardial ischaemia/defect scores were compared with the CSF data with quantitative measurements. In contrast to the previous study, only the sTPD-Cx and sscore-Cx were found to be significantly high in the patients with slow flow in the Cx coronary artery. These differences between CSF patients could be due to changes in the coronary flow reserve, which is itself a marker of microvascular function (22).

Cesar et al. (23) investigated the relationship between CSF and hemodynamic factors, and reported that of 17 patients with CSF, perfusion defect was observed in 13 (76%) patients on exercise thallium-201 MPS. In the same study, when coronary blood flow was evaluated according to heart rate, 74 patients were included with heart disease and normal coronary blood flow. The hemodynamic factors of the patients with CSF were found to be normal at a significant rate compared to the group with heart disease and normal coronary blood flow. Therefore, it was concluded that there was no correlation

between hemodynamic factors and the detection of CSF in patients thought to have ischaemia on MPS.

In another study, Demirkol et al. (24) examined 60 patients determined with CSF and 50 subjects with normal coronary flow with technetium 99m-MIBI SPECT imaging given at rest and in stress condition. Ischaemia was determined on MPS in 17 (28.3%) of the patients with CSF, and no significant correlation was determined between CSF patients with and without ischaemia in respect of TIMI frame count. MPS was then applied with dipyridamole to the patients with CSF and perfusion was seen to be corrected in all 17. As a result, it was emphasized that CSF in vessels with a diameter of <200µm was associated with pathology, and it was reported that no significant correlation was found between coronary blood flow rate and ischaemia

Dağdelen et al. (25) investigated whether there was any relationship between TIMI frame count and myocardial ischaemia with intracoronary ultrasound and MPS. The study included 24 patients determined with CSF and 13 with normal coronary flow. The patients were examined in 3 groups as CSF patients with ischaemia on MPS, CSF patients with no ischaemia on MPS and subjects with no ischaemia and normal coronary flow. The study results showed reduced coronary lumen change in cases with CSF, but no difference was found between the CSF patients with and without ischaemia. As a result of the study, there was reported to be no correlation between flow change and coronary area of ischaemia in those with CSF, the basic pathology was at the microvascular level, and the disruption in microvascular circulation was thought to lead to ischaemia. In the same study, no significant difference was determined between the two groups of CSF patients with and without ischaemia in respect of TIMI frame count. Similarly, in the current study, no significant correlation was determined between TIMI frame counts and ischaemia scores. In the previously mentioned study, only a regional comparison for LAD was made, whereas in the current study, comparisons were made by matching the scores of the regions fed by the related vessels with coronary flow rate. Thus, the confounding effect on the results of defect and ischaemia scores originating from other regions was avoided. Demirkol et al. (24) only compared the relationship between the presence of ischaemia and slow flow. Using quantitative score values together with the presence of ischaemia in the current study, it was attempted to apply a more objective evaluation. In the study by Dağdelen et al, by IVUS evaluation of IMA phasic changes, a different perspective was examined and while the results supported a relationship between atherosclerosis and slow flow, no correlation

was determined between ischaemia and slow flow. In the current study, no significant correlation was determined between CSF and the TMI frame counts and ischaemia scores.

Mangieri et al. (26) administered intracoronary infusions of 0.56 mg/kg dipyridamole and 100 µg nitroglycerine to patients determined with CSF, and reported that while nitroglycerine had no significant effect on TIMI frame count, dipyridamole resulted in a significant decrease (5). Such a result may have been due to dipyridamole having a dilation effect on vessels smaller than 200µm, rather than nitroglycerine having no effect on coronary arteries larger than 200µm, and this supports the view that the event is at the microvascular level.

Von Lierde et al. (27) measured the coronary flow reserve in a CSF patient with intracoronary Doppler ultrasound and despite very evident CSF, the coronary flow reserve was seen to be within normal limits. This was thought to be a result of epicardial coronary disease such as coronary ectasia, and not to originate from the microvascular system. Using fractional flow reserve (FFR) and IVUS, Pekdemir et al. investigated the relationship between CSF and epicardial resistance in a study of 19 patients. A significant negative correlation was found between TIMI frame count and FFR in CSF patients, and although there was a reduction in FFR in the epicardial coronary arteries of CSF patients, with IVUS in the same coronary artery, there was determined to be an increase in intimal thickness and massive calcification extending longitudinally along epicardial coronary arteries. Consequently, this was attributed to an increase in resistance in epicardial coronary arteries associated with diffuse atherosclerosis (8). In another IVUS study by Cin et al. to investigate epicardial coronary artery pathology in CSF patients, similar to the previous study, coronary angiography demonstrated widespread intimal thickening, widespread calcifications along the vessel wall and atheroma plaque not causing luminal irregularity. Flow rate was measured along the coronary vessels and there was determined to be a pressure gradient between the proximal and distal segments. It was concluded that the abnormal flow pattern observed in CSF could be a form or early stage of common atherosclerotic disease involving both the microvascular system and the epicardial coronary arteries (9).

De Bruyne et al. (28) reported that in normal coronary arteries, the gradient between distal and proximal coronary arteries was no more than 1 mmHg, and although there was no significant obstructive lesion, the gradient between the distal and proximal coronary arteries was determined to be >10mmHg, and this increase in epicardial resistance was associated with diffuse atherosclerotic disease. In addition, previous autopsy studies have

often shown the combination of small vessel disease and epicardial coronary involvement (29, 30). This has been supported in several IVUS studies with the angiographic observation of the presence of diffuse atherosclerotic disease such as intimal thickening and reduced distensibility in normal coronary arteries (31, 32). Intravascular pathologies have been evaluated in these studies with the IVUS method, which is accepted as advanced technology. In addition to the pathologies evaluated anatomically within the vessels, evaluation with scintigraphy of the functional response of the myocardial wall can be considered a contribution to these studies.

There were some limitations to this study, primarily the relatively small study population. Studies with larger patient populations would provide higher statistical power. Another basic limitation was the observational nature of the study, which did not allow for the determination of cause and effect relationships. Intravascular ultrasonography (IVUS) was not performed to determine the atherosclerotic changes in the coronary arteries of the patients, and therefore, diffuse CAD combination could not be determined as the gold standard in patients with CSF. Finally, the microvascular perfusion phenomenon was examined using the TIMI frame count method to angiographically determine the filling of epicardial coronary arteries with contrast material. This method is relatively operator-dependent. Therefore, although the angiographic contrast material was administered manually, great care was taken to provide a fixed injection speed.

CONCLUSION

When all these data are taken into consideration, it seems to be insufficient to hold only microvascular dysfunction, regardless of epicardial involvement, responsible for the explanation of the etiopathogenesis of CSF. The results of this study demonstrated that although weak, a significant correlation was determined between slow flow in the Cx coronary artery and the sTPD-Cx and sscore-Cx values reflecting the stress perfusion defect, but no significant relationships were found for LAD and RCA. As advanced technology methods such as IVUS were not available in the hospital for this study, it was not possible to evaluate on scintigraphy the functional reflection of anatomic impairments determined with IVUS in CSF.

Ethics Committee Approval: This prospective study was approved by the ethical review committee of Düzce University (OMU) Hospital (2014/94)

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Author Contributions: Concept -O K, M Z Y, Design –G A, M Z Y, Audit– M A, H A, Data Collection and/or Processing – H A, M A, Analysis and/or Interpretation –M Z Y, O K

Writing– M Z Y, Critical Review – M Z Y, O K, M A

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Evaluation of the Efficiency of Neuronavigation in Patients with Glioblastoma

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Abstract

Objective: To investigate the effect of neuronavigation use on mortality in patients with glioblastoma.

Methods: For each of the 26 patients that underwent neuronavigation-assisted supratotal resection for glioblastoma between 2018 and 2020, one patient that underwent supratotal resection without navigation was selected.

Results: Radiographic radicality was observed in 35% of the cases in the neuronavigation-assisted surgery group and 29% of those in the conventional surgery group. Absolute and relative residual tumor volumes were significantly lower in the neuronavigation-assisted surgery group. Radical tumor resection was associated with a very significant increase in survival. There was no significant difference in the survival rates between the patients that underwent surgery with and without neuronavigation. This was attributed to the small number of participants and supratotal resection being performed in all statistically determined patients The low median survival period of glioblastoma may have also contributed to this finding.

Conclusions: Surgery plays an important role in the treatment of glioblastoma. A combination of techniques including intraoperative magnetic resonance imaging, neuronavigation, ultrasound, and fluorescence guidance allows for safe and maximum surgical resection, leading to better outcomes in terms of survival and postoperative functional recovery. However, despite maximal surgical resection and adjuvant chemotherapy-radiotherapy, most cases develop tumor recurrence within 10 months, which is considered to be due to established cancer stem cells. Therefore, there is an urgent need to develop more effective treatment strategies for glioblastoma.

Key words: Neuronavigation, Glioblastoma, Supratotal resection, Mortality

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INTRODUCTION

Gliomas are the most common tumors of the central nervous system (CNS). Glial cells in CNS are composed of oligodendrocytes, astrocytes, and microglia (1,2). There are three different types of gliomas, namely ependymomas originating from glial cells in the epithelial tissue of the brain and spinal cord, oligodendrogliomas originating from oligodendrocytes, and astrocytomas originating from astrocytes (3,4). GBM is the most aggressive intracranial malignancy due to the rapid growth, inevitable recurrence, and high mortality associated with these tumors (4). Among these tumors, astrocytomas are the most common gliomas in pediatric, adolescent, and adult patients. In adults, grade IV astrocytoma or glioblastoma multiforme (GBM) accounts for approximately 15.6% of all brain tumors and 45.2% of all primary malignant brain tumors (5). GBM is the most aggressive, highly malignant tumor of the astrocytic tumor type and usually diagnosed in elderly patients (median age at diagnosis: ≥65 years) (6).

Only a small portion of cases achieve long-term survival after surgical resection and chemotherapy-radiotherapy. In patients diagnosed with GBM, the median survival is approximately 12 to 15 months (7). Despite decades of research to improve patient outcomes, GBM is still a type of tumor that is incurable and very difficult to treat. Younger age, better performance status and higher resection grade are universally considered to be the predictors of better survival in GBM. One of the challenges in GBM treatment concerns the aggressive growth characteristics of tumors. The complete surgical resection of these tumors is not possible due to their infiltrative growth and microscopic spread, and the presence of multiple lesions. Therefore, there is a pressing need for new and effective GBM therapy.

Depending on the type of intracranial lesion, pathology, and surgical approach, some craniotomy procedures can be assisted with neuronavigation based on magnetic resonance imaging or computed tomographic scans to adapt the procedure to the size of the tumor using the smallest incision possible. Neuronavigation is a modern computerized technology that can help surgeons locate a pathology more precisely by combining a series of craniofacial points in patients. Neuronavigation provides better guidance, orientation, and localization. It also offers a higher level of confidence for the surgeon and better outcomes for the patient (8).

METHODS

For each of the 26 patients that underwent neuronavigation-assisted supratotal resection for glioblastoma between 2018 and 2020, one patient who underwent supratotal resection without neuronavigation was selected. The files of a total of 52 patients (37 male, 15 female) aged 29 to 89 years were retrospectively

reviewed. The patients' demographic information (age, gender), pathology results, preoperative and follow-up magnetic resonance imaging (MRI) findings, and one-year follow-up results were recorded. The evaluation of the integrity of tumor resection, including volumetric analysis was performed with MRI in the early postoperative period. Recurrence and survival times were obtained for all the patients. The results were statistically analyzed using SSPI.

Statistical Analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) 16.0 software. The results were given as means and standard deviations. Non-parametric tests, including the Mann-Whitney U test, Fisher's exact test, chi-square test, and Kruskal-Wallis test, were used to compare the groups. Since the p-value was greater than 0.05, it was not considered significant.

RESULTS

The mean operative times were similar in the two groups, but the mean preparation time was 30 minutes longer in the navigation-assisted surgery group. Radiological radicality was detected in 35% of the cases in the neuronavigation-assisted surgery group and 29% of those in the conventional surgery group.

Table 1. Results of the chi-square test

	Value	Df	Asymp. Sig. (two-tailed)	Tam Sig. (two-tailed)	Tam Sig. (one-tailed)
Pearson chi-square median	.171[a]	1	.679		
Continuity	.005	1	.941		
Probability ratio	.171	1	.679		
Fisher's exact test				.743	.470
Number of patients	52				

[a]0 cells (0.0%) have an expected count of less than 5. The minimum expected number is 5.39
[b]Only calculated for the 2x2 table

Absolute and relative residual tumor volumes were significantly lower in the neuronavigation-assisted surgery group. Radical tumor resection was associated with a very significant increase in survival. There was no significant difference in the survival rates of the patients that underwent surgery with and without neuronavigation. This was attributed to the small number of individuals participating in the study and supratotal resection being performed in all the patients during surgery. In addition, the low median survival time of patients with glioblastoma may have contributed to this finding (Table 1).

DISCUSSION

The current gold standard of treatment for GBM is surgical resection followed by adjuvant chemotherapy and radiotherapy. Given the poor prognosis of GBM, the surgical removal of the tumor mass is often performed to reduce the

tumor burden and increase survival. It is necessary for neurosurgeons to evaluate the size and location of the tumor and the patient's functional status to determine the extent of resection (EOR) that prolongs overall survival (OS), improves the quality of life, and preserves neurological function (9). Neurosurgical options for GBM include biopsy, gross total resection (GTR), or subtotal resection (STR). GTR is defined as the maximum removal of the tumor observed on MRI. In contrast, STR refers to the removal of only a portion of the tumor, and therefore residual tumor lesions are seen on postoperative images. Han et al. showed that GTR significantly improved progression-free survival (PFS) and OS compared to STR in patients with GBM (10,11).

In recent studies, neuronavigation has been reported to help the surgeon orientate through adequate application accuracy. It facilitates the precise planning of craniotomy and the surgical vector in targeted small subcortical lesions and helps define the boundaries of the tumor and resection (12).

However, multiple tumor lesions, bilateral tumor involvement, and large-volume tumors pose clinical challenges and risks for total resection (13). STR is used as an alternative surgical approach because it is not clinically feasible. Although maximal surgical resection has been shown to improve the OS and quality of life of patients, recurrence is still inevitable.

Since the actual surgical position is related to preoperative images, a gradual recording error may be seen during intraoperative navigation due to brain shift. This situation may be further complicated by cerebral blood volume, the use of mechanical ventilators or diuretics, or retraction during surgery. Dorward et al. measured brain shift during open cranial surgery to assess the effect of post-imaging brain distortion on neuronavigation and reported a mean shift of 4.6 mm in the cortical surface after dural patency and 6.7 mm at the completion of tumor resection. This suggests that the use of more advanced navigation systems that calculate this shifting effect will make resection safer in the future.

However, there are still some uncertainties limiting the integration of augmented reality (AR) into daily practice. Currently, there is no prospective study showing a significant difference between AR-assisted and navigation-assisted procedures in terms of morbidity, mortality, and clinical efficacy.

In a study summarizing the cases in which navigation was used during various neurosurgery operations between 1996 and 2015, the rate of patients with glioblastoma was reported to be 7.17% (Table 2). This indicates that neuronavigation is used more in patients with glioblastoma than in those with other tumor types.

Table 2: Neurosurgical lesions treated with the aid of Augmented Reality

Pathology	# Lesions	% Lesions
Neoplastic lesions	**75**	**38.46**
Glioma/GBL* supratentorial	14	7.17
Glioma/GBL* infratentorial	0	0
Meningioma/supratentorial	12	6.15
Meningioma/infratentorial-skull base	7	3.58
Pituitary adenoma	12	6.15
Metastasis	11	5.64
Schwannoma, vestibular	2	1.02
Ependymoma	1	0.51
Oligodendroglioma	1	0.51
Hemangioblastoma	1	0.51
Neuroepitelial tumors	1	0.51
Other neoplastic lesions	13	6.66
Vascular lesions	**77**	**39.48**
Aneurysm ant.circul.	39	20.00
post.circul.	4	2.05
Cavernoma	20	10.25
AVM	8	4.10
Moya-Moya disease (by-pass)	3	1.53
Stroke	2	1.02
Arterial dissection (By-pass)	1	0.51
Non-neoplastic, non vascular	**1**	**0.51**
Hydrocephalus	1	0.51
Undetermined	**42**	**21.53**
Epileptogenic lesions	40	20.51
Others	2	1.02
Total	**195**	**100**

* (14)

Glioblastoma can occur in different places and in patients of different ages. For tumors located in the brainstem or adherent to supratentorial functional regions, the goal of surgery should be to maximize tumor resection while preserving important neurological functions. Therefore, it is essential to separate the tumor margin from the surrounding normal tissue for the safe removal of the lesion.

Glioblastoma surgery means striking a balance between maximizing the extent of resection and preventing postoperative neurological complications. Various surgical techniques and adjuvants can be used to identify areas of the brain that are significant either to detect tumor tissue (residual) and increase EOR (reduce residual volume) or maintain functionality. In recent years, considerable progress has been made for both ends, with numerous scientific efforts. Neurosurgeons can choose their preferred preoperative and intraoperative modalities from a wide variety of possibilities. Different modalities may be used for the same purpose, often with comparable results or without strong, prospective evidence for one modality in particular. The clinical impact for some of these modalities and patient subgroups is not always based on high-level evidence. Thus, rather large prospective studies, such as RCTs or multicenter cohort studies, are needed to compare various modalities in a multimodal setting to determine which modality is most appropriate for which patient (grade, location, etc.).

CONCLUSION

The small number of patients is a limitation of our study. The effectiveness of neuronavigation can be better evaluated in a study to be conducted with a larger number of patients. In addition, the contribution of neuronavigation to radical resection and

reduction of recurrence rates in deeply located tumors present as important topics of research.

In this study, some applications, and limitations of neuronavigation were reviewed. Advances in technology will increase the cost-benefit ratio and user-friendliness of the system and may help achieve the goal of complete cytoreductive surgery with minimal morbidity in the near future. However, considering the current state of knowledge, neuronavigation can only assist surgeons and cannot replace their experience and knowledge in neuroanatomy.

Since first developed and reported by Roberts et al. in 1986, neuronavigation has been frequently used during brain tumor resection surgery. One of the advantages of neuronavigation over glioma surgery is that it provides assistance in preoperative planning, allowing neurosurgeons to identify nuclei and white matter fibers that are not visible under a microscope, thus leading to better neurological outcomes. However, neuronavigation also has the primary disadvantage of brain shift.

In older series, it was reported that this shift was more than 10 mm (15). The range of system errors can be reduced through upgraded navigation equipment.

Recent studies have shown that neuronavigation is more effective when combined with sodium fluorescein.

Surgery plays an important role in the treatment of glioblastoma. A combination of techniques including intraoperative MRI, neuronavigation, ultrasonography, and fluorescence guidance will allow for safe and maximum surgical resection, resulting in better outcomes in terms of survival and postoperative functional recovery. However, despite maximal surgical resection and adjuvant chemotherapy-radiotherapy, tumor recurrence occurs in many cases within 10 months, which is due to established cancer stem cells. Therefore, there is a compelling need for the development of more effective treatment strategies for glioblastoma.

Ethics Committee Approval: This prospective study was approved by the ethical review committee of Lokman Hekim University Ethics Committee dated 18.10.2021 and numbered 2021/0127.

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Efficacy of Burrhole Craniostomy in Chronic Subdural Hematoma: A Retrospective 9-Year Study

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Abstract

Objective: To demonstrate the effectiveness, possible complications, and difference of Burr-hole craniostomy surgical technique applied to patients diagnosed with chronic subdural hematoma from other surgical techniques.

Methods: The surgical techniques and postoperative clinical and radiological details of 36 patients who were operated on with the diagnosis of chronic subdural hematoma in the Neurosurgery Clinic of Ordu University Training and Research Hospital between 01.01.2013 and 15.08.2022 were retrospectively analyzed. In all patients in the post-op period, control brain CT was taken within the first 24 hours and compared with the pre-op CT. Again, at the end of post-op 1st, 2nd week and 1st month, control brain CT was taken for all patients and GCS was compared with pre-op scores. After determining the post-op complications, the treatment and results of these complications were examined.

Results: One patient who was operated on with Burr-Hole developed motor dysphasia in the post-op period, and intraparenchymal hemorrhage was detected in the post-op tomography of this patient. . Post-op clinical and radiological results of patients who underwent burr-hole craniostomy were significantly better than pre-op clinical and radiological results, and the recurrence rate was low, consistent with the literature. All drains placed in the subdural area after the burr hole opened during the operation were removed before discharge.

Conclusions: Although the drainage of chronic subdural hematoma with bur-hole craniostomy has a higher recurrence rate compared to the craniotomy method, it has a lower complication rate and is a more easily applicable surgical technique. In our study, some important points about patients who underwent burrhole craniostomy for cSDH evacuation were highlighted. It was observed that our patients who underwent burrhole craniostomy had higher reoperation rates compared to our patients who underwent craniotomy. We think that the presence of residual hematoma in the controls performed with CT in the post-op period should not be the sole criterion for re-operation. We think that CT controls are sufficient if there is improvement in the neurological status of the patient and a better GCS score in the post-op follow-up.

Key words: Chronic subdural Hematoma, Burr-Hole craniostomy, Craniotomy

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INTRODUCTION

Chronic subdural hematoma (cSDH), a complex condition with an annual prevalence of 1.7-20.6 per 100,000 people, is more common among the elderly. Trauma and inflammation play a role in the pathophysiological cycle of cSDH formation and growth by promoting membrane formation with permeable neovessels (1). cSDH is a pathology characterized by blood accumulation in the subdural space and the inflammatory reaction it causes. The cSDH recurrence rate is between 9.2% and 26.5%. A distinctive membrane was identified surrounding the SDH, a source of fluid exudation and bleeding. As a result of angiogenesis, fragile blood vessels develop within the membrane walls, but continued bleeding is caused by fibrinolytic processes that prevent blood clots from forming. Membranes and subdural fluid contain a variety of inflammatory cells and markers that are expected to contribute to the propagation of an inflammatory response that supports continued membrane growth and fluid accumulation (2).

Radiological imaging shows a crescent-shaped stratification of fluid on non-contrast CT (Figure 1).

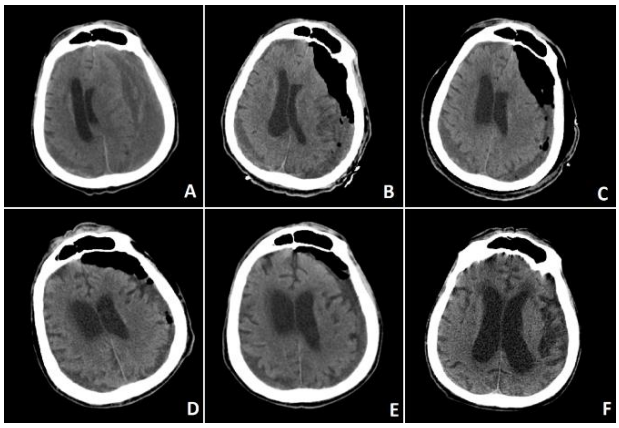


Figure 1. Cranial tomography images of the patient; A: Preoperative B: Postoperative 1st day C: Postoperative 3rd day D: Postoperative 10th day E: Postoperative 1st month F: Postoperative 3rd month

The most common symptoms of chronic subdural hematoma are headache, confusion, hemiparesis, and aphasia. Asymptomatic patients can be followed carefully, while symptomatic patients are indicated for surgical treatment (3). Tranexamic acid can be used to treat chronic subdural hematomas without requiring additional surgery. Inhibiting both the inflammatory (kinin-kallikrein) and fibrinolytic (tranexamic acid) systems at once can dissolve cSDH (4). 443 neurosurgeons practicing throughout the world responded to a survey on the non-surgical and surgical management of cSDH. Dexamethasone is sometimes used as a monotherapy by the responders, 46.2%. The majority of neurosurgeons around the world are unwilling to address cSDH with conservative therapy techniques. Which clinical characteristics of cSDH were considered to be the key indicators of surgery, according to the respondents? The Glasgow Coma Score (GCS) of less than 12

(57.8%), followed by a motor response of 5 or less on the GCS, is the most significant clinical justification for surgery. Another crucial indicator is the hematoma's size on the computed tomography (CT) scan (5).

Surgical treatment should be considered if the hematoma constituting compression in patients undergoing imaging causes clinical neurological symptoms. Surgery with irrigation, burr-hole craniostomy, or craniotomy with or without drainage is the mainstay of treatment for patients with clinical symptoms of cSDH and a large mass impact. Cranitomy, twist-drill craniostomy, and burr-hole craniostomy are the surgical procedures utilized for this. The selection of these operations, however, is debatable and mostly based on surgeon preference. Burr-hole craniostomy with drainage is the surgical technique that is most frequently utilized worldwide (6). Both craniotomy and craniostomy (burr hole/twist drill) are successful operations (7). Although endoscopic surgery for cSDH takes more time, it is effective and safe. Endoscopic treatment may be the most appropriate surgical technique for complicated cSDH (8). Neuroendovascular intervention, which is a minimally invasive procedure, is used for treatment because the condition is essentially dysfunction of the meningeal blood arteries (9). On bilateral cSDH, drilling drainage and neuroendoscopic aided surgery are effective treatments. The drilling drain has a shorter working period. For the treatment and prognosis of individuals with bilateral cSDH, neuroendoscopic assisted drainage may be more appropriate when surgical circumstances are present (10). The dissemination of level 1 support for drain use has had a favorable effect on this practice globally. After hematoma drainage, some surgeons are still hesitant to insert drains, especially when the subdural space is constrained. Placing a subperiosteal drain would be a suitable choice. However, larger research should assess its results and efficacy (11). After burr-hole drainage of a subdural hematoma, the use of drains is safe and associated with a 6-month reduction in recurrence and death (12). The drain can be placed subgaleally or subdurally. In addition, the drain may have active suction, passive suction, or continuous subdural irrigation. With standardization, passive subdural postoperative drainage is currently applied in Denmark (13). Drainage with irrigation is a risk factor for postoperative delirium and longer hospital stay. In high-risk patients with delirium, drainage without irrigation may be the most appropriate treatment for cSDH (14). Bilateral subdural hematomas should be treated as soon as feasible due to their severe symptoms, quick progression, and easy deterioration (15,16). One of the risk factors for the surgical treatment of chronic subdural hematomas is preoperative administration of

antithrombotic drugs. Craniotomy has significantly reduced the incidence of recurrence (17). The incidence of calcified or ossified CSDH is high, with a steady increase in recent years in some countries, including the United States, Japan, and Turkey. Surgery is the primary course of treatment for these individuals, and as it is uncommon following shunt in children or head trauma in adults and the clinical picture is varied, it should be taken into consideration in the differential diagnosis at the time of presentation (18). In adults with posterior fossa cSDH, the cause is often unclear. It may develop from an acute hematoma due to direct head trauma or from another type of spontaneous bleeding (19). Compared to younger patients, cSDH surgery in the elderly results in excellent neurological outcomes without an increased risk of overall complications, recurrence, or reoperation. However, older people may have a higher risk of death after surgery. (20). Neurosurgical intervention for cSDH in some non-young individuals may be a safe and advantageous technique, according to Ewbank F. et al. The patients who profited most from the procedure were those who were autonomous at home and had little medical history (21). Another type of chronic subdural hematoma is organized subdural hematoma, and the ideal surgical technique has not been determined. According to the experience of Chen K. et al., a smaller craniotomy can be considered instead of a larger craniotomy in the treatment of organized cSDH (22). Pediatric cSDH is concerning, and the doctor needs to be informed in order to look into the underlying reason and rule out child abuse. Incidences in children are rather infrequent. The main causes include child maltreatment, birth trauma, coagulopathy, and shunt operations (23). Most patients with non-traumatic cSDH are on AC (anticoagulant) or AP (antiplatelet) medication, which is an important condition and increases the risk of a poor neurological outcome. Large hematoma sizes that occur with the use of ACs worsen the conditions of cSDH patients and are more common in non-traumatic individuals. Patients with cSDH who use ACs have greater hematoma volumes and non-traumatic patients are more likely to use ACs. While AP treatment does not increase the probability of cSDH recurrence, AC treatment does (24). There may also be chronic comorbidities such as hypertension and renal failure, which may increase the risk of rebleeding in patients using antithrombotic drugs. It has been determined that these combinations, as well as antithrombotic drugs, can cause re-bleeding. After evacuation surgery, individuals using anticoagulants and antithrombotics did not have a worse mortality rate or clinical outcome (25).

METHODS

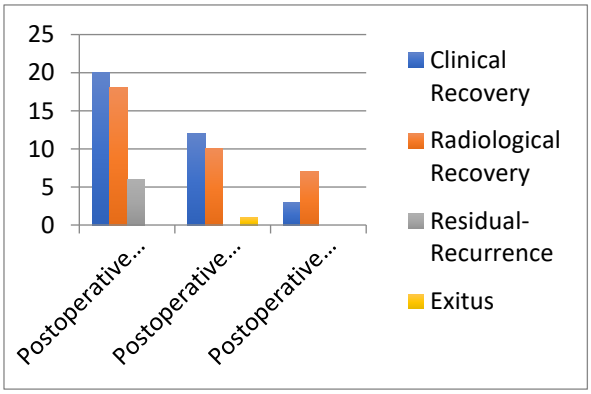
The surgical techniques and postoperative clinical and radiological details of 36 patients

who were operated on with the diagnosis of chronic subdural hematoma in the Neurosurgery Clinic of Ordu University Training and Research Hospital between 01.01.2013 and 15.08.2022 were retrospectively reviewed. As a surgical technique, 2 Burr-Hole craniostomy and 72 hour closed drainage system were applied to all patients. Craniotomy + membranectomy was performed in 6 patients who relapsed. Of the cases, 28 (77.7%) were male and 8 (22.3%) were female. The mean age was 70, with an age range of 54-87. Ten of the cases had a GCS of 15 (28%), 20 had a GCS of 13-14 (56%), and 6 had a GCS of 8-12. The most prominent complaints of the patients were headache and confusion. While 22 patients (61.1%) had a history of head trauma, 14 patients (38.9%) had no history of trauma. 9 patients (25%) had a history of anticoagulant and antiaggregant drug use.

RESULTS

Control brain CT was taken within the first 24 hours of all patients in the post-op period and compared with the pre-op CT. Again, at the end of post-op 1st, 2nd week and 1st month, control brain CT was performed for all patients and GCS was compared with pre-op scores. Craniotomy+ membranectomy was performed in 6 patients because of residual bleeding in the post-op period and no improvement in their neurological status. One of the patients who were operated on by craniotomy died due to sepsis in the later period. One patient who was operated with Burr-Hole developed motor dysphasia in the post-op period, and intraparenchymal hemorrhage was detected in the post-op tomography of this patient. This patient's dysphasia resolved at the end of the post-op 1 month. Although pneumocephalus developed in the post-op period in 9 patients who underwent burr-hole craniostomy, they did not require surgical treatment and were observed to be spontaneously resorbed.

In addition, wound infection developed in the post-op period in 4 patients who underwent burr-hole craniostomy. Appropriate antibiotic therapy was given to these patients. At the end of the first week, no residual hematoma or recurrent hematoma was observed in the radiological examination of 18 patients with CT. The radiological improvement of 10 patients was completed at the end of the 1st month and the radiological recovery of 7 patients was completed at the end of the 3rd month (Graphic 1).



Graphic 1. Postoperative clinical and radiological recovery, residual and recurrence, exit rates

DISCUSSION

There is debate over the best course of action for treating chronic subdural hematomas (cSDH). It is among the most frequent neurosurgical conditions, and it is typically treatable with straightforward and efficient surgical techniques (26). The surgical management of cSDH is burr hole craniostomy with irrigation and closed system drainage. An independent predictor of cSDH recurrence is mixed density hematoma. The main factor contributing to the recurrence of cSDH may be the existence of a thick inner neomembrane (27). More study is required due to the poor methodology of the existing studies because neither performing two burr-hole craniostomies nor one burr-hole craniostomy provides particular differences in improvement in patient outcomes after chronic subdural hematoma surgery (28). The unilateral cHSH recurrence rate is not impacted by the quantity of burr holes used. Similar to this, the length of subdural drainage has little bearing on the frequency of postoperative infection or the unilateral cSDH recurrence rate (29). Compared to patients who underwent craniotomy, Raghavan A et al showed that patients treated with burr-hole required more reoperations. The outcomes in both groups were poorer when the participants were older and scored lower on the Glasgow Coma Scale (30). For refractory cSDH without organized hematoma, middle meningeal artery embolization can be necessary. For refractory organized cSDH cases, a large craniotomy or endoscope-assisted small craniotomy may be necessary (31). Through a retrospective review, Zhang, Jibo et al. shown that endoscopic aided trepanation drainage (EATD) is a more efficient and secure approach of treating isolated chronic subdural hematoma (ICSH) than craniotomy (32). In this investigation, Idowu OE and colleagues found a strong correlation between age and death. They added that the patients' age, gender, or type of anesthesia did not affect the recurrence of cSDH (33). The majority (92%) of recurrences, according to Rauhala M et al., happened within 60 days. They stressed that CT controls should only be used for symptomatic individuals and that a 2-month follow-up time following cSDH is sufficient for the majority of patients (34). According to Hideki Nakajima and colleagues, adopting an upright position straight after surgery does not increase the likelihood of a recurrent subdural hematoma (35). Patients without neurological symptoms may not need to get a delayed cerebral CT scan throughout the recovery phase (36). Complications that may occur after surgical treatment of chronic subdural hematoma are recurrent bleeding, seizures, intraparenchymal bleeding, and infections (37). Even for older patients who have lived longer than typical, surgical treatment for cSDH can result in outcomes that are both safe

and acceptable (38).

Although the majority of patients with posterior fossa cSDH have positive results, prior research indicates that significant posterior fossa cSDH may occasionally require surgical treatment (39). According to a meta-analysis by Sherrod, Brandon A. et al., preoperative MRI T1 hypo- or isointensity cSDH signal may indicate a higher likelihood of postoperative SDH recurrence (40). Due to increased mortality and lower GCS scores in patients with cSDH without a history of head trauma, more care should be taken in the follow-up of these patients (41).

CONCLUSION

In our study, some important points about patients who underwent burr hole craniostomy for cSDH evacuation were highlighted. It was observed that our patients who underwent Burr Hole craniostomy had higher reoperation rates compared to our patients who underwent craniotomy. We think that the presence of residual hematoma in the controls performed with CT in the post-op period should not be the sole criterion for re-operation. We think that CT controls are sufficient if there is improvement in the neurological status of the patient and a better GCS score in the post-op follow-up.

Although the drainage of chronic subdural hematoma with bur-hole craniostomy has a higher recurrence rate compared to the craniotomy method, it has a lower complication rate and is a more easily applicable surgical technique.

Ethics Committee Approval: This prospective study was approved by the ethical review committee of Ordu University (OMU) Hospital (2022/195)

Peer-review: Externally peer-reviewed.

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RESEARCH ARTICLE

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Radiological Evaluation of the Relationship Between Plantar Fasciitis and Foot Arch Angles in Adults

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Abstract

Objective: The foot arch deformation increases plantar fasciitis (PF) and plantar calcaneal spur (PCS) formation. As a result, the heel fat pad becomes thinner. This study investigated the relationship between plantar fasciitis and foot arch angles.

Methods: We performed a retrospective review of patients who had PF patients (n = 53) and healthy individuals (n = 71) without PF. We have evaluated the presence of PCS and heel fat pad thickness measurements on magnetic resonance imaging (MRI), and the lateral talus-first metatarsal angle (Meary's angle), lateral talocalcaneal angle, and calcaneal inclination angle measurements were performed on X-ray images.

Results: The mean age of the PF group was significantly higher than the control group ($p = 0.001$). The degrees of Meary's angle and calcaneus inclination angle were significantly higher in the PF group ($p < 0.001$ and $p = 0.026$, respectively) than in the control group. The incidence of PCS was significantly higher in the PF group ($p = 0.028$). In the binary logistic regression analysis model, high Meary's angle and calcaneal pitch angle were found to be associated with the risk of PF. There was also a significant association between age and the presence of PCS and PF.

Conclusion: Changes in Meary's angle and calcaneal pitch angle were significant risk factors for PF.

Key words: Plantar fasciitis, heel fat pad, calcaneal spur, Meary's angle, lateral talo-calcaneal angle, calcaneal inclination angle

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INTRODUCTION

The foot is an integral component of the skeletal system in the human body and plays an important role in walking (1). Feet not only support body weight but also maintain the balance of the body against external forces. Moreover, for each step the foot takes, it simultaneously receives the pressure of approximately 80% of the bodyweight (2). The arc of the foot helps the foot adapt to a variety of surfaces and absorbs the forces exerted on the foot when performing activities in a closed kinematic chain. In addition to the above-mentioned function, the arch of the foot also acts as a solid lever during walking (3).

Plantar fasciitis is known as the most common cause of plantar heel pain (4). This problem affects approximately 10% of people at any point of time in their lives (5). With the deterioration of the arch angles of the feet, the anatomical contact of the foot with the ground is impaired; furthermore, excessive foot pronation is particularly an important risk factor for the development of plantar fasciitis. Increased plantar fascia thickness and abnormal soft tissue findings support the diagnosis of plantar fasciitis (6,7). Likewise, the contact of the bodyweight with the ground is impaired following deterioration of the arch angles of the feet (7). Pathological change can also be induced by repeatedly applied stress to the plantar fascia (8). In general, plantar fasciitis (PF) is caused by inflammation from repeated stress placed on the plantar fascia, degenerative changes due to fibrosis, microdamage of the foot heel, and excess biomechanical use such as bearing body weight for extended amounts of time (9,10). Plantar fasciitis is associated with many biomechanical factors such as pes cavus, sudden gain in body weight or obesity, increased running distance or intensity, shoes with poor cushioning, change in the walking or running surface, and tightness of the Achilles tendon (11).

To the best of our knowledge, there is no study in the literature evaluating both the thickness of the unloaded heel fat pad and the arch deformation of the foot in patients with PF. In this study, we purposed to investigate the thickness of the heel fat pad and the angles of the foot arch in patients with PF. We hypothesize that the foot arch deformation increases plantar fasciitis and plantar calcaneal spur formation. As a result, the heel fat pad becomes thinner.

METHODS

This retrospective single-center study was conducted in the Orthopedics and Traumatology Department of our hospital with the contributions of the Radiology Department between March 2020 and May 2021. This research has been approved by the IRB of the authors' affiliated institutions. The study was conducted with the principles of the Declaration of Helsinki. This study was planned with at least 52 patients in each group. The sample size was

determined using G-Power with an effect size of 0.8, a power of 0.8, and a significance level of α=0.05.

Our study evaluated the magnetic resonance imaging (MRI) findings of the ankle taken by the radiology clinic between March 2020 and May 2021. Patients diagnosed with plantar fasciitis based on these MRI findings were included in the PF group (n = 53) and those without plantar fasciitis were included in the control group (n = 71). Exclusion criteria included a history of previous foot surgery; foot injury, including any bone pathology or ligament injury; congenital deformity of the lower extremity, and patients with a space-occupying lesion. Patients who could not undergo standard true lateral X-ray and MRI, as well as patients aged under the age of 20 years, were excluded from the study. The lateral radiographs were taken with the tibia neutral on the talus and the x-ray beam centered on the lateral cuneiform and parallel to the weight-bearing surface. Unloaded heel fat pad thickness (UHPT) was measured on MRI in all patients included in the study. Scanning was performed with a 1.5 Tesla Magnetum system (SIEMENS Magnetom Amira, Germany). Sagittal, coronal, and axial images with a section thickness of 4 mm were obtained in all scans. The MRI sequences included sagittal, coronal, axial T1-weighted, and proton-weighted images. UHPT measurements were performed using the sagittal images. This measurement was accomplished by measuring the distance from the calcaneal tuberosity to the outer margin of the skin (Figure 1). The lateral talus-first metatarsal angle (Meary's angle), lateral talocalcaneal angle (LTCA), and calcaneal inclination angle (the calcaneal pitch) measurements were performed using weight-bearing lateral X-ray images of all patients (Figure 2). All measurements were made using the standardized method described by Flores et al., and were conducted using the digital radiographic viewer at our clinic with the picture archiving and communications systems (PACS) (12). The presence of plantar fasciitis and plantar calcaneal spur (PCS) was investigated by MRI. Findings such as thickening of the plantar fascia, increased signal intensity in or around the fascia in fat-suppressed sequences, and limited bone marrow edema within the medial calcaneal tuberosity were evaluated in favor of inflammation (Figure 3) (13). All X-rays and MRI were evaluated by a radiologist and orthopedic and traumatology specialist with at least 5 years of experience. Each observer evaluated the radiographs and MRI—listed differently each time—on a total of 2 occasions. The average of the measurements was recorded. In addition, the age, gender, foot side, and body mass index (BMI) data of all patients were obtained using the digital recording system.

Statistical Analysis

The sample size was based on the literature

obtained for the difference observed in the thickness of the plantar fascia in patients with plantar fasciitis. A power of 80% and a confidence level of 95% yielded the sample size. All data were analyzed using the statistical software package SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). A normality check was performed using the Kolmogorov–Smirnov test. The data were presented as mean and standard deviation for continuous variables. The categorical variables were expressed as frequency and percentage and compared using the chi-squared test. The non-normally distributed data were compared using the Mann–Whitney U test. Factors affecting the presence of plantar fasciitis were examined by binary logistic regression analysis. $P < 0.05$ was accepted as a statistically significant level. In addition, the intraclass correlation coefficient (ICC) was used as a statistical analysis to determine interobserver reliability.

