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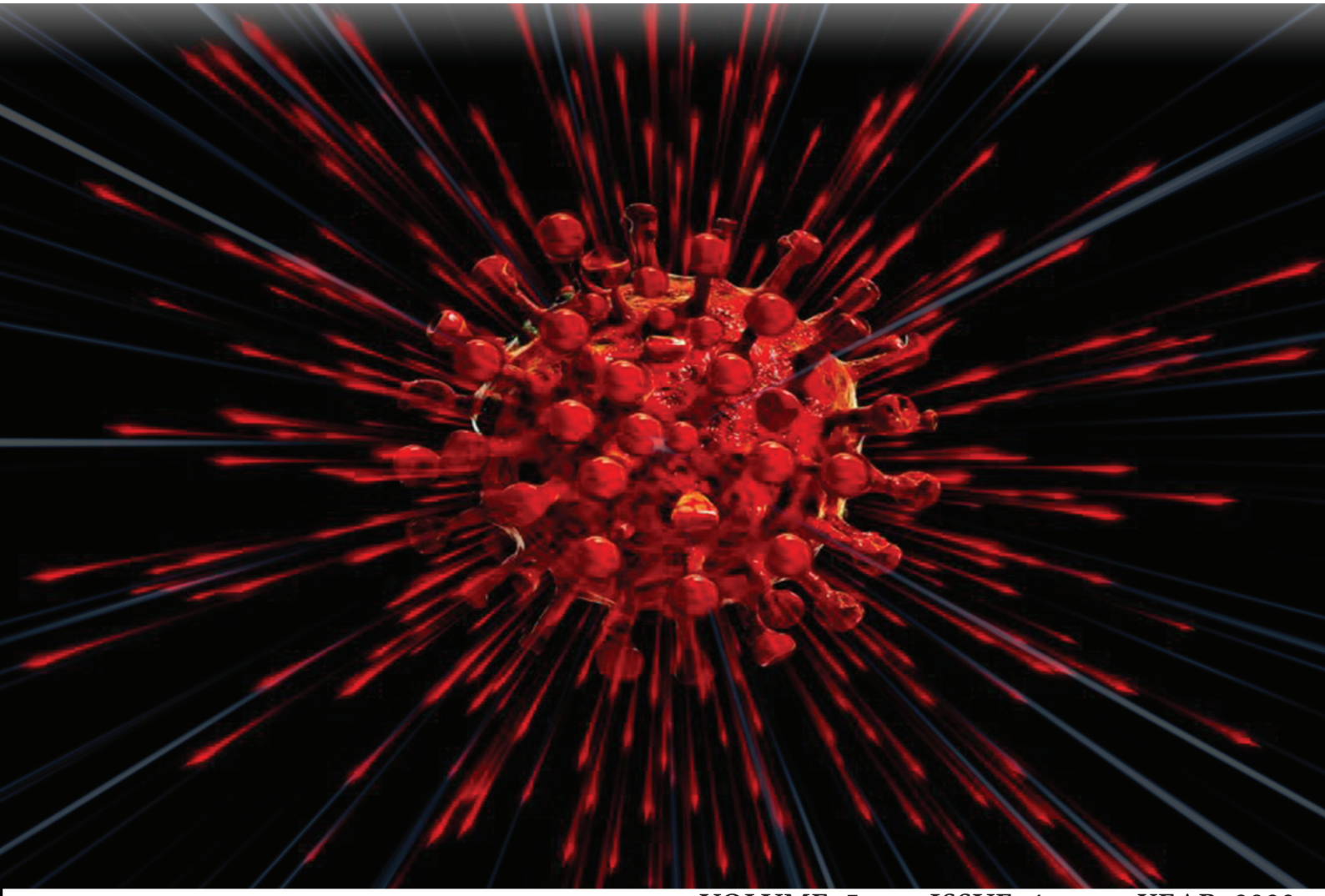

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Dear Coleagues,

We are proud to publish the fourth and final issue of Anatolian Current Medical Journal (ACMJ) in 2023. The quality of our journal is increasing day by day. We want to contribute to international literature at an increasing level and raise the bar of success for our journal by entering valuable international indexes such as SCI-Expanded, Scopus, and Pubmed in the near future. We would like to thank the editors who contributed to our journal, the authors who sent their articles, and everyone who contributed to the publication of the issue.

Sincerely

Prof. Dr. Aydın ÇİFCİ
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ANATOLIAN CURRENT MEDICAL

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Anatolian Curr Med J. 2023;5(4):305-310

The effect of thirdhand smoke belief on intention to quit smoking

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ABSTRACT

Aims: The study aimed to investigate the effect of thirdhand smoke perception (THS) on the intention to quit smoking.

Methods: We measured the perceptions of 285 smokers who admitted and did not admit to the smoking cessation outpatient clinic by the Third Hand Smoke Scale (THS) and their addiction by the Fagerström Nicotine Addiction Scale (FNAS). The factors affecting the intention to quit smoking were examined by logistic regression analysis.

Results: While 88.8% of the participants heard about passive smoking, only 14.4% stated that they had heard of thirdhand smoke. Those admitted to the smoking cessation outpatient clinic, those who had at least one smoking quitting attempt, those who wanted to quit smoking, those with high addictions, and those who were banned on smoking at home had a high THS perception, but no difference was observed in the parents.

Conclusion: Although SHS is high in smokers, we believe there is a need for the perception of THS to be supported to want and try to quit smoking. Even though the perception of THS, which has been studied for more than 10 years, is not at the desired level, it will be a strong psycho-technique with the concrete data it reveals in quitting smoking with the social training and orientations to be given.

Keywords: Perception of thirdhand smoke, perception of secondhand smoke, smoking cessation outpatient clinic

INTRODUCTION

Tobacco use is the leading preventable cause of morbidity and mortality in Turkey and the world.[1] At the same time, approximately 7-8 million people die from smoking-related causes each year (2018 WHO world health statistics), while more than 1 million people die from indirect exposure to cigarettes.[2-4] So far, the deaths due to COVID-19 are far below the deaths due to tobacco use and exposure each year.[5]

THS is residual tobacco smoke contamination left after the cigarette is put out and unlike SHS it is invisible and stays in the environment, clothing, hair of smokers.[6-8] SHS measures, such as airing the room, opening the windows, or smoking only in certain areas, cannot prevent or eliminate THS.[9,10] People are exposed to THS in 3 ways: Ingestion of nicotine-contaminated house dust, dust adhering to fabrics/surfaces absorbed through the skin, and converted into a respirable secondary polluting gas in the form of tobacco-specific nitrosamines (TSNA).[7,10]

In studies conducted, 35% of non-smokers and 57% of smokers think that THS does not harm children,[6] and those with high addiction (>10) have a low perception of THS harm.[11] On the other hand, according to the 2016 Global Adult Tobacco Survey (GATS), 83.3% of adults think that breathing someone else's smoke causes severe diseases in non-smokers, and 95.4% believe it will cause lung disease in children. These results indicate that although there is a general public awareness about the harms of SHS, people do not have enough knowledge and awareness about THS, which has been discussed since 2009, and their attitudes and beliefs are not yet developed. However, limited studies have demonstrated increasing knowledge about the adverse health effects of smoking and the belief that THS harms children. It has been shown that THS awareness would contribute to a decrease in smoking, an increase in smoking cessation attempts, and the adoption of smoke-free home/car policies.[4,6,7,9,11,12]

Although studies on thirdhand smoke are few in our country, there is not enough concrete data reported on awareness and exposure to thirdhand smoke. Hence, the present study aimed to measure the effect of thirdhand smoke belief on the intention to quit smoking in our study.

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METHODS

The study was initiated with the approval of the Bolu Abant İzzet Baysal Training and Research Hospital Ethics Committee (Date: 27.04.2021, Decision No: 2021/69). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 567 outpatients were admitted to the Family Medicine Clinic of Training and Research Hospital between March 15, 2021, and June 15, 2021. Among of them for quitting smoking admitted 270, and the other complaints admitted 297. A total of 285 outpatients was selected through stratified sampling from those who outpatient applied to the family medicine clinic as 129 (who were admitted to the smoking cessation outpatient clinic) and 156 (who were not admitted to the smoking cessation outpatient clinic). The inclusion criteria of study were to be over 18 years old, to be literate, have cognitive functions and active smoker. Those who did not comply with these conditions were not included in the study. Patients admitted to the Smoking Cessation Outpatient Clinic at the time of the study were considered as the group that wanted to quit smoking and those who smoked but never admitted to the smoking cessation outpatient clinic at the time of the study as the group that did not want to quit. It was selected through stratified sampling from those who applied to the family medicine clinic. Intention to quit smoking was measured by the survey question "Do you want to quit smoking." Information about the study was given online, and consent to participate in the survey was obtained. It was collected from voluntary participants via the Thirdhand Smoke Scale (THS) and Fagerström Nicotine Addiction Scale (FNAS).

Compliance with the normal distribution was examined based on the skewness and kurtosis coefficients and the range of ±2. Parametric data were expressed as mean±standard deviation using the Independent Sample t-test or One-way ANOVA test; nonparametric data were presented as median (min-max) deviation using the Mann-Whitney U test via SPSS18. The correlation between continuous variables was examined by Spearman correlation analysis, categorical variables was examined by Chi-Square analysis and were expressed as counts and percentages. The factors affecting the intention to quit smoking were examined by logistic regression analysis. If the cells have an expected count less than 5 was expressed Fisher-exact test. A p-value of <0.05 was considered statistically significant. Cronbach Alpha was 0.928 for THS, and 0.715 for FNAS and both scales were reliable. Also, it was decided since KMO and Bartlett's test value is 0.890, sampling adequacy is enough.

RESULTS

When **Table 1** was examined, 176(61.8%) participants were male and 195(68.4%) were married. It was stated that 183(64.2%) had at least one child, 164(57.5%) lived with their spouse and children, 174(61.1%) had smoker parents, and 246(86.4%) stated that they had full bans on smoking at home. Only 14.4%(n:41) of the participants stated that they had heard of thirdhand smoke. Participants who wanted to quit smoking (n:187, 65.6%) and those who had at least one smoking quitting attempt (n:216, 75.8%) had higher rates of applying to the smoking cessation outpatient clinic. While the mean age, years of smoking, daily smoked cigarettes, THS, and FNAS averages were higher in the group admitted to the smoking cessation outpatient clinic, the age of onset of smoking was lower.

Table 2 revealed that THS did not show a difference for the variables of gender, parentage, and parental smoking status. However, the mean THS of the participants who wanted to quit smoking, who had at least one smoking cessation attempt, who stated that they had heard of the concept of passive smoking before, and who had complete bans on smoking at home were higher. FNAS scores for the variable of having children didn't differ. While male participants, those who wanted to quit smoking, those who had at least one smoking cessation attempt, and those whose parents smoked had higher FNAS scores; those who stated that they had not heard of passive smoking before had lower FNAS score.

Table 3 indicated that THS positively correlated with the variables of wanting to quit smoking, trying to quit smoking, and hearing of passive smoking before. However, there was no correlation between hearing of thirdhand smoke before and THS. The model was compatible with the dataset as per the Hosmer and Lemeshow test result for logistic regression analysis (dependent variable: intention to quit smoking) (X_2^8:10.101, p=0.183), and the model formed by the explanatory variables was significant as per the omnibus test result (X_2^6:43.926, p<0.001). The gender of the person, whether there was a smoking cessation attempt, whether or not to smoke, and thirdhand smoke belief were effective in terms of wanting to quit smoking. The thirdhand hearing variable was not significant for this model. 19.7% of the change in the dependent variable, wanting to quit smoking, can be explained by the independent variables such as gender, attempt to quit, passive smoking, and belief in thirdhand smoke (R^2: 0.400).

Table 1. Comparison of socio-demographic characteristics of the groups (n=285)

		Who admitted to the SCOC# (n=129, %45.3)	Who not admitted to the SCOC# (n=156, %54.7)	All patients (n=285)	p-value
Gender	Female Male	43 (33.3%) 86 (66.7%)	66 (42.3%) 90 (57.7%)	109 (38.2%) 176 (61.8%)	0.121[a]
Marital status	Married Single	85 (65.9%) 44 (34.1%)	110 (70.5%) 46 (29.5%)	195 (68.4%) 90 (31.6%)	0.403[a]
Have children	Yes No	85 (65.9%) 44 (34.1%)	98 (62.8%) 58 (37.2%)	183 (64.2%) 102 (35.8%)	0.590[a]
Whom do you live with	Alone Only with spouse With wife and children Other	20 (15.5%) 17 (13.2%) 68 (52.7%) 24 (18.6%)	17 (10.9%) 21 (13.5%) 96 (61.5%) 22 (14.1%)	37 (13.0%) 38 (13.3%) 164 (57.5%) 46 (16.1%)	0.392
Parent smoking status	Yes No	78 (60.5%) 51 (30.5%)	96 (61.5%) 60 (38.5%)	174 (61.1%) 111 (38.9%)	0.853
Thirdhand hearing	Yes No	16 (12.4%) 113 (87.6%)	25 (16.0%) 131 (84.0%)	41 (14.4%) 244 (85.6%)	0.386
Smoking cessation attempt	Yes No	129 (59.7%) 0 (0%)	87 (40.3%) 69 (100.0%)	216 (75.8%) 69 (24.2%)	<0.001*[c]
Rule Regarding Smoking at Home	Yes No	116 (89.9%) 13 (10.1%)	130 (83.3%) 26 (16.7%)	246 (86.4%) 39 (13.7%)	0.121
Passive smoking hearing	Yes No	104 (80.6%) 25 (19.4%)	149 (95.5%) 7 (4.5%)	253 (88.8%) 32 (11.2%)	<0.001*
THS awareness among conscious smokers	Passive (+) Thirdhand (-) Others	88 (68.2%) 41 (31.8%)	124 (79.5%) 32 (20.5%)	212 (74.4%) 73 (25.6%)	0.030*
FNAS	Low (0-3 points) Medium (4-6 points) High (≥7 points)	30 (23.3%) 43 (33.3%) 56 (43.4%)	86 (55.1%) 48 (30.8%) 22 (14.1%)	116 (40.7%) 91 (31.9%) 78 (27.4%)	<0.001*
			$\bar{x}$±sd		
Age		39.1±12.3	36.1±8.2	37.4±10.4	0.018*[,b]
Years of smoking		20.8±12.1	16.5±8.8	18.4±10.6	0.001*[,b]
Daily number of cigarettes		23.1±11.4	14.2±8.1	18.2±10.7	<0.001*[,b]
THS		35.8±5.0	33.4±7.8	34.5±6.8	0.002*[,b]
FNAS		5.7±2.7	3.6±2.6	4.5±2.8	<0.001*[,b]
			Median (min-max)		
Age of start smoking		17 (9-41) 18.3±5.2	19 (10-39) 19.6±4.7	18 (9-41) 19.0±4.9	0.002*[,b]

#SCOC: smoking cessation outpatient clinic, [a]Chi-Square test [b]Independent Sample t test/Mann-Whitney U test [c]Fisher Exact test *Statistically significant