RESULTS

The demographic and clinical characteristics of the participants are presented in Table 1. There was no significant difference between the groups in terms of the evaluated foot side, BMI, and gender distribution ($p > 0.05$). The age of the patients with PF was significantly higher than the control group ($p = 0.001$). When the patients were evaluated according to their foot arch angles, the Meary's angle and calcaneal inclination angle degrees were significantly higher in the plantar fasciitis group ($p<0.001$ and $p=0.026$, respectively) (Table 1). No statistically significant differences were found between the groups regarding lateral talocalcaneal angle and UHPT values ($p = 0.236$ and $p = 0.231$, respectively). PCS was present in 13 (24.53%) of the patients with PF and 7 (9.86%) of the patients in the control group. The incidence of PCS was significantly higher in the PF group ($p = 0.028$).

Table 1. Demographic and clinical characteristics of the participants

Parameter	Plantar fasciitis (n = 53)	Control (n = 71)	p-value
Gender (Male/Female)	19/34 (35.85%/64.15%)	29/42 (40.85%/59.15%)	0.572[a]
Side (Right/Left)	30/23 (56.60%/43.40%)	41/30 (57.75%/42.25%)	0.899[a]
Age (years)	50.43 ± 11.59	41.92 ± 14.30	**0.001[b]**
Body mass index (kg/m^2)	23.81 ± 1.86	23.21 ± 2.08	0.120[b]
Meary's angle	4.44 ± 4.05	2.62 ± 2.17	**<0.001[b]**
Lateral talocalcaneal angle	26.92 ± 8.14	24.75 ± 5.34	0.236[b]
Calcaneal pitch angle	18.81 ± 5.58	16.60 ± 4.96	**0.026[b]**
UHPT (mm)	14.50 ± 3.00	13.86 ± 2.62	0.231[b]

Data are given as mean ± standard deviation for continuous variables and as frequency (percentage) for categorical variables. [a]Chi-square test; [b]Mann–Whitney U test. UHPT: unloaded heel fat pad thickness

In the binary logistic regression analysis model, high Meary's angle and calcaneal pitch angle were found to be associated with the risk of plantar fasciitis (95% CI: 1.070–1.537; *P* = 0.007 and 95% CI: 1.056–1.251; *p* = 0.001, respectively). There was also a significant association between age and the presence of PCS and PF (95% CI: 1.019–1.088; *p* = 0.002 and 95% CI: 1.092–9.923; *p* = 0.034, respectively) (Table 2). The measurements of all images showed almost perfect interobserver agreement, and ICC was 0.974

Table 2. Factors associated with the risk of plantar fasciitis.

Variables	Odds Ratio (95% CI)*	p-value
Age	1.053(1.019–1.088)	0.002
Meary's angle	1.282 1.070–1.537)	0.007
Calcaneal pitch angle	1.149 (1.056–1.251)	0.001
Plantar calcaneal spur	3.292 (1.092–9.923)	0.034

DISCUSSION

Plantar fasciitis is a disease frequently encountered and is the most common cause of heel pain in adults (14). Although PF is known as the local inflammation of the plantar fascia due to microtrauma, its exact etiology is yet to be clarified. Our study shows that there is a highly significant relation between PF and PCS. In addition, while changes in Meary's angle and the calcaneal pitch angle result in an increased risk of PF, whereas changes in the lateral talocalcaneal angle have no significant effect on PF. The risk of PF also increases with age.

Although the exact etiology of PF is unknown, many studies investigating its etiology are available in the literature. One of the etiological aspects emphasized the most is the relationship between obesity and PF. Frey et al. investigated the effects of obesity on foot and ankle pathologies and showed that overweight or an obese patient has a 1.4 times increased risk of PF (15). Abate et al. investigated the effect of BMI on Achilles' tendon and plantar fascia thickness, they found that the thickness of the plantar fascia increased with increasing BMI (16). They also stated that this increased the risk of PF. Caliskan et al. are demonstrated that female sex, BMI over 30 kg/m^2, higher red cell distribution width, and higher plantar fascia thickness were associated with plantar fasciitis (17). The difference of these studies, although the BMI ratio was higher in the PF group in our study, we did not determine a significant difference between BMI and PF. In addition, the female sex ratio was higher in patients with PF and this finding was consistent with the literature.

Age is among the demographic characteristics studied in the literature regarding the etiology of PF and heel pain, and contradictory results have been obtained in such studies. According to the studies by Rome et al. and Rano et al., the groups of patients with heel pain had significantly higher

ages (18, 19). Unlike these studies, Wearing et al. compared the age parameter of the groups with and without PF, no significant difference was found despite the mean age being higher in the PF group (20). In our study, the mean age in the PF group was higher than the control group, and this difference was found to be statistically significant. Additionally, according to our binary logistic regression analysis, we determined that a one-unit increase in age increases the presence of PF by 1.053 times.

In some studies, plantar calcaneal spur has also been shown in asymptomatic patients (21,22). Therefore, it is still unclear whether there is a relation between spur formation and PF. Pasapula et al. showed that calcaneal spurs can be observed in 50% of those with heel pain (21). However, the calcaneal spur can also be observed in 16% of the patients without heel pain. Johal et al. found that the incidence of PCS was 89% in the PF group and 32% in the control group (22). On the other hand, Beytemur et al. investigated the incidence of age-related calcaneal spurs (23). They evaluated the lateral ankle X-rays of 1335 patients and found the incidence of PCS to be 32.2%. In addition, they determined that the incidence of PCS increases with age, and the posterior calcaneal spur is more common in women, and there is no difference between men and women in the incidence of the plantar calcaneal spur. In our study, the incidence of PCS was found to be 24.53% in the PF group, and it was significantly higher than in the control group. This finding is consistent with the literature. In addition, according to the binary logistic regression analysis performed, we found that the presence of PCS increased the presence of PF by 3.292 times. In our study, there were no cases of posterior calcaneal spurs. Besides, 85% of the patients with PCS were female patients.

During the gait cycle, the heel is the first point of contact between the body and the ground and is covered by a special fat pad whose main function is to absorb forces from the ground. In their histological study, Jahs et al. revealed the presence of free nerve endings and Pacinian bodies in the heel fat pad (24). These findings suggest that heel pain might originate from the heel fat pad. According to Belhan et al. who investigated the relationship between heel fat pad thickness and PF using ultrasonography, the fat pad thickness of painful heels was statistically thinner than painless heels (25). In our study, unlike the literature, unloaded heel fat pads were found to be thicker in the PF group; however, this finding was not statistically significant. Hsu et al. determined that the elderly have a thicker and stiffer heel fat pad compared with young people (26). In our study, the higher mean age in the PF group may be the reason for the thicker heel fat pad.

The plantar fascia is the primary structure

that stabilizes the medial longitudinal arch (27). Therefore, abnormal arc structure may play a role in the development and progression of PF. Based on this hypothesis, the relation between PF and lower extremity biomechanics has been previously studied in the literature (28,29). In a retrospective study by Taunton et al., pes planus was found in only 19 (12%) and pes cavus in only 11 (7%) of 159 patients with PF (28). When the groups were compared in terms of Meary's angle in our study, Meary's angle was found to be higher in the PF group than in the control group, and pes planus was present in 15 (28%) of the 53 patients with PF. We did not find cavus deformity in any of our patients. Moreover, in the binary logistic regression analysis conducted, we found that a one-degree increase in Meary's angle increased the risk of PF by 1.282 times. This finding indicates that pes planus is an important risk factor for PF. In the study by Menz et al. measured calcaneal inclination angle, calcaneal-first metatarsal angle, and navicular height in their study between elderly patients with and without PCS and reported that there was no statistically significant difference between the two groups (29). As a result, they concluded that radiographic measurements were not effective on PCS formation. In our study, we measured the calcaneal inclination angle, LTCA, and Meary's angle in a group of participants aged over 20 years, including patients with and without PF. In these measurements, we found that the control group had lower values than the PF group in terms of these three angles, and the difference in Meary's angle and the calcaneal inclination angle was statistically significant. In addition, based on the binary logistic regression analysis performed, we determined that a one-degree increase in Meary's angle, and the calcaneal inclination angle increased the risk of PF by 1.282 and 1.149 times, respectively.

This study has some limitations. The first limitation relates to the retrospective nature of the study and to the fact that the cases were evaluated only radiologically; therefore, we could not evaluate other factors such as the clinical presentations, professions, daily activities of the patients, as well as for how long they stood per day. A relation can be established between the symptoms and the demographic and radiological characteristics of patients only if such evaluation is performed. The second limitation was that more angles could be measured, such as the calcaneal-first metatarsal angle. Thus, the radiological features of the patients could be evaluated from a wider perspective and more objective results could be obtained. The third limitation was that since it is a retrospective study, the mean age was different between the groups. Age-related degenerative changes in bones may affect angle measurements.

CONCLUSION

In conclusion, patients with high foot arch on X-rays have MRI changes in PF more often. In this study, we determined that changes in Meary's angle and the calcaneal inclination angle increased the risk of PF. In addition to improving current treatment strategies for PF, there is a need to prevent the onset of inflammation in the plantar fascia. For this reason, it is important to develop screening and treatment programs that will facilitate early diagnosis and treatment of foot arch disorders. The development of such a program will contribute to saving national health expenditures on PF treatment in the future and reducing the loss of the labor force. In addition, studies with larger sample groups will be useful in identifying more risk factors for PF and developing screening programs to this end.

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Are Prostate Cancer Screenings Performed in Compliance with Cancer Guidelines?

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Abstract

Objective: This study aims to examine whether our region's Prostate Cancer (PCa) screening programs comply with the European Association of Urology (EAU) Guidelines.

Method: This study was conducted as a retrospective, cross-sectional study between April 2014 and July 2022. Two hundred seventy patients who applied to our clinic for various reasons and were diagnosed with PCa were included in the study. Characteristics of the patients, such as age at diagnosis, comorbidities, age at first PSA examination, and PSA values, were recorded.

Results: The mean age of the patients at the time of cancer diagnosis was 67.42±8.64 (43-91) years. PSA value (median±IQR) at the diagnosis was 9.58±19.43 (1.83-3437) ng/ml. When the distribution of cancer according to different decades of life was examined, there were 5 (%1.8) patients in the 40-50 age range, 44 (16.1%) in the 50-60 age range, 111 (40.7%) in the 60-70 age range, 86 (31.5%) in the 70-80 age range, and 24 (8.8%) after the age of 80. While 138 patients (51.1%) had local and benign tumor features, 59 (21.9%) patients were diagnosed with metastatic findings. Only 31.3% (61/195) of the patients were under regular follow-up by a specific urology doctor.

Conclusion: It was found that the screening of prostate cancer, the most common type of cancer in men, was not performed by the guidelines, and as a result, diagnosis and treatment were delayed. It was determined that many patients lost the chance of curative treatment. In this disease, where early diagnosis is vital for effective treatment and preservation of quality of life, it is essential to follow up with aging men in accordance with the guidelines. It may be beneficial to periodically train and follow up with all health professionals interested in this issue.

Key words: EAU Guidelines, Prostate cancer, Age, Prostate Cancer Screening Programs

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INTRODUCTION

Prostate cancer (PCa) is one of the most common cancers and the leading cause of death in men. It constitutes 15% of all newly diagnosed cancers. Although it is more common in some regions, it generally constitutes a significant public health problem worldwide. Although the underlying cause has not been fully elucidated until now, its close relationship with some risk factors such as age, genetic factors, and sexually transmitted diseases is well known (1-6). Since the cause is not known precisely, we do not have a recommendation or medicine to prevent the disease. Today, all efforts are focused on early diagnosis and treatment rather than preventive measures. In cases diagnosed early, there are effective treatments such as radical prostatectomy (RP) and radiotherapy. There is a strenuous effort worldwide for the early diagnosis of cancer. Significant steps have been taken in this regard. Many international associations, such as the European Association of Urology (EAU) and the American Association of Urology (AUA), have prepared guidelines for this disease (7). In our country, especially EAU guidelines are followed in diagnosing and treating prostate cancer. However, it is not known how much these guidelines are followed in our daily practice precisely. In our own experience, these guidelines are often neglected. This study was planned to see the use of these guidelines in patients diagnosed with prostate cancer.

This study aims to examine whether patients newly diagnosed with PCa are followed up in accordance with EAU guidelines.

METHODS

This study was carried out in the Urology Clinic of Ordu Medical Faculty Training and Research Hospital. Permission was obtained from the local ethics committee for the study (No: 2022/09-214). Between April 2014 and July 2022, 270 patients diagnosed with prostate cancer in our clinic and whose data could be accessed were recorded. In this study, the data were recorded prospectively by a specialist doctor at the first encounter. Laboratory studies of all patients were completed after approximately 10 hours of fasting and before invasive procedures. All patients with primary prostate cancer, Prostate Specific Antigen (PSA) value, and pathology results, remembering the questions asked, and supporting the study were enrolled.

Patients who received treatment for prostate cancer had psychological/neurological diseases causing severe forgetfulness or cognitive impairment, did not want to participate in the study, or did not want to talk about the subject were excluded from the study. Age, body mass index (BMI), belly circumference, comorbidities, smoking, and alcohol use status of the patients were recorded. In addition, access data such as age

at the time of cancer diagnosis, serum PSA value, age at first urological examination, prostate biopsy pathology reports, and cancer stages were recorded. Our country does not have specific prostate cancer guidelines; because of its geographical features, the EAU guidelines used in Europe are also used in our country. The patient's data were compared with the EAU Prostate Cancer Guidelines.

Statistical Analysis

In the data analysis, the SPSS 20.0 package (Statistical Package for the Social Sciences, Version 20.0 SPSS Inc. Illinois, USA) program was used. In summarizing numerical data, arithmetic mean±standard deviation, median (1st Quarter-3rd Quarter), minimum and maximum values, numbers, and percentages were used in summarizing categorical data. The conformity of the data to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Relationships between categorical data were evaluated with the Chi-square test. In order to evaluate the relationship between the numerical data determined to be normally distributed and the categorical data, the independent-samples t-test was used when the categorical data were in two categories. One-Way ANOVA test was used if the categorical data were in three or more categories. Appropriate post hoc tests were performed to determine which group caused the significant difference in the One-Way ANOVA test. The Man-Whitney U test was used when the categorical data were two categories to evaluate the relationship between the numerical data determined not to be normally distributed and the categorical data. The Kruskal Wallis H test was used when categorical data were in three or more categories. Posthoc Man-Whitney U test and Bonferroni correction were performed for pairwise comparisons between groups with significant Kruskal Wallis test results. Statistically, $p<0.05$ cases were considered significant.

RESULTS

The mean age (mean ± std) of 270 patients diagnosed with prostate cancer was 67.42 ± 8.64 (43-91). BMI (mean ± std) was 27.20 ± 3.96 (17.96-39.64). The PSA value (median ± IQR) at diagnosis was 9.58 ± 19.43 (1.83-3437) ng/ml.

Table 1: Demographic Characteristics of the Patients

Decades of life	Cancer frequency n (%)
Between 40-50 years old	5 (1.8)
Between 50-60 years old	44 (16.1)
Between 60-70 years old	111 (40.7)
Between 70-80 years old	214 (79.3)
> 80 years	24 (8.8)

When the cutoff value for age at diagnosis was set to 50 years, 9 (3.3%) patients before age 50 and 261 (96.7%) patients after 50 years of age were diagnosed with cancer. When taking the age threshold of 60, 56 (20.7%)

patients were diagnosed with cancer before age 60, and 214 (79.3%) were diagnosed after age 60. For the age limit of 70 years, 175 (64.8%) patients were diagnosed before age 70 and 95 (35.2%) after age 70. When the distribution of cancer according to different decades of life was examined, there were 5 (%1.8) patients in the 40-50 age range, 44 (16.1%) in the 50-60 age range, 111 (40.7%) in the 60-70 age range, 86 (31.5%) in the 70-80 age range, and 24 (8.8%) after the age of 80.

Table 2: Distribution of cancer incidence by decades of life

Decades of life	Cancer frequency n (%)
Between 40-50 years old	5 (1.8)
Between 50-60 years old	44 (16.1)
Between 60-70 years old	111 (40.7)
Between 70-80 years old	214 (79.3)
> 80 years	24 (8.8)

One hundred thirty-eight patients (51.1%) had local and benign tumor characteristics, and 59 (21.9%) patients had signs of metastasis. For local and locally advanced PCa, 35.4% (96) of the patients were in the high-risk group according to the EAU biochemical recurrence risk classification. In other words, if 35.4% of patients were treated, a biochemical recurrence would develop. 9.9% of patients (27/75) had a family history of prostate cancer. 51.1% of patients (120/235) were taking prostate-related drugs. While a specific urologist regularly followed up only 31.3% (61/195) of patients, 68.7% (134/195) were randomly followed by more than one physician. The age of onset of symptoms in the lower urinary system (LUTS) was 62.52 ± 8.29 (40-83) years, and the age at first urological examination was 63.14 ± 8.31 (40-91). Of the patients, 61% (152/249) had the habit of smoking, and 15.3% (38/249) of the patients had the habit of using alcohol. The distribution of heart disease (CVD), diabetes mellitus, and hypertension in the group was found to be 23.4% (60/256), 14.5% (37/256), and 41.4% (106/256), respectively.

DISCUSSION

This study has the feature of being the first study in our region to determine whether male patients are followed in accordance with prostate cancer guidelines. As a result of the study, it was determined that male patients were not followed up by the rules specified in the guidelines. Accordingly, the diagnosis of cancer was delayed. This situation may cause a delay in treatments, sometimes loss of the chance of treatment, worsening prognosis, and fatal complications.

Prostate cancer is the most common malignancy in men and the second most common cause of cancer-related mortality (8). It is detected with a frequency of roughly 15-20% in autopsy studies. In a systematic review on this subject, the incidence of incidental PCa was 5% in men aged <30 years. The frequency increased 1.7 times per decade of life, reaching 59% after age 79 (9).

We have treatment options with a high chance of success in early-stage PCa. During

this stage, patients who reach appropriate treatment have a cancer-specific survival of close to 99%. Life expectancy is measured in months in patients diagnosed at an advanced stage. A significant portion of the cases (20%) present with advanced stage or metastasis at the time of diagnosis. That is, they are diagnosed late (10). Furthermore, recurrence develops in about one-third of cases with definitive treatment in the early stages (such as radical prostatectomy or radiotherapy) (11). Early detection of these patients and access to appropriate treatment are essential for survival.

In a study on this subject, patients with PCa were randomized to active treatment (RP) and Watchful Waiting arms and followed for 23.6 years. As a result of the study, RP provided superior CSS (cancer-specific survival), OS (overall survival), and PFS (progression-free survival) to the follow-up group. The 10-year CSS rates were reported as 99%, especially in patients with early diagnosis and good tumor characteristics (12). As seen in this study, delay potentially carries adverse risk factors for the patient. Metastasis and associated adverse events increase in patients with delayed or advanced stages. In a study, in patients diagnosed at a late stage, the mean survival was reported as 42 months despite all efforts (drug therapy) (13).

All efforts are directed towards early diagnosis, as there is currently no recommendation or medicine to protect men from this disease worldwide. In this regard, international urology associations have assembled and prepared a standard guideline for diagnosing and treating PCa. Prostate cancer panels; consist of an international multidisciplinary team that includes urologists, radiation oncologists, medical oncologists, radiologists, a pathologist, a geriatrician, and a patient representative. This panel meets annually, reviews recently published research and prepares a guide for professionals working on the issue. EAU guidelines, which are among them, are widely used in our country. We also follow this guide in our clinic. These guidelines ask men to be screened for prostate cancer after a certain age. With these scans, it is aimed to reduce the death associated with PCa, as well as to protect the patient`s quality of life associated with the disease. The guidelines indicate that men aged >50 years with a life expectancy of more than 10-15 years, men aged >45 years with a family history of PCa, and men of African descent are at risk for the disease. Therefore, these groups should be screened (14,15). In most patients, 10-15 years are required for the lethal effects of cancer. For this reason, PCa screenings should not be performed on every patient. In the meantime, the patient should be informed about the screening, and his consent should be obtained (16). This is because some patients do not accept this screening or subsequent procedures. It is essential to distinguish these

patients from the beginning and to protect them from unnecessary processes. Our study results determined that the rate of informing patients about the process (for example, during PSA requests) was <1%. This can cause significant legal problems.

We have highly effective and easily accessible markers for prostate cancer screening. PSA had discovered during forensic studies, and it revolutionized the diagnosis of PCa. It began widely used worldwide in the late 1980s and early 1990s. PCa, which was previously detected incidentally only during surgery for prostate enlargement (TURP), is now being understood with simple tests. While the number of prostate cancers diagnosed during TURP decreased with the use of PSA, the number of new patients increased. For example, between 1986 and 1992, the overall number of prostate cancer doubled in the USA. The widespread use of this marker has brought some negative aspects, such as the increase in the number of cancer patients. It was associated with PCa in patients who died from other causes. Despite all the negativities, PSA caused a significant change in survival. In a study conducted on this subject, the effect of PSA screening on mortality in the USA was observed and compared to the period 1950-1970, which was the pre-PSA period. Mortality was reduced by 37% (17). The cancer screenings have reduced metastatic disease incidence (approximately 28 cases to 11 cases per 100000 men). It was more effective than screening tests such as mammography, which is used for breast cancer screening. Despite the widespread use of mammography, it did not decrease (18). In summary, the literature shows that the discovery of PSA has made an essential shift in the diagnosis, treatment, and follow-up of PCa. It became an example of new markers to be used in cancer screening.

Considering the results of our study, the median age at diagnosis of PCa was 67.4 years. Also, the average PSA value was 64.9ng/ml. Considering that the critical PSA value is 2.5-3ng/ml and the screening age is 45-50 in the EAU guidelines, it is understood that our patient group was not followed according to the recommendations specified in the guidelines. This means a later diagnosis age and cancer diagnosis at a more advanced stage. Especially when the age of 60 was taken as the limit, it was observed that 79.3% of the patients in our group were diagnosed after 60. This delayed age is a significant problem not only for our country but also for the whole world (19). These patients could have been diagnosed earlier if the guidelines had been appropriately applied. This delay resulted in 21.9% (59/270) patients being diagnosed in the metastatic stage, as seen in the results. The EAU guidelines also support this by predicting biochemical recurrence after treatment (7). According to this classification, 35.4% of

patients will experience biochemical recurrence despite any treatment. Another striking problem in our study was the irregular follow-up of the patients and the lack of regular follow-up of a urology doctor. These unnecessary PSA requests potentially carry serious problems, such as missed follow-ups of risky patients. A previous study we conducted on this subject supports these results (20).

As the results of our study show, PSA levels were not checked in the majority of patients in the age range specified in prostate cancer guidelines. As a result, the patient's diagnosis was delayed. Among the reasons for this, there may be many reasons such as patient-related factors, intense working conditions in our country, ignorance and lack of education on this subject, and insufficient preventive measures in primary care. Whatever the reason, it cannot explain the late diagnosis of these patients and the risks of losing the chance of treatment. We think it is crucial for public health that the guidelines, where everything is clearly defined, are widely used in our country and that the follow-up is strictly regulated. It should be kept in mind that this disease has a severe burden, such as job loss, mental health problems, deterioration in the quality of life, and financial loss. As it is known, the most important tool in the treatment of cancers is early diagnosis and treatment. For this reason, it is vital to inform the public about these screening programs and raise awareness among health professionals and the media.

This study has some limitations. Among these, the unknown treatment outcome and follow-ups, the results from a single center, and the study's retrospective nature are the first ones that come to mind. In addition, our sample size was low in our study. However, despite the limitations of the study, we think that this study is important because it is the first study to our knowledge that examines whether patients diagnosed with PCa are followed in accordance with the guidelines and the results of the study are remarkable.

We think multicenter, prospective studies with more patients are needed on this subject. In future studies on this subject, it may be helpful to compare the survival and quality of life results of patients diagnosed randomly, which was missing in our study, and those diagnosed according to the guidelines.

CONCLUSIONS

Early prostate cancer diagnosis, an important public health issue in aging men, is vital for the treatment process. The use of prostate cancer guidelines enables early detection of patients with PCa. Therefore, it can positively affect patients' survival and quality of life. Our study found that men were not followed up according to the UAE PCa guidelines. It was observed that the patients'

diagnoses shifted to older age, and some patients lost the chance of curative treatment. We think it is essential to inform health professionals and men about this issue at specific intervals through the media and keep this disease in mind.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of Ordu University (2022/09-214).

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Efficacy and Safety of Tofacitinib in Patients with Rheumatoid Arthritis

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Abstract

Objective: The aim of our study is to show the efficacy and side effects of tofacitinib in patients with rheumatoid arthritis (RA).

Methods: 66 Patients who were followed up in the rheumatology outpatient clinic, were older than 18 years, and used tofacitinib for at least three months were included. Blood count, liver transaminase levels, cholesterol and triglyceride levels, C-reactive protein (CRP) levels, and erythrocyte sedimentation rate (ESR) were determined before and at the third and sixth months of the tofacitinib treatment. Before and after treatment, DAS 28-ESR, morning stiffness duration, and VAS score were also calculated

Results: The mean age was 54.7±12.0 years, and 84.8% were women. The mean duration of tofacitinib use was 19.0±13.5 months. Duration of morning stiffness, VAS and DAS 28-ESR scores decreased significantly after tofacitinib ($p<0.001$). The leukocyte count after treatment also decreased significantly compared to before treatment. Side effects related to tofacitinib were seen in 33.3% of the patients. Rash, cough, and nausea were the most common side effects. Tofacitinib-associated Herpes Zoster infections were seen in 13.6% of the patients. Tofacitinib treatment was discontinued in 48.5% of patients due to adverse effects, drug ineffectiveness, and disease activation.

Conclusion: There was statistically significant decrease in RA disease activity with tofacitinib treatment. It was noteworthy that 33.3% of the patients developed adverse effects and 48.5% developed a condition requiring discontinuation of tofacitinib treatment.

Keywords: Rheumatoid Arthritis, tofacitinib, efficacy

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INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic and inflammatory autoimmune disease characterized by erosive synovitis and causes progressive damage to cartilage and bone (1). The interaction between the innate immune system and the acquired immune cells (CD4+ T and B lymphocytes) and the resulting cytokines and autoantibodies play a fundamental role in the pathogenesis of the disease (2). Synovitis, which occurs as a result of synovial intimal cell hyperplasia and microvascular damage, forms the basis of the disease (3). Rheumatoid arthritis can be seen in all populations, and its prevalence is approximately 1-2%. Although its incidence increases with advanced age and female gender, it can be seen at any age. The onset of the disease peaks at the age of 4-5 decades (4).

The goals of treating rheumatoid arthritis are to suppress inflammation and prevent joint damage and extra-articular involvement. Analgesic agents, glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs), and immunosuppressive agents are used in the pharmacological treatment of RA. Disease-modifying anti-rheumatic drugs (DMARDs) are divided into synthetic or biologic DMARDs. Synthetic DMARDs consist of two groups: conventional synthetic (cs) DMARDs (Leflunomide, Sulfasalazine, Methotrexate, Hydroxychloroquine) and targeted synthetic (Tofacitinib, Baricitinib). Biological DMARDs consist of original biological (Tumor Necrosis Factor-Alpha blockers) and biosimilar agents(5).

Tofacitinib is a Janus tyrosine kinase (JAK) inhibitor and is in the targeted synthetic DMARD group. Tofacitinib exerts its efficacy by inhibiting JAK1 and JAK3 and, to a lesser extent, JAK2. As a result of Janus tyrosine kinase inhibition, cytokine signaling is inhibited, and a cytokine-dependent immune response is suppressed(6). Tofacitinib was licensed in the United States on 6 November 2012 to be used with moderate and active RA patients who have an inadequate response or intolerance to methotrexate(MTX). Phase III studies have shown that Tofacitinib 5mg and 10mg orally twice daily can be safely used as monotherapy or in combination with MTX and other non-biological DMARDs (7).

Our aim in this study is to evaluate the effect of tofacitinib on RA disease activity and the adverse effects of the drug.

METHODS

In the present study, those patients who were followed up in Ankara Yıldırım Beyazıt University- Ankara City Hospital Rheumatology Clinic had RA in accordance with the 2010 American Rheumatology Society (ACR) classification criteria and were diagnosed with RA and used tofacitinib at any period of their medical treatment, even if tofacitinib treatment was discontinued afterward were included. The group consists of

66 patients between the ages of 25 and 78 who used tofacitinib 5 mg twice a day for at least three months or were still using tofacitinib. Each patient's age, age at diagnosis, gender, baseline rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (anti-CCP), anti-nuclear antibody (ANA) values, and comorbidities were determined. Before tofacitinib treatment, the duration of morning stiffness, Disease Activity Score (DAS 28-ESR), Visual Analogue Scale (VAS) score, hemoglobin level, leukocyte count, platelet count, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride level, aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, erythrocyte sedimentation rate (ESR) and CRP levels were noted.

According to The European League Against Rheumatism (EULAR), disease activity and treatment response criteria are based on disease activity scores (DAS). The DAS28 assesses swelling and tenderness in 28 joints. A patient global health assessment is performed. In addition, the ESR or CRP value is measured. Calculating the DAS score is a complicated process that requires the help of a calculator or computer:

$$DAS28(ESR)=0.56\times\sqrt{(TJC28)}+0.28\sqrt{(SJC28)}+0.70\times\ln(ESR)+0.014\times GH(range,0–9)$$

EULAR (The European League Against Rheumatism) disease remission criteria were used for disease activity score (DAS 28-ESR). According to EULAR disease remission criteria, a DAS 28-ESR score below 2.6 was accepted as remission, 3.2-5.1 as moderate disease activity, and above 5.1 as high disease activity.In patients who used tofacitinib treatment for 3-6 months, baseline and third-month values were compared. In patients using tofacitinib for longer than six months, baseline values were compared with the third and sixth-month values. In our study, DAS 28-ESR, VAS score, and duration of morning stiffness were used to evaluate disease activity.

A visual analog scale (VAS) score was used to assess the patient's pain. DAS 28-ESR, VAS score, and duration of morning stiffness were calculated and compared before and after tofacitinib use. Statistically, significant decreases were evaluated as clinical response and increase as clinical non-response-disease activity. Adverse effects during tofacitinib use (such as rash, cough, nausea, leukopenia, pneumonia, genito-urinary infection, Cytomegalovirus (CMV) retinitis, deep vein thrombosis, right heart failure-lung edema, acute coronary syndrome), and additional diseases that developed during tofacitinib use were investigated. It was examined how many patients discontinued tofacitinib treatment due to adverse effects and which treatment was started afterward. In addition, it was investigated how many patients developed

herpes zoster and herpes simplex infections with the use of tofacitinib.

STATISTICAL ANALYSIS

Research data was evaluated by means of "SPSS (Statistical Package for Social Sciences) for Windows 22.0 (SPSS Inc, Chicago, IL)". Descriptive statistics were presented as mean±standard deviation (minimum-maximum), percentage, and frequency distribution. The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk Test). Wilcoxon Signed Ranks Test was used as a statistical method for the variables that were not found to fit the normal distribution, for statistical significance between two dependent groups, and the Friedman Test was used as a statistical method between three dependent groups. The statistical significance level was accepted as $p<0.05$.

RESULTS

A total of 66 patients who were diagnosed with RA and were given tofacitinib for three months or longer were analyzed retrospectively. The clinical and descriptive characteristics of the patients examined are presented in Table 1. The additional disease was present in 69.7% of the patients (46 patients). A total of 83 additional diseases were reported in these 46 patients. The most common comorbidity was essential hypertension, which was present in 31.8% of the patients.

Table 1. Dermographic and Clinical Characteristics of Patients with RA

	(n=66)
Age, mean±SD	54.7±12.0
Gender, n (%)	
Female	56 (84.8)
Male	10 (15.2)
Complaint Onset Age, mean±SD	42.9±11.8
Diagnosis Age, mean±SD	45.0±12.1
Time to Diagnosis, mean±SD (min-max)	2.1±3.2
ANA	
Negative	39 (59.1)
+	26 (39.4)
++	0
+++	1 (1,5)
RF n (%)	32 (48.5)
Anti-CCP Positivity, n (%)	32 (48.5)

n: Number of patients; %: Percent; SD: Standard deviation; ANA: Anti-nuclear antibody Anti-CCP: Anti cyclic citrulline protein RA: Rheumatoid Arthritis, RF: Rheumatoid factor, min: minimum, max:maximum

This was followed by diabetes mellitus at 30.3% and allergic asthma at 12.1%.

In our study, 62.1% of patients had previously used csDMARD, 37.9% had used a biologic or biosimilar DMARD in combination with csDMARD (21.2% had one, 9.1% had two, 4.5% had three, and 3.0% had four different DMARD use). When the biological biosimilar agents used before tofacitinib are examined, Etanercept was in the first place with 23.2%, followed by Abatacept with 18.6% and Tocilizumab with 10.6%. Infliximab was found to be the least used biological agent, with 3.0%. The mean duration of using tofacitinib was 19.0±13.5 (min:3-max:50) months. When the treatment regimens were examined, it was observed that 80.3% of the patients received tofacitinib in combination with at least one csDMARD and the remainder as monotherapy.

Among the combined therapy protocols, the most commonly used combination was MTX, Prednisolone, and Tofacitinib, with 15.2%, followed by Leflunomide and Tofacitinib with 12.1%. The number of patients using the combination of Tofacitinib and MTX and the combination of prednisone and tofacitinib was equal (10.6%)

The duration of using tofacitinib was 19.0±13.5 (min:3-max:50) months. When the treatment regimens were examined, it was observed that 80.3% of the patients received tofacitinib in combination with at least one csDMARD and the remainder as monotherapy.

Before and after tofacitinib treatment, DAS 28-ESR, VAS score, and duration of morning stiffness were compared, and a statistically significant difference was found for all three ($p<0.001$). The duration of morning stiffness, VAS scores, and DAS 28-ESR were significantly reduced after tofacitinib treatment (Table 2).

Table 2. Comparison of the duration of morning stiffness, DAS28 -ESR and VAS values before and after Tofacitinib treatment

(n=66)	**Before Tofacitinib Treatment** **mean±SD** (min-max)	**After Tofacitinib Treatment** **mean±SD** (min-max)	**p[1]**
Morning Stiffness (minute)	53.1±35.7 (0-120)	31.1±27.4 (0-120)	**<0,001****
DAS28-ESR	5.4±0.6 (4.2-6.7)	4.3±1.3 (2.0-7.0)	**<0,001****
VAS	72.0±14.4 (20-90)	53.9±22.7 (10-90)	**<0,001****

n: Number of patients; %: Percent; SD: Standard deviation min: minimum max :maximum VAS: visual analog scale, DAS28-ESR: disease activity scores- Erythrocyte Sedimentation Rate

ESR, CRP, leukocyte count, hemoglobin platelet, AST and ALT values were obtained in 57 of 66 patients examined before and after treatment at 3 and 6 months. Total cholesterol, HDL, LDL and triglyceride values of 24 patients were present at all three times. The leukocyte count value in the sixth month after the treatment was significantly lower than before the treatment. On the other hand, the changes in hemoglobin level, platelet count, AST, ALT, ESR, CRP, total cholesterol, HDL, LDL, and triglyceride values between the measurement times were not statistically significant ($p>0.05$) (Table 3). It was observed that 33.3% of the patients examined developed adverse effects related to tofacitinib. A total of 27 adverse events were reported. Rash was observed in 6% of the patients, cough in 4.5%, and nausea in 3%. During tofacitinib treatment, 28.8% of the patients developed Herpes Simplex and 13.6% of them developed Herpes Zoster infection. None of the patients had a previous vaccination for Herpes Simplex and Herpes Zoster infection (Table 4).

Tofacitinib treatment was discontinued in 48.5% of the patients. Treatment was discontinued in 16.7% of patients due to clinical unresponsiveness, in 12.1% of disease activation, 3% of leukopenia, and 3% of acute coronary syndrome (Table 5).

Table 3. Comparison of Laboratory Values Before and After Tofacitinib Treatment

	n	**Before Tofacitinib Treatment mean±SD** (min-max)	**3th Month of Tofacitinib Treatment mean±SD** (min-max)	**6th Month of Tofacitinib Treatment mean±SD** (min-max)	**p**[1]
Esr	57	39.0±25.2 (3-110)	38.8±22.5 (1-90)	36.7±22.8 (3-98)	0.724
Crp	57	18.4±18.7 (2.6-83.4)	14.3±17.0 (2.0-93.0)	17.2±18.7 (1.2-83.7)	0.247
Leukocyte Count	57	8.4±2.8 (3.3-15.8)[c]	8.3±2.5 (4.4-16.6)	7.5±2.5 (2.6-17.1)	**0.022***
Hemoglobın	57	12.3±1.9 (7.8-16.9)	12.3±1.6 (9.2-16.0)	12.1±1.8 (8.0-16.5)	0.296
Platelets	57	291.6±71.6 (185-534)	295.8±69.2 (168-480)	297.5±79.5 (163-569)	0.612
Ast	57	19.2±10.1 (8-68)	20.1±7.9 (11-47)	19.1±7.0 (10-44)	0.995
Alt	57	20.4±12.8 (5-82)	20.6±12.7 (7-87)	19.5±13.7 (7-87)	0.162
Total Cholesterol	24	199.2±57.9 (113-369)	210.6±50.5 (114-295)	226.7±68.3 (144-419)	0.124
Hdl	24	54.6±21.6 (29-138)	51.7±12.2 (33.4-76.0)	66.0±28.6 (38.7-175)	0.149
Ldl	24	119.9±54.3 (37-296)	125.5±49.8 (12.5-204)	138.0±68.8 (24-332)	0.137
Trıglycerıde	24	133.4±50.2 (81-262)	149.9±82.2 (68-459)	132.7±48.0 (72-267)	0.545

n: Number of patients; %: Percent; SD: Standard deviation; [1]Friedman Test; ESR: Erythrocyte Sedimentation Rate CRP: C-Reactive Protein, AST: aspartate amino transferase, ALT : alanine amino transferase, HDL: high-density lipoprotein, LDL:), low-density lipoprotein

Table 4. Adverse event rates were seen with Tofacitinib treatment

(n=66)	**n (%)**
Adverse Event Development	
No	44 (66,7)
Yes	22 (33,3)
Adverse Events	**n %**
Skin Rash	4(6,0)
Cough	3(4,5)
Nausea	2(3,0)
Acute Coronary Syndrome	2(3,0)
Leukopenia	2(3,0)
Discoid Lupus Erythematosus	1(1,5)
CMV Retinitis	1(1,5)
DVT	1(1,5)
Increase In The Number Of Rheumatoid Nodules	1(1,5)
Weakness	1(1,5)
Transaminase Elevation	1(1,5)
Anemia	1(1,5)
Pulmonary Thromboembolism	1(1,5)
Right Heart Failure And Pulmonary Edema	1(1,5)
Creatine Kinase Elevation	1(1,5)
Malignancy	1(1.5)
Pneumonia	1(1.5)
Proteinuria	1(1.5)
Genito-Urinary İnfection	1(1.5)

Table 5. Reasons for Discontinuation of Tofacitinib

(n=66)	**n (%)**
Tofacitinib Treatment	66 (100)
Continued	36 (51.5)
Discontinued	32 (48.5)
Conditions Leading to Treatment Discontinuation	
Clinical Unresponsiveness	11(16.7)
Disease Activation	8(12.1)
Leukopenia	2(3)
Myocardial Infarction	2(3)
Discoid Lupus Erythematosus	1(1.5)
Malignancy	1(1.5)
With Own Request	1(1.5)
Anemia	1(1.5)
Creatine Kinase Elevation	1(1.5)
Pneumonia	1(1.5)
Increased Number of Rheumatoid Nodules	1(1.5)
Heart Failure	1(1.5)
Proteinuria	1(1.5)

n: Number of patients; %: Percent;

DISCUSSION

In this study, we aimed to show the effect of Tofacitinib on RA patients and the adverse effects that may occur due to its use. In our study, significant improvement was observed in VAS score, DAS28-ESR, and duration of morning stiffness after tofacitinib use. These decreases in disease activity indices are an indication of the efficacy of tofacitinib on the disease, but adverse events developed in 33.3% of the patients and the treatment had to be discontinued in 48.5%.

In The European League Against Rheumatism (EULAR) guideline updated in 2021, it is recommended to start treatment with conventional synthetic DMARDs, and to use a biological/biosimilar DMARD or a targeted synthetic DMARD in patients who do not respond to conventional synthetic DMARDs (8).

A better understanding of the pathogenesis of RA; with a clearer awareness of the effects of cytokines, chemokines, and lymphocytes on joint damage, tofacitinib has taken its place in the guidelines as a targeted synthetic DMARD (9).

In our study, the duration of morning stiffness, which is one of the parameters for evaluating the clinical efficacy of tofacitinib treatment and a subjective measurement, was found to be decreased in 68.2% of the patients. While the mean duration of morning stiffness was 53.1±35.7 minutes before treatment, it decreased to 31.1±27.4 minutes after tofacitinib.

Among other parameters used to evaluate the efficacy, a mean decrease of 20.3% in the DAS 28-ESR score and 25.1% in the VAS-patient score was found. Similar to our study, in the Phase III study of Hall et al., a significant decrease was found in the first and third month DAS28-ESR scores of the RA patients who received tofacitinib compared to the placebo group. When tofacitinib was added to the placebo group in the 3rd month and re-evaluated in the 12th month, a significant decrease was observed in the DAS28-ESR scores (10). In the study of Mueller et al., similar to our study, after tofacitinib use, 53% of patients had low disease activity and 48% had remission according to the DAS28-ESR score (11). In the retrospective study of Bird et al. on patients with RA, 1300 patients using biological DMARDs and 650 patients using tofacitinib were compared. At the 18th month of treatment, 52.4% of the patients using biological DMARDs and 57.8% of the patients using Tofasitinib achieved remission in the DAS28-ESR score, and there was no statistically significant difference between the two groups in terms of remission (12).

In our study, there was a higher decrease in the duration of morning stiffness, VAS and DAS28-ESR scores of patients who did not receive a biological-biosimilar DMARD before tofacitinib; Patients who received one or more

biologic DMARDs prior to tofacitinib had a lesser reduction in these parameters. Patients who have not previously achieved remission with biologic DMARDs may have had a lower response to tofacitinib, possibly due to greater disease severity and drug resistance.

In our study, no significant change was found in hemoglobin and thrombocyte values after treatment compared to pre-treatment. There was a significant decrease in the leukocyte values in the sixth month compared to the pre-treatment level. This decrease may be an indicator of a reduction in inflammation associated with tofacitinib use. When we evaluated the other laboratory values, there were not any significant changes in liver transaminase (AST-ALT) and lipid levels. In the study of Wollenhaupt et al. 4481 RA patients using tofacitinib were retrospectively analyzed. In the follow-up of the patients, no significant changes were detected in total cholesterol, LDL, ALT, AST and serum creatinine values. During follow-up, serum creatinine phosphokinase increased by 7.6%, ALT by 4.1%, and creatinine in 3.9% of the patients(13).

In some studies in the literature, many adverse effects related to tofacitinib have been shown. These adverse effects include non-serious drug reactions (such as rash, nausea, and cough), infections due to opportunistic microorganisms, cardiovascular adverse events, thromboembolic events, and malignancies. In our study, adverse effects developed in 33.3% (22 patients) of the patients. The most common adverse effect is rash at 6%; cough at 4.5%, nausea at 3%, fatigue at 1.5%, and genito-urinary system infection at 1.5%. In a long-term study (0-9.6 years) conducted by Cohen et al. on 7061 patients receiving tofacitinib with the diagnosis of rheumatoid arthritis, the most common adverse event in patients was infections. In the study, viral upper respiratory tract infection 17.3% (1221/7061), upper respiratory tract infection 17.2% (1214/7061), urinary tract infection 11.8% (832/7061), and bronchitis 11.3% (800/7061) were detected. Serious infection events were observed in 8.2% of the patients. These included pneumonia, urinary system infection, cellulitis and herpes zoster infection(14).

Like other immunomodulatory drugs used in the treatment of RA, opportunistic infections may develop in patients using tofacitinib. In addition, the patient's age, accompanying chronic diseases and other drugs used also play a role in the risk of infection. In our study, Herpes Simplex infection developed in 28.8% of the patients, Herpes Zoster infection developed in 13.6%. In the study of Winthrop et al., 5% (239) of 4789 RA patients developed tofacitinib-associated Herpes Zoster infection, and tofacitinib treatment was discontinued in 10% of these patients(15). In the study of Curtis et al., in 8030 (83.3% women) RA patients

receiving tofacitinib, age, female gender, and concomitant use of glucocorticoids with tofacitinib have been shown to increase the risk of Herpes Zoster infection (16). In our study, the mean age of patients with Herpes Zoster infection was 50.1 years, and 7 of these patients (77.7%) were women. Five of these patients were receiving tofacitinib and prednisolone treatment together. In addition, the remaining four patients had diabetes mellitus in one, major depression in one, and hypertension in one.

In our study, cardiovascular adverse events related to tofacitinib were remarkable. Cardiovascular adverse effects developed in three (4.5%) patients. Two patients developed acute coronary syndrome and one developed pulmonary edema due to right heart failure. In a study by Kivitz et al., cardiovascular adverse effects developed in 0.6% (15) of 2435 patients with a diagnosis of RA who received a combination of tofacitinib and csDMARD and in 0.59% of 1365 patients who received tofacitinib as monotherapy (17).

In the study of Ytterberg et al., patients using tofacitinib had a significantly higher rate of major adverse cardiovascular events (MACE) compared to patients using TNF inhibitors. The most common causes of MACE were nonfatal myocardial infarction with tofacitinib. The cumulative estimated probability of MACE with combined doses of tofacitinib was 5.8% and the cumulative predictive probability of nonfatal myocardial infarction was 2.2% (18).

The thromboembolic event rate in our study was 3%. In our study, pulmonary thromboembolism (PTE) developed in one patient and deep vein thrombosis (DVT) in one patient. In the study of Cohen et al., venous thromboembolism (DVT and/or PTE) was seen in 0.8% of 7061 patients (14). In the study of Mease et al., in the first three months of follow-up of 7964 rheumatoid arthritis patients who received tofacitinib, there was no significant difference in the development of DVT and PTE compared to the placebo control group. As a result of 24-month follow-up, no significant difference was found between Tofacitinib, adalimumab, and methotrexate in terms of the development of DVT and PTE (19). Desai et al. compared the risk of thromboembolism in RA patients receiving tofacitinib and anti-TNFα therapy and found no significant difference in thromboembolism risk between those receiving tofacitinib and anti-TNF-α agents (20).

One patient in our study developed endometrial adenocarcinoma during tofacitinib use and tofacitinib treatment had to be discontinued. The patient who developed endometrial adenocarcinoma was 60 years old and had been receiving tofacitinib treatment for four months. For this reason, no clear relationship was established between tofacitinib and the development of endometrial adenocarcinoma.

Tofacitinib treatment had to be discontinued in 45.5% (30 patients) of the patients.

Tofacitinib treatment was discontinued in 16.7% of patients due to clinical unresponsiveness and in 12.1% due to disease activation. The mean age of 19 patients whose treatment was discontinued due to clinical unresponsiveness and disease activation was 56.3 years. The mean age at diagnosis of these patients was 44.2. Of the 19 patients whose treatment was discontinued, 18 were women and 15 patients had comorbidities. In addition, these patients had not previously been in remission with a variety of conventional synthetic DMARDs, and ten patients had previously used one or more biological/biosimilar DMARDs. Age, age at diagnosis, female gender, comorbidity and unresponsiveness to DMARD therapy are poor prognostic factors for RA, and these patients may have a resistance not only to tofacitinib but also to other drugs.

Limitations of our study are firstly it is retrospective; secondly the number of patients is small and thirdly, the follow-up period is short. It is possible to reach more precise information with a higher number of RA patients and longer-term follow-up studies.

CONCLUSION

In our study, the efficacy, safety, and adverse effects of tofacitinib in Rheumatoid Arthritis were investigated. Tofacitinib, which is safe and effective in the treatment of RA, can be used orally and is preferred as a monotherapy, providing an advantage over other biologic agents. However, it is necessary to be careful in terms of adverse effects that may develop due to tofacitinib.

Ethics Committee Approval: Ethics committee approval was received for this study from local ethics committee at Yıldırım Beyazıt University with file number 2019/64

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Prognostic Value of Integrated Pulmonary Index (IPI) Value in Determining Pneumonia Severity in Patients Diagnosed with COVID-19 Pneumonia in the Emergency Department

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Abstract

Objective: This study aimed to examine the predictive power regarding prognosis of the Integrated Pulmonary Index (IPI) values measured at admission for patients diagnosed with COVID-19 pneumonia in the emergency department. In addition, the correlation between CURB-65 and PSI scores and IPI values in COVID-19 pneumonia was also examined.
Methods: The study was conducted between April 2020 and December 2020 as a prospective study. We evaluated respiratory function using IPI monitoring system that includes oxygen saturation, end-tidal CO2, respiratory rate, and pulse rate. For patients diagnosed with COVID-19 pneumonia in the emergency department, the IPI value was measured at the time of admission and PSI and CURB-65 scores were calculated. The predictive power of the IPI value in patients with clinical severity and the correlations between clinical severity and PSI, CURB-65 and IPI scores were examined. All of the data that was obtained during the study was recorded in the study form and evaluated using the IBM SPSS 22.0 statistical program in which P <0.05 was considered to be statistically significant.
Results: A total of 81 patients were included in the study. When the severity of pneumonia was compared with the CURB-65, PSI and IPI values, a statistically significant difference was found between the clinical severity groups for all scores (p<0.001 for each score). Although the correlation between clinical severity, CURB-65 and PSI scores was positive and moderate (r:0.556 and r:0.613, respectively), the correlation between clinical severity and IPI value was found to be inverse and strong (r:0.824). While the IPI value was green, the sensitivity to predict mild pneumonia was 94.92%, and the specificity was 54.55%.
Conclusion: Although all of the scores showed a significant correlation with clinical severity in patients with COVID-19 pneumonia, this correlation was moderate in PSI and CURB-65 scores, while there was a strong inverse correlation between IPI value and clinical severity. Considering the ease of use of the IPI value and its correlation with the clinic, we believe that it is more successful than CURB-65 and PSI scores in predicting clinical severity in patients with COVID-19 pneumonia.
Key words: COVID-19 pneumonia, integrated pulmonary index, pneumonia severity index, CURB-65

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INTRODUCTION

Scales such as the pneumonia severity index (PSI) and CURB-65 are used to determine the severity and necessary management of the disease in patients that were admitted with pneumonia to the emergency services. However, the predictive value of these scales for COVID-19 pneumonia is unknown.

The Integrated Pulmonary Index (IPI) is a newly-adapted monitor of measurement which can be used to evaluate ventilation and oxygenation in patients, and allows for quick evaluation of the respiratory status of the patient. Arterial oxygen saturation (SpO2), end-tidal carbon dioxide (PetCO2), respiratory rate (RR) and heart rate (HR) parameters are frequently used to evaluate respiratory functions in patients. IPI is created by combining SpO2, PetCO2, SS and HR values in a mathematical model and can provide the clinician with the opportunity to evaluate all these parameters through a single data form. This index value can be followed continuously on the monitor as digital data or as a waveform (1). Through a noninvasive, dynamic and real-time measurement, IPI monitoring provides guidance in assessing the patient's respiratory status swiftly and determining whether there is a need for intervention (1).

Health systems across the world have become overburdened as an increasing number of patients have been checking as a result of the COVID-19 pandemic (2). When it comes to clinical management, a measurement tool suitable for use in triage can be very valuable for emergency physicians in identifying patients with mild clinical severity COVID-19 pneumonia who can be treated as outpatients.

The aim of this study is to examine the predictive power regarding prognosis of the IPI values measured at admission for patients diagnosed with COVID-19 pneumonia in the emergency department. In addition, the correlation between the aforementioned pneumonia scores and IPI values in COVID-19 pneumonia was also investigated.

METHODS

This study is a prospective study. It was conducted following the approval of the local ethics committee between April 2020 and December 2020 in the emergency department of a training and research hospital.

Study population

Study population was defined with consecutive sampling. Patients over the age of 18 who came to the emergency department with complaints such as fever, cough, and shortness of breath and were subsequently diagnosed with COVID-19 pneumonia were included in the study. Patients whose information could not be accessed in the hospital registry system for any reason, who were under the age of 18, had a history of recent hospitalization (within the last 2 weeks), were pregnant, and/or refused to participate in the study were excluded.

Pneumonia diagnosis criteria were defined according to the current guidelines: the patient had at least one additional symptom of lower respiratory tract disease in addition to coughing (such as phlegm, dyspnea, pleuritic chest pain, tachypnea), new focal chest findings in physical examination (rale or bronchial breath sounds), at least one systemic finding (such as sweating, aching, chills, fever, body temperature above 38º) and these complaints could not be explained in any other way (3). The diagnosis of COVID-19 pneumonia was given by the presence of positive PCR (polymerase chain reaction) results and characteristic thoracic tomography findings in addition to the existing pneumonia definition criteria.

Patients were classified as mild, moderate, or severe according to local COVID-19 pneumonia management and treatment guidelines (4). Patients with symptoms such as fever, muscle/joint pain, cough and sore throat, whose respiratory rate is <30/minute, SpO2>90 in room air with mild-moderate pneumonia findings on chest X-ray or tomography are considered to have mild-moderate pneumonia. According to the guideline, those with a respiratory rate of <24/minute and SpO2≥94% in room air was in the mild pneumonia group, and it was suggested that they could be followed up on as outpatients provided that the involvement was below 50% in thorax tomography. Pneumonia patients with respiratory rate between 24-29/minute and SpO2 level between 91-93% in room air are categorized in the moderate group, and it is recommended that these patients be admitted and followed up in the service. Severe pneumonia patients have symptoms such as fever, muscle/joint pain, cough and sore throat, their respiratory rates are ≥30/minute, SpO2 level ≤90% in room air, and have bilateral pneumonia findings of more than 50% on chest X-ray or tomography. It is recommended that these patients be monitored in the intensive care unit (ICU).

Data collection

Demographic data, physical examination findings, comorbid diseases, vital signs, consciousness at the time of admission, laboratory and radiological imaging results, pneumonia severity and clinical outcomes of the patients included in the study were recorded in a form that was prepared in advance. PSI and CURB-65 scores were calculated for all patients.

IPI measurement was carried out simultaneously as the examination and treatment began, after detailed anamnesis and physical examination was done for patients who were thought to be pre-diagnosed with COVID-19 pneumonia. This measurement was conducted with a Microstream Bedside Capnography Monitoring® device with a probe attached to the index finger of the left hand and a nasal cannula (Oridion Filter Line®) to be used for etCO2 measurement. The probe was

attached for two minutes, and the value at the end of the second minute was taken as the measurement value. The IPI value is a scoring category which uses red, yellow, and green to classify the severity of the patients' respiratory status. If the IPI value is 1-4 it is classified as red, 5-7 as yellow, and 8-10 as green (1). As the IPI value decreases, the clinical severity of the patient increases and indicates the need for urgent intervention. Score definition was given in appendix.

Appendix 1: Numeric score definition of IPI

Ipi Score	Patient's Condition
10	Normal
8-9	Close to normal
7	Close to normal but should be monitored
5-6	Should be monitored, intervention may be required
3-4	Intervention is required
1-2	Immediate intervention is required

Outcomes

Primary outcome of this study was to examine predictive power of IPI value to severity of COVID-19 pneumonia. Secondary outcome was defined as correlation of IPI value with existing pneumonia severity scores in patients with COVID-19 pneumonia.

Statistical Analysis

Data analysis was performed using SPSS for Windows 22 package program (Chicago, Illinois, USA). After determining whether the data was normally distributed with the Kolmogorov-Smirnov test, all data was presented as mean±standard deviation or the median and the interquartile range (IQR 25%-75%). Categorical variables were evaluated with the Chi-square test and continuous variables were evaluated with the Kruskal Wallis test or Mann Whitney U test. The relationship between the PSI, CURB-65 scores and IPI values of the patients was examined with the Pearson or Spearman correlation test. IPI scores and congruence with clinical severity were evaluated with the diagnostic 2x2 table. The statistical significance level was accepted as $p<0.05$ for all calculations.

Power analysis

First this study was planned as a prospective study on patients with community acquired pneumonia and sample size had been calculated according to it. Later, because of the impact of COVID-19 pandemic, most of the patients were diagnosed as COVID-19 pneumonia and analyses done at this subgroup.

This studies post hoc power analyses were done by G* Power 3 1 9 7 program (5). With the data of the study with total sample size of 81, correlation for primary outcome of the study was calculated as 0.824. For correlation analyses with %5 type I error (alpha:0.05), power of the study was calculated as 0.99.

RESULTS

A total of 81 patients diagnosed with COVID-19 pneumonia were included in the study, and the mean age of the patients was 62±15 years. A total of 45 of the patients were women. According to the severity of COVID-

19 pneumonia, 22 of the patients were in the mild, 14 in the moderate and 45 in the severe pneumonia group. Demographic data, vital signs and radiological imaging findings of the patients are shown in Table 1.

Table 1. Demographic Characteristics of the Patients, Distribution of Pneumonia Severity, Vital findings and Radiological imaging findings (n=81)

Age (Years)		**62 ± 15**
Sex		
	Female	45
	Male	36
Pneumonia Severity category		
	Mild	22
	Moderate	14
	Severe	45
Vital findings		
	Systolic blood pressure (*mmHg)*	130 (IQR 115 – 140)
	Diastolic blood pressure (*mmHg*)	80 (IQR 66 – 83)
	Pulse *(/min)*	95 ± 14,5
	Temperature *(°C)*	38 (IQR 37,25 – 38,35)
	Respiratory Rate *(/min)*	31 ± 9,4
	SpO2 *(%)*	89 (IQR 84 – 94)
	End Tidal CO2	29 (IQR 23,5 – 32)
Radiological imaging findings		
	Lobar or Segmental	58
	Consolidation	80
	Ground-Glass	10
	Infiltration	7
	Pleural Effusion	5
	Air Bronchogram	

*Data are given as n, mean ± standard deviation or median (ınterquartile range %25 – %75).

When the severity of pneumonia was compared with the CURB-65, PSI and IPI values, a statistically significant difference was found between the clinical severity groups for all scores (p<0.001 for each score). When pairwise comparisons were made in terms of all scores, it was found that the statistical difference for CURB-65 was due to the difference between mild and severe pneumonia groups. There appeared to be no significant difference between mild-moderate and moderate-severe pneumonia groups in terms of CURB-65 score (p value <0.001, 0.06, 0.06, respectively). Although there was a noteworthy difference in all subgroup analyzes (mild-moderate, moderate-severe, mild-severe) in terms of PSI and IPI values, it was shown that this difference was more significant for the IPI value (p values of mild-moderate, moderate-severe and mild-severe groups for PSI were 0.045, 0.024, <0.00, respectively; and were 0.001, <0.001, <0.001 for IPI, respectively) (Table 2).

When the correlation between the scores was analyzed, a statistically significant correlation was found between all paired groups (CURB-65 and PSI, CURB-65 and IPI, PSI and IPI) (p<0.001, r:0.828, r:-0.720 and -0.748, respectively). While the strongest correlation was found between CURB-65 and PSI as a positive correlation, a strong-level inverse correlation was found between IPI and the other two scores. The correlation of CURB-65, PSI and IPI values with the clinical severity group was also examined in the patients, and a statistically significant correlation was found for all scores (p<0.001). While the correlation between clinical severity and CURB-65 and PSI scores was positive and moderate (r:0.556 and r:0.613, respectively); the correlation between clinical severity and IPI value was found to be inverse and strong (r:0.824) (Table 3).

The IPI value is categorized as red, yellow, green. It is classified as red if the IPI value is 1-4, yellow if 5-7, and green if 8-10. The prognostic value of the color categories in terms of clinical severity was also calculated. While the IPI value was green, the sensitivity to predict mild pneumonia was 94.92%, and the specificity was 54.55%. The positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were 2.09 and 0.09, respectively. When the IPI value was red, its sensitivity was 97.78% and its specificity was 83.33% in predicting severe pneumonia. PLR and NLR were 5.87 and 0.03, respectively (Table 4).

Table 2: Relationship between CURB-65, PSI and IPI scores with disease severity of patients

Scores	All of the patients n(81)	Mild pneumonia n(22)	Moderate pneumonia n(14)	Severe pneumonia n(45)	P value
CURB-65	1 (1 – 3)	1 (0 – 1)	1 (0,75 – 2,25)	2 (1 – 3)	**<0.001**
Mild to moderate					*0.06*
Moderate to severe					*0.06*
Mild to severe					*<0,001*
PSI	77 (56,5-108,5)	53 (38,75-62,25)	65,5 (48,75-118,5)	97 (78,5-123)	**<0,001**
Mild to moderate					*0.045*
Moderate to severe					*0.024*
Mild to severe					*<0,001*
IPI	3 (1 – 6)	8 (6 – 9)	5 (3,75 – 6,5)	1 (1 – 3)	**<0,001**
Mild to moderate					*0.001*
Moderate to severe					*<0,001*
Mild to severe					*<0,001*

*Data are given as median (ınterquartile range %25 – %75).Abbreviation: CURB-65: confusion, uremia, respiratory rate, blood pressure, age older than 6, PSI: Pneumonia severity index, IPI: ıntegrated pulmonary index

Table 3: Evaluation of the correlations of the used scores with each other and with the severity of the disease

	P value	R value
Correlation between score pairs		
• CURB-65 and PSI	<0,001	0.828
• CURB-65 and IPI	<0,001	-0.720
• PSI and IPI	<0,001	-0.748
Correlation between the scores and disease severity		
• Disease severity and CURB-65	<0,001	0.556
• Disease severity and PSI	<0,001	0.613
• Disease severity and IPI	<0,001	-0.824

Table 4: Prognostic value of IPI color

	Predictive value for mild pneumonia when IPI is green	Predictive value for severe pneumonia when IPI is red
Sensitivity (%) *%95CI**	%94,92 (%85,85- %98,94)	%97,78 (%88,23- %99,94)
Specificity (%) *%95CI*	%54,55 (%32,21- %75,61)	%83,33 (%67,19- %93,63)
PLR *%95CI*	2.09 (1,32- 3,32)	5.87 (2,82- 12,2)
NLR *%95CI*	0.09 (0,03- 0,29)	0.03 (0- 0,21)
Accuracy *%95CI*	%83,95 (%74,12- %91,17)	%91,36 (%83- %96,45)

DISCUSSION

There are two main results of the study in which the effectiveness of the IPI value measured at the time of admission in patients diagnosed with COVID-19 pneumonia in the emergency department in predicting the severity of pneumonia in the patient and the correlations of the IPI value with the PSI and CURB-65 scores were studied. First and foremost, it was found out that the IPI score had gradually decreasing values in patients with mild, moderate and severe pneumonia, and a statistically significant difference was found between these groups. This result means that by using the IPI score, it is possible to have an idea about the clinical severity of the patient in the early period. Thus, with the early detection of patients with mild pneumonia, unnecessary examinations and long stays in the emergency department can be avoided. Early detection of severe pneumonia patients, on the other hand, may improve patient management by giving the physician a chance to intervene earlier on behalf of these patients.

The second result is that a highly significant, inverse correlation was found between the IPI value and the PSI and CURB-65 scores. The reason why the correlation was found to be inverse is because in the IPI score worst prognosis is given to the lowest score, not the highest score, as it is in the other two types of scores. When the correlation of the clinical severity of the cases included in the study with the IPI value, CURB-65 and PSI scores was examined, a moderately significant positive correlation was found between CURB-65 and IPI scores and pneumonia severity, although a highly significant inverse correlation was found between IPI score and pneumonia severity. Due to this fact, it is thought that in COVID-19 pneumonia, the IPI value is a better indicator in determining mild, moderate and severe pneumonia in patients compared to other scores.

COVID-19 is a disease in which viral pneumonia is the most common organ involvement, and its clinical course is often mild to moderate (81%). However, it may be severe (14%) in some patients, and might even be so severe that intensive care follow-up is required (5%) (6). Mortality rate is very high in hospitalized patients, ranging from 11-28% (7). Therefore, a marker that can be used at the time of admission to predict patients prone quickly and easily to more severe cases provide physicians with an opportunity for early intervention and appropriate resource use.

In case of pneumonias, it is strongly recommended to routinely use structured clinical decision algorithms such as the pneumonia severity index (PSI) and CURB65 in determining the severity and management of the disease in patients that were admitted (8). These algorithms allow early identification of the severe patient group and prompt commencement of absolute treatment, as well as preventing unnecessary hospitalizations of low-risk patient groups and encouraging safe discharge. There are

publications suggesting that CURB-65 and PSI scores can be used to assess clinical severity and determine the risk of 14-day mortality in COVID-19 patients (9, 10). However, the accuracy of these scores in COVID-19 pneumonia has not yet been clearly demonstrated (11). Moreover, in the present study, the correlation between clinical severity and IPI were found to be much higher than the correlation between clinical severity and CURB-65 and PSI.

Another aspect that makes the use of the IPI value advantageous is that it provides an easy interpretation by reducing many vital parameters to a single value, which makes it possible to quickly evaluate the respiratory status of the patient in triage through a noninvasive and real-time measurement (1). The PSI score includes many parameters such as patient demographics, medical history, emergency department admission vitals, laboratory parameters and radiological findings; however, it takes time to make that calculation (12). Therefore, considering the patient load created by the pandemic, the nature of it limits its use by emergency physicians. The CURB-65 score is a more convenient score that assesses patients' age, level of consciousness, respiratory rate, systolic blood pressure, and blood urea levels (13). However, these scores do not seem to take hypoxemia into account, despite the fact that it is a parameter directly related to clinical severity. Aliberti S. et al. reported that 127 out of 218 patients with a CURB-65 score of 0-1 were hospitalized, and hypoxemia was shown as the biggest reason for hospitalization (14).

When the prognostic value of IPI color was examined, it was seen that while IPI was green, its sensitivity to predict mild pneumonia was 94.92%, its specificity was 54.55%, and the PLR (positive likelihood ratio) and NLR (negative likelihood ratio) values were 2.09 and 0.09, respectively. This result suggests that the IPI value may be a good triage score for early detection and rapid discharge from the emergency department of COVID-19 patients with mild pneumonia. When IPI was red, it had a sensitivity of 97.78%, a specificity of 83.33%, and a PLR value of 5.87 and an NLR value of 0.03 in detecting severe pneumonia. IPI is an algorithm calculated based on momentary vital signs, and baseline vital values may change after initial treatment in the emergency department. Therefore, the PLR value was low, as we predicted the outcome with a single IPI value measured at the beginning. The IPI value re-measured after emergency treatment may be more effective in predicting the outcome.

LİMİTATİONS

Due to the study protocol, the IPI value was only measured at the time of admission to the emergency department. Since IPI is a value that is affected by momentary vital parameters, changes in vital signs after primary care in the emergency department may increase the IPI value. Therefore, in order to better demonstrate

the power of IPI in predicting the final outcome, conducting another measurement after the first line treatment in the emergency department would be appropriate. Second important limitation of this study was determining COVID-19 pneumonia severity according to local guidelines. Since that was a new emerged disease, there was not any global recommendation and management of the patients were done with local guidelines at every country.

CONCLUSİON

It was shown that the IPI score in patients with COVID-19 pneumonia is significantly reduced in patient groups with mild, moderate and severe pneumonia. Although all of the scores showed a significant correlation with clinical severity, this correlation was moderate in PSI and CURB-65 scores, while there was a strong inverse correlation between IPI value and clinical severity. In addition, it was shown that the IPI value predicts mild pneumonia with high sensitivity and NLR in the green category, while it predicts severe pneumonia with high sensitivity and specificity in the red category. Considering the ease of use of the IPI value and its correlation with the clinic, we believe that it is more successful than CURB-65 and PSI scores in predicting clinical severity in patients with COVID-19 pneumonia.

Ethics Committee Approval: Ethics committee approval was received for this study from local ethics committee at Keçiören Training and Research Hospital in 2019, file number 2012-KAEK-15/2010.

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Investigation of the Effect of Bleaching on Discolored Teeth After Regenerative Treatment on Fracture Resistance of Teeth

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Abstract

Objective: This study aimed to evaluate the effect of bleaching discolored teeth after regenerative endodontic treatment on fracture resistance.

Methods: Forty human maxillary incisors were selected for the study and were divided into four study groups (n=10). Group 1 was called the positive control group, and no treatment was applied. In Group 2; root canals were enlarged to size 6 peezo reamer bur to mimic an immature tooth. The disinfection protocol was performed according to the regenerative endodontic treatment protocol of the American Association of Endodontists. 3 mm thick mineral trioxide aggregate (MTA) was placed at the cementoenamel margin and restored with a temporary restorative material. The samples in group 3 were restored with glass ionomer and composite resin. In Group 4 bleaching was applied with 35% hydrogen peroxide bleaching agent to the samples before restoration with glass ionomer and composite resin. Then each group was applied to the cervical fracture resistance test with a universal testing machine. The fracture resistance values were recorded in Newtons (N), and the obtained data were analyzed statistically.

Results: While the highest fracture strength was obtained in group 1 (1092 ±9.27N), the lowest fracture resistance was observed in group 2 (493.8 ±7.60N). The difference between all groups was statistically significant ($p<0.05$).

Conclusion: 35% hydrogen peroxide bleaching agent application after regenerative endodontic treatment reduces fracture resistance of the tooth.

Key Words: Fracture Resistance, Mineral Trioxide Aggregate, Regenerative Endodontics, Tooth Bleaching

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INTRODUCTION

Regenerative endodontic treatment is an exciting up-to-date treatment protocol of endodontics that provides revascularization that allows pulp-like tissue formation in both necrotic teeth with open apex and mature teeth with extensive endodontic lesions (1).

The foundations of regenerative treatment were first laid by Nygaard Ostyb with the demonstration of the formation of new vascularized tissue at the root tip of teeth, which have open apexes with periapical lesions (2). In this treatment protocol, which aims at regeneration, first of all, disinfection of the canal is provided, and then it is aimed to thicken the root dentin and close the apical opening by placing blood clots or progenitor cells that will initiate regeneration into the canal (3**)**. Despite all the positive aspects of the treatment, it is an undesirable result that the triple antibiotic paste (TAP) used in the disinfection phase causes discoloration on the crown (4**)**. It is known that the cause of the discoloration is the minocycline in the triple antibiotic paste (5). For this reason, in some studies, different antibiotic pastes that do not cause discoloration or treatment protocols that do not have a disinfection phase have been tried (6-8). However, it was reported that TAP is used in 51% of clinical cases (9).

On the other hand, mineral trioxide aggregate (MTA), also used as a coronal barrier in regenerative treatment, is also known to cause discoloration (3). This situation is often a concern for children or adolescent patients undergoing regenerative treatment and their families (10). Therefore, the American Association of Endodontists (AAE) recommends using low amounts of TAP and white MTA as a coronal barrier (11). Discoloration that occurs despite all precautions can be treated with intracoronal bleaching (12). Hydrogen peroxide (H_2O_2), often used in bleaching, is broken down into free radicals so that colored molecules break down into smaller colorless molecules. It is an agent with high oxidizing properties that provide bleaching (13). Unfortunately, in previous studies which examined the effects on the dentin surface, dentin fracture strength decreases, dentin stretching and harmful effects occur in shear forces, the calcium-phosphate ratio decreased, and cervical root it has been reported that the resorption (14-16). This situation is worrisome in terms of cervical resorption and fracture formation, especially in thin cervical dentin teeth with incomplete maturation (17). No study in the literature examined the effect of bleaching on teeth discolored due to regenerative endodontic treatment on cervical fracture formation. This in vitro study aimed to investigate the effect of bleaching on cervical fracture formation in teeth that underwent regenerative endodontic treatment protocols. The study's null hypothesis is that bleaching

will not affect cervical fracture resistance.

METHODS

The current research was confirmed by the ethics committee of Ordu University (2022/219). The sample size was calculated based on a power analysis using G*Power software version 3.1 (Universitat, Düsseldorf, Germany) at an alpha error probability of 0.05 and a power of 95% (effect size= 0.85). The power analysis showed that a totally 40 samples were required for groups. In the present study, 40 human mature maxillary incisors of similar dimensions extracted for periodontal or prosthetic reasons, without caries, previous canal treatment, restoration, and resorption were used. Non-probable sampling method was used in this study.

The periodontal tissues on the tooth surface were cleaned with the help of a periodontal curette. Periapical radiographs were taken from the teeth and evaluated for root canal calcification. Teeth showing calcification were excluded from the study. The teeth were evaluated for the presence of resorption fractures and cracks under a stereomicroscope (Leica SP1600; Leica, Wetzlar, Germany), and teeth with fractures, cracks, or resorption were excluded from the study. The root length of each tooth was standardized by cutting 10 mm from the apical to the cementoenamel margin.

Except for the positive control group (Group 1), the access cavity was opened for the remaining teeth. Preparation was made up to the number 70 k-file. Root canals were enlarged with from 1 to 6 number peezo reamer to mimic an incomplete maturation tooth (18). The canals were irrigated with 5ml of 1.5% sodium hypochlorite (NaOCl) during instrumentation. After the preparation was completed, the triple antibiotic paste, which equal portions of metronidazole (Eczacıbası, Istanbul, Turkey), ciprofloxacin (Biofarma, Istanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany) mixed with distilled water (a powder/liquid ratio of 1:1:1/1) and with the lentulo spiral was placed in the canals. The access cavities were sealed with temporary restorative material (Cavit G; 3M ESPE, Seefeld, Germany), and the samples were held for three weeks in 100% humidity and 37 C^0 temperature. After three weeks, the TAP was removed from the root canals with conventional syringe irrigation using 17% Ethylene Diamine Tetra Acetic Acid (EDTA).

The samples were randomly divided into four groups (n=10):

***Group 1*:** This group of samples was selected as the positive control group and no treatment was applied.

Group 2: Samples were selected as the negative control group. 3 mm thick WhiteProRoot®MTA (Dentsply Sirona, York, PA, USA) was placed at the cementoenamel junction of the teeth with a pluger. Temporary restorative material was placed on it, waiting

for one day to set.

Group 3: The samples were restored with glass ionomer cement (3M ESPE, Seefeld, Germany) and composite resin (3M Filtek Z250; 3M ESPE, Seefeld, Germany) without bleaching after 3 mm thick WhiteProRoot®MTA was placed at the cementoenamel junction of the teeth with a pluger.

Group 4: After the glass ionomer cement was placed on the WhiteProRoot®MTA which was placed at the cementoenamel junction of the teeth, 35% H_2O_2 (Opalescence®Endo; Ultradent Products Inc., USA) was placed in the pulp chamber. The access cavity was covered with cotton and temporary restorative material and left for three days. After three days, the bleaching agent was replaced with fresh H_2O_2, and at the end of the second session, sufficient visible bleaching was achieved. The temporary restorative material was removed, and the teeth were restored with composite resin.

To mimic the periodontal ligament, the root surfaces were covered with wax before all teeth were embedded in acrylic resin blocks 2 cm wide and 4 cm in length. Before the acrylic polymerization was completed, the tooths were removed from the blocks, the wax was cleaned, the root surfaces were covered with polyvinyl siloxane impression material (Elite HD; Zhermack, Italy), and the roots of the teeth were again embedded in the blocks. A distance of 2 mm was left between the cementoenamel junction and the acrylic block to simulate the relationship between the alveolar bone and tooth. The samples were placed on the tip of the universal testing machine (Autograph AGS X; Shimadzu Co, Japan.) with a diameter of 3 mm in such a way that the crowns were at an angle of 135^0 degrees to perform the fracture test (Fig. 1). To mimic crown trauma, a force of 1 mm/min was applied on the palatine of the teeth, 3 mm over the cementoenamel junction. When the teeth were broken, the force was recorded in newtons (N).

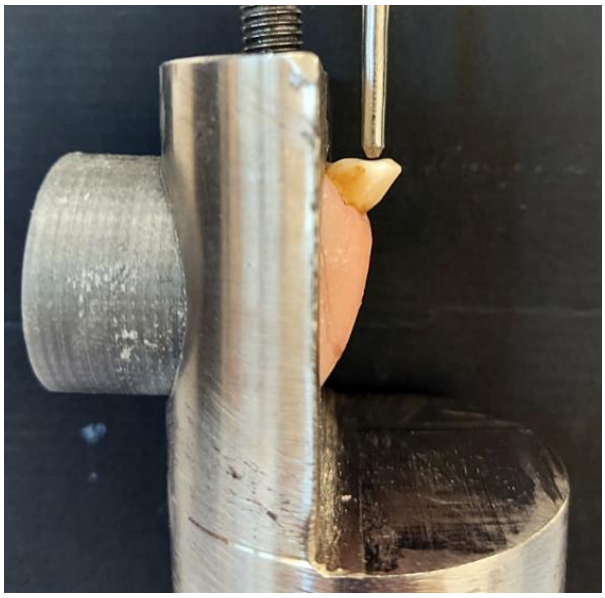

Figure 1: The samples were placed on the tip of the universal testing machine (Autograph AGS X; Shimadzu Co, Japan.)

The variables of the study are permanent restoration and bleaching application. The primary measurement of the study is the fracture resistance of teeth. According to the Shapiro-Wilk normality test, the groups'

fracture resistance values were normally distributed ($p > 0.05$). The fracture resistance results were analyzed by one-way analysis of variance. Finally, the mean values were compared using Tamhane's multiple comparison tests ($\alpha = 0.05$). All computational work was performed using SPSS statistical software (SPSS v18.0; SPSS Inc. Chicago, IL).

RESULTS

Fractures occurred in horizontal and vertical directions in all teeth. The results of the fracture test are shown in Table 1. The highest fracture resistance value was obtained in group 1 (1092 ±9.27 N), while the lowest fracture resistance value was obtained in group 2 (493.8 ±7.60 N). The fracture resistance value in group 4 with bleaching group (688.8 ±4.70 N) was lower than group 3 (887.8 ±4.46 N) without bleaching. The difference between all groups was statistically significant ($p<0.05$) (Table 1).

Table1. The mean and standard deviation (SD) of fracture resistance values (N) of test groups

GROUPS	N	Mean ± SD
Group 1	10	1092 ±9.27 [a]
Group 2	10	493.8 ±7.60 [d]
Group 3	10	887.8 ±4.46 [b]
Group 4	10	688.8 ±4.70 [c]

*Different superscripts mean statistically significant difference. Significant at $p<0.05$

DISCUSSION

Regenerative endodontic treatment protocol allows the apexification of a tooth with an infected pulp whose apex is not closed without compromising its fracture resistance (19). Although there was no standard procedure for this treatment, disinfection with antibiotic pastes is the most critical phase (20). Despite the disadvantage of discoloration, the main reason why triple antibiotic paste is widely used is that it can penetrate 350 µm into the dentinal tubules and eliminate microorganisms there (21). Although many studies showed that regenerative endodontic treatment causes discoloration, no study examined the effects of bleaching on tooth structure after regenerative endodontic treatment (3,5,12). Previous studies have shown that using hydrogen peroxide with the intracoronal bleaching method is used to bleach teeth discolored due to regenerative endodontic treatment (22-24). Previous studies which evaluated the effect of intracoronal bleaching with H_2O_2 on dentin surface reported that dentin break of strength decreased, dentin stretching and shearing in their strength damaging effects formed, the calcium-phosphate rate decreased, and cervical root resorption (14-16,25). This situation is thought to pose a fracture risk for immature teeth with thin dentin walls. In the present study, we aimed to evaluate the fracture resistance of intracoronal bleached teeth with H_2O_2 after discoloration due to regenerative treatment with artificially simulated immature roots. According to the results of the present study, the fracture resistance value of the intracoronal bleaching group was obtained statistically lower than the non-bleaching

group. Therefore, the null hypothesis of the present study was rejected. A tooth prepared for endodontic treatment is considered to be more fragile than a vital tooth. This may be due to access cavity preparation, root canal enlargement, and post-cavity preparation (24). In the present study, root fracture resistance was found to be the lowest in the group with root canal enlargement and no restoration (26).

Lado et al. stated that bleaching agents penetrate dentin and cause cervical resorption by denaturation between enamel and cementum (27). The present study's lower fracture resistance in the intracoronal bleaching group parallels this study. In previous studies, it has been shown that H_2O_2 reduces the microhardness of dentin by degrading the matrix metalloproteinase enzyme, but this has no clinical significance (28,29). These results are inconsistent with our study. This may be due to the higher concentration of hydrogen peroxide used in the present study. To avoid this adverse effect, low-concentration hydrogen peroxide might be recommended (29). However, using hydrogen peroxide in low concentration causes the tooth to be exposed to a bleaching agent for a longer period due to more treatment sessions. Therefore, in this study, a high concentration of hydrogen peroxide was used for a short time.

It has been reported that the cause of discoloration in regenerative treatments with alternative pastes instead of antibiotic pastes containing minocycline may be due to the use of MTA as a coronal barrier (30-32). To eliminate this situation, white MTA was used in the present study. However, in the clinical setting, blood in contact with MTA can also cause coronal discoloration (5). The present study under in vitro conditions suggests that the cause of the discoloration is due to the minocycline in the antibiotic paste. Although some previous studies reported that intracoronal bleaching of teeth discolored due to regenerative treatment was not satisfactory in clinical situations (33,34). Küçükekenci et al. reported that bleaching was successful in their in vitro study (24).

Samples must be placed in specially prepared moulds for fracture tests. For this purpose, auto-polymerizing acrylic or polymethyl methacrylate can be used (35). Since the forces coming to the teeth in the oral environment are buffered with periodontal tissues, in the experimental setup, the roots were covered with polyvinylsiloxane impression material to mimic the periodontal ligament and embedded in auto-polymerizing blocks that mimic bone (36). In the fracture test, a 135^0 loading angle was chosen by the relationship between the maxillary and mandibular incisors (18). In fracture resistance studies, human teeth are generally used, but the parameters should be minimized as much as possible for standardization. Therefore,

buccolingual and mesiodistal widths close to each other and standard-sized human incisors were used in this study (37).

CONCLUSIONS

This study, which shows the negative effect of bleaching of discolored teeth due to regenerative endodontic treatment on cervical fracture resistance, recommends the application of treatment protocols that preserve the original color instead of bleaching the discoloration.

Ethics Committee Approval: Ethics committee approval was received for this study from Ordu University Clinical Research Ethics Committee (2022/219)
Peer-review: Externally peer-reviewed.
Author Contributions: Idea, Design, Audit, Data Collection and/or Processing, Analysis and/or Interpretation, Writing, Critical Review are all done by F.F.K.
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Morphometric Examination of The Tracheobronchial Tree in Cases with Foreign Body Aspiration

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Abstract

Objective: Recently, few studies have evaluated bronchial morphometric parameters focusing on bronchial length, diameter, or angles. These parameters can be an important indicator in various pathological processes and are essential for simulating the flow of aspiration and inspired particle transport. The aim of this study is to determine the trachea, the diameters of the right and left main bronchus, the angle of the carina and the angulations of the right and left main bronchus; To investigate the extent to its affects the foreign body aspiration localization.