Table 3. Correlation between scales and logistic regression analysis

		Will to quit smoking	Smoking cessation attempt	Passive smoking hearing	Thirdhand hearing
Thirdhand Smoke Scale (THS) r p-value		 0.274 <0.001*	 0.215 <0.001*	 0.153 0.010*	 0.105 0.077
	Beta	**Standard Error**	**Exp (B) Odds Ratio**	**%95 CI for Exp(B) Lower - Upper**	**p-value**
Constant	-1.835	0.883	0.160	-	0.038*
Your gender	0.633	0.278	1.884	1.091-3.251	0.023*
Number of children	0.226	0.283	1.254	0.719-2.185	0.425
Passive smoking hearing	-1.844	0.615	0.158	0.047-0.528	0.003*
Thirdhand hearing	-0.183	0.375	0.833	0.399-1.738	0.626
THS	0.111	0.022	1.117	1.069-1.167	<0.001*
The total ban at home	-0.559	0.388	0.572	0.267-1.223	0.149

n=285; -2 Log Likelihood:322.904 R^2:0.143 (Cox & Snell), R^2=0.197 (Nagelkerke), r: Spearman correlation coefficient *Statistically significant

Table 2. Comparison of THS and FNAS scales by demographic characteristics

	THS	FNAS
Gender	**	**
Female	33.7±6.2	3.9±2.7
Male	34.9±7.1	4.9±2.9
p-value	0.153	0.001*
Have children	**	**
Yes	34.3±6.4	4.4±2.8
No	34.7±7.3	4.8±2.9
p-value	0.635	0.233
Will to quit smoking	**	**
Yes	35.8±5.4	4.9±2.8
No	31.9±8.3	3.7±2.8
p-value	<0.001*	0.001*
Smoking cessation attempt	**	**
Yes	35.3±5.8	4.8±2.8
No	31.9±8.7	3.8±2.8
p-value	0.003*	0.014*
Parent smoking status	**	**
Yes	34.3±6.8	4.9±2.8
No	34.8±6.7	3.9±2.9
p-value	0.498	0.009*
Passive smoking hearing	**	**
Yes	34.8±6.5	4.3±2.8
No	31.6±8.2	6.2±2.9
p-value	0.010*	<0.001*
Rule at home		
None/Partial	34.1±6.8	4.7±2.8
Yes	36.7±5.9	3.5±3.1
p-value	0.027*	0.015*
FNAS	**	-
Low (0-3 points)	34.9±6.4	
Medium (4-6 points)	34.1±6.7	
High (≥7 points)	34.2±7.4	
p-value	0.570	
The relationship between will to quit smoking and outpatient clinic application	**	**
Will (+) Plc (+)[1]	35.8±5.0	5.7±2.7
Will (+) Plc (-)[2]	35.9±6.2	3.3±2.4
No will[3]	31.9±8.3	3.7±2.8
p-value	<0.001*	<0.001*
	1,2>3 (p<0.001*)	1>2,3 (p<0.001*)
Correlation between attempt and will to quit smoking	**	**
Attempt (-) Will (+)[1]	37.9±4.7	2.2±1.8
Attempt (-) Will (-)[2]	29.9±8.8	4.3±2.9
Attempt (+)[3]	35.3±5.8	4.8±2.8
p-value	<0.001*	<0.001*
	2<1,3 (p<0.001*)	1<2,3 (p<0.001*)

* Statistically significant **Independent sample t-test/One-Way ANOVA (mean±standard deviation)

DISCUSSION

The present study is the first to investigate the effect of THS perception on the intention to quit smoking in smokers. Studies have shown that smokers' increased knowledge and beliefs about the harms of THS positively affect their intention to quit smoking.[1,12]

Similar to the studies of Escoferyy[7] and Rendon[9] 86% of the participants in our study stated that they had not heard of the concept of thirdhand smoke and did not know what it was. This situation can be perceived as proof that the awareness of THS perception has not changed over the years and that the awareness of harm perception has not yet been settled. Our study determined that the will to quit smoking was correlated with the increase in THS perception. While those who tried to quit smoking at least once constituted 20-25 percent of smokers.[13,14] this rate was close to the ratio of 18.9% in our study. We demonstrated that smokers who tried to quit smoking at least once were admitted to the outpatient clinic by taking action (who wanted to quit), had a high sensitivity to passive smoking, and had complete bans at home had high THS perceptions. The fact that the desire to quit smoking and the attempt to quit smoking occur with the increase in the perception of THS in smokers is manifested by the high rates of admission to the outpatient clinic.

It is known that avoiding smoking and exposure from a very early age will protect from cardiovascular risks, and smoking cessation is beneficial in reducing the risk even in older adults.[15] The fact that THS awareness increases as the perception of THS increases in individuals who have a long period of smoking, who start smoking at an early age, who have a high addiction, and who are elderly, reveals the importance of THS awareness.

While the average daily amount of cigarettes per capita in Turkey is 17.1 (Health Statistics Yearbook-2020), the daily consumption of those who were admitted to the polyclinic is higher in our study (mean: 23.1). Besides, while the increase in cigarette addiction along with the increase in daily cigarette consumption in studies reduces the perception of THS harm,[11] we did not find a difference in our study. However, the high sensitivity of secondhand smoke in both groups, who want to quit smoking and those who do not, suggests that the perception of harm from secondhand smoke turns into depersonalization and reduces taking an active role in smoking cessation. Besides, the high THS perceptions of those who have tried to quit at least once may provide evidence for the decision to quit smoking to turn into action.

While it is difficult to measure the extent of THS exposure precisely, children living with smokers are exposed to SHS and, inevitably, simultaneously to THS. Contrary to studies.[6,11,12,16] reporting that parents and those who

set rules about smoking at home have high THS harm perceptions, in our study, although parents and rule makers showed more smoking cessation attempts, being a parent was not significant for THS perception, but THS perception was high for rule makers. According to our study, the exposure rate of children to THS is 63.8%. Targeting parents and emphasizing the negative effects of THS on child development and health with scientific data can be a crucial element in promoting smoking bans at home for the perception of THS harm to become significant in parents.

It was stated that having a smoke-free house/car in the establishment of THS harm perception is associated with advice/direction, not asking questions about tobacco use and that THS-related messages will motivate people to make their homes smoke-free.[11,17,18] We think that the guidance of health professionals will contribute to the increase in the perception of THS harm and the intention to quit.

CONCLUSION

The present study determined that smokers who tried to quit smoking and had a high sensitivity to passive smoking had high THS perceptions. We saw that all data, including this high perception, significantly affected quitting smoking and strengthened the intention to quit smoking. We have determined that strengthening the perception of THS at all levels of smoking addiction, regardless of the degree of addiction, will play an active role in creating positive behavioral changes in smoking cessation.

Rather than repeating what is known as SHS, we think that the perception of THS, strengthened by training and awareness, will bring a new perspective and strength to the struggle to quit smoking. This, in turn, will make people who are resistant to quitting smoking more susceptible; and will brick the gap between scientific and popular understanding of the harms of THS up, which is supported by scientific evidence but not yet adopted; and will contribute to raising public health awareness.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Bolu Abant İzzet Baysal Training and Research Hospital Ethics Committee (Date: 27.04.2021, Decision No: 2021/69).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article

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What is the role of sensitization in carpal tunnel syndrome where pain impacts functional capacity?

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ABSTRACT

Aims: Expression of carpal tunnel syndrome (CTS) caused by the entrapped median nerve includes pain, paraesthesia and numbness. Extra median spread of pain can furthermore be seen as a clinical requirement defined by neuro inflammation. Central sensitization (CS) constructs a similar mechanism. This study aims to evaluate sensitization in patients diagnosed with CTS.

Methods: A total number of 152 patients diagnosed with CTS were evaluated, prospectively. Patients data such as gender, age, body mass index (BMI), disease duration, pain severity (NRS), painDETECT questionnaire, Boston CTS symptom severity scale (SSS) and functional status scale (FSS), CS scale, electroneuromyography results have been collected by the author and then the patients are divided into three groups.

Results: Regarding the age, BMI and CS rate, there was no statistical difference between the three patient groups ($p>0.05$). However, a statistically significant difference was found between these groups in disease duration, day-time and night-time NRS, Boston SSS, FSS, and pain DETECT scores ($p<0.05$). A statistically significant correlation between age, BMI, NRS daytime scores, Boston SSS, FSS, and CS existence was not found ($p>0.05$). Yet, statistically significant differences were found in a comparison of the patients with and without CS, in disease duration, NRS night scores, and painDETECT scores ($p<0.05$).

Conclusion: We conclude that the rate of CS is often undervalued in patients with CTS. CS should be considered in CTS patients with extra-median spread of pain.

Keywords: Carpal tunnel syndrome, functional capacity, central sensitization

INTRODUCTION

Carpal tunnel syndrome (CTS) is one of the common entrapment neuropathy that may be accompanied by neuropathic symptoms.[1]

Initially, pain and paresthesia occur due to compression of the median nerve in the wrist, and in the following period, loss of strength develops in the muscles innervated by the median nerve. However, some patients complain about the spread of the pain and paresthesia toward the proximal upper extremity, which does not follow the median nerve tracing. This clinical picture expressed as extra-median spread has been tried to be explained by peripheral and central sensitivity mechanisms.[2] Furthermore, Zanette et al.[2] noted that pain follows the median nerve trace in only 35% of the patients with CTS, while it occurs in the ulnar nerve trace at 5%. In another study, 45% of CTS patients had pain radiating to the elbow and shoulder region.[3] They explained this finding with neurogenic inflammation in the median nerve, sensitization mechanisms, and plasticity in the nociceptive pathways. This study also stated that neuropathic pain accompanied nociceptive pain, and the CTS patients with sensitization complained about increased pain at night and significantly reduced quality of life.[3]

Sensitization develops in many chronic painful musculoskeletal disorders.[4] It was postulated that increased nociceptive receptor sensitivity after long-term pain, peripheral sensitization due to neuroinflammation, the increased response of the nociceptive central nervous system neurons to normal or sub-threshold afferent inputs, or dysfunction of the endogenous opioid system causes central sensitization (CS). During the evaluation of CS, several methods, such as quantitative sensory tests, thermal sensitivity, and perception of vibration sensors, are used. However, these methods are difficult to apply

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in clinical practice and the evaluation process takes a long time. The CS inventory developed by Mayer et al.[5] is easy to use and provides rapid assessment, which has increased its use in clinical practice.

Delineation of the association between CS and CTS is essential for better understanding of the mechanisms of pain in CTS patients and during the decision-making processes regarding their therapeutic management. This study aimed to investigate the presence of sensitization in patients diagnosed with CTS.

METHODS

The study was initiated with the approval of Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 17/11/2021, Decision No: 2021-11/02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The current study was conducted as cross-sectional, at the medicine department of physical therapy and rehabilitation in a tertiary care centre. CTS patients aged between 18 and 75, with clinical symptoms and electroneuromyography (EMG) included in the study. Exclusion criteria were as follows: mental disability, pregnancy, cervical radiculopathy or plexopathy. Furthermore, since the conditions that are frequently accompanied by CS, patients with a previous diagnosis of migraine, fibromyalgia syndrome, chronic fatigue syndrome, restless legs syndrome, anxiety and depression, irritable bowel syndrome, multiple chemical allergies, and temporomandibular joint disorder were excluded from the study. All participants have signed informed consent documents before being included in the study.

The data of patients such as age, gender, body mass index (BMI), disease duration, and pain severity (numerical rating scale-NRS), has been recorded in the system. Neuropathic pain assessment has been performed through the painDETECT scale, and CS has been evaluated with the CS questionnaire. The Boston CTS rating scale was used to evaluate the impact of the disease on functional capacity. During the evaluation of CTS severity and electrophysiological examinations, American Electro diagnostic Medical Association guidance and diagnostic criteria were assessed.6 Patients who had prolonged distal latency in the median nerve sensory branch and reduced sensory nerve action potential amplitude were accepted as mild CTS. Yet, patients who had prolonged distal latency of the motor branch, according to these findings, were defined as having moderate CTS. Patients who had prolonged distal latency in both motor and sensory branches and reduced or absent compound muscle amplitude were defined as having severe CTS.

Levine-Katz (Boston) Questionnaire analysis (CTS Rating Scale): The scale has been developed by Levine et al., for functional and symptomatic assessment7.8 For symptomatology, the scale includes 11 questions; and for functional status, it includes 8 questions. Each question is scored on a scale of vary between 1 to 5 (i.e., mild to severe). The patient's functional and symptomatic complaints count on the higher score; if the score is high that refers to it is more severe. The outcome of the Boston Symptom Severity Scale (SSS) demonstrates symptomatic severity, yet, Boston Functional Status Scale (FSS) demonstrates its impact on functionality.

PainDETECT Questionnaire: The questionnaire is utilised for specifying the pain type.9, 10 Regarding the total questionnaire score of patients, 12 or fewer points are accepted as nociceptive pain. In contrast to this, patients with scores in the range of 13 to 18 were accepted to have a mixed type of pain with a neuropathic component, and pain scores of 19 and above reveal neuropathic pain.

Central Sensitization (CS) Inventory

This inventory consists of two sections: Section A is the CS-related symptoms, and Section B is the part regarding CS syndromes and questions whether the patient was diagnosed before or not.5 Section A contains 25 items and is scored between 0 and 100 points. In addition, each symptom is scored on a scale of 1 to 5 as never (0), rarely (1), sometimes (2), frequently (3), and always (4). Thus, relatively higher scores indicate more severe CS-related symptoms.[11]

Numerical rating scale (NRS) for pain: The scale has numerical values between 0 and 10 and the patient is requested to select the number that represents pain clearly. While a score of 0 indicates "no pain", a score of 10 indicates "unbearable pain". NRS assessments have been performed in both daytime and nighttime. These questionnaires were conducted for research goals in a face-to-face approach. All questionnaires and scales had Turkish validation.

Statistical Analysis

The SPSS (version: 22, 0) IBM SPSS statistical package program was executed through all statistical analyses in this study. Numerical data delivered as means and standard deviations revealed normal distribution. For the variables that are not offered the normal distribution, medians and interquartile ranges (IQR) were provided. Percentages and frequencies were operated for categorical variables. The results of the Shapiro-Wilk test, histogram, and q-q plots were analysed to assess the normality of data. Bonferroni, which is one of the Post Hoc tests has been employed to conduct pairwise comparisons. To compare multiple groups that did not show normal distribution, the Kruskal-Wallis test has been operated.

The Mann-Whitney U test and two-sided independent samples t-test have been performed to compare the differences between continuous variables.

The Pearson Chi-square test or Fisher's exact test has been conducted for comparing differences between categorical variables. Yet, the Phi correlation test was utilized to compare the categorical and continuous variables. For the p-value, the accepted significance level was lower than 0.05.

RESULTS

A total number of 152 participants met the inclusion criteria. The mean age of these patients was 43.35±12.71 years and 124 (81.6%) were women. The severity assessment revealed that CTS was mild in 55 (36.1%), moderate in 65 (42.8%), and severe in 32 (21.1%) patients. The CS inventory evaluation demonstrated that CS was current in 118 (77.6%) patients, and there was no CS in 34 (22.4%) patients. When the difference between the patients with and without CS regarding gender and the CTS severity (p>0.05) was examined, there was no statistically significant found. In **Table 1**, patients' demographic and clinical characteristics were presented.