Methods: In this study, pediatric patients under the age of 18 who came with a history of foreign body aspiration and underwent computerized tomography between 2017 and 2020 were included in the study. The patients were evaluated in terms of age, gender, anatomical localization of the aspirated foreign body, diameter of trachea, right main bronchus, left main bronchus, carina angle, branching angles of right and left main bronchus on CT.

Results: Of the cases in this study, 13 (56.5%) were female and 10 (43.5%) were male, It was observed that the aspirated foreign body was on the left side in 12 (52.2%) children, and on the right side in 11 (47.8%) children. The mean age of the patients was 5.52±4.86. The right bronchial angle (RBA) mean of the children with the foreign body on the left side was 39.04 ± 6.68, with a significantly wider angle compared to the mean RBA in the children with the foreign body on the right side (p=0.013).

Conclusion: The factor affecting the localization of the foreign body is the branching angle of the right main bronchus. According to the results of our study, it has been observed that if this angle is less than 37, the object will be on the right side, and if it is 37 degrees and above, the object will turn to the left.

Key Words: Tracheobronchial tree, Foreign body aspiration, Carina angle

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INTRODUCTION

The escape of any foreign body into the tracheobronchial system is defined as foreign body aspiration. Approximately 60-90% of foreign body aspirations occur in children and it is responsible for 7% of deaths in 1-3 years age group. Aspirated foreign bodies can lead to death in a short time, and if this acute period is unnoticed and remains in the tracheobronchial tree for a long time, it can cause progressive lung problems such as recurrent pneumonia, lung abscess and bronchiectasis. Therefore, early diagnosis and treatment is very important in foreign body aspirations. Half of the patients can be diagnosed within the first 24 hours, 30% within a week, and the remaining 20% within weeks or years(1,2). Although not as prominent as in adults, right bronchial tree in children is shorter, perpendicular, and its angle with the trachea is narrower than the left main bronchus. Due to this structure of the tracheobronchial tree, it has been shown in many studies that aspirated foreign bodies mostly escape to the right lung(3,4).

Recently, few studies have evaluated bronchial morphometric parameters focusing on bronchial length, diameter, or angles. These parameters can be an important indicator in various pathological processes and are essential for simulating the flow of aspiration and inspired particle transport (5,6).

To the best our knowledge, our study is one of the first studies in the literature to examine the bronchial tree morphometrically according to the foreign body location side in cases with foreign body aspiration.

The purpose of this study is to determine the trachea, the diameters of the right and left main bronchus, the angle of the carina and the angulations of the right and left main bronchus; To investigate the extent to its affects the foreign body aspiration localization.

METHODS

Patients

In this study, pediatric patients under the age of 18 who came with a anamnesis of foreign body aspiration and underwent computerized tomography between 2017 and 2020 were included in the study, with the approval of the ethics committee dated 16.01.2020 and decision numbered 19.

Patients who underwent radiography with the suspicion of foreign body, but who had no direct or indirect radiographic findings and who had CT examination (to avoid unnecessary bronchoscopy) because of the continuing clinical suspicion were included in the study. The patients were evaluated in terms of gender, age, anatomical localization of the aspirated foreign body, diameter of trachea, left and right main bronchus, carina angle, branching angles of left and right main bronchus on CT.

Patients older than 18 years of age and did not have an optimal quality radiological image, and who received emergency bronchospy due to poor general condition were excluded from

the study.

Screening protocol

CT scans were performed on the 64-slice Toshiba Aquilion (Toshiba Medical System Corporation, Otawara-Shi, Japan, Model TSX-101A, 5 mm section thickness) unit. CT images were acquired in the supine position. CT scans were performed at low dose for the pediatric age group and without any contrast agent. A lung window image array with a slice thickness of 3 mm was used for measurements.

Image Analysis

A single radiologist with 11 years of experience were analyzed CT images of the patients Anteroposterior (Tr-AP) and transvers (Tr-T) diameters of trachea were measured on axial images. Tracheal diameter measurements were made at the midclavicular level at the level of the thoracic inlet, due to its easy identification. Right bronchial angle (RBA) and left bronchial angle (LBA) measurements were made from coronal images. A vertical line was drawn through the middle of the carina in the coronal images and the angles between this line and the imaginary lines parallel to the lumen of the bronchi and passing through the middle of the bronchial lumen were measured (Figure 1). In addition, carinal angle (CA) and main bronchus diameters were measured from the coronal series.

Statistical Analysis

The normal distribution of the data to the was examined using the Shaphiro Wilk test. Variance homogenite was examined Levene test. Mean differences between two independent groups with normally distributed will be compared by Student's t-test, whereas the Mann–Whitney U test will be applied for comparisons of the not normally. In addition, the relationship between right-sided foreign body aspiration with RBA angle was examined by receiver operating characteristic (ROC). For numerical variables, mean±standard deviation are given and , number and percentage values for categorical variables are given. SPSS (**S**tatistical **P**ackage for the **S**ocial **S**ciences, SPSS Inc., Chicago, IL, USA) Windows version 22.0 program was used and $p<0.05$ was considered statistically significant.

RESULTS

Of the cases in this study, 10 (43.5%) were male and 13 (56.5%) female, It was observed that the aspirated foreign body was on the left side in 12 (52.2%) children, and on the right side in 11 (47.8%) children. The mean age of the patients was 5.52±4.86. On the aspiration side, atelectasis was observed in 2 children (8.7%), and consolidation was observed in 6 children (26.1%) (Table 1).

Table 1. General descriptive features of the cases

		n (%)
Sex	female	13 (56,5)
	male	10 (43,5)
Age(standard deviation)		5,52±4,86
side	left	12 (52,2)
	right	11 (47,8)
atelectasis	+	2 (8,7)
	-	21 (91,3)
consolidation	+	6 (26,1)
	-	17 (73,9)

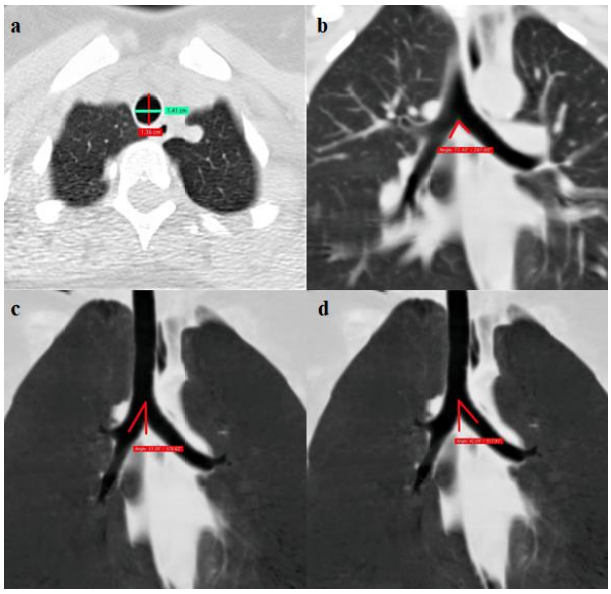

Figure 1: a. Measurement of anteroposterior and transverse diameter of the trachea. b. Measurement of carinal angle. c and d. Measurement of right and left bronchial angle.

The radiological parameters of children with a foreign body on the right side and those with a foreign body on the left side were compared (Table 2). It was observed that the right bronchial angle mean of the children with the foreign body on the left side was 39.04 ± 6.68, with a significantly wider angle compared to the mean RBA in the children with the foreign body on the right side (p=0.013). It was showed that there was no statistically difference in terms of transverse and anteroposterior diameters of the trachea, carina angle, and diameters of the left and right main bronchi in children with objects on theleft and right sides (p>0.05).

The RBA parameter was analyzed by ROC (Receiver operating characteristic) analysis to estimate the foreign body side. It was observed that the Area Under of the Curve value in determining the side of the RBA parameter was statistically significant (AUC 0.807: p=0.013) (Figure 2). It was predicted that if the RBA angle was less than 37, the object would be on the right side, and if it was 37 angle and above, the object would tend to the left (Table 3, Figure 3).

Table 2. Comparison of parameters

Variables	Left (n=12) mean±sd	Right (n=11) mean±sd	Test statistics	P
Anteroposterior tracheal diameter	7,65 ± 1,72	7,67 ± 1,17	z=-0,67	0,498
Transvers tracheal diameter	7,63 ± 2,27	7,14 ± 1,3	z=-0,19	0,853
RBA	39,04 ± 6,68	32,84 ± 3,72	t=2,71	**0,013**
LBA	40,33 ± 4,58	37,15 ± 8,42	t=1,14	0,267
CA	81,37 ± 9,97	75,66 ± 15,62	t=1,05	0,304
Right main bronchus diameter	6,2 ± 2,32	5,83 ± 1,64	z=-0,06	0,951
Left main bronchus diameter	5,67 ± 2,17	5,29 ± 1,28	z=-0,46	0,644

t value was obtained from Student t test, z value was obtained from Mann Whitney U test. sd:Standard deviation

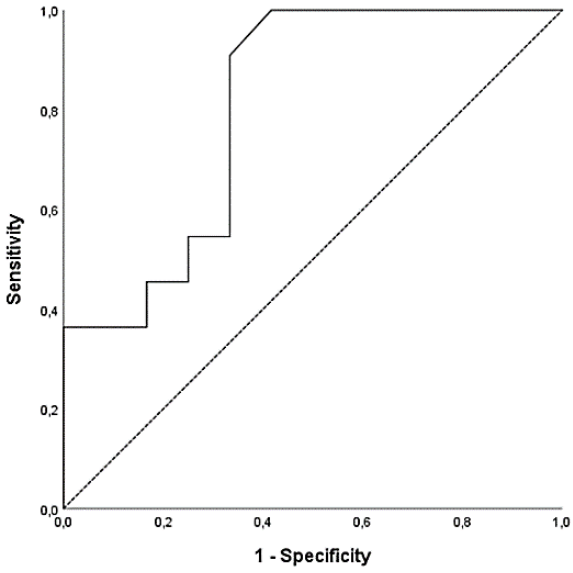

Figure 2. Roc curve of the relationship between the side of the foreign body and the RBA angle

Table 3. Determination of the relationship between the side of the foreign body and the RBA angle by Roc analysis

Cut-off	RBA<37,71
AUC (%95 CI)	0,807 (0,624 0,989)
Sensitivity	0,999
Specificity	0,583
P value	0,013

CI: confidence interval

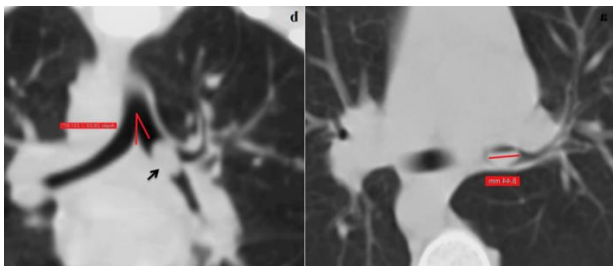

Figure 3. a and b. The right bronchial angle was measured as 28 degrees in a 9-year-old male patient with an 8.4 mm diameter foreign body (black arrow) in the right main bronchus.

DISCUSSION

Most of the morphometric studies of the bronchial tree have been performed with only posteroanterior chest radiography. There are various studies measuring the diameter, length and angles of the trachea and main bronchi at different levels and planes by computed tomography (7,8). In a morphometric study by Oliver et al. with thoracic CT, it was reported that transverse diameter of the trachea the was smaller than anterior-posterior diameter, but this difference was statistically significant only in males(9). It has been reported that the intrathoracic trachea is usually circular or oval, sometimes oblong or horseshoe-shaped; and its shape may vary according to its level. Gamsu et al. reported that the trachea can have different shapes. According to this study, it was reported that the trachea had a different shape in 22 out of 50 patients. Horseshoe-shaped trachea was present in 12 patients (24%), inverted pear-shaped trachea in 6 patients (12%), and rectangular trachea in 2 patients (4%) (10,11). In our study, there was no significant difference between the anteroposterior and transverse diameters of the trachea.

Revealed in a retrospective study that angles of tracheal bifurcation (IBA, RBA and SCA) in children younger than 18 years showed differences in different age groups. However, this difference is significant just between children over the age of 10 and under the age of 10. IBAs and SCAs expanded under age of 10. They measured mean SCA values above 80° and IBA values above 85° for children under the age of 10. In our study, almost all of our patients were under the age of 10. The mean carina angles were 81.37 ± 9.97 in cases aspirated into the right main bronchus, and 75.66 ± 15.62 in cases aspirated into the left main bronchus.

When the studies analyzing the subcarinal angle in adults and children are examined, it is showed that the subcarinal angle is higher in adults than in children (Tables III and IV). Ulusoy et al. reported that the subcarinal angle was higher in adults (12). Jit et al. and Chunder et al. study found that the subcarinal angle was higher in children (13,14). Farrukhabad et al. reported that as age progresses, chest wall and ribs ossifications is about to be completed, they become relatively stiff and direct the

undergrowth of the lungs, causing a narrowing of the subcarinal angle (15). Although different subcarinal angle values have been reported in the literature in adults and children, in our study, no statistically significant effect was found on the aspiration of the foreign body in the right or left main bronchus in the pediatric age group. However, in some studies, it has been stated that the enlargement of mediastinal structures or lung diseases can change the subcarinal angle dramatically and this can cause a change in the right main bronchus angle (16). In our study, a statistically significant relationship was found between the branching angle of the right main bronchus and the aspiration localization of the foreign body.

In a study conducted by Kubota et al., it was reported that the mean right bronchial angle was 31°±5° and the mean left bronchial angle was 49°±7°(17). No statistically significant difference was observed in the mean left bronchial angle values compared to the foreign body location side in our examination using CT images according to the side of the foreign body aspiration in cases of foreign body aspiration. However, the mean right bronchial angle was found to be 39.04 ± 6.68 in cases with aspiration to the left bronchial tree and 32.84 ± 3.72 in cases with aspiration to the right bronchial tree, which was statistically significant (p:0.013)

CONCLUSION

As a result; as far as we know, this study is the first study in the literature to examine the bronchial tree morphometrically according to the foreign body location side in cases with foreign body aspiration. Our findings showed that; the factor affecting the localization of the foreign body is the branching angle of the right main bronchus. According to the results of our study, it has been observed that if this angle is less than 37, the object will be on the right side, and if it is 37 degrees and above, the object will turn to the left. In our opinion, considering the branching angle of the right main bronchus in cases of non-opaque foreign body aspiration may be helpful in bronchoscopy.

Ethics Committee Approval: Ethics committee approval was received for this study from Hatay Mustafa Kemal University Clinical Research Ethics Committee (ethics committee date and no: 16.01.2020 and 19)

Peer-review: Externally peer-reviewed.

Author Contributions: Construction an idea for the research: F.O, M.E.C, Designing Methodology: F.O, I.K, Data collection: A.K, M.K, Analysis: I.K, M.E.C, F.O, Literature review: A.K, A.A, Writer: I.K, M.E.C, F.O, Supervision and critical review: I.K, A.A.

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Investigation of Heavy Metal Contents in Thyme *(Thymus vulgaris)* and Ginger (*Zingiber officinale)* Sold in Bingöl Herbalists

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Abstract

Objective: In our country, which is rich in medicinal plant diversity, there is an increase in heavy metal accumulation with the increase of industrial development and environmental pollution. The aim is to evaluate the health safety of heavy metal content of thyme and ginger plants, which are frequently used for therapeutic purposes, sold in herbalists.

Methods: In this study, heavy metal (Cr, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) levels in thyme (Thymus vulgaris) and ginger (Zingiber officinale) plant samples obtained from three different herbalists in Bingöl were determined by ICP-MS.

Results: Fe, Zn, Cd and Pb levels in both thyme and ginger samples, Cr levels in thyme samples and Cu levels in ginger samples were above the safe limit values for health. In the thyme and ginger samples, Co, Ni, Cr levels in ginger samples and As levels in thyme samples were found to be in the safe range for health

Conclusion: As a result; it is noteworthy that some heavy metals in medicinal plants used for therapeutic purposes in this study are above the recommended critical levels. Considering the possibility of exposure to heavy metals while consuming medicinal plants, regular monitoring of heavy metal concentrations in plants is important in order to minimize the risks that may adversely affect human health.

Keywords: Thyme, Ginger, Heavy metal, ICP-MS

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INTRODUCTION

Plants have been used throughout history to heal people and protect them from diseases. In Far Eastern countries, some plants are widely used for medicinal purposes in the treatment of diseases (1). In recent years, it is seen that the use of natural medicinal plant species has increased in western countries. In the mid-1970s, the use of herbal materials was below 5%, while at the beginning of the 20th century, more than 40% of medicines were of plant origin. On the other hand, with the increasing consumer awareness about health in the 1980s and 1990s, the demand for organic and naturally grown medicinal and aromatic plants has also increased (2).

Aromatic plants constitute 1/3 of Turkey's flora, which includes more than 10,000 plant species, and 3,000 of them are endemic. Today, there are around 300 plant varieties sold in herbalists and 70 to 100 of them are exported (3). Especially thyme, bay leaf and cumin, as well as sage, anise, acacia, fennel, sumac and rosemary are among the most important export products (4). Investigation of heavy metal accumulation in foodstuffs and medicinal plants is important for human and environmental health due to the increase in environmental pollution with technological developments (5). Consumption of medicinal and aromatic plants is low compared to other food products; however, they can be dangerous due to their possible high heavy metal content and misuse. For this reason, it is important to establish a database for the compositions of widely used medicinal and aromatic plants in terms of standardization (4). *Thymus vulgaris* (thyme), belonging to the Lamiaceae family, relieves pain types such as gastralgia, abdominalgia and cephalgia, and has carminative, digestive, diuretic, sedative properties as well as gastroenteritis, neurological diseases, trigeminal neuralgias, typhoid, diarrhea, rheumatism, epilepsy and upper respiratory tract (bronchitis, sinusitis, pharyngitis, etc.) is used in diseases (6,7). It is reported that *Zingiber officinale* (ginger) from the Zingiberaceae family has sedative, antispasmodic, antiemetic, expectorant, anticolitis, anticoagulant and regulating circulation, lowering cholesterol, and relieving abdominal pain. In addition, it provides the removal of toxins due to its diaphoretic effect against poisoning (7).

The aim of this study is to determine whether the heavy metal contents of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) sold in herbalists are among the limits recommended by International Organizations such as the World Health Organization (WHO) / Food and Agriculture Organization (FAO).

METHODS

Plant material

In this study, two different plant species, which are mostly used for treatment in upper

respiratory tract infections, were obtained from three different herbalists in Bingöl between October-November of 2019. These plants, taken from herbalists, were taxonomically defined by Bingöl University Biology Department Lecturer Alpaslan Koçak (8). The scientific names, family names, English names, Turkish names and used parts of the plants that make up the material of the study are presented in Table 1.

Table 1. Scientific, common names and used parts of medicinal plants

Scientific name	Family name	Common name	Plant Part Used
Thymus vulgaris	*Lamiaceae*	Thyme	Branches with leaves and flowers
Zingiber officinale	*Zingiberaceae*	Ginger	roots and rhizomes

Sample Preparation

In the research, different parts of thyme and ginger plants were used as material. Approximately 0.5 g of the grinded samples were weighed and placed in the CEM-Mars 6 240/50 (Corp. Mathews NC, USA) Teflon containers of the microwave unit and 10 mL of HNO_3 was added to it. It was kept in the microwave for 25 minutes at 200 ºC for 15 minutes and then the temperature was lowered to make the samples soluble. At the end of the combustion process, the volumes of 0.5 mL samples cooled to room temperature were completed by diluting up to 15 mL with 1% (HNO_3-ultrapure water).

Calibration Procedure and Analysis Method

Calibration solutions used for ICP-MS were prepared at specific concentrations by diluting commercially purchased multi-element standards with 1% nitric acid-ultrapure water. Element standards with atomic masses ^{53}Cr, ^{57}Fe, ^{59}Co,^{60}Ni, ^{63}Cu,^{66}Zn,^{75}As, ^{111}Cd, ^{202}Hg, ^{208}Pb were used. The determination of Cr, Fe, Co, Ni, Cu, Zn, As, Cd, Hg and Pb heavy metals in medicinal plant samples was performed with ICP-MS in triplicate. Perkin Elmer NexION 2000 model ICP-MS operating conditions for heavy metal analysis are given in Table 2.

Table 2. Working conditions for ICP-MS detection

Parameters	Description / Value
Nebulizer	Glass Type C
Nebulizer Flow	< 2% optimized for oxide
Nebulizer gas flow rate	0,93 L/min
Spray room and temperature	Glass, 2 ºC
Injector	2.0 mm i.d.
Deflector voltage	−12 V
Analogue step voltage	−1750 V
RF power	1600 W
Rinse Time	45 second
Standby time	50 ms
Aerosol dilution	Set to 2.5x
Sample delivery speed	350 µL/min
Threshold of discrimination	26
Alternating current bar offset	−4
Sampling Cone	Ni
The number of repetitions	3

Data Analysis

Calibration functions were determined for each element and heavy metal concentrations in plant samples were calculated using calibration functions. Heavy metal levels in plant samples are given as the heavy metal mean, three reconcentrations (µg/g) and standard deviation (mean ± standard deviation). Syngistix for ICP-MS software version 2.2 instrument software was used to control the instrument, including tuning, data acquisition, and data analysis in analyzes.

RESULTS

In this study, heavy metal amounts were determined in µg/gr in plant samples by ICP-MS method. Limit of determination (LOD), limit of measurement (LOQ) and recovery (R, %) values of the calibration obtained in the study are in Table 3, mean and standard deviation values of heavy metal concentrations of thyme and ginger plants are given in Table 4 and Table 5.

Cd in thyme samples analyzed by the device, and Hg in both thyme and ginger samples could not be read. It is seen that the thyme samples taken from the herbalist no. 3 have the highest Fe, Cr, Co, Ni, As, Pb contents, while the thyme samples taken from the herbalist no. 1 have the lowest Cr, Co, Ni, Cu contents. Likewise, it is seen that the ginger samples taken from the herbalist no. 2 have the highest content and the ginger samples taken from the no. 3 plant have the lowest Fe, Cu, Cr, Co, Ni, Cd, Pb contents (Table 4, Table 5).

Table 3. Limit of detection (LOD), limit of measurement (LOQ) and recovery (R, %) values selected for heavy metals investigated in the study

Element	LOD (mg/L)	LOQ (mg/L)	Recovery (R,%)
Cr	0,017	0,056	97,380
Fe	0,223	0,746	98,743
Co	0,00032	0,00103	96,521
Ni	0,008	0,025	98,968
Cu	0,022	0,073	96,784
Zn	0,043	0,142	97,417
As	0,001	0,004	95,826
Cd	0,000165	0,00055	97,608
Hg	0,0016	0,0054	95,950
Pb	0,010	0,033	94,882

Table 4. Mean contents of Fe, Zn, Co, Ni, Cu elements in *Thymus vulgaris* and *Zingiber officinale*

Herbalists	Plant	Fe (µg/g)	Zn (µg/g)	Co (µg/g)	Fe (µg/g)	Zn (µg/g)
Herbalist 1	Thyme 1	1785,99±1,04	44,42±0,02	0,34±0,00	0,76±0,00	2,29±0,00
	Ginger 1	535,99±0,46	13,59±0,11	0,44±0,01	2,16±0,00	4,47±0,00
Herbalist 2	Thyme 2	1740,05±2,42	37,26±0,05	0,38±0,01	0,92±0,00	3,14±0,01
	Ginger 2	700,99±1,03	62,02±0,07	0,56±0,01	2,58±0,01	5,63±0,01
Herbalist 3	Thyme 3	5109,90±6,75	42,65±0,06	0,45±0,01	1,11±0,00	2,71±0,01
	Ginger 3	415,65±0,13	75,12±0,01	0,30±0,00	0,38±0,00	3,15±0,00

Table 5. Mean contents of Cr, Cd, Hg, As, Pb elements in *Thymus vulgaris* and *Zingiber officinale*

Herbalists	Plant	Cr (µg/g)	Cd (µg/g)	Hg (µg/g)	As (µg/g)	Pb (µg/g)
Herbalist 1	Thyme 1	2,25±0,00	NR	NR	0,22±0,00	4,52±0,01
	Ginger 1	0,92±0,00	0,34±0,00	NR	1,37±0,00	11,52±0,01
Herbalist 2	Thyme 2	2,38±0,01	NR	NR	0,17±0,01	4,09±0,01
	Ginger 2	1,49±0,00	1,37±0,01	NR	1,25±0,00	16,40±0,02
Herbalist 3	Thyme 3	2,56±0,01	NR	NR	0,27±0,00	6,28±0,01
	Ginger 3	0,83±0,00	0,08±0,00	NR	0,93±0,00	6,12±0,00

DISCUSSION

The use of natural medicinal plants is increasing day by day in our country as well as in the world. Medicinal plants are mostly consumed in autumn and winter by brewing (infusion) and boiling (decoction) methods (1). In addition to many health benefits of medicinal plants, mistakes made in their use, excessive consumption, and the risk of contamination due to uncontrolled collection, diagnosis and stocking pose a threat to health. The presence of factors such as industrial pollution, pesticides, traffic pollution increases the exposure of plants to heavy metals. The different concentrations seen in plants vary depending on the type of plant, the parts used, soil content, water pollution, air pollution, industrial activities, and the use of fertilizers as well as other chemicals (9).

Elements such as iron, chromium, copper, zinc, cobalt, manganese and nickel, which play an important role in biological processes in metabolism, are vitally essential. Heavy metals such as mercury, lead and cadmium can be toxic even at very low concentrations. Heavy metals such as lead, cadmium, chromium and arsenic are recognized as potential pollutants commonly found in our environment (10). Heavy metal contamination with medicinal plants is attributed to environmental pollution and should be limited as they have the potential to pose a health hazard (11-16). Plants grown in areas contaminated with heavy metals may have high metal contents (17). In addition, the use of cadmium-containing fertilizers and the use of organic mercury or lead-containing pesticides in agricultural lands increase the heavy metal rates in grown plants (18). Heavy metals taken in high doses through the food chain also affect human health negatively (19,20).

Elements that fall into the category of micronutrients that the human body needs less than 100 mg/day are considered to be vanadium, chromium, manganese, iron, cobalt, copper, zinc, molybdenum, selenium, fluorine and iodine. Micronutrients such as Cu, Cr, Mo, Ni, Se, and Zn can be toxic at high concentrations (21-24). High zinc intake from contaminated food or drink and acute zinc poisoning have been associated with nonspecific gastrointestinal symptoms such as abdominal pain, diarrhea, nausea and vomiting. In addition, exposure to high zinc interferes

with the metabolism of other trace elements such as copper absorption (25). The mechanism of cellular and tissue damage of excess iron is thought to be modulated by Fenton reaction, as it is a potent free radical generator. In vivo free radical generation has been associated with cancer, cardiovascular disease and arthritis (26).

In the study, thyme and ginger samples, whose heavy metal content was determined, were dissolved in the microwave, then content analysis was performed with ICP-MS and the results are given in Table 4 and Table 5. Permissible limit values for Fe and Zn determined by FAO/WHO in edible plants are 20 ppm and 27.4 ppm (1ppm=µg/g), respectively (27). Fe and Zn values detected in thyme and ginger samples were above the limit values allowed by FAO/WHO. While Fe values of thyme and ginger samples reported in the literature were mostly above the limit values allowed by FAO/WHO (4,28-31), Okut et al. found that Fe and Zn values were within the limit values in the thyme sample they studied (30).

High copper levels can cause hair and skin discoloration, dermatitis, upper respiratory tract irritation, metallic taste in the mouth and nausea. Copper deficiency results in anemia and congenital failure (11). Chronic exposure to chromium can cause liver, kidney, stomach and lung damage (31).

In edible plants, 3.0 ppm for Cu by FAO/WHO (27) and 2.0 ppm for Cr by WHO (32) are allowable limit values. Except for ginger Cr levels and thyme 1st and 3rd Cu values, chromium and copper values of all thyme and ginger were found above the determined limits. In addition to the studies in which the Cu values in thyme samples were found below the limit values allowed by FAO/WHO (30), there are also studies that were found above the limit values (4,29). In the literature, while the Cr values in some ginger samples are below the limit values allowed by WHO (4,29), there are also studies that are above the limit values (33).

It has been reported that acute exposure of the human body to nickel can cause liver, kidney, spleen, brain and tissue damage, vesicular eczema, lung and nose cancer (25,31). The systemic effects of cobalt are mainly characterized by a complex clinical syndrome involving neurological (hearing and visual impairment), cardiovascular and endocrine (34).

While the limit value allowed for Ni by FAO/WHO (31) is 1.63 ppm, there is no regulatory limit for Co. However, in a study conducted on seven plants by Başgel and Erdemoğlu in Turkey, it was reported that the Co concentration ranged between 0.14 ppm and 0.48 ppm. Ni values determined in thyme samples were found below the limit value (35). There are studies in which Ni values detected in thyme and ginger samples reported in the

literature are above the value allowed by FAO/WHO (4, 28). The daily Co intake determined by the Agence Française de Sécurité Sanitaire des Aliments (AFSSA) is 1.6–8 µg/kg body weight/day (36). Except for the ginger 2 plant sample obtained from Herbalist 2, the cobalt contents of all samples were within the determined safe range. In the literature, Co values in thyme samples were found to be between 0.00285-0.66 mg/kg (4,28,29). In the literature, ginger Co content has been determined between 0.16-0.41 mg/kg (4,28).

On the other hand, it is known that toxic elements such as As, Cd, Hg and Pb have harmful effects on human health (24). Lead accumulates mainly in the skeletal system and soft tissue. Also, lead tends to accumulate in gray matter and certain centers in the central nervous system. Lead inhibits porphobilinogen synthase and ferrochelatase, preventing the formation of porphobilinogen, incorporation of iron into protoporphyrin IX, causing ineffective heme synthesis and microcytic anemia. In addition, lead poisoning negatively affects the kidney, liver, vascular and immune systems (33). Low levels of Cd exposure can cause damage to the kidney, liver, skeletal and cardiovascular systems, as well as impaired vision and hearing. Cadmium, which is known to have teratogenic and mutagenic effects, also causes delay in puberty and menarche, steroidogenesis, menstrual cycle, reproductive hormone disorders, miscarriage, preterm birth and low birth weight (37). Elemental and methyl mercury are toxic to the central and peripheral nervous systems, its inorganic salts are corrosive to the skin, eyes, and gastrointestinal tract, and can cause kidney toxicity when taken orally (25). Exposure to inorganic arsenic is associated with an increased risk of skin lesions and developing skin, lung, liver, and kidney cancer (38).

The limit values allowed for Cd and Pb by FAO/WHO (27) are 0.21ppm and 0.43 ppm, respectively, while the limit allowed for As by FAO/WHO (39) is 0.9- 1.1 ppm. It was determined that cadmium content was high in one of the ginger plant samples, while the cadmium content was found in the reference range in thyme samples. The amount of Pb was found above these allowable limit values in both thyme and ginger samples. The amount of As in thyme samples was determined below the limit value. It was determined that arsenic content was high in one of the ginger plant samples. Cd values detected in ginger samples reported in the literature ranged between 0.05-2.39 mg/kg (4, 28). While thyme Pb values are in the range of 0.001-1.02 ppm in the literature (4, 30), it has been determined as 0.05-1.6 mg/kg for ginger samples (4, 40). In the literature, As values determined in thyme samples were determined as 0.001-0.38 ppm (28, 29, 30), and in ginger samples as 0.03-0.84µg/g (28)

Heavy metals pollute soil and water through the use of coal as fuel, industrial fertilizers, pesticides and industrial flue gases, exhaust fumes, industrial oxidation and water pipes. Plants have the capacity to collect heavy metals from soil and water throughout their development (20). Based on the findings of our study, high heavy metal contents in plants exposed to environmental pollutants can reach toxic levels for humans and animals.

CONCLUSION

As a result; it is noteworthy that some heavy metals in medicinal plants used for therapeutic purposes in this study are above the recommended critical levels. Adverse environmental conditions in which medicinal plants are grown and stocked can lead to heavy metal contamination that is harmful to human health. Considering geographical conditions and different factors in industrial activities, metal levels in plants should be carefully monitored. Herbal materials, including medicinal plants and imported products sold in herbalists, should be checked regularly and heavy metal contamination risks should be evaluated continuously.

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Investigation of The Learning and Memory Enhancing Effects of 0.25 mA and 0.5 mA Anodal and Cathodal Transcranial Direct Current Stimulations in Healthy Rats

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Abstract

Objective: Our aim in this study was to investigate the effects of tDCS, which is known to be effective on AMPA and NMDA, with different anodal and cathodal stimulation types and 0.25 mA and 0.5 mA current intensities on learning and memory by behavioral and molecular mechanisms.

Methods: 50 male Wistar rats weighing 290-310 g were divided into 5 groups as control, C1-tDCS, C2-tDCS, A1-tDCS and A2-tDCS. In the C1-tDCS group, 0.25 mA cathodal tDCS stimulation for 30 min per day for 5 days, in the C2-tDCS group for 30 min per day for 0.5 mA cathodal tDCS stimulation for 5 days, in the A1-tDCS group for 5 days with 0.25 mA anodal tDCS stimulation for 30 min per day and A2-tDCS group The tDCS group received 0.5 mA anodal tDCS stimulation for 30 minutes per day for 5 days. On the 6th and 7th days of the experiment, the locomotor activity, learning and memory behaviors of the rats were evaluated by open field test, y maze test and object localization test. In addition, glutamate levels were measured in hippocampus tissues by ELISA method.

Results: It was observed that there were non-significant decreases in the results of the C1-tDCS and C2-tDCS groups in which cathodal stimulation was applied compared to the control group in locomotor activity, learning and memory data. On the other hand, an increase was observed in the data of the A1-tDCS and A2-tDCS groups in which anodal stimulation was applied, and the increase in the data of the A2-tDCS group from these groups was found to be statistically significant compared to the control ($p<0.05$). Similar results were also seen in glutamate levels. A non-significant decrease in glutamate levels was observed in the C1-tDCS and C2-tDCS groups compared to the control, while an insignificant increase in glutamate levels in the A1-tDCS group was observed. On the other hand, there was a significant increase in glutamate level in the A1-tDCS group compared to the control group ($p<0.05$).

Conclusion: In conclusion, our data showed that 0.5 mA anodal tDCS stimulation for 30 min for 5 days can enhance learning and memory on the glutamatergic pathway.

Key words: Glutamate, Hippocampus, Learning, Memory, tDCS

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INTRODUCTION

The brain is the organ responsible for important physiological functions such as seeing, smelling, walking, running, thinking, feeling, attention, learning and memory (1). One of the most important functions of the brain is to obtain information about what is happening around people and to store the information they have acquired for later use. Learning is a process involving the acquisition of information about the environment and its changes on the behavior of such information (1). Memory, on the other hand, is the ability to store what is experienced and learned in the mind by establishing a relationship with the past, and it is shown as a trace of learning that remains in neural networks (1). Glutamate is the most important excitatory neurotransmitter in the central nervous system, which plays an active role in learning and memory formation in the hippocampus (1). Glutamate activity, the main neurotransmitter of learning, and N-methyl-D-aspartate (NMDA) receptor activity, which is the ionotropic glutamate receptor, are modulated by transcranial direct current stimulation (tDCS). Studies have reported that anodal tDCS improves behavioral performance and has an excitatory effect, while cathodal tDCS has an inhibitory effect on performance or neuronal activation (2-4).

tDCS, a non-invasive brain stimulation technique, is cheap and easy to use, and has no negative side effects (5). This technique is applied superficially to the cerebral cortex by applying constant and low-intensity current from the skull and the direct current given from the active electrode placed in the skull reaches the reference electrode by passing through the cerebrum tissue (5). tDCS is caused by the passage of a constant DC in the cerebrum after the two terminals of a battery-based stimulator are placed in the cerebrum (6). Weak current transmitted by tDCS, could not trigger a rapid depolarization of the electric field in the brain tissue, not directly create action potentials in cortical neurons (6). Therefore, tDCS can be considered to have a neuromodulatory effect. The current flow direction determines the effects of electrical excitation (6). tDCS has useful effects in an extensive variety of clinical pathologies like epilepsy (7), stroke (8, 9), and various pain conditions, and also psychiatric conditions such as depression and addiction (4, 10). In recent studies, it has been shown to affect cell excitability and epileptic discharges by changing membrane potential without creating an action potential in neurons (11, 12). Furthermore, tDCS has noninvasive clinical neuroprotective effects, which is preferred, especially for the treatment of learning and memory disorders (13, 14). In addition, tDCS is preferred in studies investigating the effects of cognitive functions such as learning, memory, and decision-making in healthy individuals (15). The duration, polarity and direction of the direct current applied determine the degree of change in the

brain (16). The current passing through the brain tissue creates increasing or decreasing excitable effects in the cortical area (17). The excitability is determined by the intensity of the current and the polarity with anodal stimulation or cathodal stimulation (17). There are two types of tDCS as anodal and cathodal tDCS. Anodal stimulation (A-tDCS) creates a depolarizing response with an excitatory effect, whereas cathodal stimulation (C-tDCS) induces a hyperpolarized response with an inhibitory effect (2-4). In studies related to the efficiency of tDCS stimulation, it has been shown the activation or inhibition effects last up to 90 minutes depending on the duration and location of the stimulation, and these effects continue even longer after regenerative stimulus (18, 19). It is known that A-tDCS application creates depolarization in neuron membranes and increases the excitability of cortical neurons by activation of Na+-Ca2+ dependent channels in neurons (17). Anodal tDCS shows its effects by modulation at both GABAergic (short interval intracortical inhibition) and glutaminergic (intracortical facilitation) synapses, while cathodal tDCS exerts its effects only through glutaminergic synapse modulation (16, 17) (Figure 1). It has been observed in the literature that the effects of tDCS are long-term rather than short-term (5, 20). It has been reported that tDCS activates NMDA receptors in the long term and that resulting effect can spread to neuronal networks in the area where it is applied (4, 17, 21). Nitsche and Paulus (22) reported that tDCS causes subthreshold stimulation in membrane polarization rather than presynaptic or postsynaptic cell stimulation. tDCS shows its effect through the activation of Na^{+}-$Ca2^{+}$ dependent ion channels and through long-term potentiation and depression-like changes through N-methyl-D-aspartate (NMDA) receptor activity (23).

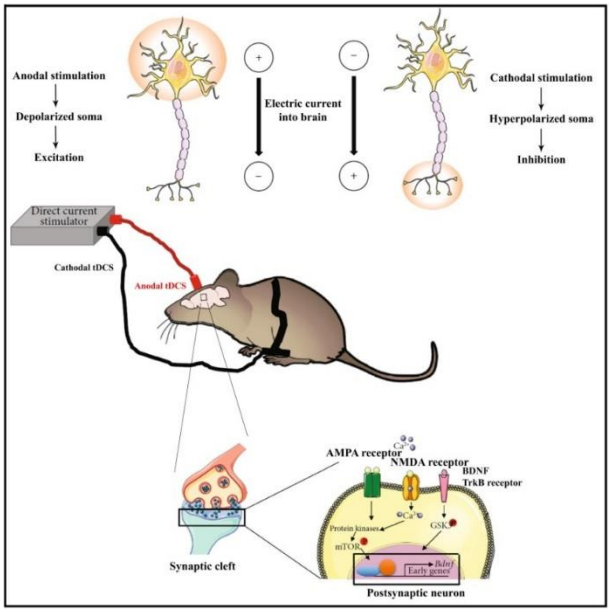

Figure 1. Schematic representation of transcranial direct current stimulation (tDCS). While A-tDCS increases excitability by acting on the neuronal membrane potential by depolarizing, C-tDCS decreases excitability by affecting hyperpolarization. A-tDCS depolarizes the presynaptic neuronal membrane and glutamate, and glutamate binds to AMPA and NMDA receptors. It regulates the neuronal signaling pathway that leads to transcriptional changes by activating protein kinases with the increase of intracellular Ca2+ in the postsynaptic neuron. Also, tDCS modulates the BDNF signaling pathway.

Abbreviations: AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; A-tDCS: anodal-tDCS, BDNF: brain-derived neurotrophic factor; CBP: CREB-binding protein; C-tDCS: cathodal-tDCS, CREB: cAMP response element binding protein; GSK3: glycogen synthase kinase 3; LTP: long-term potentiation; mTOR: mammalian target of rapamycin; NMDA: N-methyl-D-aspartate; TrkB: tropomyosin receptor kinase B. Adapted from Cavaleiro et. al. (30).

METHODS

Our study was carried out in Akdeniz University Experimental Animals Unit. Rats obtained from Akdeniz University Experimental Animals Application and Research Center with the approval of Akdeniz University Animal Experiments Local Ethics Committee (Decision No 40) were used in the study.

Experimental Groups and Protocol

Experiments were carried out by dividing 50 male Wistar albino rats, weighing 290-310 gr, into 5 groups:

Group 1: Control (n=10), sham tDCS stimulation was applied 30 min a day for 5 days (n=10),

Group 2: C1-tDCS, 0.25mA cathodal stimulation was applied for 30 minutes during the day (n=10),

Group 3: C2-tDCS, 0.5mA cathodal stimulation was applied 30 min a day for 5 days (n=10),

Group 4: A1-tDCS, 0.25mA anodal stimulation was applied 30 min a day for 5 days (n=10),

Group 5: A2-tDCS, 0.5mA anodal stimulation was applied 30 min a day for 5 days (n=10).

Throughout the experiment, animals were kept in a 12-hour dark/light cycle with 5 animals in each cage. During the experiment, animals were fed with commercial rat chow and tap water. Starting from the 3rd day of the experiment, handling was applied for 5 minutes, 3 times a day for 2 days. On the 6th day of the experiment, the rats were taken to open field (OF) and Y-maze tests, and the object localization test (OLT) was taken on the 7th day (Figure 2). On the 7th day of the experiment, the subjects were sacrificed and their brain tissues were taken, and glutamate measurement was made in the hippocampus tissue by ELISA method.

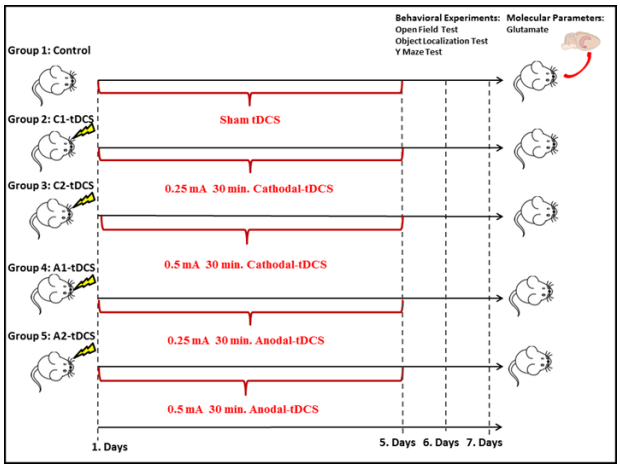

Figure 2. Experimental protocol

tDCS Stimulation

For tDCS application, Animal DCS Stimulator (model 2100) device with temporal resolution of 1 min was used. In our study, tDCS stimulation was applied to all groups, except the control group, for 30 minutes for 5 days. During the tDCS application, a superficial disc electrode was used, the maximum current intensity was ±1000 μA, and the current resolution was set as 0.01mA (Figure 3).

Behavioral Experiments:

Open Field Test

The open field test is used to evaluate locomotor activity. Open field experiments were performed in an 80x80x40 cm setup (Figure 4). Rats were placed in the central area of this area

and their movements were recorded for 5 minutes using a camera system. The odor cues were eliminated by cleaning the open field setup with 70% ethanol for each rat. Locomotor activity was evaluated with the parameters total distance (cm) and frequency (24).

Figure 3. tDCS applications (24)

Figure 4. Open field test (24)

Y-Maze Test

The Y-maze test is used to investigate short-term memory and spatial memory. The Y-maze test was performed on a black plastic assembly consisting of three arms (50 cm long, 20 cm wall high, 10 cm wide) at a 120° angle from each other in a room including a variety of distinct distal cues (Figure 5). In training phase, the rats were left at the end of the starting arm, and each rat was given 15 minutes to freely examine the other arms while the new arm was completely closed. IN testing phase, the rats were removed from maze and the Y-maze assembly was cleaned with 70% ethanol to prevent their movements according to the sense of smell during the experiment. One hour after the first session was opened arms and all three arms of rats were allowed to explore freely for 5 minutes. In this second session, subjects with spatial memory are expected to make the first turn into the "novel arm" and spend more time exploring this arm. The number of new arm entries and the time spent exploring the new arm were recorded. The behavior of the rats was monitored by a camera system.

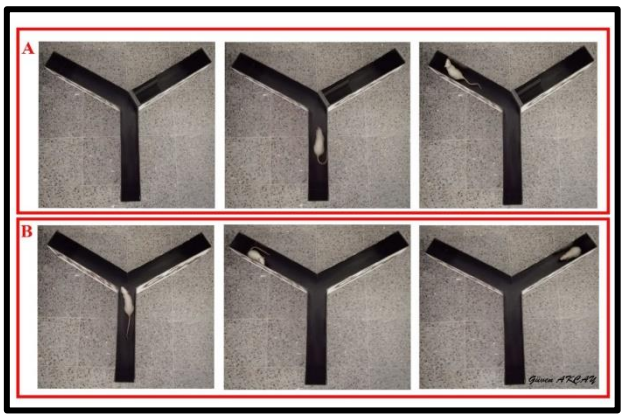

Figure 5. Y maze test procedure A) Training phase B) Testing phase

Object Localization Test

The object localization test is used especially in short-term and spatial memory studies. It consists of three stages: habituation, training and retention. On habituation phase, rats center of the arena (40 cm high, 80 x 80 cm in size) were placed and allowed to explore for 5

minutes without any object (Figure 6). In the training phase, the rats were allowed to set the media from the center and 5 minutes for the two objects are expected review. The time spent exploring each object was recorded. The maze was cleaned with 70% ethanol to prevent their movements according to the sense of smell during the experiment. In the testing phase, one of the objects was relocated and the rats were allowed to explore the objects for 5 minutes. It is expected to spend more time to examine the objects of the subjects relocated. The total exploration time and the total number of touches to the displaced object were recorded with the camera system (25).

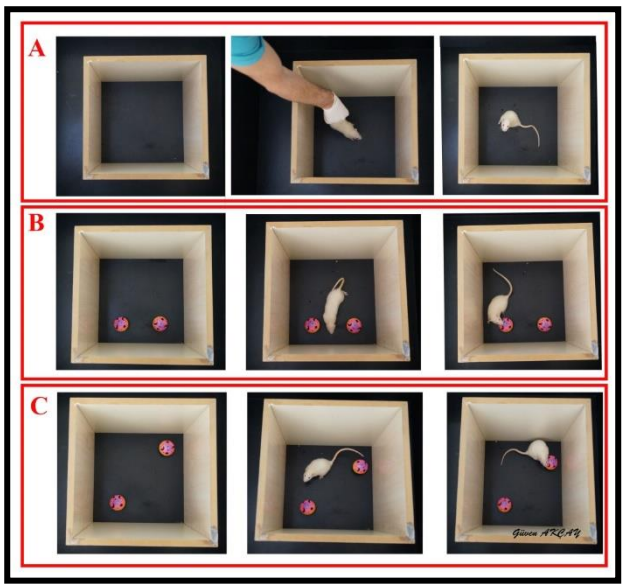

Figure 6. Object localization test procedure A) Habituation phase B) Training phase C) Testing phase

Biochemical Method:

Glutamate level

Measurements were performed according to the protocol specified in the commercially purchased solid phase sandwich enzyme immunoassay (Glutamate Assay Kit ab83389-ELISA) kit. Standard solutions and samples prepared in decreasing concentrations by serial dilutions were loaded into 96-well plates containing specific monoclonal antibodies against rat glutamate. In this method, which is based on antigen-antibody binding, glutamate molecules in the samples were bound to the antibody. It was incubated in an incubator set at 37 °C for 90 min. At the end of the incubation, concentrated biotinylated detection Ab was diluted 1/100 with biotinylated detection dilution solution and 100 µl was added to each well. After incubation at 37 °C for 1 hour, 750 ml of distilled water was added to the concentrated wash buffer and washed 3 times with a wash buffer solution, and unbound molecules were removed. Then, peroxidase (Horseradish Peroxidase-HRP) conjugate bound with 100 µl of streptavidin was added and incubated at 37 °C for 30 minutes. After incubation, this time it will be washed 5 times and substrate solution is added. Color change will be observed in direct proportion to the glutamate concentration in the samples and 50 µl of stop solution was added to each well to stop the reaction. The absorbance value of each well was determined by reading in a microplate reader at 450 nm.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software. Results are given as mean±SEM. Statistical significance was

accepted as p <0.05. Open field test data were evaluated with one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test was used as posthoc test.

RESULTS

The total distance and frequency in open field in A2-tDCS group (p<0.05) was significantly increased compared with that in Control group (Figure 7). When the locomotor activity parameters were compared between the control and A1-tDCS groups, no significant difference was found. Similarly, there was no difference between C1-tDCS and C2-tDCS groups.

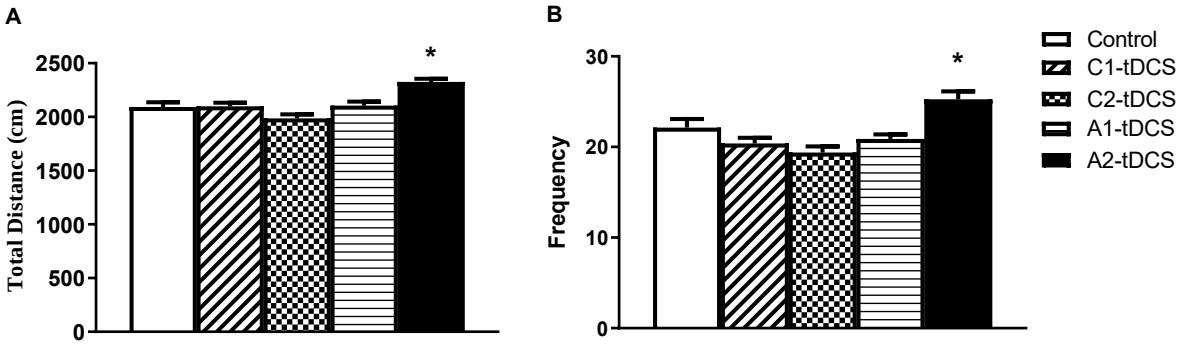

Figure 7. Open field test results of experimental groups. A) Total distance (cm), B) Frequency. (n=10, for each group; * p<0.05 shows the difference compared to the Control group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± SEM.

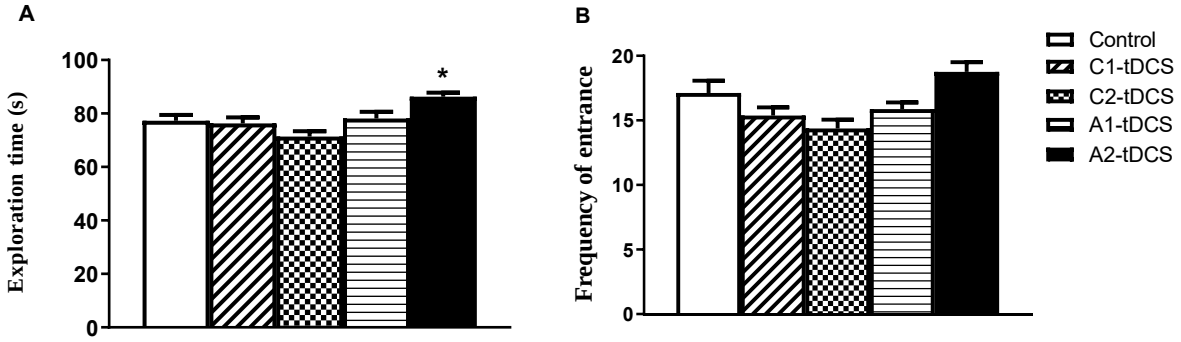

Figure 8. The effect of 5-day tDCS stimulation on the spatial memory. A) Exploration time to the novel arm (s) and B) Frequency of entrance to the novel arm (n = 10 for each group; *p < 0.05compared to Control, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± SEM.

The exploration time to the novel arm (s) and frequency of entrance to the novel arm were evaluated using Y maze (Figure 8). Our results showed that there was a decrease in the exploration time to the novel arm (s) and frequency of entrance to the novel arm in the C1-tDCS and C2-tDCS group compared to the Control group. Y-maze test values of the A2-tDCS group was significantly increased compared to the Control

group. Similarly, there was no difference between C1-tDCS and C2-tDCS groups.

The exploration time and number of touches to the relocated object were evaluated by object localization test (Figure 9). The results showed that there was not a significant decrease in both the exploration time and number of touches of the relocated in the C1-tDCS and C2-tDCS groups compared to the control group. The results showed that both exploration time and number of touches were significantly higher in A2+tDCS group as compared to control group (Figure 9).

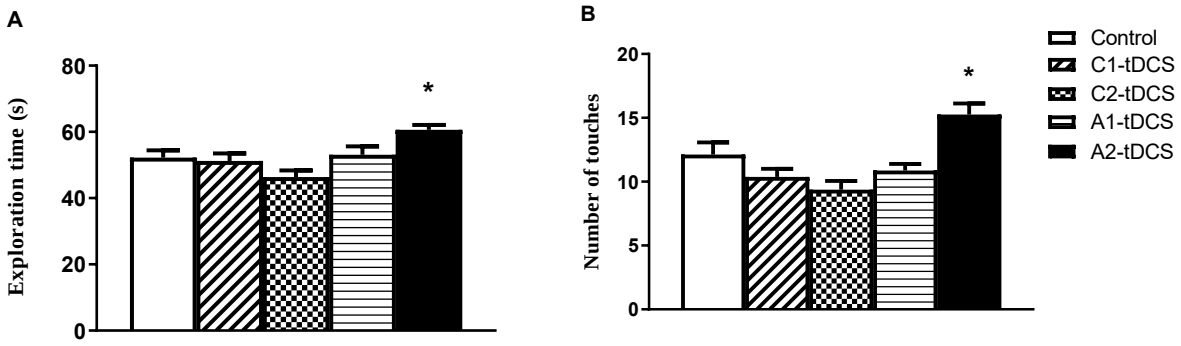

Figure 9. The effect of 6-day tDCS stimulation on the spatial memory. A) Exploration time of the relocated object (s), B) Number of touches (n = 10 for each group; * p < 0.05 compared to Control Sham, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± SEM.

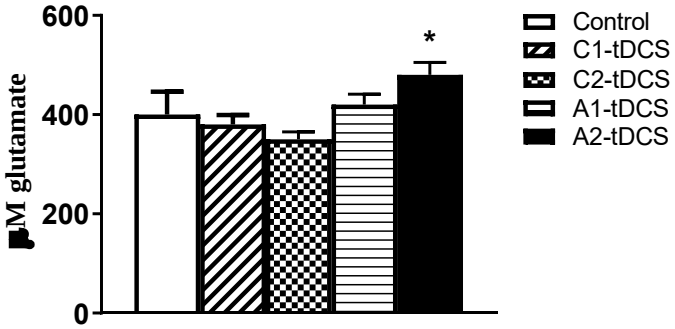

Figure 10. The effect of 7-day tDCS stimulation on the glutamate levels in the hippocampus tissue. * p<0.05 Control, (n =10). The data are means ± SEM. Statistical analyses are One-way ANOVA followed by Tukey's multiple comparison test against the indicated group.

Figure 10 shows glutamate levels in hippocampus tissue. When the glutamate levels in the hippocampus were evaluated, there was no difference between the Control and C-tDCS groups, while and glutamate (Figure 10) levels of the A2-tDCS group increased significantly compared to the Control group.

DISCUSSION

The brain is responsible for many important physiological functions such as learning, memory, speech, thinking and decision making. The learning and memory area of the brain is the hippocampal area, and the most important excitatory neurotransmitter in the central

nervous system is glutamate (1). There are different treatments and techniques to strengthen learning and memory. However, in recent years, neuromodulation and the regulation of the neuronal membrane potential of the brain have become widespread. One of these techniques transcranial direct current stimulation. tDCS is a non-invasive neuromodulation technique that delivers a constant low-intensity sub-threshold direct current to specific areas of the brain through electrodes placed on the scalp, thereby regulating cell transmembrane potential depolarization and hyperpolarization, and altering neuronal activity and excitability of the cerebral cortex (2, 26). Some clinical and basic studies have found that tDCS treatment can improve memory and cognitive dysfunction in patients and animals. Studies have shown that different tDCS current density and stimulus types have different effects on learning and memory (27-32). However, there are few studies investigating the effects of tDCS stimulation of different types and current intensity on learning and memory, and little is known about the mechanism of action. Preclinical studies investigating behavioral and molecular mechanisms are needed to fully understand the mechanisms of action of tDCS. Therefore, in our study, the effects of anodal and cathodal stimulation types of tDCS and different current intensities of 0.25 and 0.5 mA on learning and memory in the hippocampal glutamatergic pathway were investigated behaviorally and molecularly. Mehrsafar et al. conducted a study on 12 male archers on the dorsolateral prefrontal cortex for 20 minutes (33). They showed that the application of 2 mA anodal tDCS caused an increase in archers to feel more energetic and decreased anxiety feelings such as tension and fatigue. In their study on humans, Bogdanov and Schwabe reported that the application of 1.075 mA anodal tDCS could be a potential new method to prevent working memory disorders caused by acute stress (34). Luo et al. (28) reported that 0.15 mA Anodal tDCS stimulation for 2 weeks improves spatial learning and memory in the early stage of Alzheimer's disease in transgenic mice. Yu et al. (31) showed that 0.1 mA and 0.2 mA repetitive anodal tDCS can improve spatial learning and memory dysfunction in Alzheimer's mice, depending on current intensity. Au et al. (29) showed that 25 min with a current intensity of 2 mA tDCS on the left dorsolateral pFC strengthens long-term learning and memory consolidation in aging and improves performance in multiple memory areas. Zhang et al. (32) reported that after 0.5 mA 15 min and 5 days of cathodal tDCS treatment, it did not affect motor functions, learning and memory ability, and had no effect on neurotransmitter levels. Roostaei et al. (30) applied 0.2 mA 20 min 2-day anodal and cathodal tDCS stimulation in their study and reported that 0.2 mA anodal tDCS was effective

on memory via dopaminergic pathway, but cathodal tDCS had no effect on memory. Our findings are in line with the results of Roostaei et al. (30), and it was determined that 0.5 mA anodal tDCS application increased the locomotor activation in the open field test, and learning and memory parameters in the object localization tests with y maze. In our study, it was demonstrated that 5-day 0.25 mA and 0.5 mA cathodal stimulation had a reducing effect on both learning and memory behavior experiment results and glutamate level, and this activity may also be related to the current intensity of tDCS. As a matter of fact, 0.5 mA 30 min 5 days tDCS was used instead of 0.2 mA 20 min 2 days tDCS used by Roostaei et al. (30) in our study, and it was evaluated that the application time and current intensity of tDCS are important in the therapeutic efficacy associated with tDCS application.

Glutamate, a metabolite in the glutamatergic pathway and the main excitatory neurotransmitter of the central nervous system, is involved in learning and memory processes and can be affected by tDCS; thus, they present themselves as potential biomarkers for tDCS-induced behavioral gains due to neuroplasticity processes (27). Studies have reported that tDCS stimulation on learning and memory is beneficial by affecting AMPA and NMDA in the glutamatergic pathway (2, 24). Therefore, it has been stated that tDCS application to strengthen learning and memory may be related to the glutamatergic system, which is known to play a role in its pathophysiology. Although the reason for the shorter duration of action of NMDA receptor antagonists is not yet known, it is thought to be caused by changes in glutamate levels in the synaptic gap. In our study, it was concluded that anodal tDCS can consolidate learning and memory via the hippocampal glutamatergic pathway.

CONCLUSION

These results suggest that tDCS may have an enhancing effect on learning and memory, possibly via the glutamatergic pathway. It is thought that future studies researching especially glutamate transporters and receptor levels, as well as behavioral experiments and glutamate levels, will make great contributions in order to better understand the effects of tDCS.

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Nurses' Covid-19 Vaccine Hesitancy: A Qualitative Study

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Abstract

Objectives: This qualitative study aimed to conduct an in-depth analysis of the opinions of nurses who did not receive the COVID-19 vaccine during the pandemic regarding the issue.

Methods: This research was designed as a qualitative research in the case study design and based on the guidelines proposed by the COREQ checklist. It was conducted in nurses working in various healthcare institutions and units providing preventive and therapeutic services. In the study, 10 nurses who did not receive the COVID-19 vaccine were interviewed. The data of the study were collected through an introductory "Information form" and "Semi-structured interview form" by conducting in-depth interviews. The data were analyzed by the content analysis method.

Results: In the study, 3 main themes and 8 sub-themes were determined after the thematic analysis. Themes were determined as follows: (1) Extreme skepticism (a. mistrust, b. rumor, c. anxiety), (2) Perceived risk (a. low risk perception, b. experiences), and (3) Self-others (a. individual freedom, b. inconsistency, c. perception of social benefit).

Conclusion: It was found that nurses refused to receive the COVID-19 vaccine, which is an important strategy in the fight against the pandemic, based on various reasons and inferences. Developing a standard recommendation for all nurses in the world on vaccine hesitancy can be difficult. But the strategic efforts to increase confidence in vaccines should focus on the invisible barriers to vaccine hesitancy.

Keywords: COVID-19 vaccines, nurses, qualitative study, vaccine hesitancy.

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INTRODUCTION

Vaccine hesitancy, which is considered to be one of the most serious problems of our age, is referred to as a delay or refusal in the acceptance of vaccines, although many comprehensive services on vaccination are carried out by the World Health Organization (1). Vaccine hesitancy is a major threat to public health and a long-standing global challenge (1-5). Vaccine hesitancy is also an important threat to the fight against the pandemic. The fight against the COVID-19 pandemic, which has become a global crisis, continues in all aspects. COVID-19 vaccinations, which contribute to the fight as an important force, are being carried out rapidly all over the world. However, studies on the subject have reported that there are some important problems with the acceptance of COVID-19 vaccines by healthcare professionals (4-9). As a matter of fact, in a scoping review examining COVID-19 vaccine hesitancy, it was determined that the prevalence of vaccine hesitancy varied between 4.3% and 72% among healthcare professionals (10). However, it is a known fact that if healthcare workers are infected, it affects not only their own health but also the health of the society, for which they provide services, causing disruptions in the healthcare service provision of countries (6-11). It is believed that the COVID-19 vaccine hesitancy and refusal of healthcare professionals will seriously hinder the fight against the pandemic (4,8,12,13).

It is important to understand the emotions, thoughts and behaviors of healthcare professionals, as they are at the forefront of combating epidemics and carry out various services such as vaccination (9, 14). In addition, the attitude of health workers towards vaccination; vaccination services are also considered important in terms of vaccination programs and reducing indecision about vaccination (15). WHO emphasized the important role of healthcare professionals, especially nurses, in influencing the public's trust in vaccines, and studies have revealed that nurses are an important role model in this regard (10, 15-16). International Council of Nurses (ICN) nurses' role in vaccination; It has reported that it covers a wide area such as awareness raising, public advocacy, active health education, reducing myths, administering vaccines, prescribing vaccines, supervising vaccination programs, consulting on immunization programs and strategies (17).

Studies conducted in nurses who are believed to have critical roles in vaccination and thus should be more cautious, similarly showed that vaccine acceptance rates were low (6,18). In a study carried out in Palestine, 40% of the nurses intended to get vaccinated, while 41% stated that they would get vaccinated only if adequate protection and safety measures were provided, and 18% stated that

they would never get vaccinated (5). In a study conducted in Turkey, it was found that 40% of the nurses were hesitant about getting the COVID-19 vaccine, while 14.3% of them did not consider getting the vaccine at all (19).

Opinions of nurses about vaccines can affect both public perception and acceptance of vaccines. In the light of this information, it is believed that it is a necessity to conduct studies on vaccine hesitancy among nurses. The aim of this study was to thoroughly examine the opinions of nurses who did not receive the COVID-19 vaccine during the pandemic regarding the issue.

Research Questions

What are the nurses' feelings, opinions, and actions about the COVID-19 vaccine?

METHODS

Study Design: This research was designed as qualitative research in the case study design. This study was conducted according to the guidelines of the COREQ checklist.

Participants and setting: The sample of this study included nurses serving in different healthcare institutions in a province of Türkiye. Nurses from institutions providing preventive and therapeutic services from urban and rural areas were included in the research in order to provide maximum data variety. Participants were selected by the purposive sampling method, taking into account the inclusion criteria of the study, and selection continued until data saturation was achieved.

A total of 10 nurses were interviewed in the study. The ages of these nurses were in the range of 21 to 43 years; 8 of them were women, 6 of them were single, 4 of them lived alone, and 9 of them did not have any chronic disease. It was found that 6 of the nurses were working in a department related to COVID-19, one was serving in primary care at the time, and 10 of them had provided care to COVID-19 patients while working in preventive and therapeutic services during different periods of the pandemic. It was determined that 7 of the participating nurses experienced COVID-19.

Data Collection Tools

In the research, data were collected with an introductory information form and a semi-structured interview form. The introductory information form includes questions about the nurses' socio-demographic characteristics such as gender, age, education level and job-related characteristics such as the department in which they were working, total years of working in the profession, and characteristics of the job. The semi-structured interview form includes questions about thoughts about COVID-19 vaccine, experiences with COVID-19 vaccine, views about hesitancy about COVID-19 vaccines.

Data Collection

The data were collected by the researchers between June 03 and June 13, 2021 using a personal information form and a semi-structured interview form that included open-

ended questions and evaluated the views of nurses on vaccine refusal. Due to the physical restrictions associated with the pandemic, the interviews were carried out on telephone or by a video conference application. Initial interviews with the individuals were conducted over the phone to obtain participants' consent, the participants were informed about the reasons and objectives of conducting the study, and the subsequent interviews were scheduled.

The participants were determined with the first author contacting the nurses who refused vaccination in personal interviews performed during clinical practices of students. Following the first participant found by the first author, the preliminary interviews were performed, and the other participants were contacted in turn. The participants were informed about the study and reminded that their interviews would be recorded by the first author. Interviews were conducted out of working hours, on days when the nurses were on leave.

Interviews were conducted by the fourth author, who is experienced in qualitative research. Audio or video recording was made after obtaining the permission of the participants. The interviews took approximately 35-40 minutes. Interviews were continued until data saturation was achieved and ended when data saturation was achieved. Care was taken to conduct the interviews in an online-at video conference where the researcher and the participant could see each other easily and there was no noise and interruption, which allowed for comfortable communication.

A semi-structured interview form was used to ensure that the questions were equivalent for all participants. Following the opening question, participants were asked six key questions: (1) "What do you think about the COVID-19 vaccine?"; (2) "What did you think when you first found out about the COVID-19 vaccinations in the world and in Turkey?"; (3) "What are your experiences with the COVID-19 vaccine?"; and (4) "When you consider the measures for prevention of COVID-19, where do you think is the place of vaccination among these measures?", (5) "What are your views on the factors that prevent you from getting the COVID-19 vaccine?", and (6) "What are your views on individuals who are hesitant about getting the COVID-19 vaccine?" They were also asked to respond to investigative questions such as "Can you clarify this?" and "How do you feel about this?". At the end of the interview, the question "Is there anything you want to add?" was addressed to the participants. The interviews were exactly transcribed by the interviewer, and the accuracy of the content was checked by both the interviewer and other authors.

Data Evaluation

Quantitative data in the personal information form were evaluated in the

computer and expressed in numbers. Qualitative data audiotapes were transcribed by the researchers. A total of 371 pages of interview text served as the raw data for analysis. Qualitative data were analyzed by content analysis. The data of the study were divided into categories by two different researchers via coding, and then themes and sub-themes were created by revealing the relationships between the categories. Expert opinion was sought from two independent researchers with qualitative research training and experience regarding the validity of the themes and sub-themes. After obtaining the expert opinions, unnecessary codes were removed, the ones connected to each other were regrouped, the main idea in the statements was identified, and the themes and sub-themes were finalized. Themes were supported with direct citations when necessary. Citations were shown by participant's number and age (*P1-36, P2-32* etc.).

Credibility and Trustworthiness of Qualitative Data

Long interviews, participant confirmation, and expert review methods were used to ensure the credibility of the study. For participant confirmation, the data obtained by the researcher were summarized to the individuals and they were asked to state their opinions about the accuracy of these data at the end of the interview. In addition, the participants were asked whether they had an opinion that they would finally like to add. The additional statements were recorded and the interview was ended. During the planning phase of the study, expert opinion was sought regarding the questions in the interview form and the themes created. Thus, attempts were made to ensure credibility by seeking expert opinion from the beginning to the end of the study. Researcher triangulation and methodological triangulation were used to ensure trustworthiness in the study. For the sake of confirmability, the interview notes and notes regarding the statements of the participants were taken during the interview as raw data, and the statements of the participants were directly included in the study report. The fact that the study results obtained from the interviews with this sample group can be used in different settings and in similar sample groups ensures transferability.

Reflexivity

The self-reflective knowledge of the researchers in this study was as follows: the first author had scientific research experience on immunization and COVID-19 vaccine acceptance of nurses. It is believed that the fact that the researcher conducting the interviews was a psychiatric nurse contributed to effective communication with nurses and to the sustainability of the interviews. All researchers work in the nursing departments of different universities in Turkey.

Ethical Consideration

Before starting the research, the approval of the Scientific Research Board of the Ministry of Health (2021-05-22T08_10_09) and the ethics committee approval (Decree no: 2021/130) from Ordu University were obtained. The purpose of the study was explained to the participants individually and approval was obtained from the participants for participating in the study. The names of the participants were kept confidential, and the participant number and age were indicated instead of name in the statements of the individuals (*P10-33* etc.).

RESULTS

Following the thematic content analysis, 3 main and 8 sub-themes were determined in the study. Main themes were: "Extreme skepticism", "Perceived risk", and "Self-others". The themes and sub-themes obtained in the study were presented in Table 1.

Table 1. Themes emerging from the interviews

Thema	Subthema
Extreme Skepticism	Mistrust Rumor Anxiety
Perceived risk	Low risk perception Experiences
Self-others	Individual freedom Inconsistency Perception of social benefit

Theme 1. Extreme Skepticism

a. Mistrust: It was observed that almost all of the nurses in the study experienced many different mistrust issues related to the COVID-19 vaccine. It has been determined that the most common mistrust issue was related to vaccine manufacturers, companies, vaccine trials they conducted, and politicians.

"Childhood vaccines were developed over a long period of time, but this happened very quickly Also, vaccine companies do not accept responsibility for deaths caused by the vaccines, and there is a clause in consent forms regarding this" (P7-42).

"Actually, I also thought it was a game of the pharmaceutical industry……." (P10-33).

"I don't find vaccines very safe. Foreign countries found the vaccine, and the disease had emerged from them, too. That's why I feel mistrustful. That's why I haven't received it myself. I can't see what it will bring me in 10 years from now. The fact that the virus emerged from foreign countries and the vaccine was also found in foreign countries reduced my trust in the vaccine." (P2-43).

b. Rumor: It was observed that some of the participants believed the rumors they heard about the COVID-19 vaccine and took them seriously. It was also found that these rumors, the source of which is unknown and which have been understood to have been heard from people around, were generally related to foreign countries, vaccines, vaccine manufacturers, and the virus.

"The foreign films I watched also had an effect. The rumors that the world population has increased too much and that this virus was

made to reduce it affected me" (P2-43).

"The vaccine companies or the people who claim to have found the vaccine do not get vaccinated themselves" (P7-42).

c. Anxiety: During the interviews, it was observed that most of the nurses often expressed their anxiety about vaccination. It was found that this anxiety was mostly related to fertility and having children in the future.

"I haven't considered it, because I am planning to get married and have children. Let's say I have been vaccinated and I get pregnant right after that, will there be any problems during my pregnancy or will it harm the baby? There has been no study about this. My fear concerns this issue" (P1-41).

"...I am an individual of childbearing age and I do not know what effect the vaccine will have on me in the future. What if I become infertile, what do I do then?" (P3-25).

"Yes, I believe in the protection of the vaccine. I think it is protective, but I do not know what effect the vaccine will have on me after 5-10 years" (P3-25).

Theme 2. Perceived Risk

a. Low risk perception: The risk perception of the nurses was evaluated to be low for various reasons. It was observed that the perceived risk of some of the nurses was low because they had already had COVID-19 and had antibodies.

"The reasons why I didn't get vaccinated are because I am young, I don't have a chronic disorder, I live alone, and I pay attention to mask and hygiene issues" (P9-21).

"So, how is a vaccine made against a virus that is constantly mutating? We have now found the vaccine, but this virus has already mutated." (P7-42).

"...For me, the vaccine comes after mask and distance, it is of secondary importance, we must first take our individual precautions. I believe that the vaccine will prove to be less important than the trio of mask, distance and hygiene" (P5-30).

b. Experiences: Most of the nurses reported that they were affected by their experiences with individuals who had the disease although they were vaccinated, and this pushed them not to get vaccinated.

".... I met a lot of people who got vaccinated but got sick. There were people who got sick even though they had received double doses of the vaccine" (P8-26).

Theme 3. Self-Others

a. Individual freedom: During the interviews, it was observed that some of the nurses emphasized the importance of individual freedoms regarding the issue of not getting vaccinated.

"I read a news article about classifying individuals into who were and were not vaccinated, and I was sad to hear that. This is because it is our decision whether to get vaccinated or not. Our free will" (P3-25).

"For example, it is very strange that the

Ministry of Health puts directives in front of us to get vaccinated… It seems to me that people are obligated rather than choose to get vaccinated, because people are forced to get vaccinated and there are statements such as those who do not get vaccinated cannot enter such and such places" (P4-32).

b. Inconsistency: It was observed that the nurses made quite inconsistent statements, had ups and downs, and made contradictory speeches while expressing their opinions on vaccination and vaccine counseling.

"I can't believe the reality of the vaccine…. Accordingly, I thought vaccination was more important than wearing masks. It is good for COVID-19, but we don't know what it may cause in the future. Vaccination may be a little more important than others for now"(P6-26).

c. **Perception of social benefit:** During the interview, it was found that nurses' perceptions of social benefit with respect to vaccination and counseling varied.

"I leave people who are hesitant to their own devices. I say whatever you feel comfortable with. And there have been people whom I asked whether they ever had covid, or told to determine the risks before getting vaccinated and the risks after getting vaccinated about catching covid and decide accordingly"(P6-26).

DISCUSSION

It has been observed that one of the main reasons behind vaccine refusal of the nurses is excessive skepticism. In the study, all nurses stated that they had a mistrust of the COVID-19 vaccine for various reasons, and this mistrust made them skeptical. In a qualitative study related to the COVID-19 vaccine, healthcare professionals stated that they believed government decisions regarding vaccination were not supported by evidence-based studies, and this lowered their trust in the COVID-19 vaccination program (8). In this study, some of the nurses stated that they did not trust pharmaceutical companies, scientists, the government, politicians, foreign countries, and this mistrust pushed nurses to look for untrue things in everything that was done.

Another reason that made nurses extremely skeptical was their reliance on rumors about the COVID-19 vaccine. In a study conducted in Switzerland, it was found that nurses tended to believe in practices related to traditional health beliefs rather than evidence-based medicine in their decision to refuse the flu vaccine (20). In a study on the COVID-19 vaccination program, it was found that misinformation spread on the internet affected the attitudes of healthcare professionals towards vaccination (8). In this respect, it has been reported that social media is a significant component for the acceptance of the COVID-19 vaccine (21). In this study, the majority of nurses stated that they believed in the rumors about the source of the virus, manufacturing

process of the vaccine, and the long-term effects of the vaccine, which were shared especially on social media and which did not have any scientific basis.

In the study, it was observed that the last reason that made nurses skeptical about the COVID-19 vaccine was anxiety. In a study in Philadelphia, it was found that more than 80% of healthcare professionals experiencing vaccine hesitancy were anxious about the side effects of the vaccine and the novelty of the vaccine (4). Other studies in healthcare workers have found that reasons for rejecting the COVID-19 vaccine include concerns about the safety and side effects of the COVID-19 vaccine (5, 22). Similarly, in our study, the majority of nurses stated that they were concerned about the side effects of vaccines in the short and long term. In this study, it was determined that some of the concerns of the nurses increased their skepticism. One of them was the statement that the vaccine negatively affects fertility. It was observed that some female nurses were skeptical of the vaccine because they were worried about being infertile and thought that it would affect their fertility. In this respect, it was observed that the nurses stated that they were worried based on a rumor, that they felt they had to protect their fertility and wanted to have children.

Previous research shows that a number of cultural factors, including politics and religion, are significantly associated with anti-vaccine attitudes (23). As a matter of fact, in the study, the roles expected from the gender, women feel obliged to protect their fertility and meanings attributed to childbearing It was thought to be related to the cultural reasons underlying the anti-vaccination. In the study, some nurses stated that they believed in the protection of the vaccine but were worried about its possible long-term effects.

Individuals who believe that the severity of COVID-19 has been overdone may perceive the vaccine risk as greater than the risk of infection (3). In this study, one of the factors affecting the attitudes of nurses towards the COVID-19 vaccine was their perceptions of the disease and virus risk. It was found that nurses see themselves at a lower risk than other individuals in society due to reasons such as having had the disease, being young, and not having a chronic disease. There has been evidence of greater acceptance of the vaccine among healthcare workers caring for hospitalized COVID-19 patients, possibly due to an accurate perception of the severity of the disease (10,24). The nurses in our study worked in different departments, and although all provided care to COVID-19 patients and some even worked in the COVID-19 intensive care unit, they refused to get vaccinated. Similarly, the fact that nurses stated that they were hesitant about vaccination based on their experiences of losing their patients who had been vaccinated, suggested that their

experiences played a role in their decisions. In a qualitative study involving healthcare professionals from European countries, negative experiences of healthcare professionals were similarly found to have had a role in their decisions on vaccination (11). This suggested that although nurses took an active role in healthcare services during the pandemic, they could not follow scientific studies, visual or written events, and reports on COVID-19.

In this study, it was found that some participants believed that masks, distance, and hygiene measures are more effective than vaccination, they prefer natural immunity to acquired immunity, and a more considerable amount of immunity will be provided than that to be provided by the vaccine if the majority of the society is infected with the virus. However, it has been noted in studies that waiting for herd immunity would increase morbidity and mortality (25,26). For example, although a large part of the population (76%) was infected with the virus in the Brazilian city of Manaus, it has been reported that the city's healthcare services were shattered and the city turned into a grave due to the population structure, poverty, and non-pharmaceutical interventions (26).

Globally, data from seroprevalence studies show that less than 10% of workers have been infected, meaning that the vast majority of the world's population remains susceptible to this virus (27). It has also been demonstrated by scientific results that the vaccine, which provides acquired immunity, is highly effective (the only method to achieve success) in the fight against COVID-19 (28,29). In this respect, it can be said that the reason behind the low-risk perception of the nurses regarding COVID-19 is that they deal with the issue at an individual level and express their opinions based on experiences, and not on scientific results.

The study of Chankod et al. showed that COVID-19 vaccine hesitancy and the decision to vaccinate against COVID-19 involve ongoing and unresolved internal conflicts about COVID-19 vaccines (30). The fact that it is an important inconsistency that nurses did not receive the vaccine themselves, although they referred to the vaccine positively during the interviews. In a study conducted with healthcare professionals in Turkey, it was found that 17% of them still did not have the COVID-19 vaccine despite having a positive attitude towards the vaccine (31). It was remarkable that participants emphasized their individual freedoms among the key reasons underlying their refusal to get vaccinated and made inconsistent statements. This suggested that nurses experienced a dilemma of individual freedom versus social welfare. Although such libertarian and anti-paternalist arguments are often cited regarding vaccine refusal, it has been reported that these

arguments will be invalid considering the deadly threat that vaccine-preventable diseases pose to the whole society (32).

In the literature, nurses are the most major source of information for patients who make decisions about vaccines in the fight against epidemics (33,34). In a study on the COVID-19 vaccine, the majority of healthcare professionals stated that they felt encouraged to motivate their patients to get vaccinated, advocate vaccination, or would join conversations about vaccination with others (8).

In another study, it has been shown that the acceptance of the vaccine by only ½ of the healthcare professionals, whom the society sees as role models, will affect the success of controlling the COVID-19 pandemic (13). In this study, it was shown that the participants refrained from expressing their opinions on vaccinating the society and the individuals they counseled and adopted different attitudes. The fact that some of the nurses stated that the choice of getting vaccinated should be left to the individual and that it is a right to choose this suggested that they ignored the social aspect of vaccination. In addition, the fact that a few nurses stated that whether or not to get vaccinated is an individual decision was another indication that suggested that they ignore the social aspect of the issue. In addition, the fact that the nurses stated that individuals should find out and decide about the vaccine on their own suggested that the negative attitude of the nurses towards the vaccine makes it difficult for them to fulfill their educational and counseling roles on vaccines.

Limitations

The study was limited to this sample group, because it was conducted in one province of Turkey and with nurses who could be contacted.

CONCLUSION

In this study, it was found that the nurses were overly skeptical of the COVID-19 vaccine and their risk perceptions were affected for various reasons. It is believed that the factors collected under these two themes cause nurses to ignore the social benefit and refuse to get vaccinated. It has been observed that this attitude is not only limited to the refusal of nurses to get vaccinated, but also has negative consequences on their duty of providing counseling and education to the society as healthcare professionals, and that the nurses make their decision based on the self and others approach.

It is important for nurses to experience vaccination hesitancy, both in terms of maintaining health services and affecting the education and counseling services they provide. Therefore, in the fight against vaccine hesitancy; it is important to develop effective and transparent health and education policies covering the whole society, especially for

health workers and nurses. It is recommended to support activities for nurses to follow the literature, read and evaluate research results, and participate in research, especially about vaccine applications and vaccine indecision, and to plan evidence-based practices.

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May the Systemic Immune-Inflammation Index be an Indicator of Premature Ovarian Insufficiency?

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Abstract

Objective: This study aimed to determine whether there was a correlation between the systemic immune-inflammation index and ovarian reserve markers such as follicle stimulant hormone, estradiol, and anti-mullerian hormone

Methods: The study comprised 65 people with premature ovarian insufficiency and 71 controls with similar demographics. The concentrations of hemoglobin, hematocrit, platelets, white blood cells, neutrophils, and lymphocytes were evaluated. The neutrophil leukocyte ratio, platelet lymphocyte ratio, and systemic immune-inflammation index were calculated. The antral follicle count reserves of all patients were evaluated by transvaginal ultrasonography. An independent t-test was used for the comparison of the study and control groups. Correlations between variables were analyzed using Pearson's correlation test. A p value of 0.05 was considered significant.