Table 1. The demographic and clinical characteristics of the patients

Female/male	124 (81.6)/28 (18.4)
Age, (years)	43.35±12.71
BMI, (kg/m2)	29.48±4.62
Disease duration,(years) (years)	5.00±3.86
NRS	
Daytime	4.08±2.04
Night-time	5.76±2.65
Boston-SSS	3.00±0.73
Boston-FSS	2.38±0.86
CS scale	50.46±14.87
PainDETECT	16.02±4.44

Date presented as mean ± standard deviation or number (%)
BMI: Body masss index, NRS: Numerical pain scale, Boston SSS: Boston symptoms Severity scale, Boston-FSS: Boston functional status scale, CS: Central sensitization

Daytime and night-time NRS values of the patients were 4.0 (0.0- 8.0) and 6.00 (0.0-10.0). According to the outcome of the pain DETECT questionnaire, 28 (18.4%) patients had nociceptive pain; 99 (65.1%) patients had mixed-type pain and 25 (16.4%) patients had neuropathic pain.

In terms of age and BMI, it was determined that there was no significant difference between the groups. However, there were significant differences between these patient groups regarding disease duration, daytime and night-time NRS scores, Boston SSS, FSS, and painDETECT scores. In addition, there was a positive correlation between disease severity and all other parameters, as shown in **Table 2**.

Table 2. Relationship between CTS severity, demographics data, and clinical evaluation results

	CTS severity			r	p
	Mild	Moderate	Severe		
Age, (years)	42.0 (19.0)	43.0 (19.0)	41.0 (21.5)		0.781
BMI, (kg/m2)	28.07 (7.05)	29.97 (6.97)	30.43 (4.51)		0.151
Disease duration, (years)	3.0 (5.0)	9.0 (6.0)	5.5 (4.75)	.279	0.002
NRS					
Daytime	3.0 (2.0)	4.0 (2.5)	5.0 (2.75)	.314	0.000
Night	4.0 (2.0)	8.0 (3.0)	7.0 (3.0)	.483	0.000
Boston-SSS	2.36 (0.71)	3.09 (0.8)	3.71 (.78)	.636	0.000
Boston-FSS	1.87 (1.0)	2.5 (1.0)	3.6 (1.0)	.738	0.000
Pain DETECT	14.0 (4.0)	17.0 (3.5)	17.0 (6.75)	.485	0.000

Data presented as median (interquartile range), *p<0.05 is significant
CTS: Carpal tunnel syndrome BMI: Body mass index, NRS: Numerical pain scale, Boston SSS: Boston symptoms severity scale, Boston-FSS: Boston functional status scale,

Age, BMI, NRS daytime scores, Boston-SSS, FSS, and the presence of CS (p>0.05) was examined and, no statistically significant correlation was found. However, patients' comparison with and without CS revealed statistically significant differences in disease duration, NRS night scores, and painDETECT scores (p<0.05). The relationship of CS with demographic data and other variables is presented in **Table 3**.

Table 3. Relationship between CS inventory results and clinical variables

	Presence of CS		r	p
	Absence of CS	Presence of CS		
Age, (years)	43.0 (19.50)	43.0 (18.25)		0.464
BMI, (kg/m2)	28.30 (7.37)	29.01 (6.18)		0.430
Disease duration, (years)	2.5 (6.0)	5.0 (5.0)	.163	0.045
NRS				
Daytime	4.0 (3.0)	4.0 (2.25)	.226	0.153
Night	4.0 (1.25)	6.0 (3.25)		0.005
Boston-SSS	2.81 (0.62)	3.0 (1.0)		0.076
Boston-FSS	2.0 (0.90)	2.0 (1.13)		0.995
Pain DETECT	13.50 (6.0)	16.0 (3.0)	.347	0.000

Data presented as median (interquartile range), *p<0.05 is significant
BMI: Body mass index, NRS: Numerical pain scale, Boston SSS: Boston symptoms Severity scale, Boston-FSS: Boston functional status scale, CS: Central sensitization

In the numerical evaluation of the CS scale, there was a weakly positive correlation with disease severity (r=0.170). However, a statistically significant difference was not found between the mild, moderate, and severe CTS groups regarding the presence or absence of CS as per categorical evaluation (p>0.05). The data regarding the relationship between CTS severity and CS are shown in **Table 4**.

Table 4. Relationship between CTS and CS inventory results

	CTS severity			p
	Mild	Moderate	Severe	
Absence of CS	10	20	4	0.082
Presence of CS	45	45	28	

*p<0.05 is significant, CTS: Carpal tunnel syndrome, CS: Central sensitization

DISCUSSION

In musculoskeletal diseases that cause chronic pain, long-term nociceptive input leads to changes in pain transmission pathways and activation of peripheral and central sensitization mechanisms. Therefore, multi-dimensional evaluation is required in managing these diseases in addition to standard medical treatment.[12]

A positive correlation and significant difference were found between disease duration, the severity of nocturnal pain, neuropathic pain scores, and the presence of CS. However, no statistically significant difference was found between the disease severity and the presence of CS.

İt was well-known that age and obesity are the risk factors for CTS, and CTS is most common in patients aged between 40 and 60.[13] In a case-control study, it was stated that morbid obesity was a risk factor for CTS,[14] in another study including total of 109 patients, no statistically significant association was found between CTS severity and obesity.[15] In obesity, an increase in inflammatory mediators such as interleukins and the calcitonin gene-related peptide has been detected.[16] These mediators also play a role in the pathogenesis of CS. Although the impact of obesity on pain perception was previously analysed, the relationship between obesity and CS has not been evaluated.[17]

In this study, it was determined that there was no statistically significant difference was found between age and obesity, the severity of CTS, and the presence of CS. In CTS, repetitive exposure to painful stimuli and the prolongation of the disease duration cause an increase in pain intensity.[18] The CS mechanisms have an impact on the pathogenesis of refractory and chronic pain. Prolongation of the disease duration and the persistence of neuro inflammation leads to chronic pain and modulate pain sensitivity.[19]

In this study, disease duration was positively correlated with CS. This finding aligns with the other studies advising that disease duration and sensitization mechanisms should be taking into consideration during management of the treatment.[12]

The Boston CTS scale has been utilized in a meta-analysis study before and after CTS treatment to evaluate symptom severity and functional capacity.[20] The authors emphasized the effectiveness of manual therapy techniques based on soft tissue and neurodynamic mobilizations on isolation, pain, physical function and nerve conduction studies in patients with CTS.

In this study, we found a positive correlation and significant difference between disease severity and CTS scale results. However, it was not found to be a significant difference between patients with or without CS regarding Boston CTS scale results.

In CTS, it has been reported that the incidence of neuropathic pain was in the range of 31-77%.[21] occurring during the development of neuropathic pain triggered both peripheral and CS mechanisms.[22] In many clinical conditions, such as post herpetic neuralgia, complex regional pain syndrome, or traumatic nerve injury, neuropathic pain is accompanied by CS in the later stages.[23] In our study, we uncovered a statistically significant positive correlation between the painDETECT questionnaire scores and the CS. Pathophysiological changes that occur in the formation of CS and neuro inflammation mechanisms that cause an extra median spread of pain deliver common features.

The CS should be assessed for CTS patients with severe pain, parenthetic complaints, and extra-median spread of pain, appropriate treatment must be chosen.

In a study including a total number of 53 patients with chronic pain due to knee osteoarthritis, the researchers worked on CS.[24] In addition to evaluating the functional capacity of the patients, these authors conducted pain distribution mapping by operating a unique device. In this study, the increased extent of knee pain was accepted as evidence of diffuse hyperalgesia and CS. In another study involving a total number of 91 patients with knee osteoarthritis who experienced total arthroplasty, CS was detected in 44 patients, and relatively less pain palliation was determined in patients with CS in the postoperative period.[25] In a study number of 31 patients with CS who originated myofascial trigger points after whiplash, local anaesthetic was injected in 15 patients, and saline was injected as a placebo in 16 patients. Consequently, an increased pain threshold was observed in the local anaesthetic group.[26] In addition, a review hypothesized that manual therapy may inhibit CS mechanisms by reducing abnormal afferent input, inflammation, and oxidative stress.[27]

In another study, including a number of 140 female patients with CTS, participants were requested to map the pain.[28] Among these patients, 124 reported extra-median symptoms; however, no relationship was determined between pain severity and clinical and psychological factors. In addition, no relationship was found between the lateralization and spread of pain and the pain severity. These authors clarified the extra-median spread of the pain with CS.

In this study, the diagnosis of CTS was revealed by clinical presentation and EMG outcomes, and the pain distribution pattern was not questioned. Being found CS in most of our patients (i.e., 77.6%) indicates that sensitization should be evaluated in these cases.

A small number of subjects, the absence of a control group, and the lack of pain distribution mapping can be considered as the limitations of our study.

CONCLUSION

The CS rate is often undervalued in patients with CTS. CS should be considered in CTS patients with atypical pain, pain intensity and extra-median spread of pain. We advise that the examination of the co-existence of CTS and CS will be required due to the relief of symptoms caused by CS that may contribute to CTS treatment. Large series of studies that evaluate the effects of CTS treatments on CS is required to reach definitive conclusions.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 17/11/2021, Decision No: 2021-11/02).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Exploring the impacts of Pycnogenol on pentraxin-3 levels in the heart tissue of rats administered with gentamicin

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ABSTRACT

Aims: The present study explored if pentraxin-3 (PTX-3) levels, which would be boosted due to cardiac damage by gentamicin, can be regressed thanks to Pycnogenol, which was also previously shown to have desirable impacts on cardiovascular diseases.

Methods: In the study, we recruited 28 8-10-week-old male Sprague-Dawley rats into four groups: control, gentamicin, gentamycin+Pycnogenol, and Pycnogenol. We stained the tissue samples with hematoxylin-eosin and Masson's trichome dye for histopathological analysis. Then, malondialdehyde (MDA) levels were measured using the spectrophotometric technique. In addition, we measured PTX-3 levels in the heart tissues by an immunohistochemical method.

Results: We discovered the heart tissue samples of the rats in the control and Pycnogenol groups were histologically normal. As well as mononuclear cell increase and degeneration of cardiac muscle cells, we observed mild congestion in the gentamicin group compared to the control group. Despite more significant damage to the heart tissue in the gentamicin+Pycnogenol group compared to the control group, we found that the histopathological damage regressed in this group compared to the gentamicin group. While PTX-3 immunoreactivity was similar between the control and Pycnogenol groups, it was significantly elevated in the gentamicin group compared to the control group ($p < 0.001$). Moreover, the gentamicin+Pycnogenol group had decreased PTX-3 immunoreactivity than the gentamicin group. While MDA values followed a similar pattern between the control and Pycnogenol groups, these values were found to be significantly increased in the gentamicin group compared to the control group ($p < 0.001$). These values, however, were decreased in the gentamicin+Pycnogenol group compared to the gentamicin group.

Conclusion: In a nutshell, the present study was able to demonstrate that gentamicin may lead to cardiac damage by boosting PTX-3 levels and that the damage can be regressed thanks to the Pycnogenol treatment.

Keywords: Pycnogenol, gentamicin, pentraxin 3 (PTX-3), malondialdehyde (MDA), cardiotoxicity

INTRODUCTION

Pycnogenol, a phenolic compound, represents a nutritional product utilized as a bioactive phytochemical medication across the world. It was coined as a scientific term for the class of polyphenols; however, it currently refers to the extract of pine bark in France.[1] The resulting extract product is a fine, reddish-brown water-soluble powder. The consistent extract of Pycnogenol consists of phenolic components such as monomers (taxifolin, epicatechin, and catechin), condensed flavonoids (grouped as procyanidins and proanthocyanins), and phenolic acids (cinnamic acids and some glycosides). It bears a protective effect against inflammatory diseases, hypertension, diabetes mellitus (DM), and obesity.[2] In addition, it extends benefits to lung fibrosis and was previously shown to yield positive impacts on cognitive ability and cardiovascular diseases.[3-5]

Gentamicin is an aminoglycoside-group antibiotic adopted in the treatment of diverse bacterial infections. It is bactericidal that acts as a protein synthesis inhibitor by binding to the 30s subunit of the bacterial ribosome.[6] Aminoglycoside antibiotics often demonstrate three main toxic effects: nephrotoxicity, ototoxicity, and blocking neuromuscular-ganglionic transmission. The previous study also reported depression of cardiac function in various animal species, including rats, following aminoglycosides administration.[7] Acute hypotension emerging after intravenous (IV) administration of aminoglycosides leads to an adverse inotropic effect on the heart.[8,9] In addition, administration of aminoglycosides was associated with the weakening of hemodynamic parameters and even cardiovascular collapse.

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Pentraxin-3 (PTX-3), C-reactive protein (CRP), and Serum amyloid P component (SAP, an acute phase protein in rodents) are members of the pentraxin superfamily, but PTX-3 is characterized by the presence of an N-terminal domain in addition to a C-terminal pentraxin-like domain (the family's distinctive feature).[10] Expression of PTX3 is induced by proinflammatory signals such as IL-1β, TNF-α, and Toll-like receptor (TLR) agonists through both MyD88 and TRIF-dependent pathways.[10-13] Similar to CRP, plasmatic levels of PTX-3 are rapidly elevated in various pathological conditions of inflammatory and/or infectious etiology, including acute myocardial infarction, sepsis, and SARS-CoV-2 infections, and the higher levels are associated with disease severity and risk of mortality.[14-18] Therefore, the present study explored if pentraxin-3 (PTX-3) levels, which would be boosted due to cardiac damage by gentamicin, can be regressed thanks to Pycnogenol, which was also previously shown to have desirable impacts on cardiovascular diseases.

METHODS

We initiated this study with the approval of the Fırat University animal Experimentation Ethics Committee (Date: 18.04.2023, Decision No: 07-03). In all stages of the study, we strictly adhered to the rules and the principles set forth in the Declaration of Helsinki. We performed the experimental procedures on the research animals at the Experimental Research Center. At the end of the procedures, we studied the tissue samples in Faculty of Medicine, Histology and Embryology Department and serum samples in the Medical Biochemistry Laboratory of the Faculty of Medicine.

Procurement of the Rats

In this study, we studied the serum and tissue samples of 28 8-10-week-old Sprague-Dawley male rats weighing between 200-220 g. They were divided into four groups, with seven rats in each, and housed in rooms in a 12:12 light-dark (LD) cycle at 22±20 °C room temperature, with food and water ad-libitum during the 1-week adaptation and 9-day experiment period.

Experimental Groups

The groups were composed as listed below:

Group I (control group, n=7): The rats received orally 1 mL/kg saline throughout the experimental period.