Results: The results of the neutrophil-to-lymphocyte ratio and the platelet-lymphocyte ratio showed a significant difference between the groups ($p = 0.043$). The Systemic Immune Inflammation Index value was the statistically significant difference found between the groups. There was a significant positive correlation between the systemic immune-inflammation index, neutrophil-to-lymphocyte ratio, platelet-lymphocyte ratio, and follicle stimulant hormone, while a significant negative correlation was found between the systemic immune-inflammation index, neutrophil-to-lymphocyte ratio, platelet-lymphocyte ratio, antral follicle count, and anti-mullerian hormone. In ROC analysis for SII at a cut-off level of 441.35, the sensitivity was 72.1% and the specificity was 68.9.

Conclusion: Our study was the first in this field to reveal the relationship between premature ovarian failure and the systemic immune-inflammation index. According to our study results, the systemic immune-inflammation index, neutrophil-to-lymphocyte ratio, and platelet-lymphocyte ratio are significantly higher in individuals with ovarian failure.

Key words: Complete blood count, neutrophil-to-lymphocyte ratio, systemic immune-inflammation index, platelet-lymphocyte ratio, premature ovarian insufficiency

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INTRODUCTION

Indirectly measuring ovarian reserve is possible by the analysis of hormone levels or ultrasound imaging of the ovaries (1). The quantity of oocytes in an ovary is considered its ovarian reserve. The average number of oocytes in a female at birth is between half a million and one million. With follicular atresia and ovulation, the number of oocytes begins to deplete over time, followed by menopause. Although ovarian reserve declines with age, even among women of the same chronological age, there is a wide range in ovarian reserve (2). Early follicular phase assessment of follicle stimulant hormone (FSH), estradiol (E2), and inhibin B, measurement of anti-mullerian hormone (AMH) independent of cycle day, and a clomiphene citrate challenge test are biochemical measures of ovarian reserve (1).

In women under the age of 40, diminished ovarian function is called primary ovarian insufficiency (POI), and it appears as oligomenorrhea or amenorrhea, subfertility or infertility, depletion of residual follicles in the gonads, decreased estradiol levels, and increased FSH levels (3,4). POI is characterized by steroid deficiency (5). By blocking several pro-inflammatory immune pathways and inflammatory tissue responses, estrogen exerts its anti-inflammatory effect. Decreased estrogen levels result in a change in the direction of inflammation (6).

There are a few well-known hematological indicators of systemic inflammation that correlate with markers of a pro-inflammatory state, including the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR). The systemic immune-inflammation index (SII) is a new inflammatory marker that combines NLR with platelets (7–11). There are studies reporting that it is used in SII premature rupture of membranes, in demonstrating the prognosis of tumors, and in the follow-up of cranial hemorrhages (7–10).

This study aimed to determine whether SII is related to ovarian reserve tests and whether it can be used as a valid indicator in the diagnosis of POI.

METHODS

In this retrospective study, 136 women who were followed up in the gynecology polyclinic of a tertiary Training and Research Hospital between January 2018 and July 2019 were included. The study protocol was approved by the local ethics committee of our hospital. Patient file data were accessed from the hospital's digital archive system. All techniques performed on human subjects in studies conformed to the ethical norms of the institutional and/or national research committee and to the 1964 Helsinki

declaration and its later revisions or other equivalent ethical standards.

Medical diseases and conditions that may affect ovarian reserve, such as pregnancy, polycystic ovarian syndrome, chronic medical diseases, endocrine disorders, excessive exercise, poor caloric intake, pituitary or hypothalamic adenomas, chemotherapy, radiotherapy, and ovarian surgery, were excluded. Hematological, cardiovascular, renal-liver illness, asthma, arthritis, neoplastic disorders such as androgen-secreting tumors, ovarian tumors, glucocorticoid usage, infectious, and parasitic diseases were also ruled out since they could impact the results of a complete blood count.

The study comprised 65 women with POI and 71 controls with similar demographics. POI was diagnosed in women younger than 40 years of age with at least 4 months of amenorrhea, FSH levels ≥ 40 mIU/milliliter, and no or few follicles on transvaginal ultrasonography. All POI patients had 46 XX karyotypes.

The concentrations of hemoglobin, platelets, neutrophils, and lymphocytes were evaluated. The NLR was determined by dividing neutrophils by lymphocytes. Calculating PLR included splitting platelets into lymphocytes. SII was calculated using the neutrophil-platelet-lymphocyte formula.

Demographic data such as age, body mass index, and gravida for all cases were recorded. Ovarian reserves of all patients were evaluated by transvaginal ultrasonography (Samsung HS70) between the second and fifth days of menstruation. Follicles between 2 and 10 mm in both ovaries (antral follicle count) were counted and recorded. AMH and FSH were studied from 10 cc of antecubital venous blood. AMH levels, 3rd-day hormone profiles, and total blood counts of all individuals were studied. The NLR, PLR, and RPR values of both patients and controls were determined. An automated hematology analyzer was used to measure the complete blood count (Beckman UniCel DXL 600 Coulter Cellular, California, United States). The electrochemiluminescence immunological test (CLIA) was used to measure the serum AMH level (Beckman UniCel Dxl 600 Immunoassay, California, United States).

Statistical Analysis

SPSS version 23.0 was used for statistical analysis (SPSS, Chicago, IL, USA). Data were given as mean + standard deviation (SD). The compatibility of the data set with the normal distribution was confirmed by the Kolmogorov-Smirnov test. An independent t-test was used for the comparison of the study and control groups. Pearson's correlation test was used to examine correlations between variables. The confidence interval for the difference analysis was 95%, and the confidence interval for the correlation analysis was between 95% and 99%. A p value of 0.05 was regarded as

statistically significant. The ROC curve was analyzed to determine the best threshold for SII.

RESULTS

There were a total of 130 participants, including 65 people with POI and 65 healthy controls. In terms of age, body mass index, neutrophils, lymphocytes, and platelets, there was no significant difference between the groups ($p > 0.05$) (Table 1). The comparison of FSH, AMH, and AFC revealed, as was to be expected, that there was a difference between the groups (p 0.05). NLR was determined by dividing the mean of the neutrophils by the mean of the lymphocytes, and the results showed that there was a significant difference between the groups ($p = 0.043$). There was a significant difference between the groups in the PLR that was obtained by dividing the mean of the platelets by the mean of the lymphocytes ($p = 0.041$) (Table 1). To obtain SII, the neutrophil x platelet / lymphocyte formula was used, the SII value was calculated, and A statistically significant difference was found between the groups. There was a significant positive correlation between SII, NLR, and PLR and FSH, while a significant negative correlation was found between SII, NLR, and PLR and AFC and AMH (Table 2). In ROC analysis for SII at a cut-off level of 441.35, the sensitivity was 72.1% and the specificity was 68.9 (Figure 1).

Table 1. The table shows the demographic data of the groups

	Study (n=65)	Control (n=71)	P value*
Age (year)	33.71±4.12	34.40±3.93	0.228
BMI (kg/m^2)	25.31±4.12	24.90±4.02	0.199
Hemoglobin (g/dL)	12.18 ± 1.89	11.89 ± 1.73	0.213
Neutrophile count (10e3/uL)	3.75 ± 1.30	3.43 ± 1.17	0.143
Lymphocyte count (10e3/uL)	2.24 ± 0.52	2.43 ± 0.57	0.164
Platelet count (10e3/uL)	257.81 ± 53.25	244.70 ± 57.78	0.112
FSH (mIU/ml)	23.56 ± 3.89	7.19 ± 1.29	***0.001***
AMH (mIU/ml)	0.75 ± 0.21	3.10 ± 1.43	***0.001***
AFC	2.25 ± 0.35	9.23 ± 3.27	***0.001***
NLR	1.69 ± 0.68	1.41 ± 0.61	***0.043***
PLR	118.61 ± 32.85	103.54 ± 21.25	***0.041***
SII	433.45 ± 61.23	348.45 ± 63.72	***0.021***

Data is presented mean ± std. *Independent t test. AMH: Anti-müllerian Hormone, FSH: Follicle stimulating hormone, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio, SII: Systemic Immune-Inflammation Index.

Tablo 2. Correlations between ovarian reserve tests and inflammation markers

	FSH		AMH		AFC	
	r	p	r	P	r	p
NLR	0.32	***0.042***	-0.24	***0.023***	-0.31	***0.032***
PLR	0.21	***0.052***	-0.19	***0.051***	-0.21	***0.021***
SII	0.41	***0.001***	-0.45	***0.002***	-0.28	***0.005***

AMH: Anti-müllerian Hormone, FSH: Follicle stimulating hormone, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio, SII: Systemic Immune-Inflammation Index.

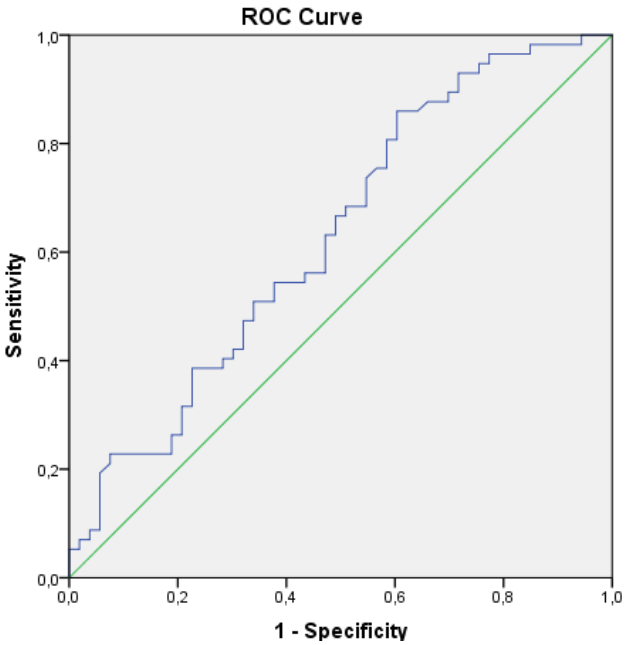

Figure 1. ROC curve. AUC:0.630 (95% CI: 0.525–0.734). (AUC:area under the curve, CI: confidence interval, ROC:receiver operating characteristic).

DISCUSSION

To our knowledge, this study provides the first information about POI and its effect on NLR, PLR, and SII levels. According to our findings, it increases SII in POI. This is the first study in the literature examining the relationship between SII and POI.

There are studies reporting that inflammation reduces ovarian reserve (12). When the inflammatory process in the ovary was reduced, the reproductive window was prolonged, and ovarian aging was postponed, according to a study conducted on animals to investigate the potential influence of inflammation on ovarian reserve. In parallel, it has been shown that rising inflammation in the ovary consumes the follicles over time and causes ovarian aging (12).

Ilhan et al studied the relationship between NLR, PLR, and RDW and premature ovarian failure. They measured FSH and AMH for the diagnosis of ovarian reserve. They found a positive correlation between NLR and FSH and a negative correlation between NLR and AMH (13). Although there was a positive correlation between NLR and FSH and a negative correlation between NLR and AMH, similar to theirs, in our study, we studied a new inflammation index, SII, and added AFC to ovarian reserve tests.

NLR has been frequently used to evaluate the intensity of inflammation and has been demonstrated to be raised in a variety of disorders (14–21). PLR was identified as an independent risk factor for decreased survival

in individuals with a variety of cancers (22–25). Some studies have evaluated the value of some blood cell count indices, especially NLR and PLR, and reported that these biomarkers may be related to POI (3,13).

Although an increased FSH level is a reliable indicator of decreased ovarian reserve, it cannot reveal an earlier loss in ovarian reserve. AMH levels begin to decrease before FSH levels begin to increase (26). When compared to levels of early follicular phase hormone, AMH levels are a more sensitive indicator of ovarian reserve than those levels. Numerous studies have concluded that AFC and AMH are equally effective in providing evidence of ovarian reserve (27). As a result, when conducting research on ovarian reserve tests, we examined not only FSH but also AMH and AFC to determine how closely these factors are related to inflammation.

In the etiopathogenesis of POI, one can identify genetic abnormalities, metabolic illnesses, autoimmune conditions, iatrogenic events such as chemotherapy and infections, and environmental variables. Idiopathic describes conditions for which the etiological origin is uncertain (28). Recent research has demonstrated that inflammation plays a crucial role in the etiology of POI. Inflammatory cells linked with lymphocytic infiltration and other immune responses have been demonstrated in ovarian biopsies (12,29). Aging causes the appearance of inflammatory cells in the ovaries, which play a significant role in idiopathic and unknown cases (30). In addition, studies have shown that lower estrogen levels stimulate proinflammatory processes, whereas estrogen supplementation reduces inflammatory cell levels (6).

Demir et al. reported that 47 patients with POI had substantially higher than average white blood cell and MPV values, platelet counts, and lymphocyte counts. However, they did not report significant differences between groups for NLR and PLR. They also did not detect any correlation between blood parameters and hormone levels. In our results, NLR, PLR, and SII were significantly higher in the POI group (31).

Ovarian aging causes a decline in estrogen production and an increase in inflammatory mediators. Thus, the ovarian reserve is diminished, and the number of inflammatory cells increases. When the ovarian reserve diminishes, fertility declines. The effectiveness of in vitro fertilization may even be predicted by measuring inflammatory indicators in the patient's complete blood count, according to some research (32).

One of our limitations was the retrospective nature of our study and the relatively small number of cases. In addition, although diseases and conditions that may affect the CBC's inflammation markers have been excluded, they may have been affected by unpredictable conditions. Another limitation was that the

ultrasonography image can be affected by conditions such as weight, gas, device resolution, and the interpretation of the person performing the measurement in the AFC count. Our research results should be supported by a large number of prospective studies.

CONCLUSION

In conclusion, our study was the first in this field to reveal the relationship between premature ovarian failure and SII. According to our study results, SII, NLR, and PLR are significantly higher in individuals with ovarian failure. It can be used as a marker for the diagnosis of POI when supported by well-designed, forward-looking, large-scale studies.

Ethics Committee Approval: Ethics committee approval was received for this study from local ethics committee at SBU Trabzon Kanuni Training and Research Hospital with file number 2019/48.

Peer-review: Externally peer-reviewed.

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Hypernatremia in Critically Ill COVID-19 Patients: Is it a manifestation of COVID-19 or acquired in the ICU?

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Abstract

Objective: It has been noted that COVID-19 patients experienced electrolyte problems more frequently, and these disturbances were linked to unfavorable results. The purpose of this study was to investigate the incidence and consequences of hypernatremia in severely ill COVID-19 patients receiving intensive care (ICU).

Methods: Retrospective data analysis was done on COVID-19 patients who were admitted to ICUs over a six-month period at two centers.

Results: Data from 270 patients were collected in total. 138 (51%) patients developed hypernatremia (Na >145 mmol/l) during ICU stay. Hypernatremia was observed to be more in older or ventilated patients, whereas less in patients with chronic kidney disease. However, in patients with and without hypernatremia, unfavorable outcomes like length of stay (LOS) or mortality were comparable. Frequency of hypertension, septic shock as well as SOFA score, and serum BUN levels were significantly higher in moderate to severe hypernatremic (Na ≥150 mmol/l) vs mild hypernatremic (Na=146-149 mmol/l) group. Moderate to severe hypernatremia had worse prognosis than the mild group: ICU LOS (12 vs 9-day, p=0.033), ICU mortality (86% vs 61%, p=0.001 and 28-day mortality (89% vs 68%, p=0.004). Elevated serum BUN levels and moderate to severe hypernatremia were independent predictors of both ICU and 28-day mortality.

Conclusion: Critically ill COVID-19 patients experienced hypernatremia more frequently than expected, suggesting that hypernatremia may be a manifestation of systemic involvement of COVID-19 rather than iatrogenic. Patients with and without hypernatremia were found to have similar outcomes.

Key words: COVID-19, hypernatremia, critically ill patient, mortality.

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INTRODUCTION

It has been well established that that Coronavirus disease (COVID-19) affects many organs, including the lungs, heart, blood vessels, brain, liver, kidney, skin, gastrointestinal system, and eyes. However, recently, electrolyte disturbance associated with the disease has also been described. Among these, sodium disorders are the most frequently reported. In noncritically ill hospitalized patients, hypernatremia is uncommon, with prevalence rates of 0–2% at the time of admission and 1% for patients who develop it while being treated there. Hypernatremia is, nevertheless, a disorder that affects critically sick patients in ICUs far more frequently. Up to 6% of patients admitted to the ICU had hypernatraemia already (1, 2). In the literature, the frequency of hypernatremia in ICUs varies between 6-26% (3), while this rate rises up to 50% for COVID 19 patients (2). One of the potential mechanisms put forth is that the renin-angiotensin-aldosterone system (RAAS), which is increased and activated by the Angiotensin II molecule and cannot be metabolized due to virus invasion of the angiotensin-converting enzyme 2 (ACE2) receptors, increases potassium excretion and sodium reabsorption (3).

In COVID 19 patients, hypernatremia has been linked to an increased need for a ventilator, a prolonged stay in the ICU, a decline in mental health, and an increased mortality rate (4). When the serum sodium concentration is above 150 mmol/L, mortality of up to 48% has been reported (3, 5). However, the literature about sodium disturbances and its relationship with COVID-19 is limited. The aim of the study was to determine the prevalence and clinical consequences of hypernatremia in critically ill COVID-19 patients. It also sought to investigate whether hypernatremia is associated with COVID-19 itself or is acquired in ICU.

METHODS

Patients and study design

Patients with COVID-19 pneumonia who were admitted to the medical ICUs of Ondokuz Mayıs University Hospital and Ondokuz Mayıs State Hospital between 01.10.2020 and 31.03.2021 were enrolled for the study. Clinical and laboratory data were taken out of each patient's electronic medical record. Patients who developed hypernatremia during the intensive care follow-up were analyzed retrospectively. Those who did not develop hypernatremia were considered as the control group. Control group included both patient with normonatremia or hyponatremia. A serum sodium level over 145 mmol/l was considered hypernatremia. Despite hypernatremia being classified as mild (146-149 mmol/l) moderate (150-159 mmol/l) and severe (above 159 mmol/l) (6), in this study patients with hypernatremia were split into two groups for this study: those with mild hypernatremia (146-

149 mmol/l) and those with moderate to severe hypernatremia (150 mmol/l) (7). The time point for the first peak of hypernatremia during ICU stay was determined. Accordingly, serum sodium peak level (Na-peak) and time from ICU admission to first Na-peak level were recorded.

The following were the exclusion criteria: Below the age of 18, readmission to ICU, negative polymerase chain reaction (PCR) for COVID-19, ICU stay shorter than 48 hours, the time interval between COVID-19 diagnosis and ICU admission longer than 14 days, presence of the moribund state. COVID-19 diagnosis time was considered as the date of COVID-19 PCR positivity.

The study been approved by the Turkish Republic Health Ministry General Directorate of Health Services, COVID-19 Scientific Research Evaluation Commission, and Ondokuz Mayıs University Ethics Committee (Approval number: 2021/213). Since the study was retrospective, informed consent was waived.

Data Collection

Demographic, clinical, and laboratory parameters at admission were recorded. These data were as follows: age, gender, place of admission (ward, emergency department); comorbidities including hypertension (HT), cardiovascular disease, obstructive pulmonary disease, chronic kidney disease (CKD), malignancy, cerebrovascular disease; severity of disease represented by the Acute Physiology and Chronic Health Evaluation II (APACHE II) score and the sequential organ failure assessment (SOFA) score; medications including corticosteroid therapy, thiazide, and loop diuretics; biochemical parameters including blood urea nitrogen (BUN), creatinine, C-reactive protein (CRP) and, serum glucose level; blood gas analysis variables including pH, partial pressure of arterial carbon dioxide (PaCO2) and standard base excess (SBE). Interventions at admission or during ICU stay such as invasive mechanical ventilation (IMV), administered intravenous fluid therapy, and renal replacement therapy (RRT), complications developed at admission, or during ICU stay such as septic shock or acute kidney injury (AKI) were also recorded. Outcomes were determined as hospital length of stay (HLOS), ICU- LOS as well as ICU and 28-day mortality.

Kidney Disease: Improving Global Outcome (KDIGO) guidelines were used to define AKI (8). The presence of fluid-refractory hypotension needing vasopressor therapy together with accompanying tissue hypoperfusion (lactate > 2 mmol/L) was described as septic shock (9).

We divided steroid therapy into two options high dose (≥ 40 mg of methylprednisolone or ≥ 6 mg of dexamethasone per day) and pulse dose (≥ 250 mg of methylprednisolone per day for at least 3 days) (10). Since liberal fluid delivery is

linked to poor clinical and organ-specific outcomes in critically ill patients, restrictive fluid strategy is used to prevent positive fluid balance in ARDS patients. Patients in our practice who cannot or should not receive enteral feeding only received intravenous dextrose 30% fluid as hypocaloric nutrition.

Statistical Analysis

The SPSS 21 program was used to analyze the data.Categorical data were presented as frequencies and percentages. For numerical data that was normally distributed, mean and standard deviation were used; for non-normally distributed data, median and interquartile ranges were used. Comparisons between categorical variables were made with Chi-square (x2) or Fisher's exact test. The Student's t-test for normally distributed independent variables and the Mann Whitney U test for non-normally distributed independent variables were both used to compare continuous variables. In order to determine the independent predictors of hypernatremia, binary logistic regression analysis was used to examine the significant factors with a p-value 0.25 in univariable analysis. Among the hypernatremic patients, subgroup analysis was performed between mild and moderate to severe hypernatremic patients to identify the risk factors for mortality and severity of hypernatremia using multivariable logistic regression analysis. Continuous variables which are independently related with ICU and 28-day mortality were assessed using receiver operating characteristics (ROC) and areas under curve (AUC). Cut-off values were calculated for sensitivity and specificity. The treshold for statistical significance was set at P 0.05.

RESULTS

270 patients in total were included at two centers during six months of hospitalization.138 (51%) of them developed hypernatremia during their ICU stay. Baseline characteristics of patients with and without hypernatremia are shown in table. In the group with hypernatremia, the median age was older (71 vs. 68 y, p=0.041). Among the comorbidities, CKD was more common in patients without hypernatremia (18 vs 9%, p=0.036) than in patients with hypernatremia. The rates of patients who underwent intubation and IMV were higher in hypernatremic patients than in those who did not (79 vs 67%, p=0.023). Other variables along with hospital and ICU outcomes including LOS and mortality were comparable between the two groups (Table-1). On multivariable logistic regression analysis, both advanced age [adjusted OR, 1.02; 95% CI, 1.01-1.05; p=0.030] and IMV [adjusted OR, 1.77; 95% CI, 1.02-3.08; p=0.044] were independent risk factors for hypernatremia whereas presence of CKD [adjusted OR,0.41; 95% CI, 0.20-0.87; p=0.019] was associated with a lower risk of hypernatremia compared with the absence of CKD. Subgroup analysis

Table 1. Baseline characteristics of the study patients and risk factors for development of hypernatremia

Variables[a]	All patients N=270	Patients without hypenatremia N=132	Patients with hypernatremia N=138	Univariate *p*-value	Multivariate *p*-value, OR (95 % CI)
Age	70 (63, 77)	68 (62, 75)	71 (64, 78)	**0.041**	**0.030**, 1.02 (1.01-1.05)
Male gender	161 (60 %)	84 (64 %)	77 (56 %)	0.189	
Place of admission				0.354	
Emergency Department	157 (58 %)	73 (55 %)	84 (61 %)		
Ward	113 (42 %)	59 (45 %)	54 (39 %)		
Preexisting Conditions					
Hypertension	150 (56 %)	73 (55 %)	77 (56 %)	0,935	
Diabetes Mellitus	106 (39 %)	54 (41 %)	52 (38 %)	0.587	
Obstructive lung disease	53 (20 %)	26 (20 %)	27 (20 %)	0.978	
Cardiovascular disease	68 (25 %)	40 (30 %)	28 (20 %)	0,058	
Chronic kidney disease	37 (14 %)	24 (18 %)	13 (9 %)	**0,036**	**0.019**, 0.41 (0.20-0.87
Malignancy	24 (9 %)	13 (10 %)	11 (8 %)	0.588	
Cerebrovascular disease	17 (6 %)	8 (6 %)	9 (7 %)	0.876	
Severity of Disease					
SOFA score	4 (3, 6)	4 (3, 6)	4 (3, 7)	0.187	
APACHE II score	20 (16, 27)	20 (16, 26)	21 (17, 27)	0.467	
Time interval, day					
COVID 19 diagnosis to ICU admission	5 (1, 9)	5 (1, 9)	6 (1, 8)	0.958	
Medications					
High dose steroid	153 (57 %)	71 (54 %)	82 (59 %)	0.351	
Standart dose steroid	102 (38 %)	51 (39 %)	51 (37 %)	0.776	
Furosemid	94 (34 %)	49 (37 %)	42 (30 %)	0.227	
Thiazide	28 (10 %)	16 (12 %)	12 (9 %)	0.356	
Laboratories at admission					
BUN, mg/dL	43 (27, 66)	40 (24, 66)	46 (28, 66)	0.276	
Glucose, mg/dL	179 (125, 277)	180 (117, 269)	176 (130, 289)	0.541	
Creatinin mg/dL,	1.1 (0.84, 1.73	1.1 (0.8, 1.9)	1.2 (0.8, 1.7)	0.562	
CRP, mg/dL	117 (57, 191)	108 (50, 162)	130 (64, 215)	0.062	
pH	7.41 (7.35, 7.46)	7.42 (7.35, 7.47)	7.40 (7.35, 7.46)	0.095	
$PaCO_2$, mmHg	37 (32, 44)	37 (32, 43)	37 (32, 44)	0.824	
SBE, mmol/L	-1 (-4.4, +2.4)	-0.35 (-3.6, +2.8)	-2 (-4.8, +1.7)	0.079	
Interventions					
IMV	197 (73 %)	88 (67%)	109 (79 %)	**0,023**	**0.044**, 1.77 (1.02-3.08)
RRT	47 (17 %)	29 (22 %)	18 (13 %)	0.053	
Complications					
Septic shock	179 (66 %)	84 (64 %)	95 (69%)	0.366	
AKI	133 (49 %)	59 (45 %)	74 (54%)	0.143	
Outcomes					
Hospital LOS day	16 (11, 25)	17 (10, 27)	15 (11-24)	0.387	
ICU LOS day	9 (6, 15)	9 (5, 14)	10 (7-15)	0.241	
ICU mortality	207 (77 %)	98 (74 %)	109 (79%)	0.357	
28 day mortality	218 (81 %)	103 (78%)	115 (83%)	0.269	

showed significant differences between mild hypernatremic patients vs moderate to severe hypernatremic patients in terms of median age (69 vs 72 years, p= 0.036), presence of hypertension (40% vs 62%, p=0.017), median SOFA score (3 vs 4 points, p=0.003), serum levels of BUN (35 vs 49 mg/dl, p=0.001), creatinine (0.9 vs 1.3 mg/dl, p= 0.001), CRP (93, 144 mg/dl, p=0.017), pH (7.43 vs 7.39, p=0.023) and SBE (-0.2, -2.2 mmol/l, p=0.012), AKI (40 % vs 75 %, p=0.040) as well as septic shock (53% vs 75%, p=0.011) (Table-1). However, multivariable analysis confirmed the presence of hypertension [adjusted OR, 2.52; 95% CI, 1.16-5.49; p=0.020], median SOFA score [adjusted OR, 1.27 ; 95% CI, 1.01-1.60; p=0.037], median serum BUN level [adjusted OR, 1.03; 95% CI, 1.01-1.05; p=0.043] and

presence of septic shock [adjusted OR, 2.46; 95% CI, 1.10-5.51; p=0.029] were significantly associated with moderate to severe hypernatremia.

Table 2 Baseline characteristics of the hypernatremic patients and predisposing factors for moderate to severe hypernatremia.

Variables[a]	**Mild hypernatremia** 146-149 mmol/L N=38(28 %)	**Moderate to severe hypernatremia** ≥150 mmol/L N=100 (72 %)	**Univariate *p***	**Multivariate *p*,** OR (CI, 95%)
Age, year	69 (62,75)	72 (65, 79)	**0.036**	NS
Male gender	23 (61 %)	54 (54 %)	0.49	
Place of admission			0.659	
Emergency Department	22 (58 %)	62 (62 %)		
Ward	16 (42 %)	38 (38 %)		
Preexisting Conditions				
Hypertension	15 (40 %)	62 (62 %)	**0.017**	**0.020, 2.52 (1.16-5.49)**
	12 (32 %)	40 (40%)	0.362	
Obstructive lung disease	7 (18 %)	20 (20 %)	0.835	
Cardiovascular disease	5 (13 %)	23 (23 %)	0.199	
Chronic kidney disease	2 (5 %)	11 (11 %)	0.515	
Malignancy	4 (11 %)	7 (7 %)	0.495	
Cerebrovascular disease	1 (3%)	8 (8 %)	0.444	
Severity of Disease				
SOFA score	3 (2, 6)	4 (3, 7)	**0.003**	**0.037, 1.27 (1.01-1.60)**
APACHE II score	18 (14, 26)	21 (18, 27)	0.137	
Time interval, day				
ICU admission to Na_{peak} level	5 (3, 8)	6 (4, 10)	0.088	
COVID 19 diagnosis to ICU admission	5 (2, 9)	6 (1, 8)	0.070	
Medications				
High dose steroid	18 (47%)	64 (64 %)	0.076	
Standart dose steroid	18 (47%)	33 (33 %)	0.118	
Furosemid	13 (34%)	29 (29 %)	0.552	
Thiazide	3 (8%)	9 (9 %)	0.569	
Peak Na level	147 (146, 148)	153 (151, 156)	**<001**	NA
Laboratories at admission				
Glucose, mg/dl	164 (132, 235)	184 (129, 302)	0.309	
BUN, mg/dl	35 (22, 49)	49 (33,74)	**0.001**	**0.043, 1.03(1.01-1.05)**
Creatinine, mg/dl	0.9 (0.8, 1.3)	1.3 (0.9, 1.8)	**0.001**	NS
CRP	93 (27, 182)	144 (83, 223)	**0.017**	NS
pH	7.43 (7.38, 7.47)	7.39 (7.31, 7.44)	**0.023**	NS
PCO2, mmHg	38 (34, 47))	37 (31, 44)	0.386	
SBE, mmol/l	-0.2 (-3.8, +4.1)	-2.2 (-5.7, +1.0)	**0.012**	NS
Interventions				
IMV	26 (68 %)	83 (83%)	0.060	
RRT	6 (16 %)	12 (12 %)	0.577	
Complications				
Septic shock	20 (53 %)	75 (75 %)	**0.011**	**0.029, 2.46 (1.10-5.51)**
AKI	15 (40 %)	59 (59 %)	**0.040**	NS
Outcome				
Hospital LOS, day	14 (10, 23)	15 (11, 25)	0.551	
ICU LOS day	9 (6, 12)	12 (7, 17)	**0.033**	NA
Mortality in ICU	23 (61 %)	86 (86 %)	**0.001**	NA
28 day mortality	26 (68 %)	89 (89 %)	**0.004**	NA

[a] Categorical variables are presented as frequency and percentage, continous variables are presented as median and inter-quartile ratio.

OR, odds ratio; *SOFA*, sequential organ failure assessment; *APACHE II*, Acute Physiology and Chronic Health Evaluation II; *COVID 19*, Coronavirus disease 2019; *ICU*, intensive care unit; *BUN*, blood urea nitrogen; *CRP*, C-reactive protein; *$PaCO_2$*, partial pressure of arterial carbon dioxide; *SBE*, standard base excess; *IMV*, invasive mechanical ventilation ; *RRT*, renal replacement therapy; *AKI*, acute kidney injury; *LOS*, length of stay; *NA*, not applicable; *NS*, nonsignificant.

Table 3 Demographic and clinical variables in survivors and nonsurvivors among hypernatremic patients

Variables[a]	ICU outcome				Hospital outcome			
	Survivors N=29 %)	Nonsurvivors N=109	Univariate p value	Multivariate p- value OR (CI 95	Survivors N=23 %)	Nonsurvivors N=115	Univariate p value	Multivariate p- value OR (CI 95
Male gender	12 (41%)	65 (60%)	0.079	NS	8 (34 %)	69 (60 %)	0.026	**0.023** 3.7 (1.24, 11.2)
Hypernatremia			0.001	**0.045**			0.004	**0.047**
Mild	15 (52 %)	23 (21 %)		2.58 (1.03-	12 (52 %)	26 (23 %)		2.9 (1.1,
Moderate to severe	14 (48 %)	86 (79 %)		6.49)	11 (48 %)	89 (77 %)		8.5)
Place of admission			0.004	NS			0.019	NS
Emergency ward	11 (38 %)	73 (67 %)			9 (39 %)	75 (65 %)		
Ward	18 (62 %)	36 (23 %)			14 (61 %)	40 (35 %)		
Laboratory at admission								
BUN, mg/dL	24 (19, 44)	49 (34, 70)	<0.001	**0.012** 1.03 (1.01- 1.06)	23 (19, 31)	49 (34, 68)	<0.001	**0.014** 1.04 (1.02- 1.08)
SBE, mmol/l	1.5 (-4.8, 5.5)	-2.2 (-4.9, 0.8)	0.015	NS	1.6 (-4.1, 6.4)	-2.2 (-5.1, 0.8)	0.015	NS
SOFA	3.0 (2.0, 5.5)	5.0 (5.0, 5.0)	0.001	NS	3.0 (2.0, 4.0)	5.0 (5.0, 5.0)	0.001	NS
APACHE II	18 (14, 24)	21 (21, 21)	0.049	NS	17 (14, 22)	21 (21, 22)	0.049	NS
Pulse steroid	9 (31 %)	73 (67 %)	0.001	NS	9 (39 %)	73 (64 %)	0.030	NS
Standart steroid	19 (66 %)	32 (29 %)	0.001	NS	13 (57 %)	38 (33 %)	0.033	NS
IMV	18 (62 %)	91 (84 %)	0.012	NS	14 (61 %)	95 (83 %)	0.019	NS
Septic shock	15 (52 %)	80 (73 %)	0.028	NS	11 (48 %)	84 (73 %)	0.017	NS

[a] Categorical variables are presented as frequency and percentage, continous variables are presented as median and inter-quartile ratio.

OR, odds ratio; *SOFA*, sequential organ failure assessment; *APACHE II*, Acute Physiology and Chronic Health Evaluation II; *ICU*, intensive care unit; *BUN*, blood urea nitrogen; *SBE*, standard base excess; *IMV*, invasive mechanical ventilation; *NS*, nonsignificant

Outcomes including ICU LOS (12 vs 9, p=0.033), ICU mortality (86% vs 61%, p=0.001 and 28-day mortality (89% vs 68%, p=0.004) were significantly worse in moderate to severe hypernatremic patients compared to the mild hypernatremic group while hospital LOS (15 vs 14 days, p=0.551) was similar in both groups (Table-2).

On multivariable logistic regression analysis, the patients with moderate to severe hypernatremia and high serum BUN levels were significantly associated with either ICU [adjusted OR, 2.58; 95% CI, 1.03-6.49; p=0.045 and adjusted OR, 1.03; 95% CI, 1.01-1.06; p=0.012 respectively] and 28-day mortality [adjusted OR, 2.93; 95% CI, 1.10-8.52; p=0.047, and adjusted OR, 1.03; 95% CI, 1.02-1.08; p=0.014, respectively] whereas male gender [adjusted OR, 3.70; 95% CI, 1.24-11.21; p=0.023] had significant impact just on 28-day mortality (Table-3).

Diagnostic accuracy of BUN levels above 31 mg/dL for predicting ICU and 28-day mortality were 83% sensitivity, 69% specificity, AUC 0.78 (95 % CI, 0.67-0.89) p<0.001 (Figure-1) and 82% sensitivity, 79% specificity, AUC 0.81(95%CI, 0.69-0.93) p<0.001 (Figure-2), respectively.

DISCUSSION

The present study demonstrated that nearly half of the critically ill COVID-19 patients developed hypernatremia during ICU stay. No difference was observed in ICU and hospital outcomes between hypernatremic versus non-hypernatremic groups. However, patients with moderate to severe hypernatremia had a worse prognosis than the mild group. In critically ill COVID-19 patients, hypernatremia could be a manifestation of the disease.

Age is a well-known independent risk factor for hypernatremia (11). Underlying mechanisms are age-related physiological changes such as impaired thirst drive, decreased renal concentrating ability, and compromised adaptability to fluid losses. Decreased water intake may exacerbate the condition. As mentioned in our results advanced age predicts hypernatremia strongly. In ventilated patients, hypernatremia may develop due to failure to provide adequate free water as well as increased insensible loss of fluid (12). We figured out that patients on IMV had 1.7 fold increased odds of hypernatremia compared to non-intubated patients.

Since the renal ability to concentrate and dilute urine is impaired in CKD, the susceptibility to dysnatremia increases. In the early stages of CKD, the kidneys are capable of excreting a normal ingested load of sodium and other solutes but it achieves this by excreting large volumes of water due to its reduced concentration capacity (13, 14). However, as the glomerular filtration rate decreases over time, the renal water excretion ability decreases, thus it becomes less possible to develop hypernatremia (13). Nevertheless, in advanced stages of CKD predisposition to both hypernatremia and hyponatremia may be possible. The prevalence of hypernatremia in studies of patients with CKD shows great variability as 3.5 to 24.7%, depending on the methodological differences (15, 16). According to our data proportion of patients with CKD in the hypernatremic group was 9% which was only half of the nonhypernatremic group.

Dysnatremia, either hyponatremia or hypernatremia is demonstrated to be associated with poor outcomes including increased mortality and length of hospital stay both in COVID-19 era according to the the current literatüre (3, 17, 18). Unlike the previous studies, we were unable to show a relationship between elevated serum sodium levels and prognosis in patients admitted to the ICU with a diagnosis of COVID-19. We could not explain this result however it may be attributed to the small size of the study sample. Also, the severity of illness regarding APACHE score, SOFA score, and the frequency of adverse events such as septic shock or AKI were similar in both groups which may contribute to a similar prognosis between groups. However, the subgroup analysis revealed that patients with moderate to severe hypernatremia had a

higher death rate and longer stay in the ICU than those with mild hypernatremia. This result implies that the noticeable effect of hypernatremia occurs after 150 mmol/l. In patients with moderate to severe hypernatremia, both ICU and 28-day mortality were increased up to 2.58 and 2.93 times of mild group respectively. ICU length of stay is also prolonged much more in moderate to severe hypernatremic patients than in mild hypernatremic ones.

The presence of hypertension, high SOFA score, increased serum BUN levels at admission and septic shock in the follow-up were predisposing factors for the development of moderate to severe hypernatremia. Of these the most surprising data is the link between hypertension and moderate to severe hypernatremia. Several possible mechanisms may explain this relationship. Nearly half of the patients with essential hypertension are sensitive to salt. This sensitivity gets stronger with aging. As a result, the relationship between arterial blood pressure and sodium excretion is altered, thus higher blood pressure is needed to ensure salt excretion (19). In critically ill patients with a history of hypertension, due to factors related to the environment, such as sedation, diuretics, increased insensible fluid loss, sepsis, etc., blood pressure may not increase in response to increased salt load. This suggests that inadequate reduction of sodium excretion in these patients leads to hypernatremia. Furthermore, hypertension is the most common comorbidity recorded in patients with COVID-19 patients (20, 21). The significance of hypernatremia as a risk factor for severe COVID-19 outcomes has been reported from multiple observational and retrospective studies (20,22). If hypernatremia is a manifestation of COVID-19 as we suppose, the presence of hypertension might also increase the severity of hypernatremia. Association between hypernatremia and sepsis is not fully elucidated. However, the positive correlation between hypernatremia and septic shock has been shown in some studies (23, 24). As sepsis may contribute to hypernatremia, it may also be a manifestation of sepsis, especially in elderly patients (23). We report more than twofold risk of moderate to severe hypernatremia in patients with septic shock than patients without septic shock. Osmotic diuresis may directly lead to or contribute to the development of hypernatremia by causing severe fluid loss. Osmotic diuresis due to urea is a well-known cause of hypernatraemia in intensive care unit (25). Our findings suggest that high BUN levels may play an important role in predicting the severity of hypernatremia and mortality among the critically ill COVID-19 patients with hypernatremia. Several studies concluded that the male gender is strongly associated with a higher risk of death in hospitalized COVID-19 patients (25,26). On contrary, we could not demonstrate the same

association in this cohort. However, among hypernatremic patients with COVID-19, the male gender had a higher odds ratio (3.7 fold) of 28-day mortality in comparison with females. This finding may imply that hypernatremia increases the male gender's contribution to COVID-19 mortality. Surprisingly, the use of corticosteroids had no impact on the development of hypernatremia in the study population. Corticosteroids may facilitate hypernatremia by mineralocorticoid effect and sodium retention as a consequence. It can induce hypernatremia not only in this way but also by causing electrolyte-free fluid loss (27). The underlying mechanism is known as excess corticosteroids cause urinary concentration defect and polyuria by down-regulating urea transporters without affecting the aquaporin channels (28). Furthermore, corticosteroids lead to osmotic urea diuresis by elevating BUN levels via catabolism. The relation between steroid therapy and hypernatremia is not proven fully in clinical studies. Despite some studies revealing that corticosteroids are associated with a higher risk of hypernatremia (5, 27, 28), a recent study showed a significant association between ICU acquired hypernatremia and high dose steroid therapy, but no association between pulse steroid therapy and hypernatremia (29). In this cohort, 95 % of the patients with COVID-19 had received either a high dose or pulse steroid for COVID-19 pneumonia. The proportion of patients who received steroid treatment was similar in both the hypernatremic and non-hypernatremic groups. The lack of expected effect of corticosteroids on water sodium balance may be explained by the critical illness-related corticosteroid insufficiency (CIRCI) state. In CIRCI, target tissue resistance to corticosteroids may occur despite high levels of serum cortisol. Evidence from studies of severe acute respiratory syndrome (SARS) suggests that COVID-19 which is an infection with SARS-CoV-2 is associated with CIRCI (30). This study is mainly limited by its retrospective design. Despite being conducted at two centers our results cannot be generalized due to the small size of the study population. Since we had missing data on sodium input, we could not establish whether there was a relationship between sodium input and hypernatremia. Nevertheless, our data is valuable regarding its contribution to the limited data on the impact of COVID-19 on sodium water balance and thereby the association with adverse outcomes. Also, detailed subgroup analysis of hypernatremic patients with COVID-19 provided valuable information on the comparison of the risk factors and outcomes between mild and moderate to severe hypernatremia.