Group II (gentamicin group, n=7): A single dose of 80 mg/kg gentamicin was administered to the rats at the beginning of the experiment.[19]

Group III (treatment group, gentamicin+Pycnogenol, n=7): The rats received a single dose of 80 mg/kg gentamicin at the beginning of the study19 and 50 mg/kg Pycnogenol dissolved in 1 mL of saline for 9 days.[20]

Group IV (Pycnogenol group, n=7): Rats were administered orally 50 mg/kg Pycnogenol dissolved in 1 mL of saline for 9 days.[20]

Collection of Tissue and Serum Samples

The rats in all groups were anesthetized using xylazine (10 mg/kg in a %2 solution) and ketamine (75 mg/kg in a %10 solution) and decapitated at the end of the 24-hour study period. Next, we rapidly removed their hearts. Some of the serum samples were stored at -20 ºC till biochemical analysis, and the remaining heart tissues were stored in fixative (10% formaldehyde) for immunohistochemical examination.

Immunohistochemical Evaluation

We applied the avidin-biotin-peroxidase (ABC) complex with minor modifications upon an immunohistochemical staining method. Sections of 4-6 µm thickness were taken from the tissue samples blocked with this method and deparaffinized. We used the primary antibody PTX-3 (Rabbit monoclonal IgG antibody, sc-373951 Santa Cruz Biotechnology, Inc.) diluted 1/200 with the TermoScientific™ TP-015-HA commercial kit. After applying AEC Chromogen, we performed staining using Mayer's hematoxylin and examined it under a light microscope. The preparations were then examined, assessed, and photographed using the Leica DM500 microscope (LeicaDFC295). Finally, we generated a histoscoring based on the prevalence (0.1=<25%; 0.4=26-50%; 0.6=51-75%; 0.9=76-100%) and severity of immunoreactivity in staining (0=No staining; +0.5= Very faint staining; +1=Faint staining; +2=Moderate staining, +3=Intense staining) (i.e., histoscore=prevalence x severity).[21,22]

Histopathological Analysis

We embedded renal tissues fixed with 10% formaldehyde in paraffin blocks after routine tissue follow-ups. The preparations prepared with hematoxylin-eosin (H&E) and mason trichome-staining on 4-6 µm sections from the paraffin blocks were examined through the semi-quantitative assessment proposed by Kuloğlu et al.[23] and photographed under a light microscope (Leica DFC295).

Biochemical Analysis

MDA (Malondialdehyde) Measurement: We measured MDA levels by adding 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N HCl (1:1:1,w/v) onto 500 µL of homogenate, as proposed by Placer et al. We next heated the mixture in a water bath at 100°C for 30 minutes. It was then cooled to room temperature and centrifuged at 15,000 g for 15 minutes. Finally, we determined MDA levels by analyzing the supernatant samples using a spectrophotometer at 532 nm. MDA levels are expressed as nmol/g wet tissue weight.[24]

Statistical Analysis

Initially, we resorted to the Kolmogorov-Smirnov (K-S) test to explore if the data showed a normal distribution. Accordingly, the data were presented as means and standard deviations and analyzed using one-way analysis of variance (ANOVA). We performed the pairwise analysis of the data appearing as statistically significant in ANOVA with a post-hoc test (Tukey). All analyses were performed in SPSS 26.0, and a p-value of <0.05 was considered statistically significant.

RESULTS

Histological Findings

The examination of Masson's trichrome- and hematoxylin-eosin-stained preparations of all groups showed that the heart tissue of the control (**Figure 1a**, **Figure 2a**) and Pycnogenol groups (**Figure 1d**, **Figure 2**d) had a typical appearance. Compared to the control group, the gentamicin group (**Figure 1b**, **Figure 2b**) had mild congestion (black arrow), mononuclear cell increase (red star), and degeneration of heart muscle cells (red arrow). Although the gentamicin+Pycnogenol group had greater damage to the heart tissue (**Figure 1c**, **Figure 2c**) compared to the control group, the findings yielded that the severity of histopathological damage was reduced in this group compared to the gentamicin group.

Immunohistochemical Findings

We examined immunohistochemical staining for PTX-3 immunoreactivity under light microscopy. The findings revealed PTX-3 immunoreactivity in heart tissue (black arrow), and it was similar in the control (**Figure 3a**) and Pycnogenol (**Figure 3d**) groups. Compared to the control group, PTX-3 immunoreactivity was found to be significantly increased in the gentamicin group ($p < 0.001$; **Figure 3b**). Yet, PTX-3 immunoreactivity was decreased in the gentamicin+Pycnogenol group than in the gentamicin group (**Figure 3c**; **Table 1**; **Figure 4**).

Table 1. Group Differences of PTX3 Histoscores and MDA Levels

	Control[a]	Gentamicin[b]	Pycnogenol[a]	Gentamicin-pycnogenol[c]	p
PTX3	0.34±0.12	1.05±0.18	0.31±0.13	0.70±0.12	<0.001
MDA	9.28±0.97	16.6±2.57	8.95±0.79	11.5±1.20	<0.001

Variables are expressed as means (M) and standard deviations (SD); the data were analyzed with one-way ANOVA; the Tukey test was utilized for pairwise comparisons; PTX-3=Pentraxin-3, MDA=Malondialdehyde. *a, b, are c are different in pairwise comparisons.

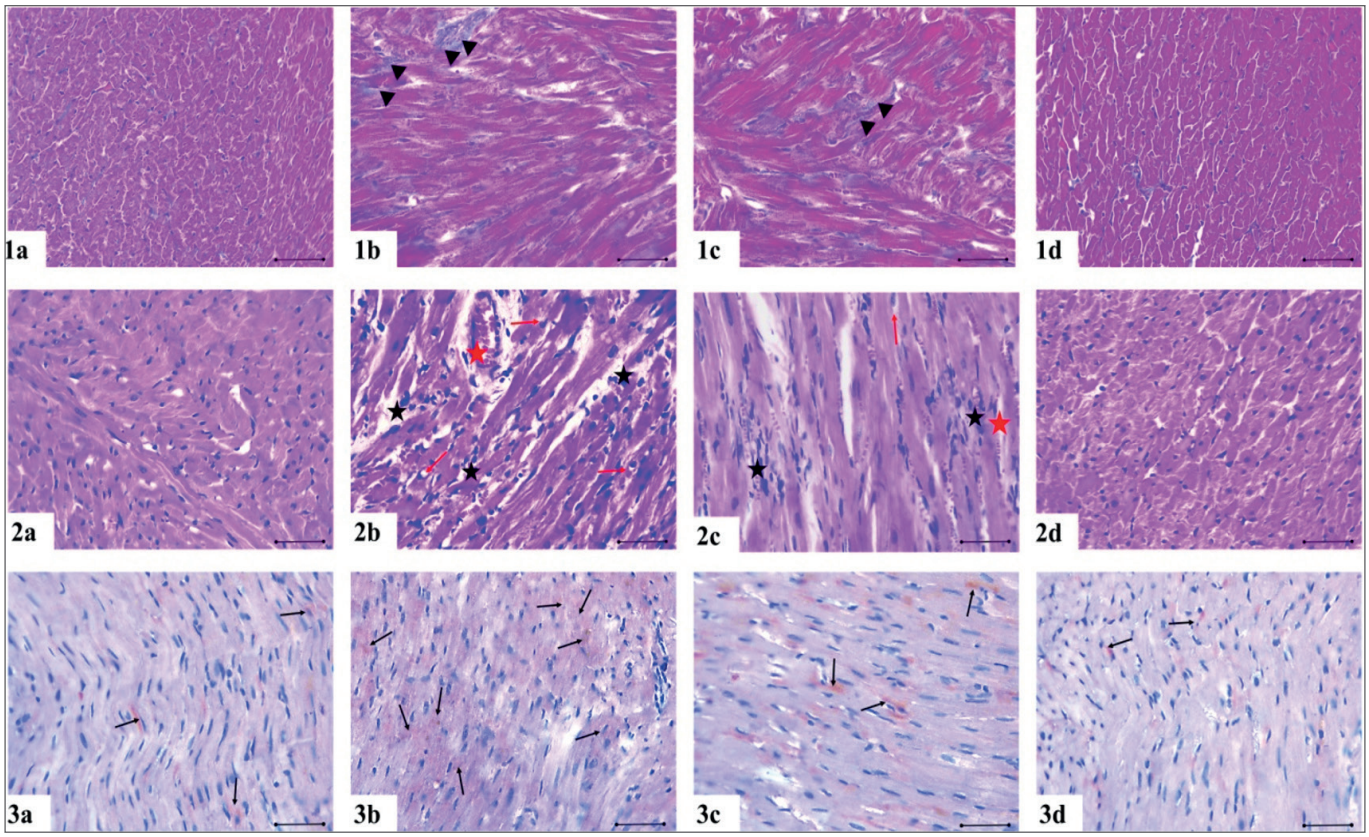

Figure 1. Cardiac tissue stained with Masson's trichome shows congestion (black arrow), mononuclear cell increase (red star), fibrosis (black star), and degeneration of cardiac muscle cells (red arrow); Scale bar=100 µm; a=control group, b= gentamicin group, c=gentamicin+Pycnogenol group, d =Pycnogenol group.
Figure 2. Heart tissue stained with hematoxylin-eosin shows mononuclear cell increase (red star) and degeneration of cardiac muscle cells (red arrow); Scale bar=100 µm; a=control group, b=gentamicin group, c=gentamicin+Pycnogenol group, d=Pycnogenol group.
Figure 3. PTX-3 immunoreactivity in heart tissue with immunohistochemical staining (black arrow); a =control group, b=gentamicin group, c=gentamicin+Pycnogenol group, d=Pycnogenol group; AEC chromogen; Mayer's hematoxylin; Scale bar=100 µm.

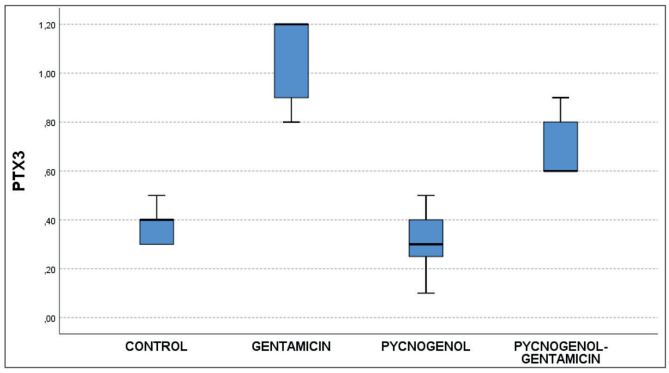

Figure 4. Box-plot of PTX3 levels of the groups (PTX-3: Pentraxin-3)

Biochemical Findings

MDA levels, a noteworthy parameter in demonstrating oxidative damage and an indicator of lipid peroxidation in tissue, were similar in the control and Pycnogenol groups. However, they were found to be significantly increased in the gentamicin group than in the control group ($p < 0.001$). We discovered that MDA levels were decreased in the gentamicin+Pycnogenol group compared to the gentamicin group (**Table 1**; **Figure 5**).

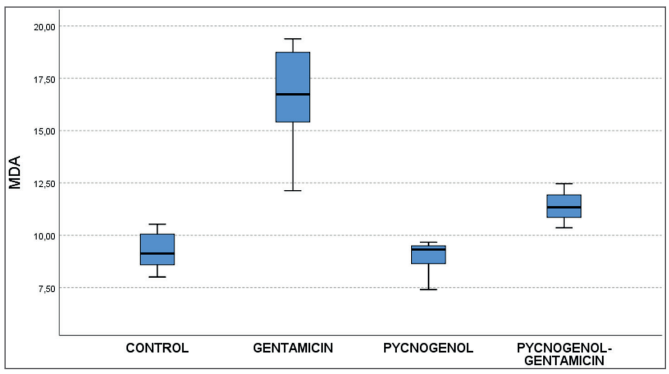

Figure 5. Box-plot of the spectrophotometrically-measured MDA levels of the groups (MDA: Malondialdehyde)

DISCUSSION

In the present study, we found that Pycnogenol, known for its protective properties, reduced PTX-3 and MDA levels in rats with gentamicin-induced cardiac damage. Therefore, we can now count cardiac protection among the protective properties of Pycnogenol.

Plant polyphenols act as natural antioxidants through a variety of processes, including free radical scavenging, metal chelation, and protein binding.[25] Among the natural polyphenols, Pycnogenol is an extract of a generic French pine bark (Pinus pinaster Aiton) containing polymeric (70%) and monomeric flavonoids (30%).[1] The high flavonoid concentration in Pycnogenol can be attributed to a wide range of antioxidant effects against both free oxygen radicals and nitrogen species.[20] In addition, Pycnogenol exhibits anti-inflammatory[26] and anticancer activities.[27,28] Orally administered Pycnogenol increases plasma antioxidant capacity, expressed as oxygen radical absorbance capacity,[29] and reduces plasma oxidative stress, measured as plasma free radicals.[30] Pycnogenol has further been shown to protect lipids from peroxidation by free radicals in the elderly and people with coronary artery disease.[31,32] Pycnogenol can improve endothelial function. It is thought that Pycnogenol exerts this effect by activating endothelial nitric oxide synthase (eNOS). In this way amplifying the nitric oxide generation from L-arginine, eventually leading to an increase in vessel lumen and adequate tissue perfusion. People with coronary artery disease, endothelial function was assessed by measuring the flow-mediated dilatation (FMD) of the brachial artery; 200 mg Pycnogenol per day was supplemented in a randomised, double-blind, placebo-controlled cross-over study for 8 weeks.[31] Several studies reported that the efficiency of Pycnogenol on blood vessels depends on the endothelium, as it could be abolished by administration of an endothelium-specific nitric oxide synthase inhibitor or by removing the endothelial lining.[33,34] Nishioka et al.[33] investigated the pharmacological effects of Pycnogenol on the endothelium-dependent vasodilation via nitric oxide production by measuring the forearm blood flow in response to acetylcholine (an endothelium-dependent vasodilator). They supported the beneficial effects of Pycnogenol on endothelial function with their study. Pycnogenol bears a protective effect against inflammatory diseases, hypertension, DM, and obesity.[2] Our findings were able to demonstrate the anti-inflammatory effect of Pycnogenol on cardiac cells, as it led to a decrease in PTX-3 levels in the treatment group; therefore, it should be noted that Pycnogenol may be adopted as a treatment option in case of cardiotoxicity.

PTX-3, identified as a cognate molecule of CRP, is a multifunctional protein with complex regulatory roles in inflammation, extracellular matrix organization, and remodeling.[10] In humans and rats, PTX-3 levels rise rapidly and dramatically in pathological conditions of inflammatory and/or infectious origin. The hallmark of PTX-3 may be that it rises more quickly than CRP (the peak within 6-8 hours for PTX-3 and within 24-48 hours for CRP), most likely due to local and systemic production of two proteins.[10] In addition, a previous study examined the heart tissues of patients who died from myocardial infarction and concluded specific immunostaining for PTX-3.[35] In our study, the high PTX-3 levels in the group administered gentamicin imply that we successfully generated cardiac damage in that group. In addition, reduced PTX-3 in the gentamicin+Pycnogenol group may be recognized as evidence that Pycnogenol alleviated cardiac damage.

MDA is the primary metabolite developing due to the oxidation and deterioration of cell lipids and is accepted as an index of lipid peroxidation.[36] Therefore, any tissue damage leads to an elevation in MDA levels. In our study, we determined that MDA levels were significantly higher in the groups receiving gentamicin compared to the others. On the other hand, reduced MDA levels in the treatment group may support the therapeutic effect of Pycnogenol.

Aminoglycosides (gentamicin and kanamycin), glycopeptide antibiotics (vancomycin), and quinolones (ciprofloxacin) are widely utilized in orthopedic surgery to prevent or treat associated infections.[37] Yet, gentamicin was previously reported to adversely affect cardiac function in various animal experiments.[7] The IV administration of aminoglycoside was also documented to cause acute hypotension and adverse inotropic effect on the heart.[8,9] It can initiate a pathological process that can lead to the weakening of hemodynamic parameters and even cardiovascular collapse. However, the mechanisms by which aminoglycoside antibiotics exert detrimental effects are largely speculative.[38] In their study, de la Chapelle-Groz and Athias[38] confirmed that gentamicin acts as a competitive inhibitor for the invasion of extracellular membrane calcium sites. This process may be the basis of early cardiac dysfunction following aminoglycoside therapy. However, penetration of gentamicin into cardiac cells may cause irreversible damage. Our study supported the previous findings that aminoglycosides cause cardiac damage by showing significant increases in PTX-3 and MDA levels in the gentamicin group. Our results also apparently underscored that gentamicin can be utilized as a cardiotoxic agent in other animal experiments and that it should be administered carefully in cardiac patients due to its cardiotoxicity. Moreover, we can assert that Pycnogenol can be considered a treatment option in clinical cases where cardiotoxicity is suspected (e.g., acute hypotension, cardiogenic shock).

Limitations

The present study is not free of a few limitations. This was an experimental study not carried out with humans. Moreover, we adopted only PTX-3 and MDA levels as indicators of cardiac damage, not other biochemical parameters (e.g., TAS, TOS, Caspaca-3, etc.).

CONCLUSION

We showed that Pycnogenol is an effective treatment method for agents causing cardiac toxicity. Furthermore, we can confidently propose that Pycnogenol should be counted in the treatment for increased proinflammatory parameters in patients treated with agents with well-known toxicity, such as gentamicin.

ETHICAL DECLARATIONS

Ethics Committee Approval: We initiated this study with the approval of the Fırat University animal Experimentation Ethics Committee (Date: 18.04.2023, Decision No: 07-03).

Informed Consent: Because the study was an animal experiment, no written informed consent form was obtained.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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

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Original Article

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The relationship between insomnia and acute postoperative pain: a case-control study on laparoscopic cholecystectomy patients

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ABSTRACT

Aims: Psychological factors play a significant role in predicting postoperative pain. However, the impact of insomnia on acute postoperative pain is little known. The aim of this study was to investigate the relationship between insomnia and acute postoperative pain.

Methods: We performed a case-control study in patients undergoing elective laparoscopic cholecystectomy. Patients with an Insomnia Severity Index (ISI) score >14 were allocated to an insomnia group (n=35) and those with an ISI score <7 to a control group (n=35). All patients were asked to rate their current level of pain on a numeric rating scale (NRS) at 1, 2, 4, 8, 12, and 18 hours postoperatively.

Results: There was no between-group differences in age, gender, body mass index, American Society of Anesthesiologists score, or operating time. The patients in the insomnia group had higher NRS scores and requested significantly greater amounts of tramadol (269.4±33 mg vs. 235.0±36 mg; p<0.001) and rescue analgesia (paracetamol; 1.06±0.8 g vs. 0.40±0.7 g; p<0.001) postoperatively than did the controls. Analysis of covariance revealed a significant interaction between insomnia and the preoperative experience of pain (F=6.62; p=0.013) and significant impact of insomnia on the mean NRS score (F=15.47; p<0.001).

Conclusion: Patients who experienced preoperative insomnia and underwent elective cholecystectomy have a reduced threshold for postoperative pain, which increases the need for analgesics.

Keywords: Insomnia, sleep disorder, postoperative pain, pain threshold predictor, cholecystectomy

INTRODUCTION

More than 230 million patients undergo surgery each year worldwide.[1] Surgical procedures are often followed by acute postoperative pain, which should be relieved to promote healing and prevent complications.[2] However, despite efforts to improve the management of acute postoperative pain, a high number of patients experience moderate to severe pain during the immediate postoperative period.[3]

Insomnia is a common chronic disorder, defined as a subjective report of difficulty with initiation, duration, consolidation, or quality of sleep that occurs despite adequate opportunity for sleep and causes substantial distress and impairment of daytime functioning.[4,5] Psychiatric and medical comorbidities commonly present with insomnia.[6,7] Chronic pain is one of the most important of these comorbidities and may increase the burden on health care. There is strong evidence that sleep disturbances and chronic pain have a reciprocal relationship.[8-10] Symptoms of insomnia not only increase the risk of developing pain in the future but also increase the severity of existing pain. However, the severity of pain may also predict the severity of subsequent insomnia.[11-14]

Acute postoperative pain differs from chronic pain in its etiology, mechanism, and treatment.[15] Beyond incisional pain, hyperalgesia, the mechanism of ischemic pain, and central neuronal sensitization, and psychologic factors

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can be associated with the experience of postsurgical pain.[16] We hypothesized if patients with insomnia experienced increased postoperative pain. Therefore, we performed a case-control study to investigate the experience of postoperative pain in patients with insomnia undergoing elective laparoscopic cholecystectomy.

METHODS

After approval by İstanbul Training and Research Hospital Clinical Researches Ethics Committee (Date: 01.03.2019, Decision No: 1731), and obtaining informed consent from all enrolled subjects, we performed this prospective observational case-control study in patients scheduled for elective laparoscopic cholecystectomy between January 2017 and January 2020 at the İstanbul Training and Research Hospital. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Patients with renal failure, thyroid dysfunction, morbid obesity, obstructive sleep apnea, neurologic dysfunction, or alcoholism were excluded, as were patients receiving antidepressant therapy, those scheduled for urgent surgery, and those taking anticonvulsants or opioids.

To assess the nature, severity, and impact of insomnia, the patients completed the Turkish version of the 7 item Insomnia Severity Index (ISI) questionnaire.[17,18] The dimensions evaluated were as follows: severity of sleep onset, sleep maintenance, early morning awakening problems, sleep dissatisfaction, interference of sleep difficulties with daytime functioning, noticeability of sleep problems by others, and distress caused by sleep difficulties. A 5-point Likert scale was used to rate each item (0, no problems; 4, very severe problems) and yielded a total score ranging from 0 to 28. The total score was interpreted as follows: absence of insomnia (0-7); sub-threshold insomnia (8-14); moderate insomnia (15-21); or severe insomnia (22-28). The patients were divided into two groups according to the results of the ISI questionnaire. Thirty-five patients with moderate to severe insomnia (ISI >14) and sleep problems lasting for more than 3 months were allocated to an insomnia group and 35 with an ISI score ≤7 were allocated to a control group.

To assess the preoperative experience of pain, the patients were asked if they have experienced any pain lasting for 1 day or more in the previous month. If pain was reported, the patient was asked to describe the pain on a two-sided blank body manikin (2 front and back) before surgery. Pain that was located on both sides of the body above and below the waistline and in the axial skeleton was defined as ''widespread pain''. Pain experiences that did not meet all the criteria for widespread pain were defined as ''some pain''.

Postoperative pain was assessed by a different anesthesiologist who was blinded to group allocation. To evaluate the postoperative pain, the patients were asked to rate their current level of abdominal pain using a numeric rating scale (NRS; 0, no pain; 10, the worst imaginable pain) at 1, 2, 4, 8, 12, and 18 hours after surgery. Patients with a mean NRS score ≥4 were considered to have moderate to severe pain.

None of the patients received a premedication agent. Propofol 2 mg/kg, fentanyl 1 μg/kg, and rocuronium 0.5 mg/kg were used for the induction of anesthesia in all patients. No further fentanyl was administered intraoperatively. Anesthesia was maintained using sevoflurane in a mixture of oxygen 2 L/min and N_2O_2 L/min. The anesthesia technique during the operations was standardized.

An intravenous patient-controlled analgesia (PCA) device (CADD-Legacy Patient Control Analgesia Device Model 6300; Ambulatory Infusion Pump Smith Medical ASD, Dublin, OH, USA) was used in all patients for postoperative pain management. All patients were informed about how to use the device and how to control the pump before and after the operation. Each pump contained 300 mg tramadol diluted in 100 mL of 0.9% saline solution. The PCA device was set to deliver a continuous infusion rate of 10 mg per hour, with a bolus dose of 10 mg and a lock-out interval of 15 minutes. During treatment with PCA, when analgesia was inadequate, rescue analgesia (paracetamol 1 g) was provided.

Statistical Analysis

The power analysis was performed using the difference in NRS scores between the 2 groups and calculation was based on a findings of a previous study (19). We assumed that the mean NRS scores in the insomnia group and control group would be 3.6 and 2.0, with a standard deviation (SD) of ± 2.0 and calculated sample size for each group of 32 (α =0.05, β= 0.8). Therefore, 35 subjects were included in each group to allow for a dropout rate of 10%. Normality of distributions was tested with the Shapiro-Wilk test. Numerical data are expressed as the mean ± SD, and the categorical data as the number (percentage). The parametric data (age, body mass index [BMI]) were analyzed using the Student's t-test, nonparametric data (operating time, postoperative tramadol and paracetamol doses, postoperative NRS scores) with the Mann-Whitney U test, and categorical data (sex, preoperative experience of pain, American Society of Anesthesiologists (ASA) score, mean NRS score) with the chi-square test. We

also performed an analysis of covariance to assess the main effects of insomnia, age, sex, BMI, and preoperative experience of pain and their interaction with regard to the postoperative mean NRS score. The statistical analysis was performed using SPSS for Windows software (version 21; IBM Corp., Armonk, NY, USA). A P-value <0.05 was considered statistically significant.

RESULTS

The inclusion criteria were met by 110 of the 470 patients operated on by the same surgeon. (**Figure 1**) Due to technical problems with the device and drug inadequacy during the study, 40 of the 110 participants were eliminated from the study. The patients' descriptive data are summarized in **Table 1**. There was no statistically significant between-group difference in sex, age, BMI, operating time, or ASA physical classification. The preoperative experience of pain was more common in the insomnia group than in the control group (p=0.04). Nine patients in the insomnia group had some pain and 4 had widespread pain whereas 2 patients in the control group had some pain and 1 had widespread pain. The postoperative NRS scores at 1, 2, 4, 8, 12, and 18 hours after surgery was significantly higher in the insomnia group (**Table 2**). Tramadol consumption was also significantly higher in the insomnia group (269.4 ± 33 mg) than in the control group (235.0 ± 36 mg; p<0.001, **Table 3**). A rescue dose of paracetamol 1 g was required in 26 patients in the insomnia group and 12 in the control group (p=0.001). The mean NRS score was also calculated according to the results checked after surgery. Twenty-seven patients in the insomnia group (77.1%) and 4 in the control group (11.4%) experienced moderate or severe postoperative pain (p<0.001; **Table 3**).

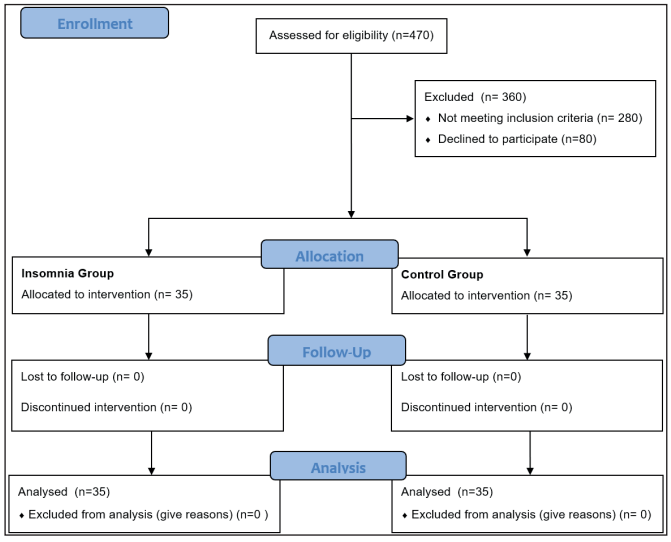

Figure 1. Flow Diagram of the Patient Selection

Table 1. Baseline descriptive and clinical characteristics

	Insomnia group	Control group	p
Age (years)	49.5±9	49.5±9	0.968
Gender (f/m)	27/8	24/11	0.420
BMI (kg/m²)	28.7 ±4	29.8 ±5	0.286
Duration of surgery (minute)	45.6±12	49.4 ±12	0.102
Preoperative pain experience (y/n)	13/22	3/32	0.04
ASA 1/ASA 2	18/17	18/17	1.0

f/m=female/male; BMI=Body mass index; y/n=yes/no ; ASA: American Society of Anesthesiologists, Data are presented as mean ±SD and number.

Table 2. Postoperative NRS Scores

	1h	2h	4h	8h	12h	18h
Insomnia group	8.37±2.1	7.43±2.2	6.00±2.1	4.49±1.9	3.09±1.6	2.34±1.6
Control group	7.06±2.3	5.57±2.1	3.29±2.1	1.63±2.0	0.54±1.3	0.43±1.3
P	0.02	<0.001	<0.001	<0.001	<0.001	<0.001

h=hour (Time after operation); Data are presented as mean ± SD

Table 3. Postoperative analgesic consumption and mean NRS scores

	Insomnia group	Control group	p
Tramadol consumption dose(mg)*	269.4 ±33	235.0±36	<0.001
Rescue dose of paracetamol (gr)*	1.06±0.8	0.40±0.7	<0.001
Mean NRS score ≥4**	27(77.1)	4(11.4)	<0.001

*Data are presented as mean ±SD; ** Data are presented as number (%)

To elucidate the interplay between demographic factors, insomnia, preoperative experience of pain, and mean NRS score, we performed an analysis of covariance (with the mean NRS score as the dependent variable, insomnia, preoperative experience of pain, and sex as independent variables, and age, BMI, and operating time as co-variables). We found a significant association between insomnia and preoperative experience of pain (F=6.62; p=0.013) and a significant impact of insomnia on the mean postoperative NRS score (F=15.47; p<0.001).

DISCUSSION

A number of experimental and clinical studies have documented the relationship between sleep disruption and hyperalgesia.[20-24] Sleep disruption is a stressor, and preoperative stressors may contribute to postsurgical hypersensitivity. In animal models, brief sleep deprivation before surgery has been shown to significantly worsen the severity of subsequent pain.[25] However, the relationship between insomnia disorder and acute postoperative pain is poorly understood.[26]

The results of this case-control study show that there is an impact of insomnia on the acute postsurgical experience of pain; 77.1% of our patients suffering from insomnia experienced moderate to severe pain

after elective cholecystectomy. We found that patients suffering from chronic insomnia reported higher pain scores than the controls at 1, 2, 4, 8, 12, and 18 hours postoperatively. There was an association between insomnia and postoperative analgesic consumption in patients with insomnia who requested more tramadol and rescue analgesics than by the controls.