CONCLUSION

Hypernatremia was recorded at a higher frequency than expected in patients with COVID-19 pneumonia admitted to the

intensive care unit. This unusual finding may be a primary manifestation of the SARS-CoV-2 virus, which involves multiple systems. Elevated BUN levels and moderate to severe hypernatremia may predict mortality in critically ill COVID-19 patients who developed hypernatremia. However, further investigations are needed by large scale studies.

Ethics Committee Approval: Ethics committee approval was received for this study from local ethics committee at Ordu University with file number 2021/213

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RESEARCH ARTICLE

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Evaluation of Sleep Quality of Anesthesiologists Working in Turkey

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Abstract

Objective: Sleep is one of the basic daily activities that are important for an individual with physical, mental, social, and intellectual needs to be in good physical and mental health. Although many definitions are made, sleep; is a complex and physiological event affected by pathophysiological, physical, psychological, and environmental factors. Anesthesiologists often work in closed environments and with long working hours, which can cause severe, chronic sleep loss and sleep disruption. Sleeplessness and low sleep quality can cause decreased attention during the day, impaired judgment, and delays in decision-making. This study, it is aimed to determine the sleep quality of Anesthesiology and Reanimation specialists working in Turkey.

Methods: The study included all anesthesiologists whose e-mail addresses were registered with the Turkish Society of Anesthesiology and Reanimation and who worked in Turkey. Survey questions, which the participants can answer electronically, were sent to the participant's e-mail addresses. To increase the number of participants, a reminder email was sent three weeks after the first email. The data were expressed as a number, percentage, mean, and standard deviation and the correlation between the parameters will be evaluated at a 95% confidence interval by performing Pearson correlation analysis; $p< 0.05$ was accepted as significant.

Results: Due to seven people with sleep disorders being excluded from the study, 210 out of 217 people who answered the questionnaires sent by email to the anesthetists who are members of the Turkish Society of Anesthesiology and Reanimation were included in the study. Of the respondents who worked in the following fields: 30% (n = 63) State Hospital, 33.3% (n = 70) University Hospital, 17.1% (n = 36) Ministry of Health Affiliate Hospital, 19.5% (n = 41) Private Hospital, and 73.3% (n = 154) at the Operating Room, 10% (n = 21) at the Intensive Care, 15.2% (n = 32) at the Operating Room + Intensive Care, and 1.4% (n = 3) at other departments, 48.4% (n = 105) were male, 51.6% (n = 112) were female, with a mean age of 41.5±7.6 years. The average hourly shift for the participants, 61% of whom were on the night shift, was 50 hours per month, and 25% were working more than 45 hours per week.

Conclusion: The sleep quality of anesthesiologists is poor, which causes daytime dysfunction. It is thought that the reason for poor sleep quality, in general, is the adverse effects of their duties and responsibilities, working environment, and operating conditions on sleep quality.

Key words: sleep quality, Pittsburgh sleep quality index, anesthesiologist, night shift

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INTRODUCTION

Sleep is a state of unconsciousness in which mental and physical activity is suspended, as well as a period of restructuring during which our bodies store energy (1,2). It is known that quality sleep is needed to be healthy both physically and mentally (2,3). Sleep quality includes people's level of alertness and sleep duration while sleeping, in addition to their level of physical and emotional comfort. The Pittsburgh Sleep Quality Index (PSQI), a psychometric and non-polysomnographic test, is used to evaluate sleep quality (4,5).

Sleep and sleep quality are influenced by a variety of physical, psychological, and environmental factors. Sleeplessness and poor sleep quality can lead to decreased attention during the day, impaired judgment, delays in decision-making, and a general decrease in job performance (6–8). Anesthesiologists often work in closed environments and with long working hours, which can cause severe, chronic sleep loss and sleep disruption. Sleeplessness and low sleep quality can cause decreased attention during the day, impaired judgment and delays in decision making.

In this study, it is aimed to determine the sleep quality of Anesthesiology and Reanimation specialists working in Turkey.

METHODS

Ethical approval for our study was obtained from the Clinical Research Ethics Committee of Ordu University at the meeting of 26.04.2018 with decision number CREC 2018/96.

This research is a descriptive study conducted to evaluate the sleep quality of anesthesiologists whose e-mail addresses are registered with the Turkish Society of Anesthesiology and Reanimation and who work in Turkey.

Survey questions, including descriptive questions and PSQI questions that they could answer electronically, were sent to the participants' e-mail addresses. Emails sent were responded to by 217 people and evaluated. Due to seven people with sleep disorders being excluded from the study, 210 out of 217 people who answered the questionnaires sent by email were included in the study.

Pittsburgh Sleep Quality Index

PSQI is a self-report test that evaluates sleep quality and sleep disturbance over a one-month time interval. Firstly, its reliability and validity were demonstrated by Buysse et al. (4). Agargün et al. determined the index's validity and reliability in Turkish society (5). PSQI consists of seven components: subjective sleep quality, sleep latency, sleep duration, habitual

sleep efficiency, sleep disturbance, use of sleeping medications, and daytime dysfunction. Component 1: Subject refers to sleep quality, Component 2: Sleep latency, Component 3: Sleep duration, Component 4: Habitual sleep efficiency, Component 6: Use of sleeping medications, Component 7: Daytime dysfunction. The sum of all components forms the total PSQI score. A total PSQI score above 5 indicates poor sleep quality, and below 5 indicates good sleep quality (5).

Statistical Analysis

The analysis of the research data was conducted using the Jamovi for Linux (Open Stats R based) 1.6.3 package program, and the quantitative data were given as percentages and the qualitative data as mean±standard deviation. Mann-Whitney U and Kruskal-Wallis tests were used to compare dependent and independent variables. Pearson or Spearman correlation analysis was performed for correlation between data. The results were expressed as 95% confidence interval and $p<0.05$ as significant.

RESULTS

Due to seven people with sleep disorders being excluded from the study, 210 out of 217 people who answered the questionnaires sent by email to the anesthetists who are members of the Turkish Society of Anesthesiology and Reanimation were included in the study. Of the respondents who worked in the following fields: 30% (n = 63) State Hospital, 33.3% (n = 70) University Hospital, 17.1% (n = 36) Ministry of Health Affiliate Hospital, 19.5% (n = 41) Private Hospital, and 73.3% (n = 154) at the Operating Room, 10% (n = 21) at the Intensive Care, 15.2% (n = 32) at the Operating Room + Intensive Care, and 1.4% (n = 3) at other departments, 48.4% (n = 105) were male, 51.6% (n = 112) were female, with a mean age of 41.5±7.6 years. The average hourly shift for the participants, 61% of whom were on the night shift, was 50 hours per month, and 25% were working more than 45 hours per week (Table 1).

Table 1. Characteristics of the Participants

Categories	*n*	*% or Mean±SD*
Sex		
Man	100	47.6 %
Women	110	100.0 %
Hospital		
Government Hospital	63	30.0 %
University Hospital	70	33.3 %
*M.H Affiliate Hospital**	36	17.1 %
Private Hospital	41	19.5 %
Departments		
Operating Room	154	30.0 %
*ICU***	21	33.3 %
Operating Room+ICU	32	17.1 %
Other	3	19.5 %
Working Time		
Over 45 h/week	118	56,2 %
Under 45 h/week	92	43,8 %
Night shift		
Yes	128	61.0 %
No	82	39.0 %
Age	210	41,35±7,41
Night shift hour/mount	210	51±13,1
PSQI**	210	6,73±3,11
Sleep time/hour	210	6,35±1,05
Bedtime/minutes	210	417,40±61,66

*Ministry of Health
** Intensive Care Unit
*** Pittsburg Sleep Quality Index

While the mean total PSQI score was 6.73 ± 3.1, 75.2% of respondents had a PSQI total score greater than 5. There was no statistical

difference in total PSQI scores between male and female anesthesiologists (p = 0.08). The total PSQI for those on the night shift and those working more than 45 hours per week was assessed as high (p=0,007, p<0,001). When the participants' departments were compared, no statistical difference in total PSQI scores was found (p=0,51). When the PSQI components of those on the night shift and non-night shift were compared separately, the difference between the scores of components 1, 5, and 7 were found to be significant (p=0.037, p=0,023, p<0,001). When the components were compared separately between male and female anesthesiologists, only the difference between component 5 scores was significant (p=0.001) (Table 2).

Tablo 2. Differences in Pittsburgh Sleep Quality Index Scores According to Participants' General Characteristics

Variables	*Categories*	*n*	*Global PSQI*	*Comp. 1*	*Comp. 2*	*Comp. 3*	*Comp. 4*	*Comp. 5*	*Comp. 6*	*Comp. 7*
			M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
Sex	Male	100	6,31±2,94	1,29±0,72	1,04±0,94	0,89±0,81	0,30±0,67	1,34±0,60	0,11±0,46	1,34±0,93
	Female	110	7,11±3,21	1,41±0,65	1,21±0,95	0,79±0,77	0,33±0,70	1,63±0,63	0,19±0,67	1,55±1
	*p**		0,079	0,324	0,173	0,414	0,682	0,001	0,508	0,123
Hospital	Government Hospital	63	6,84±2,97	1,44±0,61	1,17±0,95	0,78±0,81	0,29±0,63	1,54±0,66	0,06±0,35	1,56±0,94
	University Hospital	70	6,4±3,34	1,23±0,70	1,03±0,91	0,87±0,77	0,23±0,64	1,44±0,65	0,19±0,64	1,41±1,0
	M.H Affiliate Hospital[α]	36	6,78±2,92	1,36±0,63	1,14±0,96	0,81±0,78	0,42±0,84	1,44±0,60	0,11±0,52	1,5±0,73
	Private Hospital	41	7,07±3,10	1,41±0,80	1,22±0,96	0,9±0,83	0,41±0,70	1,54±0,59	0,27±0,775	1,32±1,03
	*p***		0,714	0,297	0,726	0,849	0,417	0,762	0,324	0,641
Departments	Operating Room	154	6,73±2,99	1,38±0,65	1,08±0,94	0,86±0,801	0,27±0,63	1,47±0,62	0,18±0,63	1,49±0,95
	ICU**	21	7,52±3,84	1,43±0,67	1,38±0,92	0,81±0,81	0,62±1,07	1,62±0,66	0,19±0,68	1,48±0,98
	Operating Room+ICU	32	6,22±3,23	1,22±0,87	1,19±0,93	0,69±0,78	0,34±0,60	1,56±0,669	0	1,22±1,09
	Other	3	6,33±2,08	1	1	1,33±0,57	0,33±0,57	1	0	1,67±0,57
	*p***		0,518	0,493	0,57	0,48	0,178	0,356	0,415	0,52
Working time	Under 45 h/week	92	5,84±3,03	1,16±0,61	1,08±0,98	0,57±0,66	0,25±0,62	1,43±0,63	0,08±0,42	1,27±0,92
	Over 45 h/week	118	7,42±2,99	1,5±0,71	1,17±0,90	1,05±0,82	0,36±0,73	1,53±0,63	0,21±0,67	1,59±0,98
	*p**		<0,001	<0,001	0,39	<0,001	0,204	0,147	0,056	0,016
Night shift	Yes	128	7,19±3	1,43±0,64	1,19±0,91	0,86±0,81	0,3±0,65	1,57±0,64	0,17±0,62	1,66±0,89
	No	82	6,01±3,14	1,23±0,74	1,04±0,98	0,8±0,77	0,33±0,73	1,37±0,59	0,12±0,507	1,12±0,99
	*p**		0,007	0,037	0,211	0,689	0,926	0,02	0,624	<0,001

*U Mann-Whitney test; **Kruskal-Walli's test; α Ministry of Health

In the correlation analysis, there was no relationship between age, hospital, and department of work and PSQI (r=0.054 p=0.440, r=0,032 p=0,641, r=0,037 p=0,595), while a weak correlation was found between working time, night shift, and total monthly night shift time (r=0.254 p<0.001; r=0.185 p=). 0.007, r=0.217 p=0.002) (Table 3).

DISCUSSION

Anesthesiologists often work in closed environments and with long working hours, which can cause severe, chronic sleep loss and sleep disruption. Sleeplessness and low sleep quality can cause decreased attention during the day, impaired judgment and delays in decision making. There is evidence that working at night

disrupts the circadian rhythm, sleep-wake balance, and nutritional status (9-11). It has even been reported to be associated with metabolic disorders such as cardiovascular system diseases, depression, anxiety, obesity, and diabetes (9,10,12,13). It is pointed out that working at night causes serious problems in terms of employee health as well as serious problems related to patient care and safety (7,13). Short sleep, frequent awakenings, and a long time to fall asleep, all have an impact on sleep quality, as do many environmental, physical, psychological, and physiological factors. (12). It is known that poor sleep quality caused by sleepiness has negative effects such as drowsiness, deterioration in cognitive functions, staying awake during work, and difficulty concentrating (13,14). In our study, the total PSQI for those on the night shift and those working more than 45 hours per week indicates is high that keeping watch and overworking reduces their sleep quality. In addition, it is thought that those on the night shift and those working more than 45 hours per week, experience daytime dysfunction, and this is due to poor sleep quality in general.

Tablo 3. Correlations of the Participants Characteristics with Pittsburgh Sleep Quality Index Scores

Correlation Matrix		Age	Hospital	Departments	Working Time	Night shift	Night shift hour/mount	Bedtime /minutes	PSQI
Age	Pearson's r	—							
	p-value	—							
Hospital	Pearson's r	0.217	—						
	p-value	0.002	—						
Departments	Pearson's r	-0.110	0.024	—					
	p-value	0.111	0.729	—					
Working Time	Pearson's r	0.068	0.292	-0.082	—				
	p-value	0.325	< .001	0.237	—				
Night shift	Pearson's r	0.288	0.157	0.040	-0.061	—			
	p-value	< .001	0.023	0.562	0.383	—			
Night shift hour/mount	Pearson's r	-0.396	-0.117	-0.016	0.144	-0.782	—		
	p-value	< .001	0.091	0.813	0.037	< .001	—		
Bedtime/minutes	Pearson's r	-0.042	-0.064	0.060	-0.288	0.024	-0.095	—	
	p-value	0.543	0.357	0.383	< .001	0.727	0.169	—	
PSQI	Pearson's r	-0.054	0.032	-0.037	0.254	-0.185	0.217	-0.126	—
	p-value	0.440	0.641	0.589	< .001	0.007	0.002	0.068	—

It has been said that falling asleep between 16 to 30 minutes in all age groups shows good sleep quality, which is an objective assessment (15-17). Although the participants included in the study were found to have an average of 20 minutes to fall asleep, the fact that the total PSQI score was above 5 suggests that falling

asleep alone was not enough to indicate good or bad sleep quality.

In adults, seven hours or more of sleep per day is adequate for physiological needs (18). The fact that the average sleep time of the study participants was less than seven hours shows that it contributed to poor sleep quality.

There is a two-sided interaction between working conditions and sleep. Just as sleep has positive and negative effects on work performance, working conditions also have positive or negative effects on sleep quality (8,10). The fact that there is no difference between the sleep quality of the anesthesiologists working in different hospitals and different departments participating in our study gives the impression that the poor sleep quality, in general, is due to the work done. However, this effect is difficult to demonstrate by this study alone.

CONCLUSION

As a result, anesthesiologists have poor sleep quality, and this leads to daytime dysfunction. In general, it is thought that the reason for poor sleep quality is that their duties and responsibilities, working environment, and working conditions have negative effects on sleep quality.

Ethics Committee Approval: Ethics committee approval was received for this study from local ethics committee at Ordu University with file number 2018-96

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RESEARCH ARTICLE

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GCF Levels of Osteoclastogenesis-Related Cytokines in Periodontitis in Relation to Smoking During Non-Surgical Periodontal Therapy

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Abstract

Objective: Interleukins (IL) -1β, -34, receptor activator of nuclear factor-kB ligand (RANKL), and osteoprotegerin (OPG) play a crucial role in osteoclastogenesis and bone resorption through modulating inflammatory processes and osteoclastogenesis. Smoking is the major risk factor in the initiation and progression of the periodontitis, and adversely affects the outcomes of non-surgical periodontal therapy. To date, there is no study investigating both gingival crevicular fluid (GCF) IL-1β, IL-34, RANKL, and OPG levels before and after non-surgical periodontal therapy in smoking and non-smoking patients with periodontitis stage 3, grade B and C. The aim of current research was to examine the GCF levels of some osteoclastogenesis-related cytokines in periodontitis in relation to smoking before and after periodontal therapy.

Methods: At baseline, full-mouth periodontal status together with GCF samples were collected from 116 individuals, including 60 periodontitis patients (30 smokers and 30 nonsmokers) and 56 periodontally healthy controls (28 smokers and 28 nonsmokers). Non-surgical periodontal therapy, consisting of instruction for daily plaque control and scaling and root planing (SRP), was performed. GCF sampling and full-mouth periodontal measurements were repeated 6 weeks after completion of SRP. The GCF levels of biomarkers were measured by enzyme-linked immunosorbent assay.

Results: The periodontitis groups exhibited significant improvement in clinical parameters. At baseline, the GCF IL-1β levels in periodontitis groups were significantly higher than periodontally healthy controls ($p<0.05$) and it was significantly decreased in periodontitis groups after non-surgical periodontal therapy. At baseline, the GCF IL-34 levels in periodontitis groups were significantly higher than periodontal healthy controls($p<0.05$) and the GCF IL-34 level was significantly decreased in non-smoking periodontitis patients. At baseline and after periodontal therapy, the GCF RANKL levels were similar in all groups. The GCF OPG level was significantly lowest in non-smoking periodontitis patients at baseline and the GCF OPG level was significantly increased in smoking and non-smoking periodontitis patients after non-surgical periodontal therapy.

Conclusion: In the periodontal inflammation process, GCF IL-34 level followed a similar pathway to GCF IL-1β, suggesting that IL-34 may be a marker in the pathogenesis of periodontal disease. The significant decrease in GCF IL-34 and a significant increase in GCF OPG level in the non-smoker periodontitis group after periodontal therapy suggest the negative effect of smoking on the response to periodontal therapy. More comprehensive studies are needed by increasing the number of samples included in the study groups in order to better understand the pathogenesis of periodontitis.

Key words: Periodontitis, cytokine, non-surgical periodontal therapy, smoking

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INTRODUCTION

Periodontitis is an inflammatory and microbial disease defined by pathologic damage of the periodontium and alveolar bone (1). Systemic immune response, genetic and environmental risk determinants contribute to the establishment and improvement of periodontitis (2). Periodontal disease is under the influence of environmental factors and smoking is one of the most important of these. The prevalence and severity of periodontal destruction boost due to smoking. In addition, smoking negatively affects the response to periodontal therapy (3).

Osteoclasts, which are produced through a differentiation process called osteoclastogenesis, are regulated by numerous cytokines. Osteoprotegerin (OPG) inhibits osteoclast differentiation whereas osteoclastogenic cytokines, like receptor activator of nuclear factor-kB ligand (RANKL), interleukin-1beta (IL-1 β), and interleukin-34 (IL-34) induces this process (4). These cytokines have been related to bone loss in patients with periodontal diseases (5-7). In patients with periodontitis, it is identified that the gingival crevicular fluid (GCF) contains reduced OPG levels and elevated levels of RANKLwith which RANKL interacts during the inflammatory process. IL-1β plays a potent key role by inducing the expression of RANKL in some cells. In the results of previous studies, it was reported that the RANKL/OPG ratio showed significant increases in individuals with periodontitis compared to the control group (8, 9). There is no study evaluating the response after initial periodontal treatment in GCF IL-1β, IL-34, RANKL, OPG levels in Stage III, grade B and C periodontitis with smoking status. Our hypothesis in this clinical trial is GCF IL-1β, IL-34, and RANKL levels reduction at 6-week. The objectives of our clinical research were to determine the association of GCF levels of cytokines IL-1β, IL-34, RANKL, and OPG with the clinical substantiation of the periodontium and also to compare the cytokines for the estimation of the pathophysiological status of Stage III grade B and C periodontitis and improved answer of non-surgical periodontal therapy in relation to smoking.

METHODS

Design of the clinical trial

The study procedure was approved by the Medical Research Ethics Committee of Sivas Cumhuriyet University with regard to the Declaration of Helsinki (2018-07/02). All patients accepted and signed the detailed informed consent form. A total of 116 participants were concerned, including 60 periodontitis patients (30 smokers, 30

nonsmokers) and 56 periodontally healthy controls (28 smokers, 28 nonsmokers) (Figure 1). Patients with certain exclusion criteria were not included in the study. These exclusion criteria are listed below.

Patients who have conservative or prosthetic restorations in the sample site, patients who were not systemically healthy, in pregnancy or lactation process, patients who require antibiotic prophylaxis, patients who used anti-inflammatory or antibiotic drug in the last six months, patients who were treated periodontally within six months.

The patients were categorized according to the 2017 classification. The H group took place of participants with at least twenty teeth in their mouths. They mustn't have a history of periodontitis. With these criteria, bleeding on probing (BOP) <10% and probing pocket depth (PPD) of 3 mm or less, and besides, they have healthy gingiva without clinical inflammation, no loss of alveolar bone, and attachment. In addition, the smoking habits of the individuals were examined. Those who smoke 10 or more cigarettes a day were considered active cigarette users. The H group was divided into two groups smoking 10 < or non-smoking 10 > status.

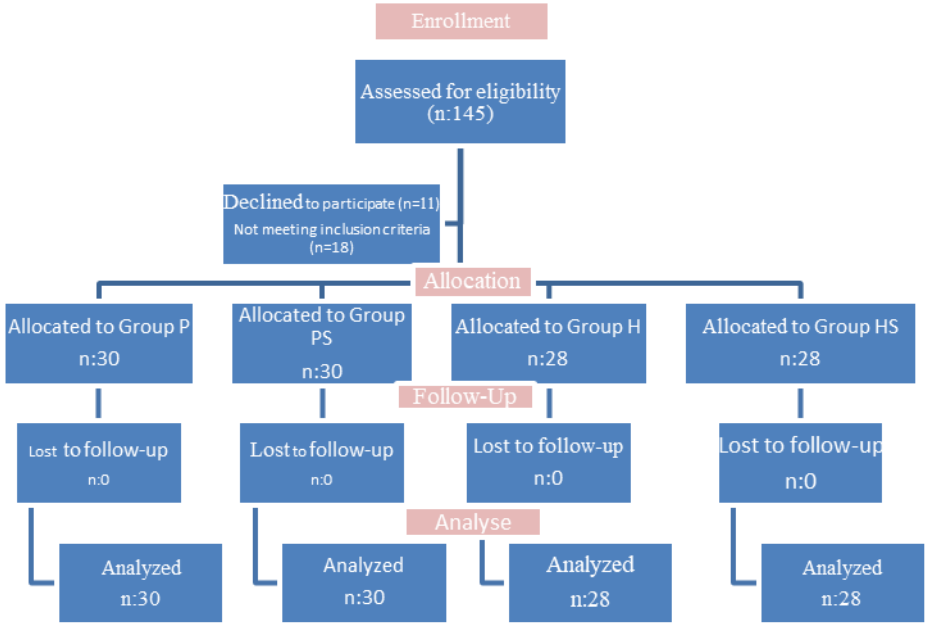

Figure 1. Flow diagram of patients' recruitment and follow-up

The Stage III -grade B periodontitis patients indicated clinical attachment loss (CAL) ≥5 mm and PPD ≥6 mm on at least two non-adjacent teeth. When we evaluated the bone loss radiographically, patients with not exceeding ≥30% of the teeth and up to the

middle or apical third of the root were included in this group. Since we do not have long-term follow-up radiographs of the patients, the grade of periodontitis patients has been defined by radiographic bone loss/age. The percentage of root length was determined by reference to the tooth with the highest radiographic bone loss. Grade B was determined the ratio of bone loss/age was 0.25–1.00 and the smoking status 10 >. The Stage III -grade C periodontitis group was similar to Stage III-grade B except for tooth loss (<5) due to periodontal disease. Destruction inconsistent with biofilm, a ratio of the percentage of root bone loss to age bigger than one, and smokers more than 10 cigarettes are other factors that determine the grade C periodontitis.

In periodontal measurements, we used a periodontal probe with indicator lines up to 15 mm (William's probe, Hu-Friedy, Chicago, USA). In order to optimize the CAL measurement, the individual acrylic stents, for which we determined the reference points in all periodontitis patients, were made of cold acrylic. Accordingly, the group definitions are as follows:

Group P: Periodontitis; Stage III, Grade B; Non-smoker

Group PS: Periodontitis; Stage III, Grade C; Smoker

Group H: Periodontal Healthy; Non-smoker

Group HS: Periodontal Healthy; Smoker

Clinical measurement and non-surgical periodontal therapy

The four quadrant clinical measurements of GI, PI, PD, and CAL were taken by all study participants. PD and CAL were measured at six points per tooth. The PI was based on the supragingival plaque, while the GI was based on gingival inflammation (10). A single expert (NA) performed the collection of clinical parameters and samples to ensure standardization. Clinicians' measurements are proven to be reliable on repeated measurements (≥98%). All clinical measurements of whole participants incorporated in the research were recorded before proceeding to the treatment phase. Firstly, periodontitis patients in stage III, grade B and C groups were given oral hygiene instructions within the scope of Phase 1 treatment. Then scaling and root planning (SRP) was applied within two weeks by using Gracey curettes with different forms (Hu-Friedy, Chicago, USA). Antibiotics and mouthwash were not prescribed to any of the patients. The patients were recalled 6 weeks later and whole-mouth clinical measurements were taken. GCF samples were retrieved from sample sites.

GCF sampling and ELISA analysis

GCF exemplification was performed by using the absorbent paper strip method in mesial or distal sites of the sampled tooth (OraFlow Inc., Amityville, NY, USA). For the collection of GCF specimens, three non-

abutting proximal sampling sites with a 5-6 mm probing pocket depth were preferred in the upper anterior region, which is easier to isolate and access. The periopaper was placed into pathologic periodontal pocket in periodontitis patients and gingival sulcus in periodontal healthy groups until a slight resistance was felt. We preferred to keep the periopaper in the pocket for 30 seconds. We have criteria for the exclusion of samples. These are blood and saliva contamination due to isolation, irritation, or excessive inflammation. All periopaper strips were placed into eppendorf tubes which were encoded with numbers.

The GCF samples, which were kept at -80 until the analysis time, were first left at room temperature, then 250 µL of phosphate buffer solution was annexed into the eppendorf tubes and mixed with vortex for 2 minutes to transfer the contents of the strip. Cytokine levels in GCF were analyzed via ELISA kits(FineTest, Wuhan, China) according to the prospectus. After the steps suggested by the company, the plates were read at 450 nm wavelength in the spectrometer, and the standard curves were used for GCF cytokine levels calculation. The data were presented as the total amount (picogram/site). As a result of the analysis, it was seen that there were regions with low cytokine levels than the detectability limits of the test, and these regions, which were relatively few, were given a score of 0.

Statistical Analysis

The SPSS program (IBM SPSS v22.0, IBM, USA) was preferred for statistical analysis. The Kolmogorov-Smirnov test was preferred to determine the distribution of data. While the Mann-Whitney test was preferred for pairwise comparisons, the numerical data were compared in groups by the Kruskal-Wallis ANOVA. Wilcoxon test was used to compare data during the treatment process. The chi-square test was preferred for evaluating frequencies. The p-value was set as <0.05. The computation of the sample size was performed under a 5% error considering a required sample size of 30 in each group, with a statistical power of 80% .

RESULTS

Demographic features and clinical periodontal measurements

The mean age P, PS, H, and HS groups ranged 39.5±8.8, 41.6±7.5, 36.2±10.9 and 37.9±8.1 years, respectively. The female/male ratio was 16/14 in P, 19/11 in PS, 17/11 in H, and 18/10 in the HS group. In the study groups, the mean age and gender distribution were homogeneous (p>0.05). Our primary outcome measure was changed in GCF biomarker levels from baseline to post-treatment assessment in groups based on smoking status. The secondary outcome measure was the changes in clinical parameters caused by the non-surgical therapy process.

Clinical Parameters

PI, PPD, and CAL values of clinical parameters before non-surgical periodontal therapy were higher in group PS than in group P, but only PPD values were statistically significant ($p<0.05$). GI values were decreased in group PS compared to group P but they were not statistically significant ($p>0.05$). All clinical parameters in 6th week were significantly decreased than at baseline levels.($p<0.05$)(Table 1).

Biochemical Parameters

At baseline GCF IL-1β levels in periodontitis groups were significantly higher than periodontal healthy groups ($p<0.05$). However, GCF IL-1β levels were higher in group PS than in group P, but the difference was not statistically significant ($p>0.05$). Significant reductions were observed in periodontitis groups in 6th week after non-surgical periodontal therapy ($p<0.05$). GCF IL-1β levels in the HS group were significantly higher than the H group ($p<0.05$).

At baseline GCF IL-34 levels in periodontitis groups were significantly higher than in periodontally healthy groups ($p<0.05$). However, the periodontally healthy group's GCF IL-34 levels were higher in group HS than in group H, but the difference was not statistically insignificant ($p>0.05$). Reductions were observed in periodontitis groups at the 6th week after non-surgical periodontal therapy but the difference was statistically significant in the P group only ($p<0.05$).

At baseline and 6-week after non-surgical periodontal therapy, GCF RANKL levels differences were insignificant between all groups ($p>0.05$).

At baseline GCF OPG levels in the PS group were significantly higher than in the P group ($p<0.05$). Increases were observed in periodontitis groups at the 6th week however they were insignificant ($p>0,05$) (Table 2).

Correlations

At baseline GCF IL-34 levels were negatively correlated with GCF OPG levels($r = -0.533$, $p <0.05$) in the PS group. There was a negative correlation between GCF RANKL and OPG levels($r =-0.642$, $p <0.05$) in the H group at baseline. There was a positive correlation between GCF IL-34 and RANKL levels ($r=0.617$, $p <0.05$) in the HS group at baseline.

Table 1. Comparison of the clinical data of the sample tooth region of the patients participating in the study before and after periodontal treatment and between groups

			Stage 3 Grade C	Stage 3 Grade B	Control Smoking	Control	*p*
GI	Baseline	mean ± SD	1.86±0.50[c,b]	2±0.74[b,c]	0.32±0.27	0.17±0.24	0.001*
		Med	2.00	2.00	0.50	0.00	
		Min	1.00	1.00	0	0.00	
		Max	3.00	3.00	1.00	0.50	
	6-week	mean ± SD	1.34±0.41[a,c,b]	1.46±0.57[a,b,c]			
		Med	1.10	1.00	0.50	0.00	
		Min	1.00	1.00	0.00	0.00	
		Max	2.00	3.00	1.00	0.50	
PI	Baseline	mean ± SD	2.25±0.25 [c,b]	2.10±0.40 [c,b]	0.39±0.41[b]	0.17±0.27	0.001*
		Med	2.25	2.00	0.50	0.00	
		Min	2.00	1.00	0.00	0.00	
		Max	2.50	3.00	1.00	1.00	
	6-week	mean ± SD	1.25±0.25[a,c,b]	1.11±0.21[a,c,b]			
		Med	1.25	1.00	0.50	0.00	
		Min	1.00	1.00	0.00	0.00	
		Max	1.50	1.50	1.00	1.00	
PD	Baseline	mean ± SD	5.15±0.26 [c,b]	4,61±0,58 [d,c,b]	1,73±0,56	1,57±0,48	0.001*
		Med	5.00	4.50	2.00	1.50	
		Min	5.00	4.00	1.00	1.00	
		Max	6.00	5.50	2.50	2.50	
	6-week	mean ± SD	2.23±0.25[a,c,b]	2.95±0.42[a,d,c,b]			
		Med	2.00	3.00	2.00	1.50	
		Min	2.00	2.00	1.00	1.00	
		Max	2.50	3.50	2.50	2.50	
CAL	Baseline	mean ± SD	10.06±0.34	9.31±1.13			0.001*
		Med	10.00	9.25			
		Min	9.50	5.00			
		Max	11.00	11.00			
	6-week	mean ± SD	8.83±0.44[a]	8.50±1.11[a]			0.191
		Med	9.00	8.50			
		Min	8.00	4.00			
		Max	9.50	10.00			

[a]: different from baseline
[b]: different from control non-smoking
[c]: different from control smoking
[d]: different from stage 3 grade C periodontitis group
mean±SD(standard deviation), med: median, min: minimum, max: maximum
* $p<0.05$
Wilcoxon test was used to compare data during the treatment process (baseline-6th week).
Comparisons of numeric variables of the study groups were evaluated by the Kruskal-Wallis ANOVA test with post hoc Mann-Whitney test for pairwise comparisons.

Table 2. Comparison of cytokine levels of patients participating in the study before and after periodontal treatment and between groups

			Stage 3 Grade C	Stage 3 Grade B	Control Smoking	Control	*p*
IL-1β	Baseline	mean ±SD	147.79±70.83[c,b]	140.94±70.36[c,b]	79.52±58.82[b]	36.19±25.92[c]	0.001*
		Med	134.727	122.415	66.354	37.000	
		Min	33.168	11.310	12.372	5.030	
		Max	244.053	244.053	232.018	97.680	
	6-week	mean± SD	85.09±52.99[a,b]	88.09±65.62[a,b]			
		Med	82.725	73.619			
		Min	5.912	11.664			
		Max	222.549	228.566			
IL-34	Baseline	mean± SD	0.98±0.06 [c,b]	1.03±0.08 [c,b]	0.88±0.031	-0.89±0.07	0.001*
		Med	0.958	1.003	-0.959	-0.914	
		Min	0.936	0.943	-1.029	-0.984	
		Max	1.151	1.268	0.673	-0.672	
	6-week	mean± SD	0.96±0.005 [c,b]	0.98±0.005 [a,c,b]			0.108
		Med	0.946	0.955			
		Min	0.932	0.934			
		Max	1.223	1.200			
RANKL	Baseline	mean± SD	1.46±0.13	1.44±0.006	1.43±0.45	1.44±0.06	0.108
		Med	1.405	1.422	1.422	1.424	
		Min	1.397	1.400	1.398	1.390	
		Max	1.830	1.675	1.598	1.636	
	6-week	mean± SD	1.43±0.007	1.43±0.003			0.347
		Med	1.408	1.413			
		Min	1.397	1.398			
		Max	1.726	1.561			
OPG	Baseline	mean± SD	168.45±15.11	141.42±15.33[c,b,d]	178.14±56.95	177.54±14.62	0.001*
		Med	169.889	137.228	170.181	182.347	
		Min	134.544	121.254	121.416	128.542	
		Max	191.295	174.395	394.299	187.729	
	6-week	mean± SD	175.02±56.60	171.27±12.24[a]			0.086
		Med	173.416	175.384			
		Min	121.720	133.578			
		Max	449.430	186.852			

[a]: different from baseline
[b]: different from control non-smoking
[c]: different from control smoking
[d]: different from stage 3 grade C periodontitis group
mean±SD(**standard deviation**), **med: median, min: minimum, max: maximum**
*** *p*<0,05**
Wilcoxon test was used to compare data during the treatment process(baseline-6th week).
Comparisons of numeric variables of the study groups were evaluated by the Kruskal-Wallis ANOVA test with post hoc Mann-Whitney test for pairwise comparisons.

DISCUSSION

We evaluated the predictive value of biomarkers IL-1 β, IL-34, RANKL, and OPG detected in the GCF of smoking and non-smoking stage 3 grade B and C periodontitis patients during the appraisal of the severity of periodontal diseases and the effect of non-surgical periodontal therapy. Considering the literature on periodontitis, it is a pioneering study that evaluated the success of periodontal treatment via full-mouth clinical measurements and biomarkers of GCF IL-1, IL-34, RANKL, and OPG in patients with smoking habits. As expected, the mean PI, GI, PD, and CAL values in the pre-treatment periodontitis groups were found to be statistically highly significant when

compared with the control groups. Again, as a predictable outcome, significant healing in clinical parameters was observed after treatment. In our study, the fact that the GI was lower in the smoking periodontitis group compared to the non-smoker periodontitis group is a result that supports the suppression of inflammation by smoking.

Periodontal disease is a multidirectional chronic disease that grounds the destruction of the periodontium (11). Periodontopathogens are necessary for the formation and progression of periodontitis and genetic predispositions, acquired diseases, and environmental factors determine the severity of periodontal destruction (12). The cytokine network is of great importance in revealing the molecular mechanisms of inflammatory diseases accompanied by active and passive periods, such as periodontal diseases. In periodontitis, pro-inflammatory cytokines are considered potential inflammatory markers and play a pioneering role in the inception and enhancement of the inflammatory response (13). Thus, the current study evaluated the role of pro-inflammatory cytokines as markers of bone resorption in different stages and grades of periodontitis patients.

Cytokines which are produced locally in the periodontal tissues are included in gingival crevicular fluid. The GCF is a non-invasive approach that consists of components of molecules involved in the host response network in the inflammatory process and is a reflection of the oral ecology located in the gingival sulcus. GCF is a unique model for the measurement of various potential inflammatory biomarkers, and investigation of periodontal immunoinflammatory and we can see the reflection of the host response (14). In the method of our study, it is among our priorities to evaluate the total amount of cytokine changes in GCF before and after periodontal treatment with smoking. In our study, 2 samples were taken from each patient and stored in the same eppendorf tube to support the detection rate in the analysis of biomarkers. Since many parameters affect the concentration in the presentation of GCF samples, the presentation of total cytokine activity is considered to be a good indicator (15).

Smoking may be at the top of the environmental risk factors for periodontitis, and a recent study reported that smoking is responsible for more than %50 of periodontitis cases (16). Studies have emphasized that there is a linear relationship between the inflammatory process of periodontitis and smoking habits (17, 18). While symptoms such as bleeding can be masked with smoking, an increase in plaque accumulation and the progression of the disease can accelerate. When smokers and non-smokers with similar plaque accumulation rates were compared, it was observed that the bleeding areas, the percentage of gingival discoloration, and the volume of

GCF were lower in the smokers (19, 20). According to our study findings, in smoker periodontitis patients PI, PPD, and CAL were higher and GI was lower than in non-smoker periodontitis patients. Our results support that is consistent with studies advocating the efficacy of smoking on increasing the amount of periodontal destruction (21). It is assumed that the destruction of periodontal tissues and alveolar bone loss is more common in actively smoking patients than in non-smokers. As a matter of fact, in our study, the level of GCF IL-1 β was found to be higher in all smoking groups.

IL-1β is a potent biomarker most studied in periodontal pathogenesis. Studies have shown that GCF IL-1β levels are dependent on the severity of inflammation, that the GCF IL-1β levels of periodontally healthy individuals are lower than those with periodontitis, and that these levels decrease in individuals with gingivitis and periodontitis after nonsurgical periodontal therapy (22, 23). Opposite to our result, Rawlinson et al. (24) announced lower GCF IL-1β levels in smokers. Differences in GCF IL-1β levels among smokers vary due to differences in host response, smoking-related variables, and the use of different techniques in GCF collection and analysis (25). Consistent with the outcomes of the studies introduced down there, in our study, GCF IL-1β level decreased after non-surgical periodontal treatment of P patients. IL-1β induces bone resorption and suppresses bone formation. Although it is a powerful pro-inflammatory cytokine involved in the pathophysiology of periodontitis, also it is comprised of inflammatory processes such as cell proliferation, differentiation, and apoptosis (26). Many studies have shown an interrelation between GCF and periodontal tissue IL-1β levels and the inflammatory state of periodontitis. In a study of periodontitis patients, it was reported that the IL-1β level decreased in the 2nd and 4th-month measurements compared to the initial measurements in shallow and deep pockets (22). Toker et al. reported that clinical complaints improved significantly with successful periodontal treatment and this clinical improvement was consistent with the reduced IL-1β in GCF (27).

IL-34 can completely substitute macrophage-colony stimulating factor in RANKL-induced osteoclastogenesis and this makes known the essential factor of IL-34 in inflammation-driven alveolar bone loss (28). In this research, before non-surgical periodontal therapy, the GCF IL-34 level was noticed to be higher in stage 3, grade B and C periodontitis groups compared to the smokers and non-smokers periodontally healthy groups. After non-surgical periodontal therapy, the GCF level of IL-34 in the periodontitis groups was reduced. Even after the non-surgical periodontal therapy, higher values were

observed in stage 3 grade B and C periodontitis groups compared to the controls. In support of our findings, in three different studies in which chronic periodontitis and healthy groups were evaluated together with obesity, smoking, and type-2 diabetes, they revealed that IL-34 level was uppermost in obese chronic periodontitis patients, followed by chronic periodontitis patients who smoked and then chronic periodontitis with type 2 diabetes mellitus (29). The IL-34 level was the lowest in systemically healthy periodontitis patients. (29,30). Guruprasad et al. assessed the GCF and plasma IL-34 levels in patients with chronic periodontitis and the efficacy of non-surgical periodontal treatment on GCF and plasma IL-34 levels. They informed that IL-34 levels decreased in measurements after non-surgical periodontal treatment (6). While there was no difference between GCF IL-34 levels between smoking and non-smoking control groups, the significant decrease in GCF IL-34 levels after 6-week in the stage 3 grade B periodontitis group can be the indication that smoking suppressed the response to treatment. In osteoclastogenesis, IL-34 plays an active role in the association of RANKL and ensures the adhesion and proliferation of osteoclast precursor cells. (34). In our previous study, we evaluated GCF IL-34 in chronic and aggressive periodontitis. We were informed that GCF IL-34 level was increased in the aggressive periodontitis group after initial periodontal therapy while this was the opposite in the chronic periodontitis group (31). When we look at the results of the current study, GCF IL-34 levels were significantly higher in the periodontitis groups compared with the periodontal healthy groups at the beginning. GCF IL-34 levels decreased in all periodontitis groups at 6-week. This is consistent with the results of other studies with IL-34 (5,32-33).