Sleep and pain are inter-related. Sleep disturbances may exacerbate existing pain and predict new-onset pain.[27-29] Overlapping mechanisms may be involved in this complex relationship. Symptoms of insomnia may trigger a cascade of neuronal changes leading to central sensitization, which in turn may contribute to hyperalgesia.[30-32] A dysfunction in the mesolimbic dopaminergic system may underlie the comorbidity of insomnia and pain.[33] Furthermore, sleep deprivation has been shown to affect the serotoninergic system, which also plays a central role in the descending pain inhibitory control system.[34-37] The interplay of psychological factors, such as stress, anxiety, depression, and insomnia, may also contribute to hyperalgesia.[8] There is evidence suggesting that deregulation of the hypothalamic-pituitary-adrenal system may play a central role in this complex relationship.[40,41]

Acute pain also serves the important function of signaling harm to the body's integrity. In rats, acute sleep disruption preoperatively has been shown to worsen postoperative pain.[23] However, there is an important distinction between acute sleep disturbance and insomnia disorder in terms of their courses and trajectories.[42,43] Previous research showed that up to 30% of patients who underwent surgery had pain scores >3 on a visual analog scale of 10.[3] The fact that 77.1% of patients in our insomnia group and only 11.4% of those in the control group had moderate to severe postoperative pain underscores the strong impact of severity of insomnia on acute postoperative pain.

Early studies identified preoperative experience of pain as an independent factor in predicting acute postsurgical pain; however, experimental pain assessment studies could not identify patients at risk for acute postsurgical pain.[44,-46] Our study revealed an interaction between insomnia and preoperative pain, but we did not find any association between preoperative experience of pain and acute postsurgical pain. Overlapping complex mechanisms in the central nervous system rather than preoperative experience of pain in itself might play a significant role in predicting acute postsurgical pain.

ISI is a valuable and valid instrument that can not only be used as a screening tool for insomnia disorder but can also be an effective instrument in identifying patients with significant insomnia disorder.[25,26-47] We did not simply compare patients and controls by diagnosis but investigated the severity of insomnia in all subjects.

The duration, intensity, and location of postoperative pain differs between surgical procedures. To limit the potential impact of heterogeneity in surgical pathophysiology and procedures on the effects of insomnia on acute postsurgical pain, we selected patients with a history of cholelithiasis who were undergoing elective laparoscopic cholecystectomy.

To avoid confounding results, psychological factors other than insomnia are not considered in this paper. The aim of our study was to evaluate the ability of chronic insomnia to predict postoperative pain rather than the role of other mostly interrelated psychological factors.

Although our study included a homogenous sample, it is limited by its cross-sectional design. Pain following a single operation type was assessed because the study's participants were all homogeneous. After various operations, further studies with larger patient populations are required. Another limitation of the study is that it was not double-blind; only the pain assessor was not aware of group allocation.

CONCLUSION

The data presented here show that the severity of insomnia prior to surgery may worsen the experience of acute postoperative pain. Our results emphasize the relevance of preoperative sleep management in clinical care. Efforts to prevent and treat acute postoperative pain may be well served to target insomnia as a point of intervention.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul Training and Research Hospital Clinical Researches Ethics Committee (Date: 01.03.2019, Decision No: 1731).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Hemoglobin red cell distribution width ratio as a prognostic marker in patients with locally advanced lung adenocarcinoma

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ABSTRACT

Aims: Hemoglobin/red cell distribution width ratio (HRR) has been defined as an effective prognostic factor in various malignancies. The aim of this study is to investigate the prognostic role of HRR in locally advanced lung adenocarcinoma.

Methods: 626 patients diagnosed with locally advanced lung adenocarcinoma were screened. The best cut-off point of HRR for overall survival (OS) and progression free survival (PFS) was determined by ROC analysis. A HRR cut-off value was determined, patients were classified as having lower or higher HRR. Both groups' clinical, demographic, laboratory values were compared. To identify independent predictors of prognosis, multivariate cox regression analysis was used.

Results: A total of119 patients were included. The best cut-off point of HRR in determining OS was 0.963%.HRR below the cut-off value increased mortality by 2.2fold.The group with HRR<0.963% had higher mean age and RDW and lower mean albumin. At the same time, the 5-year OS value of the group with HRR ≥0.9632 was 52.6% and the median OS of this group was 62.7months,while the 5year OS vale of the group with HRR <0.9632 was 24.7% and the median OS was 32.4 months. In multivariate cox-regression analysis, HRR was not independent predictor of mortality risk.

Conclusion: HRR is a potential biomarker for prognosis in patients with locally advanced lung adenocarcinoma.

Keywords: Hemoglobin red cell distribution width ratio, mortality, OS

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INTRODUCTION

RDW is thought to be a potential marker for oxidative stress, endothelial damage, and the inflammatory process that leads to neoplasm development.[1] A high RDW value is associated with systemic inflammation and therefore with aggressive tumor behavior.[2] In studies, high RDW has been associated with increased mortality in lung cancer.[3] At the same time, detection of anemia before treatment is associated with poor outcomes in oncology patients .[4]

Although low Hb and high RDW values are associated with poor progression free survival (PFS) and overall survival (OS) in a variety of cancers, it is debatable whether these values alone can predict tumor behavior because they are influenced by the inflammatory process. As a result, the Hb/ RDW (HRR) can help to reduce potential bias.[5-8]

HRR was first proposed as a prognostic factor biomarker to determine overall survivor in esophageal squamous cell carcinoma patients, followed by a similar study in lung small cell carcinoma patients, and in 2021, the usefulness of preoperative HRR values on resected lung adenocarcinoma as a biomarker to determine patient prognosis was emphasized.[9-11]

The HRR value is a potential biomarker as it is simple to calculate and the parameters that comprise this ratio are inexpensive. In our study, we examined the prognostic sensitivity of HRR in patients with locally advanced lung adenocarcinoma by excluding factors that could cause an inflammatory response and thus affect the HRR value.

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METHODS

The study was carried out with the permission of Ankara Atatürk Sanatorium Training and Research Hospital Ethics Committee (Date: 26.04.2023 Decision No: 2628). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was planned retrospectively. 4,785 lung adenocarcinoma patients who applied to our hospital between January 2010 and January 2020 were screened. 626 patients had locally advanced lung adenocarcinoma. Considering the inclusion criteria, 119 patients were included in the study.

These criteria were that the patient should be older than 18 years of age, all clinical, laboratory, treatment, pathology and imaging data should be available, the patient should have locally advanced lung adenocarcinoma according to the 8th TNM staging, and there should be no comorbidities such as diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), coronary artery disease (CAD), secondary cancer, concomitant infection, inflammatory disease or lymphoproliferative disease that may affect the HRR value.[8,9] Gender, age, smoking history, malignancy stage, treatments received, total follow-up time, PFS, OS values, laboratory values (albumin g/dl, hemoglobin g/dl, neutrophil mcl, lymphocyte mcl, platelet u/ml, RDW fL) were recorded. Hemoglobin/RDW% (HRR), neutrophil/lymphocyte % (NLR), platelet/lymphocyte % (PLR) values were calculated.

The best cut-off point of HRR value in determining OS and PFS was determined according to ROC analysis. Patients were divided into two groups according to the cut-off value. Clinical characteristics, OS and PFS times were compared.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). The optimal threshold for HRR in order to predict prognosis (i.e., progression free and overall survival) was evaluated ROC analysis. Sensitivity, specificity, positive and negative predicted values, and accuracy levels for HRR were also calculated. While the mean differences between groups were compared Student's t test, otherwise the Mann Whitney U test was applied for comparisons of not normally distributed variable. Cumulative survival rates for 1, 3, and 5 years, mean expected duration of life and 95% confidence intervals were computed by Kaplan-Meier survival analysis. Whether the potential factors were statistically significant effect on prognosis or not was investigated univariate Cox's proportional hazard regression models. Multiple Cox's proportional hazard regression model was obtained to determine the best independent predictors which mostly affected on prognosis after adjustment for clinically important factors. A p value less than 0.05 was considered statistically significant.

RESULTS

Among 626 locally advanced lung adenocarcinoma patients, 356 patients were excluded from the study due to comorbidities that would affect the HRR value. 119 patients were included in the study. 87.4 % of the cases were male. The mean age was 60.4±9.5 years. The most common stage in the study was Stage IIIA with 40.4 % (**Table 1**). Mean RDW value was 15.1 fL (±1.67), albumin value was 3.8 g/dl (3.5-4.2), NLR value was 2.71 (1.96-3.59)%, PLR value was 115.6 (82.6-180.8) %, and HRR value was 0.94 (±0.16) % (**Table 1**). The mean PFS was 36.5 months, and the mean OS was 44.3 months. The best cut-off point of HRR in determining OS and PFS was obtained by ROC analysis (**Table 2**). The best cut off point of HRR for OS was 0.963% (p=0.042) (**Figure**). HRR below the cut off value was associated with a 2.2-fold increase in mortality (**Table 3**). Demographic and clinical characteristic of the patients were compared according to the HRR cut-off value (**Table 4**). The group with HRR<0.963 had higher mean age and RDW fL (p=0.020 and p<0.001) and lower mean albumin g/dl (p<0.001) (**Table 4**). At the same time, the 5-year OS of the group with HRR≥0.9632 was 52.6% and the mean OS of this group was 62.7 months (95% CI: 48.0-77.4), while the 5-year OS of the group with HRR<0.9632 was 24.7% and the mean OS was 32.4 months (95% CI: 23.6-41.1).

In multivariate cox-regression analysis, stage, lack of treatment, and advanced age were independent factors in determining OS, whereas HRR was not an independent factor in determining OS and PFS. (**Table 3**).

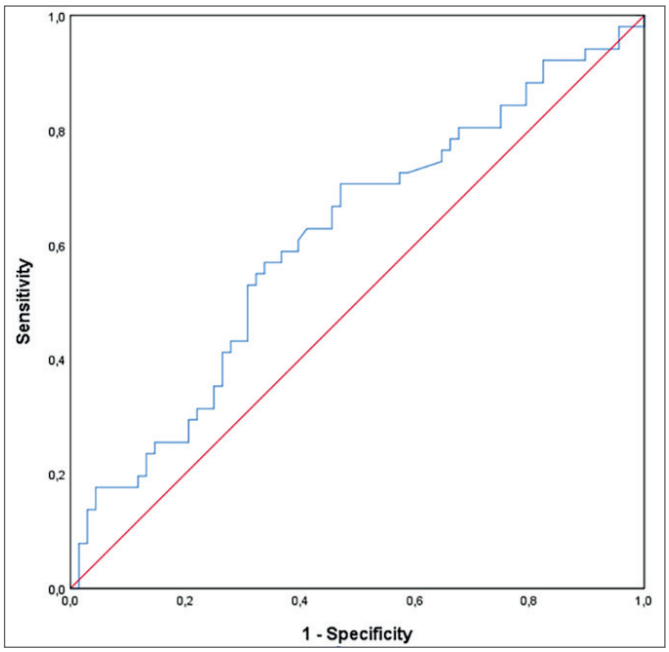

Figure. ROC curve of HRR measurements

Table 1. Demographic and clinical characteristics of the patients

	n=119
Age (year)	60.4±9.5 (35-86)
Sex	
Female	15 (12.6%)
Male	104 (87.4%)
Stage	
IIA	4 (3.4%)
IIB	24 (20.2%)
IIIA	48 (40.4%)
IIIB	30 (25.1%)
IIIC	13 (10.9%)
Treatment	
None	23 (19.3%)
Operated	12 (10.1%)
Chemoradiotherapy	18 (15.1%)
Radiotherapy	2 (1.7%)
Chemotherapy	27 (22.7%)
Operated and Chemoradiotherapy	34 (28.6%)
Operated and Chemoradiotherapy	3 (2.5%)
Smoking History	
None	18 (15.1%)
Quit	64 (53.8%)
Smoking	37 (31.1%)
RDW*	15.1±1.67
Albumin	3.8 (3.5-4.2)
NLR*	2.71 (1.96-3.59)
PLR*	110.6 (81.2-167.2)
HRR*	0.94±0.16
Progression	44 (37.0%)
Total Follow-up Duration	12.3 (4.8-31.7)
Recovery	
Alive	68 (57.1%)
Deceased	42.9%)

*RDW: Red cell distribution width NLR: Neutrophil-lymphocyte ratio PLR: Platelet-lymphocyte ratio HRR: Hemoglobin Red Cell Distribution Width Ratio

Table 2. ROC analysis of HRR as best cutoff to determine OS and PFS

	PFS	OS
AUC	0.525	0.609
95% CI	0.414-0.636	0.506-0.713
p-volume	0.652	0.042
Best cut off	-	<0.9632
Sensitivity	-	70.6%
Specificity	-	52.9%
PPV	-	52.9%
NPV	-	70.6%
Accuracy	-	60.5%

PFS: Progression free survival, OS: Overall survival, AUC: Area under the curve, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value

Table 4. Comparison of demographic and clinical characteristics of HRR groups

	HRR ≥0.9632 (n=51)	HRR < 0.9632 (n=68)	p value
Age (year)	58.1±7.7	62.1±10.4	0.020†
Sex			0.028‡
Female	2 (3.9%)	13 (19.1%)	
Male	49 (96.1%)	55 (80.9%)	
Stage			0.673¶
IIA	3 (5.9%)	1 (1.5%)	
IIB	9 (17.7%)	15 (22.1%)	
IIIA	22 (43.1%)	26 (38.2%)	
IIIB	12 (23.5%)	18 (26.5%)	
IIIC	5 (9.8%)	8 (11.7%)	
Treatment			
None	7 (13.7%)	16 (23.5%)	0.269‡
Surgery	26 (51.0%)	23 33.8%)	0.090‡
Chemoradiotherapy	11 (21.6%)	10 (14.7%)	0.466‡
Radiotherapy	0 (0.0%)	2 (2.9%)	0.506¥
Chemotherapy	25 (49.0%)	36 (52.9%)	0.812‡
Smoking History			0.373¶
None	5 (9.8%)	13 (19.1%)	
Quit	29 (56.9%)	35 (51.5%)	
Smoking	17 (33.3%)	20 (29.4%)	
RDW	14.2±1.03	15.8±1.73	<0.001†
Albumin	4.0 (3.7-4.4)	3.6 (3.4-4.0)	<0.001§
NLR	2.65 (1.78-3.53)	2.79 (2.06-3.65)	0.342§
PLR	105.6 (82.6-152.4)	113.1 (76.4-179.0)	0.599§

† Student's t test, ‡ Continuity corrected $\chi 2$ test, ¶ Pearson's $\chi 2$ test, ¥ Fisher's exact test, § Mann Whitney U test.

Table 3. Results of univariate and multivariate Cox's proportional hazards regression analysis on overall survival

	Univariate			Multivariate		
	HR (95% CI)	Wald	p-value	HR (95% CI)	Wald	p-value
Age	1.045 (1.016-1.075)	9.187	0.002	1.036 (1.001-1.072)	4.156	0.041
Male	0.897 (0.381-2.111)	0.062	0.803	-	-	-
Stage	1.301 (0.983-1.722)	3.389	0.066	1.538 (1.124-2.105)	7.239	0.007
No treatment	3.140 (1.708-5.771)	13.566	<0.001	2.509 (1.246-5.054)	6.632	0.010
Surgery	0.224 (0.111-0.452)	17.412	<0.001	-	-	-
Chemotherapy	0.586 (0.336-1.023)	3.539	0.060	-	-	-
Radiotherapy	5.179 (0.682-39.354)	2.526	0.112	-	-	-
Chemoradiotherapy	0.836 (0.404-1.729)	0.233	0.629	-	-	-
Smoking History	1.357 (0.538-3.421)	0.417	0.518	-	-	-
RDW	1.152 (0.983-1.349)	3.053	0.081	0.963 (0.793-1.171)	0.141	0.707
Albumin	0.665 (0.393-1.125)	2.309	0.129	-	-	-
NLR	1.006 (0.846-1.195)	0.004	0.948	-	-	-
PLR	1.002 (0.999-1.006)	1.613	0.204	-	-	-
HRR	0.189 (0.032-1.110)	3.402	0.065	-	-	-
HRR <0.9632	2.182 (1.188-4.010)	6.322	0.012	1.994 (0.975-4.077)	3.576	0.059

RDW: Red cell distribution width NLR: Neutrophil-lymphocyte ratio PLR: Platelet-lymphocyte ratio HRR: Hemoglobin Red Cell Distribution Width RatioHR: Hazard ratio, CI: Confidence interval.

DISCUSSION

It was determined in the study that HRR <0.963 increased the mortality rate by 2.2 times in patients with locally advanced lung adenocarcinoma. Patients with lower HRR values had a shorter 5-year OS, lower albumin, and higher RDW values.

Lung adenocarcinoma constitutes 60% of non-small cell lung cancers and new prognostic markers are needed in diagnosis and follow-up due to high mutation rates.[14] RDW has recently been used in cancer patients as a prognostic marker in addition to determining the type of anemia. A 2017 meta-analysis of the prognostic impact of RDW on cancers, which included 16 articles and 4,267 patients, discovered that high RDW was associated with poor OS and PFS.[15] There are conflicting results in lung cancer. Koma et al.[16] in a study of 332 lung cancers showed that high RDW reflected inflammation and malnutrition and was associated with poor OS. In another study conducted in 2016, no statistically significant results were found for RDW as a prognostic marker in lung cancer.

The prognostic effect of HRR on patients with squamous cell carcinoma of the esophagus was first investigated by Sun et al.[9] In the study of 362 patients, the group with HRR <0.989 had lower 5-year OS (33.7% vs. 55.5%) and median OS (89.8 months vs. 81.7 months) than the group with HRR ≥ 0.989. Although HRR below the cut off value increased the mortality rate 1.416 times, it was found that NLR, PLR and RDW values were higher in the group with HRR<0.989.[9] In our study, there was no significant difference in NLR and PLR values between the low HRR (<0.963) and high HRR (<0.963) groups, while albumin value, which indicates increased inflammation, tumor burden, and malnutrition, was lower and RDW value was higher. The finding of low albumin and high RDW in the group with low HRR supports the association of HRR with mortality. There have been few studies on the value of HRR in predicting prognosis in lung cancer patients. Low HRR was discovered to be an independent factor in determining OS and PFS in a study of 153 patients with advanced non-small extracellular lung carcinoma.[18] In a study conducted by Ergur et al.[19] on 840 small cell lung cancers, it was found that low HRR increased the risk of death by 1.6 times. Petrella et al.[11] reported that preoperative HRR value was an effective prognostic factor for disease-free survival with pathologic lymph node involvement in resected lung adenocarcinoma patients.[13] While the cut-off value of HRR was 0.88 in the study of Bozkaya et al.[18] and 1.01 in the study of Petrella et al.[11] and 0.580 in the study of Ergur et al.[19] in our study, the HRR value was 0.963.

Finally, in a meta-analysis of 11 studies involving 2,985 patients, it was found that a low HRR value increased the risk of death and recurrence by twofold in cancer patients in 2022.[20] Since lung cancer has many subtypes and high mutation diversity, this study focused on locally advanced lung adenocarcinoma. By excluding all comorbidities and secondary malignancies that would affect the Hb and RDW parameters that comprise the HRR value, the effect of HRR on a specific malignancy group, such as locally advanced lung adenocarcinoma, was more clearly seen. The limitations of our study are the retrospective nature of the study and the lack of a standard cut-off value for HRR.

CONCLUSION

Low HRR value is associated with lower OS in patients with Locally Advanced Lung Adenocarcinoma. HRR is a potential biomarker. To show that it is an independent factor, many more prospective studies are needed in which all factors are taken into account.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Atatürk Sanatorium Training and Research Hospital Ethics Committee (Date: 26.04.2023, Decision No: 2628).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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The initial symptoms in multiple sclerosis: clinical and demographic data of Çorum province

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ABSTRACT

Aims: Multiple sclerosis, which has individual and societal effects such as being observed in young and middle-aged people and its long and expensive treatment process, has become an important public health issue.

Methods: Between January 2022 and January 2023, 103 patients with MS were evaluated using anamnesis, neurological examinations, and neuroimaging results. The patients' initial findings upon their MS diagnosis, the duration of diagnosis, their neurological observations in the past month, and their clinical categorization were examined.

Results: Out of the 103 patients, 70 (67.96%) were female, and 33 (32.04%) were male, which was detected as a female-to-male ratio of 2.12/1. The average age of the patients was 34.41±8.4 years, and the average disease duration was 8±5.8 years. The initial findings in females were as follows; 25 patients (35.7%) had sensory, 13 patients (18.6%) had motor (pyramidal), 17 patients (24.3%) had brain stem-cerebellar, 10 patients (14.3%) had a visual impairment, and 5 patients (7.1%) had other findings. The distributions of the initial symptoms in males were as follows; 12 patients (36.4%) were motor (pyramidal), 10 patients (30.3%) were sensory, 5 patients (15.1%) were brain stem-cerebellar, 5 patients (15.1%) had visual impairment, and 1 patient (.3.1%) had other findings. The mean Expanded Disability Status Scale (EDSS) at the time of initial diagnosis was 2.5± 1.5. Among the 60 patients with RRMS who were first diagnosed, the duration elapsed between initial symptom onset and diagnosis was 12.8± 5.7 months.

Conclusion: Demographic information of the MS patients followed up in our clinic, their initial complaints, frequency of clinical subtypes, differences between clinical subtypes, their clinical status in the last month, and their EDDS at their initial diagnosis and last follow-up are presented. As this represents the first data on the epidemiology of MS in our city, we believe it will contribute to the national data of Turkey and help raise MS awareness among clinicians.

Keywords: Multiple sclerosis, symptom, disability

INTRODUCTION

Multiple sclerosis (MS) is a chronic progressive disease observed during early adulthood. Depending on the localization of the lesion, it can manifest with diverse symptomatology. The disability caused by the progression of the disease after the initial symptom gives rise to novel challenges and uncertainties in numerous aspects of the patient's personal and social life. The loss of an emerging workforce and the prolonged, costly treatment procedure also position MS as a significant public health issue. With the advancements in neuroimaging, since they are faster and more accessible, and due to the precision of diagnostic criteria, the diagnosis of MS can be achieved with greater certainty and speed.[1] Numerous disease-modifying treatments with significant effects in controlling disease activity and reducing long-term morbidity are currently employed.[2] In terms of the initial symptoms, exercising caution at primary care centers can help to shorten the diagnostic process.

METHODS

The study was carried out with the permission of Hitit University Medical Faculty Clinical Researches Ethics Committee (Date: 14.06.2023, Decision No: 20323-75). All procedures were carried out by the ethical rules and the principles of the Declaration of Helsinki.

Between January 2022 and January 2023,103 patients who were followed up with the MS diagnosis in the neurology clinic, diagnosed per the 2018 McDonald criteria, and had regular data in their patient follow-up files were examined. Patients were evaluated with anamnesis, neurological examinations, and neuroimaging results.

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The initial symptoms observed in patients diagnosed with MS were classified as sensory, pyramidal, cerebellar and brain stem, and visual pathway-related findings; additionally, cognitive or mental function-related symptoms under the category of other functions, bladder-bowel and sexual dysfunctions, and other neurological manifestations associated with MS. Based on the clinical course, the patients were categorized into primary progressive MS (PPMS), secondary progressive MS (SPMS), and relapsing-remitting MS (RRMS) and their neurological functioning was assessed using the Expanded Disability Status Scale (EDSS) scores during the initial and final outpatient clinic evaluations.

RESULTS

Out of the 103 patients, 70 (67.96%) were female, and 33 (32.04%) were male, which was detected as a female-to-male ratio of 2.12/1. The average age of onset for the patients was 34.41±8.4 years, the average age of onset for males was 29.84±6.9 years, and the average age of onset for females was 30.30±5.6 years. The average duration of the disease was 8±5.8 years. Regarding the clinical course, 62 (60.2%) patients were determined to be followed up with the diagnosis of RRMS, 31 (30.1%) of SPMS, and 10 (9.7%) of PPMS. Analyzing the clinical course specifically in female patients, 42 (60%) were identified as RRMS, 21 (30%) as SPMS, and 7 (10%) as PPMS. Regarding male patients, 20 (60.6%) were diagnosed with RRMS, 10 (30.3%) with SPMS, and 3 (9.1%) with PPMS. When the clinical classification of the patients was made according to their gender, it was observed that the incidence rates of RRMS in both sexes were high (**Table 1**). When we look at the initial symptoms; it was detected that 35 patients (34%) presented with sensory findings, 25 patients (24.3%) with motor (pyramidal) findings, 22 patients (21.3%) with brain stem - cerebellar findings, 15 patients (14.6%) with visual impairment and 6 patients (5.8%) with other findings (pain, mental changes, sphincter disorders). The initial findings in females were as follows; 25 patients (35.7%) had sensory, 13 patients (18.6%) had motor (pyramidal), 17 patients (24.3%) had brain stem-cerebellar, 10 patients (14.3%) had visual impairment, and 5 patients (7.1%) had other findings. The distributions of the initial symptoms in males were as follows; 12 patients (36.4%) were motor (pyramidal), 10 patients (30.3%) were sensory, 5 patients (15.1%) were brain stem-cerebellar, 5 patients (15.1%) had visual impairment, and 1 patient (.3.1%) had other findings (**Table 2**). While the most sensory findings in females were paresthesia and hypoesthesia in the extremities and body, mono paresis was frequently detected among the motor (pyramidal) symptoms. In males, hemiparesis and hemihypoesthesia as a sensory or motor (pyramidal) symptom were more prominent. A deterioration in visual acuity was more common as a visual impairment. Other results in the females included L-hermitte and trigeminal neuralgia in two patients, cognitive regression in two patients, inability to control due to urinary incontinence in one patient, and in males, neuropathic pain in the left upper and lower extremities was detected as the initial symptom in one patient (**Table 3**). When comparing the symptoms of onset based on gender, it was observed that motor (pyramidal) symptoms and onset rates were more prevalent in male patients, while other systemic manifestations were predominant in the female gender ($p<0.001$). The mean EDSS at the time of initial diagnosis was 2.5±1.5. Among the 60 patients with RRMS who were first diagnosed, the duration elapsed between initial symptom onset and diagnosis was 12.8±5.7 months. Our SPMS and PPMS patients had previously been diagnosed at external facilities and were now under our clinic's follow-up. The average time to diagnosis for these patients was 26.1±9.7 months. During the outpatient examinations conducted in the last month, the mean EDSS score 0-3 was 60 (58.25%), with 33 (32.05%) patients scoring between 3.5-6 on the EDSS, and 10 (59.70) patients scoring between 6.5-8 (**Table 4**). When SPMS and RRMS were compared; the mean disease duration was longer in SPMS than in RRMS. The mean initial EDSS score and the mean EDSS score in the final outpatient clinical control were higher in SPMS than in RRMS. Onset with motor (pyramidal) symptoms was detected more in SPMS compared to RRMS, while PPMS had a significant superiority in favor of PPMS over SPMS ($p<0.001$). When RRMS and PPMS were compared; in terms of motor (pyramidal) symptom and onset, PPMS was again significantly superior to RRMS in favor of PPMS ($p<0.001$). Positive family histories were detected in seven patients (6.79%). Of these, one of the siblings of 3 patients, one of the cousins of 3 patients, and the mother of one patient were detected to have MS.

Table 1. MS clinical classification based on sex

SEX	RRMS	PPMS	SPMS
Female	42 (60%)	7 (10%)	21 (30%)
Male	20 (60.6%)	3 (9.1%)	10 (30.3%)
	62 (60.2%)	10 (9.7%)	31 (30.1%)

RRMS: relapsing-remitting multiple sclerosis, PPMS: primary progressive multiple sclerosis, SPMS: secondary progressive multiple sclerosis, MS: multiple sclerosis

Table 2. Initial symptom distribution based on sex

SEX	Motor symptoms	Sensory symptoms	Brain stem-cerebellar symptoms	Symptoms related to vision	Other symptoms
Female	13 (18.6%)	25 (35.7%)	17 (24.3%)	10 (14.3%)	5 (7.1%)
Male	12 (36.4%)	10 (30.3)	5 (15.1%)	5 (15.1%)	1 (3.1%)

Table 3. Other symptoms

Sex	Symptom
Female (n=70)	L-hermitte 1
	Trigeminal neuralgia 1
	Cognitive regression 2
	Urinary incontinence 1
Male (n=33)	Neuropathic pain 1

Table 4. EDSS values of the patients at the last outpatient clinic controls

EDSS	0-3	3,5-6	6,5-8
Patient (n=103)	60 (58.25%)	33 (32.05%)	10 (59.70)

EDSS expanded disability status scale

DISCUSSION

The clinical onset of MS typically occurs between the ages of 20 and 50, although the prodromal phase may begin years earlier, and its clinical manifestations are likely to differ among individuals.[3-5] In industrialized societies, the female-to-male ratio becomes more pronounced, increasing up to 3/1, which was approximately twice as high in our study. The exact etiology remains unknown, but genetics, environmental factors, nutrition, smoking, viral infections, and vitamin deficiencies impact the development and prognosis of the disease. The global prevalence of MS ranges from 5-300/100,000 individuals.[6,7]

The rise in the prevalence of MS further supports the contribution of genetic factors in its etiology. Extensive mapping studies have identified numerous distinct genetic variations associated with an elevated risk of MS, with the HLA-DR1 1501 allele being the most prevalent risk factor linked to MS. MS is an autoimmune disorder caused by self-reactive cells that trespass the blood-brain barrier and attack the central nervous system (CNS). Principal subsets of T cells implicated in MS comprise CD8+ T cells, CD4+ Th1 cells, and Th17 cells. Interferon-gamma, IL-17, and granulocyte-macrophage colony-stimulating factors are cytokines produced by self-reactive T cells that potentially contribute to the pathophysiology of MS. Elevated levels of immunoglobulin in the cerebrospinal fluid (CSF) indicate the involvement of B cells in MS. A majority of B cells in the CSF and brain parenchyma are CD27+ memory B cells.[8-11]