Osteoclastogenesis proceeds through the in vitro binding of RANK (reseptör of RANKL) with RANKL, resulting in a series of cellular events such as monocyte/macrophage progenitor differentiation and activation of mature osteoclasts. OPG inhibits osteoclast differentiation and bone resorption as a result of winning the race with the receptor of RANKL (34). In the literature, although the findings of studies examining RANKL and OPG levels in periodontitis patients differ, generally the results were that; the gingival tissues and GCF RANKL levels of periodontitis patients were statistically highly significant and OPG levels were decreased (8, 35-37). In a previous study, it was reported that smoking and increased periodontal inflammation did not affect the GCF RANKL level in the periodontitis group without any systemic disease (38). Similarly, in our study when the GCF RANKL levels before periodontal therapy were compared between the groups, the difference was insignificant.

Before periodontal therapy, GCF OPG levels were lower in Stage 3 groups against

periodontal healthy groups. However, the difference was significant only in non-smoking periodontitis patients. In addition, GCF OPG levels were statistically significantly higher in the smoker periodontitis patients than the non-smoker periodontitis patients. In the smoker periodontitis group, GCF IL-34 and OPG (r = 0.533) levels were negatively correlated. This correlation may indicate that cytokines simultaneously take an active role in the etiopathogenesis of periodontitis and the maintenance of periodontal health.

CONCLUSION

In the periodontal inflammation process, GCF IL-34 level followed a similar pathway to GCF IL-1β, suggesting that IL-34 may be a marker in the manner of development of periodontitis. The changes in IL-34 and OPG levels in the non-smoker periodontitis group after periodontal treatment suggest the negative effect of smoking on the response to periodontal treatment. More comprehensive studies are needed by increasing the number of samples included in the study groups to recognize the role of these bone destruction markers and smoking in the pathogenesis of periodontitis.

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Relationship of Thyroid Function with Metabolic Parameters in Euthyroid Adults

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Abstract

Objective: Thyroid hormones have a significant effect on carbohydrate, lipid metabolism disorders, and insulin resistance (HOMA-IR) development. Vitamin D (25(OH)D) has been shown also can affect not only the musculoskeletal system, but also almost all tissues in the body, including the thyroid in recent years. In the study, we aim of this study is to investigate the relationship between the levels of thyroid-stimulating hormone (TSH) within the reference range and metabolic parameters in adults.

Methods: 561 adult outpatients were divided into 2 groups low normal range (0.27-2.5 mIU/mL) and high normal range (2.5-4.2 mIU/mL) according to TSH, and HOMA-IR, 25(OH)D, and lipid levels were compared.

Results: A statistically significant positive correlation was found between TSH and HOMA-IR in both the low normal range group (r = 0.123, p = 0.041) and the high normal range group (r = 0.196, p = 0.001). In the high normal range group, the relationship between TSH with vitamin D (r =-0.200, p =0.003), cholesterol (r =0.143, p =0.024), LDL cholesterol (r =0.154, p =0.018), non-HDL cholesterol (r = 0.134, p = 0.035) levels was statistically significant.

Conclusion: Our study shows that high normal TSH levels in euthyroid adults are related to higher insulin resistance and lower 25(OH)D levels, and this interaction is a major contributor to dyslipidemia. Thyroid hormones explain the metabolic disorder in the early stages of T2DM. Therefore, we believe that screening TSH levels and determining the optimal TSH target will be beneficial.

Keywords: Euthyroidism, insulin resistance, lipid profile, vitamin D

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INTRODUCTION

Insulin resistance is a metabolic disorder in which cells fail to respond adequately to normal or increased insulin levels, causing impaired glucose uptake and utilization (1). Insulin resistance may occur with hypertriglyceridemia, hypercholesterolemia, glucose intolerance, obesity, Type 2 DM, and cardiovascular diseases. Thyroid hormones are also one of the important determinants involved in glucose homeostasis (2). Contrary to a general opinion that insulin is the main hormone responsible for blood glycemic control, there is reported that the synergistic effects of insulin and triiodothyronine affect glucose and lipid metabolism (3). In studies, it has been shown that patients with overt hypothyroidism and hyperthyroidism are more likely to develop insulin resistance and dysglycemia (2). However, it has also been stated that thyroid hormones are associated with insulin resistance in individuals with subclinical hypothyroidism and hyperthyroidism with mild thyroid dysfunction (4). This shows that abnormal thyroid hormones and thyroid-stimulating hormone (TSH) levels are effective on glucose metabolism and insulin resistance. The seriousness of the illness picture is proportional to the seriousness of the disorders. The development of insulin resistance in hypothyroidism is related to decreased blood flow, gluconeogenesis, lipolysis, and basal insulin secretion in peripheral tissues (2).

Overt hypothyroidism is also identified as a possible risk factor for increased risk of coronary artery disease (CAD) and death, it is often related to hyperlipidemia (5). Similarly, asymptomatic subclinical hypothyroidism, characterized by serum free T4 concentrations at normal levels and slightly elevated serum TSH concentrations are also accompanied by a risk of CAD, increased cholesterol, and LDL cholesterol levels (6). It has been indicated that the total cholesterol and LDL cholesterol levels of these individuals can be reduced by thyroxine treatment (7).

Vitamin D is involved in bone mineral homeostasis. However, high expression of Vitamin D receptors (VDR) has been shown in many tissues, including the thyroid gland in recent years (8). It has been shown that vitamin D is an effective hormone not only in the skeletal system but also in many target tissues and also affects the secretion of pituitary hormones (9). In addition, it has also been stated that vitamin D has anti-proliferative effects on thyrocytes in vitro. In the studies conducted, the correlation between low Vitamin D levels and subclinical hypothyroidism was drawn attention (10).

In the levels of thyroid hormones and TSH are observed differences which are documented with little individual variation within the normal range (11). Similarly, substantial

variation in thyroid function can be seen between populations. Such variations are due to a combination of genetics and environmental factors, for which the level of iodine intake is of great importance. Studies have conducted an increased risk of developing thyroid dysfunction in people with high normal TSH levels (12). It has been shown that the TSH value has above 2.5 mIU/L in about 9% of the population and the risk of developing hypothyroidism in the future is high. Therefore, although the optimal level for serum TSH levels is not clear, the general trend in recent years is to narrow the optimal TSH range (13).

TSH levels harm insulin resistance and cardiovascular risk factors. In addition, in euthyroid individuals, even small differences in thyroid function have a possible contribution to these negative effects. Therefore, the question of whether there is an **association** between TSH levels in the normal range with metabolic parameters and vitamin D levels will allow the identification of early new markers of cardiovascular risk. This study was conducted to investigate the potential relationship of TSH levels in the reference range with insulin resistance, serum 25(OH)D, and lipid levels in healthy, euthyroid adults.

METHODS

Study design and participants

This study was approved by the ethics committee of Istanbul Başakşehir Çam and Sakura City Hospital (No.2021.06.120). Due to the retrospective and observational character of the study design, the requirement for informed consent has been waived.

The records of 561 healthy, euthyroid adult patients who applied to the Internal Medicine Outpatient Clinic of Başakşehir Çam and Sakura City Hospital in Istanbul between September 2020-December 2020 were retrospectively analyzed. Those with diabetes mellitus, thyroid dysfunction, pregnancy, infection, and cardiovascular disease were not included in the study. The normal ranges of TSH are between 0.3-2.5 mIU/mL in 95 percent of the population (13). According to the TSH levels, the cases were divided into 2 groups as group 1 (low normal range (0.27-2.5 mIU/mL), (n=285) and group 2 (high normal range (2.5-4.2 mIU/mL), (n=276)). Fasting glucose, insulin, HOMA-IR, 25(OH)D levels, and lipid levels were compared between individuals with high normal and low normal TSH levels.

Data collection and analysis

Age, gender, body mass index (BMI), and biochemical data obtained retrospectively from the hospital information system of the patients who had simultaneous measurements of fasting glucose, insulin, TSH, 25(OH)D, and lipid profile were analyzed and compared. All tests were analyzed in Istanbul Başakşehir Çam and Sakura City Hospital Central Laboratory. The analysis of biochemical tests was carried out with serum obtained. Serum glucose concentrations with enzymatic reference

hexokinase, urea level with kinetic urease and glutamate dehydrogenase, creatinine level with kinetic colorimetric, cholesterol and triglyceride with the enzymatic colorimetric method, HDL with homogeneous enzymatic colorimetric method were measured on the Roche Cobas 8000 (Roche Indianapolis/America) analyzer. Serum TSH and insulin concentrations quantitatively with sandwich principle, fT4 and 25 (OH) D concentrations with competition principle method were measured on the Roche Diagnostics Cobas 8000 (Roche Indianapolis/America) analyzer. Serum LDL cholesterol levels were calculated. Evaluation of insulin resistance is based on simultaneous measurements of glucose and insulin. HOMA-IR= fasting glucose (mmol/L) x fasting insulin (µu/ml)/22.5 **(**4).

Statıstıcal Analysıs

In calculating the sample size of this study a Power analysis was determined by taking at least 80% and Type-1 error of 5 % for each variable. Kolmogorov-Smirnov and Skewness-Kurtosis tests were determined to check whether the continuous measurements in the study were normally distributed, and Parametric tests were applied because the measurements were distributed normally. The ”Independent T-test“ was used to compare continuous measurements according to the ”TSH groups". Pearson correlation coefficients were calculated to determine the relationships between continuous measurements. The chi-square test was used to determine the relationships between categorical variables. The SPSS (IBM SPSS for Windows, ver.26) statistical package program was used for analysis, and the statistical significance level was taken as 5 % in the calculations.

RESULTS

Basic and metabolic characteristics of groups formed according to TSH levels

A total of 561 adult participants were included in this study. TSH levels of all individuals were within normal limits (0.27–4.2 ml/mL). 285 patients had low normal TSH levels and 276 patients had high normal TSH levels. The mean age was 37.47±12.43 years in the low normal TSH group and 37.89 ± 12.84 years in the high normal TSH group. 73.7% and 75.7% of the patients were women, while 26.3% and 24.3% were men, respectively. There was no statistically significant relationship between the groups in terms of gender (p<0.05) (Table 1).

Table 1. The relationship and distribution of gender and TSH groups

Gender	TSH (0.27-2.5mIU/mL)			TSH (2.5-4.2 mIU/mL)			
	N	*Row %*	*Column %*	*N*	*Row %*	*Column %*	**p.*
F	210	50,1%	73,7%	209	49,9%	75,7%	,578
M	75	52,8%	26,3%	67	47,2%	24,3%	

** Significance level according to Chi-square test results,F: female, M: male*

In the table below; The biochemical measurements of the patients "according to

TSH groups" were compared and the results were given (Table 2).

Table 2: Comparison results of metabolic data according to TSH groups

	TSH (0.27-2.5 mIU/mL) Mean± SD	TSH (2.5-4.2 mIU/mL) Mean± SD	*p
Age, years	37.47±12.43	37.89±12.84	*0.691*
Glucose (mg/dL)	94.91±9.40	97.41±11.76	***0.005***
Insulin, uIU/mL	12.68±6.74	18.12±11.27	***0.001***
HOMA-IR	3.02±1.74	4.50±3.15	***0.001***
BMI, kg/m²	27.12±5.43	31.62±7.81	***0.001***
FT4, ng/dL	1.26±.17	1.09±0.12	***0.001***
25 (OH) D, ng/mL	21.06±11.36	16.39±7.99	***0.001***
Urea, mg/dL	23.96±7.05	24.56±10.70	*0.435*
Creatinine, mg/dL	0.74±0.16	0.77±0.54	*0.427*
Triglyceride, mg/dL	111.65±62.93	148.54±115.48	***0.001***
Cholesterol, mg/dL	185.17±40.34	195.06±38.98	***0.005***
HDL, mg/dL	53.02±14.83	49.93±13.08	***0.014***
LDL, mg/dL	110.31±33.59	116.50±30.90	***0.036***
Non-HDL Cholesterol, mg/dL	134.09±40.06	146.34±39.65	***0.001***

** Data are presented as mean ± standard deviation (SD), BMI = Body mass index. Significance levels according to Independent T-test results*

The fasting, insulin, glucose, and HOMA-IR levels of the patients changed according to the groups and were found to be statistically significantly higher in group 2 than in group 1 ($p<0.05$) (Figure 1 a, b, c). The BMI value of the patients was found to be statistically significantly increased while Vitamin D, and HDL significantly decreased in group 2 ($p<0.05$) (Figure 1 d, e). Similarly, the triglyceride, cholesterol, LDL, and non-HDL cholesterol levels of the patients were found to be significantly increased in group 2 compared to group 1 ($p<0.05$) (Figure 1 e).

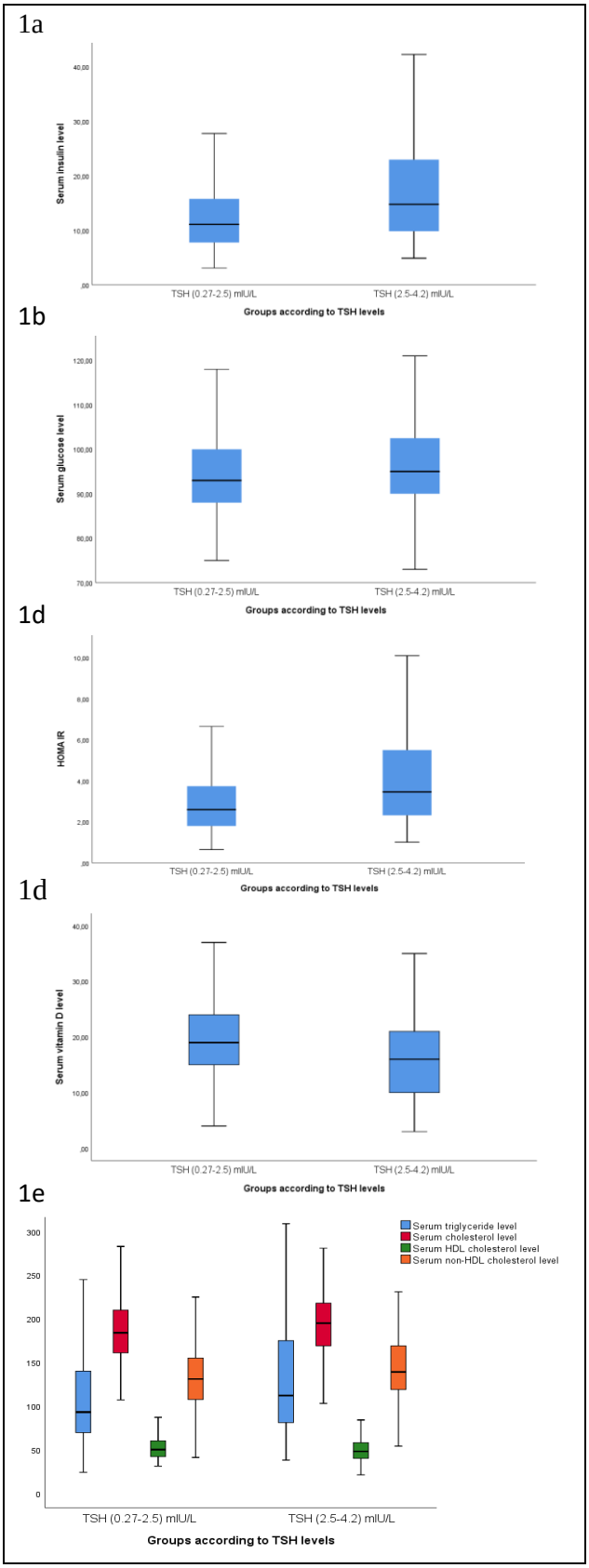

Figure 1 (a-e): Serum insulin, glucose, HOMA-IR, Vitamin D and Lipid levels according to TSH levels

Correlation analysis

TSH and insulin resistance

In the table below; the results of correlation analysis between "TSH" and "HOMA-IR" measurements were shown separately in the groups (Table 3). Accordingly; a statistically significant positive correlation (12.3%) was found between the "TSH" and "HOMA-IR" measurements in the "low normal range" group ($p<0.05$) (Figure 2 a). Similarly; a statistically significant positive correlation (19.6%) was found between "TSH" and "HOMA-IR" measurements in the "high normal range" group ($p<0.05$) (Figure 2 b).

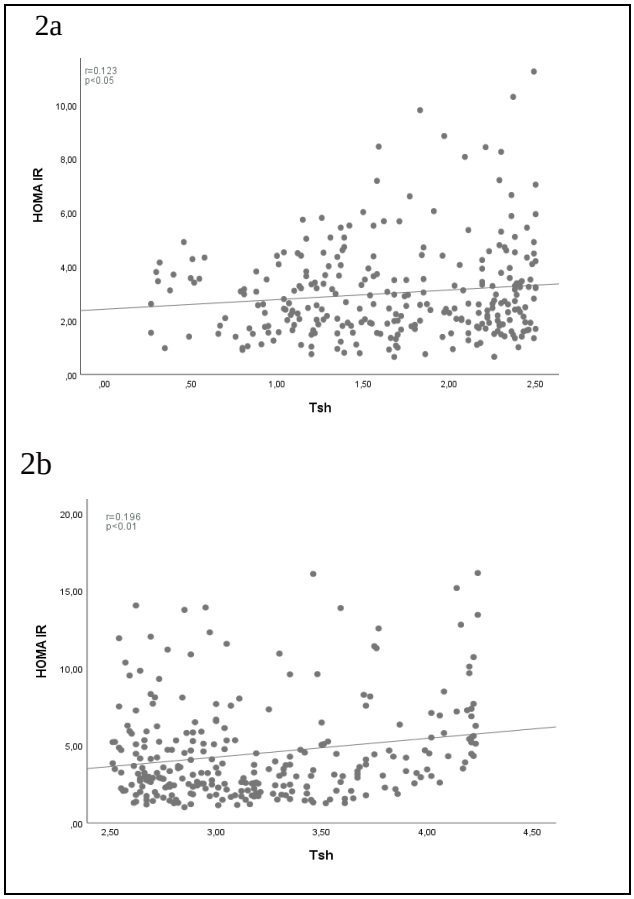

Figure 2 (a, b): Relationship between "TSH" and "HOMA-IR" in the low normal and high normal range group

Table 3: Correlation analysis between "TSH"and "HOMA-IR" measurements in groups

HOMA-IR	(0.27-2.5 mIU/mL)	(2.5-4.2 mIU/mL)	(0.27-4.2 mIU/mL)
	TSH	TSH	TSH
r	0.123	0.196	0.314
p.	***0.041***	***0.001***	***0.001***

** Pearson correlation*

TSH and other data

There was a significant positive correlation between TSH and HOMA-IR (r =0.123, p = 0.041), fT4 (r = -0.404, p =0.040) in the low normal range group. The relationship between vitamin D and cholesterol (r =0.141, p =0.045), LDL cholesterol (r =0.162, p =0.025), non-HDL cholesterol (r =0.157, p =0.027), HOMA-IR and glucose (r =0.443, p <0.001), BMI (r =0.329, p <0.001), insulin (r =0.982, p <0.001), HDL cholesterol (r =-0.203, p =0.001), non-HDL cholesterol (r =0.132, p = 0.040), triglyceride (r = 0.405, p <0.001) levels was statistically significant. There was a positive correlation between glucose and insulin (r =0.295, p <0.001), triglyceride (r =0.168, p =0.007), non-HDL cholesterol (r =0.141, p =0.025), BMI (r =0.289, p =0.001) levels was also statistically significant.

The relationship between TSH and glucose (r =0.231, p <0.001), insulin (r =0.165, p =0.006), HOMA-IR (r =0.196, p <0.001), fT4 (r =-0.477, p =0.025), vitamin D (r =-0.200, p =0.003), cholesterol (r =0.143, p =0.024), LDL cholesterol (r =0.154, p =0.018), non-HDL cholesterol (r = 0.134, p = 0.035) levels was statistically significant in the high normal range group. There was a significant positive

correlation between HOMA-IR with glucose (r =0.560, p <0.001), insulin (r =0.979, p <0.001), BMI (r =0.426, p <0.001), triglyceride (r =0.276, p <0.001), HDL cholesterol (r =-0.291, p <0.001), non-HDL cholesterol (r =0.159, p =0.013) levels was statistically significant. The relationship between glucose and insulin (r =0.413, p <0.001), triglyceride (r =0.231, p <0.001), HDL cholesterol (r =-0.224, p <0.001), non-HDL cholesterol (r =0.185, p =0.003) levels was also statistically significant.

DISCUSSION

As far as we know, this is the first study aimed at assessing whether TSH levels within the reference range are simultaneously related to insulin resistance, lipid profile, and vitamin D levels. In our study, the mean serum TSH level of all our patients was within the normal range. Our results showed that the increase in serum TSH levels within the reference range was positively associated with insulin resistance and BMI. The correlation between TSH and HOMA-IR was better in the high normal TSH group than in the low normal TSH group. In addition, cholesterol, LDL, and non-HDL cholesterol levels were found to be positively correlated with TSH levels, and fT4 and vitamin D were negatively associated. These data suggest that thyroid hormones are associated with metabolic status and may lead to increased insulin resistance and related diseases. Our data are similar to several studies showing that TSH levels in adults may be associated with insulin resistance (12,14).

Serum TSH concentrations were found to be positively correlated with fasting and post-loading insulin levels and negatively correlated with insulin sensitivity in euthyroid individuals (15). Ping Zhu et al. reported that TSH levels in 447 euthyroid individuals were positively and linearly related to HOMA-IR in both diabetic and nondiabetic groups (16). Mueller et al. also in a study, found a correlation between insulin resistance with TSH in polycystic ovary syndrome, independent of age and BMI (14). In our study, we found that increases in TSH levels in the reference range were positively associated with HOMA-IR, as reported by Vaia Lambadiari et al. (17). Studies suggest that even small deviations in thyroid hormone levels in the physiological range can lead to insulin resistance (2). Thyroid hormones control metabolic rate, core body temperature, appetite, and sympathetic activity as well as regulate insulin secretion and destruction (17). Studies conducted showed that thyroid hormones may affect insulin sensitivity by affecting the activation or expression of β adrenergic, and gamma receptors (18). Brenta et al. have reported that patients with hypothyroidism showed much lower glucose utilization during intravenous insulin tolerance tests (19). Thyroid hormone therapy has been reported to increase insulin sensitivity in obese diabetic rodents (20). In tissue cultures from rats, pose

to the thyroid hormone has been shown to cause raised GLUT 4 expression and glucose transport rate in the precursor cells of brown adipocytes. As a result, even mild hypothyroidism can trigger insulin resistance by causing reduced transcription of glucose transporters such as GLUT4 (18).

It has been stated that thyroid function is positively related to insulin resistance in obese individuals (21). Insulin resistance is determined by adipose tissue and associated inflammatory changes. As a possible key factor in the development of IR, it has also been suggested that leptin, which causes obesity, may be related to TSH (14). Studies conducted on euthyroid individuals have shown a positive relationship between serum TSH and BMI (12). Even minor changes in thyroid function have possible implications for the risk of developing obesity. Our results are consistent with the results of Mueller et al., in which BMI is positively correlated with TSH levels in the reference range (14). TSH receptors are found in a variety of body cells, including adipocytes. TSH mediates leptin secretion by binding to receptors on adipocytes (16). Leptin also has a possible role in the regulation of thyroid function through the stimulation of TRH. Moreover, thyroid hormones, and their metabolites also cause adiposity and weight gain. Mechanisms are related to adenosine triphosphate utilization, synthesis, direct impact on mitochondrial biogenesis, and its inotropic and chronotropic effects (22). This condition the idea that changes in the thyroid function are the main ones, and changes in BMI through changes in energy expenditure are secondarily effective. The increase in BMI and fat mass can also subsequently cause an increase in serum leptin (12). Since ectopic fat has a critical role in the formation of insulin resistance, fat cells may be an important factor in the relationship between TSH and insulin resistance (16). Our data confirm the results of some studies showing a close relationship between BMI and HOMA-IR and that development of obesity is a risk factor for the formation of IR (23,24).

Thyroid hormones are associated with dyslipidemia as they act as stimulators in the synthesis and destruction of lipids (25). Asvold et al. in a study conducted with people without known thyroid disease; reported that TSH increases in the normal range were positively, and significantly correlated with serum cholesterol, triglyceride, LDL, and non-HDL cholesterol levels (26). In our study, as in some studies conducted in individuals without significant thyroid dysfunction, increases in TSH showed an association with total cholesterol (27, 28) LDL cholesterol (27, 28) non-HDL cholesterol (27), and TG (28) levels. Thyroid hormones induce lipolysis in adipocytes and thus rise fatty acid levels in vivo. It also stimulates the re-esterification of free fatty acids to triacylglycerol, fatty acid

oxidation in the liver, and de novo lipogenesis from glucose metabolism (22). The higher total cholesterol and LDL cholesterol levels we detected may be due to less cell surface receptor expression for LDL leading to a reduction in LDL catabolism (26). Decreased lipoprotein lipase activity and impaired clearance of lipoproteins due to less LDL receptor function may also outcome in high triglycerides in patients with high TSH (18). Increased accumulation of triglyceride in muscle tissue of type 2 diabetics and obese has been related to IR (29). It is also known that insulin resistance is accompanied by high hepatic cholesterol, very low-density lipoprotein production, precursor LDL particles, and increased HDL cholesterol clearance. Since LDL particles have a lower affinity than the LDL receptor, there is a retardation in their clearance (30). Studies have shown that thyroid hormone replacement reduces total serum cholesterol and LDL cholesterol levels in persons with TSH in the upper limit of the reference range (31).

Data on the direct interactions among circulating TSH and vitamin D levels are still insufficient. Tamer et al. found the frequency of vitamin D deficiency to be higher in those with obvious hypothyroidism (94%) or subclinical hypothyroidism (98%) than in those with euthyroidism (86%) (32). Our results showed that TSH levels at the upper limits of the normal range were related to lower vitamin D. Barchetta et al. in his study, which examined the relationship of TSH levels with the seasons in euthyroid adults, revealed for the first time that vitamin D deficiency was related with higher TSH levels, and showed that the relationship between serum TSH levels with vitamin D status was independent of the season (33). Liu et al. found that mice that received intraperitoneal injection of calcitriol before sensitization with porcine thyroglobulin did not show signs of inflammation of the thyroid, and reported that vitamin D had a protective role against the formation of thyroiditis (34). Gelbard et al. VDRs have been detected in the pituitary gland and it has been shown that vitamin D regulates the secretion of pituitary hormones in experimental studies (35). Vitamin D levels can affect the hypothalamus-pituitary-thyroid axis exerting a direct effect on thyrocytes as well as VDR expression in hypothalamic and thyrotropic pituitary cells. Vitamin D insufficiency may result in decreased sensitivity of thyrocytes to TSH stimuli and increased serum TSH levels (33).

There is concurrence that many persons with high normal TSH are probably to have early signs of thyroid dysfunction**.** Some authors state that the reference range for TSH must keep as 0.4 -4.0 mIU/l, as there is not sufficient cause for lowering the upper limit of the reference range for TSH (36). Mueller et al. in their study, associated the 2 mIU/l TSH threshold for IR detection with the best sensitivity and specificity in women with PCOS (14). Some

studies suggest that individuals with a TSH value above 2.5 mIU/L are at risk in terms of thyroid disorders (13). Studies in euthyroid individuals have detected that increased thyroid antibodies correlate with TSH levels (31). In addition, the results of the 20-year follow-up of study also showed that TSH elevations greater than 2 mU/l were related to an increased hypothyroidism risk (37). The American Society of Clinical Endocrinologists stated that it would be appropriate to change the upper limit of the TSH reference range for adults to 3.0 mIU/L (38).

Our study has some limitations. The sample size of the study was relatively small, retrospective, and cross-sectional. Our findings indicate that multicenter, larger prospective studies are needed for the relationship between insulin resistance, hyperlipidemia, and vitamin D in people with TSH levels within the normal range.

In conclusion, our study showed that increased TSH levels within the reference range were positively correlated with insulin resistance and cholesterol, LDL cholesterol, and non-HDL cholesterol levels, and negatively correlated with vitamin D and fT4 levels. These data suggest that even TSH levels showing clinically normal thyroid function may be associated with low vitamin D levels and may conduce to the development of IR and dyslipidemia, which are effective in the formation of Type 2 DM and CAD. Therefore, we believe that determining the optimal TSH target and regularly reviewing serum TSH levels with thyroid status can reduce cardiometabolic risk and related morbidity and mortality.

Ethics Committee Approval: This study was approved by the ethics committee of Istanbul Başakşehir Çam and Sakura City Hospital (No.2021.06.120). Due to the retrospective and observational character of the study design, the requirement for informed consent has been waived.

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Author Contributions: Concept: Design:. Literature search: Data Collection and Processing: Analysis or Interpretation: Writing: G. Ş.

Conflict of Interest: The author declares that they have no conflict of interest.

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RESEARCH ARTICLE

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The Effect of Vitamin Use During Pregnancy on First Tooth Eruption Time in Postpartum Period

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Abstract

Objective: Vitamin supplements are commonly used to support the nutritional status of pregnant women during gestation. In this multidisciplinary study, we discussed the relationship between the three most used vitamin supplements in the Turkish population (vitamin D, vitamin B complexes, and multivitamins prepared for pregnant women) during pregnancy and tooth eruption time in babies.

Methods: The data of approximately 1,000 patients who gave birth in the obstetrics and gynecology clinic of our university were retrospectively reviewed. The babies of 145 patients who could be reached and who met the inclusion criteria were followed up in their postnatal period. Babies' first tooth eruption time, presence of caries, use of breast milk, and number of missing or excess teeth according to the month were determined.

Results: No statistically significant differences were found between the groups regarding maternal age, infant age, number of caries, breast milk use, or the number of missing/excessive teeth. However, it was found that patients who took vitamin D + B and patients who took only vitamin B had significantly earlier first tooth eruption times than those who used all three vitamins. The patients who used three vitamins had the latest tooth eruption time. There was no statistically significant difference between first tooth eruption time and maternal age or breast milk use.

Conclusion: The use of vitamins in pregnant women can affect babies' oral–dental systems, which develop while they are still in their mother's womb. More detailed information on the use of vitamins is needed in the future.

Keywords: Vitamins, tooth, pregnancy, health.

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INTRODUCTION

Tooth development and eruption in infants is affected by many biological factors (1). The earliest histological symptom of tooth development starts with the thickening of the oral epithelium on Day 11 of pregnancy and permanent dentition begins in week 20 of gestation, but the enamel matrix secretion for permanent teeth begins after birth (2). Micronutrient deficiencies at these two key time points can directly affect matrix secretion in hard dental tissues. These deficiencies can also impair tooth formation, mineralization, and the eruption process (3).

Vitamins are also micronutrients. In the literature, there are lots of studies about vitamins and dental development. However, its role in oral health or tooth eruption is not clear yet (4). It is generally known that an imbalance of vitamins leads to malnutrition, and they have great impact on dental health. Also, their deficiency causes oral problems and diseases (5). Vitamins greatly affect the nutritional status of hard dental tissues during developmental stages and, therefore, vitamin deficiency (6).

It is known that the time of the first tooth eruption is multifactorial and is affected by many variables. Besides personal characteristics such as genetics, ethnicity, and gender, other factors affect the time of first tooth eruption, including smoking and excessive caffeine consumption during pregnancy, birth weight, nutritional status of the baby at birth, gestational week, socioeconomic status, systemic diseases, obesity, and the environmental factors (7,8).

Vitamins are essential substances and we can only supply small amounts from food, so they are often provided through food supplements (5,9). Today, vitamin supplements are commonly used to support the nutritional status of pregnant women during gestation. Since the use of vitamin D and multivitamins in pregnancy period is supported by the Ministry of Health, it is very common in the Turkish population. Other vitamin supplements most commonly used during pregnancy are pyridoxine and other B complex vitamin supplements to reduce pregnancy-related nausea and vomiting, especially hyperemesis gravidarum (10). In this multidisciplinary study, we discussed the relationship between the three most used vitamin supplements in the Turkish population (vitamin D, B and multivitamins) during pregnancy and tooth eruption time in babies. We aimed to discern whether there is a relationship between vitamin supplements used during pregnancy and the baby's teething time.

METHODS

Design of the Study

This study, numbered 9/17(05.11.2020), was planned with the permission of the ethics committee of our university's faculty of medicine. The files of approximately 1,000

patients who gave birth in the obstetrics and gynecology clinic of our university between 2019–2020 were retrospectively reviewed, and their information on the pregnancy process was accessed for our study. The babies of 145 patients who could be reached, met the inclusion criteria, and accepted to participate, were followed up in the postnatal period. Babies' first tooth eruption time, presence of caries, use of breast milk, and number of missing or excess teeth according to the month were determined. First tooth eruption time is accepted as the day when the tooth appeared by piercing the gum and recorded as the baby' age at the time of eruption by month. Missing or excess teeth is determined by dental examination and accepted by determining how many teeth are missing or extra according to the number of teeth that should be at the age of the baby. Oral and written consent was obtained from all patients.

The inclusion criteria in the study were to have given birth in our obstetrics and gynecology clinic; for mothers to be between 18 and 45 years old without chronic systemic disease, gestational diabetes, diabetes mellitus, or a disease that could cause malabsorption; and to have a body mass index between 18 and 25.

Each baby was examined by a pedodontist with 10 years of professional experience. Babies older than 2.5 years were not included in the study. The data obtained from the mother and child were recorded in a computer environment and collected by obstetrician and two dentists.

Statistical Analysis

The analyses were performed with the IBM SPSS 20 (Statistical Package for and Social Sciences 20) statistical analysis program (SPPS Inc., Chicago, IL, USA). The data were prepared as the mean, standard deviation, median, minimum, maximum, percentage, and number. The normal distribution of continuous variables was analyzed with the Shapiro–Wilk and the Kolmogorov–Smirnov test. The ANOVA test was used for normal distribution, and the Kruskal–Wallis test was used for non-normal distribution to compare continuous variables with more than two independent groups. Following the ANOVA test, post-hoc tests were performed using the Tukey test when the variances were homogeneous. If the variances were not homogeneous, post-hoc tests were conducted using Tamhane's test. The Kruskal–Wallis one-way ANOVA (k samples) test was used for post-hoc tests, following the Kruskal–Wallis test. Statistical significance was $p < 0.05$.

RESULTS

A total of 145 patients were divided into seven groups: those who took only vitamin D (D vit, n:24), only vitamin B (B vit, n:41), only a multivitamin (Multi vit, n:13), vitamins D and B (D + B vit), vitamin D and a multivitamin (D + multi vit, n:9), vitamin B and a multivitamin (B + multi vit, n:9), and vitamins B and D and

a multivitamin together (D + B + multi vit, n:14) during their pregnancies. The demographic characteristics of the patients are shown in Table 1.

No statistically significant difference was found between the groups in maternal age (P = 0.561), infant age (P = 0.056), and the number of missing/excessive teeth (P = 0.448) according to age in the statistical study. There was also no statistically significant difference between the groups regarding the number of caries and breast milk use. Considering the first tooth eruption time of the babies, it was found that patients who took vitamins D + B and patients who took only vitamin B had significantly earlier first tooth eruption times than those who used all three vitamins, as shown in Table 2 (P = 0.009).

Table 1. Demographic characteristics of patients by groups

	D vit (n:24)	B vit (n:41)	Multi vit (n:13)	D+B vit (n:35)	D+Multi vit (n:9)	B+Multi vit (n:9)	D+B+Multi vit (n:14)
Mother age (year) Min-max mean±SD	23-39 30±4	22-40 30±5	16-40 31±7	23-44 30±4	19-36 28±6	23-34 28±4	18-36 28±4
Baby age (month) Min-max mean±SD	3-30 19±7	3-32 13±8	3-37 14±9	2-25 14±7	3-26 14±9	4-24 13±6	7-30 17±6
less/more number of teeth Min-max mean±SD	0-8 3±3	0-10 3±3	2*-8 3±3	0-16 4±3	0-6 4±2	0-10 5±4	2*-9 4±3
First tooth eruption time(month) Min-max mean±SD	4-12 7±4	5-13 5±4	4-12 7±5	3-11 5±4	2-10 6±4	4-13 6±5	5-12 10±2
Presence of caries No-yes (N-N)	21-3	39-2	12-1	34-1	9-0	9-0	13-1
Breast milk use No-yes (N-N)	11-13	17-24	5-8	16-19	4-5	3-6	5-9

SD: standard deviation
Min-max: Minimum-maximum
*: One patient each from the Multi vit group and the D+B+Multi vit group had two more teeth for their age.

Table 2. First tooth eruption times (month) according to the groups

Groups	First tooth eruption time (month)				Post-hoc test results
	Minimum	Maximum	Median	Mean ± Standart deviation	
D vit (n:24)[a]	4	12	8	7 ± 4	B vit – D+B+Multi vit (p=0.009) D+B vit – D+B+Multi vit (p=0.038)
B vit (n:41)[b]	5	13	6	5 ± 4	
Multi vit (n:13)[a]	4	12	7	7 ± 5	
D+B vit (n:35)[b]	3	11	7	5 ± 4	
D+Multi vit (n:9)[a]	2	10	7	6 ± 4	
B+Multi vit (n:9)[a]	4	13	8	6 ± 5	
D+B+Multi vit (n:14)[a,c]	5	12	10	10± 2	
p value = 0.009 (Anova)					

Similar letters demonstate no statistical significance

DISCUSSION

This retrospective study of a limited population demonstrated that vitamins B and D and multivitamins used by the expectant mother during pregnancy and their combination may influence babies' first tooth eruption time. The literature reports that this situation influenced tooth eruption in babies of pregnant women whose vitamin D levels were measured. Therefore, vitamin D is known to be related to bone biology and affects dentofacial development (11). Milk tooth movement through the alveolar bone is a complex issue about which there are only hypotheses. Many known and unknown factors, such as hormonal conditions, systemic diseases of the mother and baby, viral diseases in pregnancy and the baby, syndromes, systemic disorders, and hereditary and genetic factors, can affect the eruption of the first tooth (12). No relationship was found between maternal age and tooth eruption time in this study. One study found a relationship between maternal age and tooth eruption time (13). The small sample size of this research may have caused an unspecific relationship between maternal age and tooth eruption. The uncertainty of the mechanisms underlying the various first months of primary toothing in infants has been noted in some research (14). Many studies have examined the effects of smoking during pregnancy, low birth weight, breastfeeding, and vital effects on tooth eruption and have found divergent results (15,16). This study did not find a proportional relationship between breast milk use and tooth eruption. The literature examines the effects of metabolic and systemic diseases, hormonal problems, and obesity on tooth eruption (17). However, this study did not include these groups in its sample. This work found no relationship between vitamin use and early childhood caries. However, unlike this research, some studies have found a relationship between vitamin D levels and caries in infants (17). But this study did not include these groups in its sample. This study found no relation between vitamin use and early childhood caries. However, contrary to this study, there are studies that found a relationship between vitamin D levels and caries in infants (18).

There are studies in the literature stating that vitamin D supports the acceleration of tooth movement, in local applications. Again, there are also studies stating that tooth movement slows down due to vitamin D deficiency (19.20). In a study conducted by Xaviyer et al., it has been found that the serum level of vitamin D in the group with persistence of primary teeth was considerably lower than the control group. They determined a considerable delay in eruption in the group with vitamin D deficiency (12).

A literature search reveals that limited studies have evaluated the relationship between the vitamin supplements used in pregnancy and

the first tooth eruption time. Vitamins are steroid compounds that act like hormones because of their capacity to function far from where they are synthesized. The effects of the use of vitamin B and multiple vitamins on tooth eruption are not known, and there are hardly any studies on this subject (21).

This study evaluated the first tooth eruption time of newborns in the cases of using vitamin D, vitamin B, and multivitamins alone or together during pregnancy, and determined that tooth eruption occurs earlier in those who use only vitamin B and those who use vitamins D and B together compared to patients who use vitamins D + B + multivitamin together. However, no disparity was observed between the other groups. Consequently, it can be said that the time of tooth eruption is delayed if vitamin D and multivitamins are used together.

This study has some limitations. In this research, which evaluates the use of vitamins in pregnant women and the first tooth eruption time in babies, the vitamin levels of pregnant women before and after vitamin use are missing. The study, conducted with a small sample group, presented only preliminary information about the use of vitamins and first tooth eruption. The genetic pathways of vitamin synthesis in the patients were not included in the study. Nonetheless, this study attempts to illuminate the multifactorial nature of first tooth eruption in the use of vitamins.

CONCLUSİON

According to the outcomes acquiered from this study, the use of vitamins in pregnant women can affect babies' oral–dental systems that develop while they are still in the mother's womb. This retrospective study of a limited population depicted that the vitamins B and D, as well as multivitamins used by the expectant mother during pregnancy and their combination, may affect the eruption time of the first tooth in babies.More detailed information on the use of vitamins is needed for the problems that pregnant women and their babies may encounter. Simultaneously, it can be an important parameter to monitor in pregnant women and their babies.

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