The diagnosis of MS relies on the revised McDonald criteria, which encompass a combination of clinical findings, neuroimaging, and laboratory data.[12] MS is a disease in which demyelination and inflammation can affect gray matter and cortical region or medulla spinalis, although mostly white matter in the brain, and symptoms may vary according to localization of the lesion. After the acute phase, MS lesions turn into a chronic phase in which a combination of remyelination, inflammation, and myelin degeneration can be observed. With this transformation, new symptoms or changes in the severity of symptoms are seen in the clinic. MS can be observed as a clinically isolated condition in patients and may present with single or multiple symptoms depending on the localization of the lesion. Brain stem, spinal cord syndrome, and optic neuritis are among the common clinical presentations; however, a variety of clinical symptoms may be observed, including cortical manifestations such as dominant parietal lobe syndromes. Numerous clinical manifestations are indicative of MS, yet only a few are pathognomonic. MS episodes typically emerge over hours or days, with gradual improvement observed over weeks. Following this healing process, residual effects may remain. The clinical symptoms, results, and progression of MS are assessed across a broad spectrum. MS is characterized by intermittent periods of neurological abnormalities, referred to as "attacks," that may exhibit remission and initially occur more frequently. In the subsequent periods, a process called SPMS may develop in which permanent neurological deficits develop and clinical disability occurs. And PPMS is characterized by a progressive course from the onset. The most prevalent form, as we detected also in our study, is RRMS. It is recognized that this clinical course entails periods of remission periods between the periods of attacks. Due to CNS damage in MS patients; visual symptoms such as weakness in the extremities, sensory symptoms, ataxia, bladder problems, fatigue, diplopia, blurred vision, dysarthria, memory-concentration-attention disorder can be seen, while movement disorders, epileptic seizures, headaches, cognitive impairment, cortical symptoms, hearing loss, amyotrophy are among the rare symptoms and findings.[13-16] Acute demyelinating optic neuritis is the symptom of application in 20% of MS patients and affects approximately half of MS patients at some point in the course of the disease.[17] Some eye movement abnormalities can be observed in MS patients. These eye movement abnormalities may present with diplopia or oscillopsia.[18] Vertigo is seen in 30-50% of MS patients. In a retrospective study, the most prevalent cause of vertigo in MS patients has been identified as benign positional paroxysmal vertigo.[19] Motor signs and symptoms typically manifest as paraparesis, quadriparesis, hemiparesis, or monoparesis. They are often accompanied by other symptoms. Spasticity is frequently present, with a more pronounced manifestation observed in the lower extremities compared to the upper extremities.[20] Sensory symptoms are frequent initial manifestations and occur in nearly all patients during the disease. The disease causes both positive and negative sensory symptoms. Sensory loss, paresthesias, dysesthesias, and hyperesthesias are commonly reported. Impairment in vibration and proprioception may result from demyelination in the posterior cord. Rarely, a condition resembling Brown-

sequard syndrome, characterized by a spinal cord half-incision-like presentation, may occur. The most common finding is L-hermitte, which is observed with a rate of 3% as the initial symptom and 30-40% in the whole period.[21] Bowel dysfunction is reported in approximately 50 percent of patients with MS, and bladder dysfunction is reported up to 75 percent.[22] Sexual dysfunction is observed frequently in MS patients.[23] However, due to societal and social reasons, there is not much application as it being the first symptom. Rare cases of MS have been reported in the literature.[24] Among the cases we followed, neuropathic pain occurred as the first symptom in a total of 3 cases. In female patients, L-hermitte and neuralgia in the trigeminal region were described, while in male patients, neuropathic pain in the left hemibody was reported. In the literature, publications on pain associated with MS under the title of neuropathic pain syndrome show that we should forget that the first symptom in these patients may be pain.[25,26] MS patients presenting with uncommon yet significant symptoms such as incontinence and cognitive impairment also underscore the importance of considering MS in the distinctive diagnosis for patients referred from different clinics.[27-28] EDSS is utilized to assess and monitor clinical manifestations in MS. In addition to the disease's clinical course, numerous factors influence the EDSS score. Disease activity and progression are typically assessed through relapses, MRI activity, and disability progression. However, it is employed to track increased disease activity and newly formed lesions on MRI scans.[29] While various studies in the literature discuss the duration from initial symptoms to the conclusive diagnosis of MS in MS patients, this duration diminishes with advancements in accessibility and technology of neuroimaging, yet disregarding sensory symptoms frequently leads to an extended period between the onset of the initial symptom and the actual diagnosis.[30] We think that this process takes long in patients with an old diagnosis in the patient group we follow, because of the referral of patients to external facilities for MS and due to social reasons. The most important limitations of our study are that we could not perform genetic analysis and it is a single-centered study. However, we have observed that our results are directly proportional to the literature data.

CONCLUSION

Demographic information of the MS patients followed up in our clinic, their initial complaints, frequency of clinical subtypes, differences between clinical subtypes, and their EDDS are presented. As this represents the first data on the epidemiology of MS in our city, we believe it will contribute to the national data of Turkey and help raise MS awareness among clinicians.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Medical Faculty Clinical Researches Ethics Committee (Date:14.06.2023 Decision No:20323-75).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Nitric oxide therapy in COVID-19 patients with acute respiratory distress in intensive care unit

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ABSTRACT

Aims: The administration of inhaled nitric oxide (iNO) is a promising and new approach to treat viral load while increasing oxygenation directly. This research aimed to elucidate the clinical and laboratory response to the treatment of the patients diagnosed with Coronavirus disease-19 (COVID-19) in the intensive care unit (ICU) and followed up due to respiratory failure and given iNO.

Methods: A total of 46 individuals who were diagnosed with COVID-19 and developed severe respiratory failure were followed up with or without intubation, had previously received standard care were evaluated within the study's scope. iNO initiation time in the ICU, whether the patients were intubated, clinical and laboratory parameters before and after iNO treatment were obtained from hospital records.

Results: A statistically significant difference has been achieved in arterial partial pressure of oxygen (PaO_2), peripheral oxygen saturation (SpO_2), and the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) ratios before and after iNO ($p<0.05$). While significant differences were observed in oxygenation with iNO treatment, no significant differences were observed in other parameters. When iNO onset times were evaluated, it was determined that the initiation time of iNO treatment was significantly later in patients who died. The relationship between the duration of mechanical ventilation, the duration of stay in the ICU, and the onset of iNO was a statistically significant relationship between all measurements ($p<0.05$).

Conclusion: iNO has been suggested as an alternative rescue method before invasive treatment in guidelines, especially for the relief of hypoxemia. However, the effective dose and safety of iNO is still not clear.

Keywords: Nitric oxide, inhaled nitric oxide, COVID-19, acute respiratory distress syndrome, intensive care unit

INTRODUCTION

COVID-19, caused by the SARS-CoV-2 virus, is a worldwide pandemic described clinically as viral pneumonia. While some COVID-19 patients have a mild course, like a cold, many may develop acute respiratory distress syndrome (ARDS), which progresses to more severe lung damage and requires intensive care admission. The coronavirus epidemic; constitutes an international public health emergency. COVID-19 pandemic has caused an intense loss of human life worldwide and presents an extraordinary challenge to public health systems and the world economy. It created an abnormal burden on health workers and was very emotionally worn out.[1]

ARDS is a frightening complication in COVID-19 patients.[2] Unlike ARDS, the main mechanism is due to the ACE-2 receptor. The ACE-2 receptor is the key receptor that binds with the SARS-CoV-2 protein. ACE-2 plays an important role in the progression of ARDS. Binding to the cell surface, SARS-CoV-2 suppresses ACE-2 expression. As a result of the decreased ACE-2 level, the conversion of angiotensin 2 to angiotensin 1 is reduced, and unopposed Angiotensin 2 dominance occurs. Undesirable effects of increased angiotensin 2, such as pulmonary vasoconstriction, increased vascular permeability, pulmonary edema in hypoxic conditions, inflammatory cytokine release in the lung, and increased apoptosis, can be achieved.[3,4]

In the pathogenesis of COVID-associated ARDS or, in other words, CARDS, pro-inflammatory cytokines are first released from monocytes, and pneumocyte apoptosis is induced. Other cytokines are released from monocytes, increasing capillary permeability, and neutrophil migration begins. Alveolo-capillary membrane destruction is

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initiated via neutrophils. As a result, protein-rich interstitial and alveolar edema occurs.[5] Pathophysiologically, this condition resembles the typical ARDS, however, there are some differences between typical ARDS and CARDS. Typical Berlin criteria defined ARDS onset time as 'new or worsening respiratory distress occurring within the first week.6 However, studies have shown that the onset time of ARDS in COVID-19 is later than this period.[7]

In a retrospective study conducted on 191 patients in China, the mean ARDS onset time was reported as approximately day 12.7. CARDS have worse outcomes than ARDS from other causes. The mortality of typical ARDS in the ICU is 35%, and hospital mortality is 40%. However, mortality ranges between 26% to 61.5% in COVID-19 patients admitted to ICU due to ARDS, while the mortality rate in mechanically ventilated patients could vary between 65.7% and 94%.[8] Risk factors for poor outcomes include the presence of comorbidities such as advanced age, hypertension, cardiovascular disease, chronic renal failure, diabetes mellitus, low lymphocyte counts, and high D-dimer levels.[9]

The use of iNO in ARDS has been suggested in the literature. Since iNO does not contribute to survival, its routine use in ARDS is not recommended.[10] However, it can be used in severe respiratory failure that does not respond despite the use of advanced ventilation techniques.[11] It is advised to utilize iNO in cases of severe hypoxemia brought on by CARDS because of its good effect on oxygenation in the literature.[12,13] One study showed that iNO temporarily improved oxygenation and reduced the rate of severe respiratory failure, but did not reduce mortality or length of stay in the ICU or hospital.[14] The aim of this research was to elucidate the clinical and laboratory response to the treatment of the patients who were diagnosed with COVID-19 in the ICU and followed up due to respiratory failure and given iNO.

METHODS

A total of 46 patients hospitalized in the ICU due to ARDS with a confirmed diagnosis of COVID-19 between 01.03.2020 to 01.03.2022 and treated with iNO have been enrolled in this retrospective analysis. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. As this was a retrospective analysis, informed consent was not mandatory from enrolled patients. Informed consent was obtained from all participants. The study was carried out with the permission of Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 17.08.2022 Decision No: E1-22-2747).

Individuals diagnosed with COVID-19 and developed severe respiratory failure were followed up with or without intubation and had previously administered treatments according to guidelines were evaluated within the scope of the study. Gender, age, comorbidity, intensive care scores (SOFA, APACHE), and length of stay in the ICU were recorded. After the diagnosis of COVID-19, the onset time of iNO and whether the patients were intubated were obtained from hospital records. The dose of iNO given continuously to intubated and non-intubated patients was 20 ppm. Demographic data were collected together with the treatment regimens. Ferritin, interleukin-6 (IL-6), C-reactive protein (CRP), procalcitonin (PCT), D-dimer, fibrinogen, troponin, urea, creatinine, alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) on the day before iNO administration and three days after iNO administration, lymphocyte count, neutrophil count, platelet count, lactate, oxygen values (PaO_2, SpO_2, $PaCO_2$, FiO_2/PaO_2) were investigated.

Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 23.0 (IBM Corp., Armonk, NY) package program.

Frequency and percentage were given for categorical data and median, minimum, and maximum descriptive values for continuous data. "Mann Whitney U Test" was used for comparisons between groups, "The Friedman Test" for the comparison of measurement values, "Fisher's Exact Test" for the comparison of categorical variables, and "The Spearman Correlation Analysis" for the evaluation of the relationship between continuous data.

Correlation coefficient (The fact that it is between 0.90 and 1.00 indicates that the relationship between the two variables is very strong.)

- Weak in terms of being between 0.00 - 0.29,
- Between 0.30 and 0.49, the relationship between the two variables is low,
- Between 0.50 and 0.69, the relationship between the two variables is medium,
- Between 0.70 and 0.89, the relationship between the two variables is strong.

The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

Within the scope of the study, 46 patients, 30.4% (n=14) female and 69.6% (n=32) male, were evaluated. The ages of the patients ranged from 20 to 80 years, with a mean age of 55 years (**Table 1**).

Table 1. Distribution of demographic and clinical findings of the patients

Characteristics (n=46)	n (%) or Median (Min - Max)
Age, years	55 (20-80)
Gender	
Female	14 (30.4)
Male	32 (69.6)
BMI (kg/m²)	26,5 (17-35)
Comorbid disease	36 (78.3)
Hypertension	24 (52.2)
DM	16 (34.8)
CAD	3 (6.5)
COPD	5 (10.9)
Malignity	3 (6.5)
Heart failure	4 (8.7)
Immune deficiency	3 (6.5)
Pulmonary emboli	2 (4.3)
Transplantation	4 (8.7)
Renal failure	2 (4.3)
Other	6 (13)
Epilepsy	1 (2.2)
Pregnancy	1 (2.2)
Hyperthyroidism	1 (2.2)
Idiopathic pulmonary fibrosis	1 (2.2)
Obesity	1 (2.2)
Cerebrovascular disease	1 (2.2)
Computerized tomography CORADS	
Low	2 (4.3)
Suspicious	18 (39.1)
High	25 (54.3)
Extremely high	1 (2.2)
Apache - 2 Scores	18 (6-43)
SOFA scores	4 (2-12)
Intubation	44 (95.7)
Day of intubation	5,5 (1-50)
Duration of mechanical ventilation, day	19 (0-89)
Length of ICU stay, days	22.5 (2-97)
Nitric oxide initiation time (when positive), days	16 (3-68)
Nitric oxide initiation time (after ICU entry), days	9.5 (1-57)
Nitric oxide delivery time	3 (1-16)
Vasopressor/inotrope requirement before nitric oxide	12 (26.1)
Need for vasopressor/inotrope after nitric oxide	32 (69.6)
Sepsis before nitric oxide	10 (21.7)
Cytokine filter	14 (30.4)
ECMO	5 (10.9)
ARF	14 (30.4)
Dialysis	9 (19.6)
Mortality	39 (84.8)
Mortality (first month)	22 (47.8)
Cause of death	
Multiple organ failure	2 (5.1)
Septic shock	34 (87.2)
Shortness of breath	3 (7.7)
Tracheostomy	6 (13)
PNX	9 (19.6)

There was at least one comorbid disease in 78.3% (n=36) of the patients. The most common comorbid disease was hypertension with 52.2% (n=24). While the mortality rate of the patients in the first month was 47.8% (n=22), it was determined that the mortality rate was 84.8% (n=39) during the study period (**Table 1**).

The distribution of laboratory parameters of the patients included in the evaluation before iNO, on the first, second and third days after iNO treatment were elaborated in **Table 2**. When the table was examined, it was observed that there was a statistically significant difference in the ratios of PaO_2, SpO_2 and FiO_2/PaO_2, which are laboratory parameters measured before and after iNO ($p<0.05$).

The distribution of the relationship between the duration of mechanical ventilation, the duration of stay in the ICU, and the onset of İno was denoted in **Table 3**. When the table was examined, it was seen that there was a statistically significant relationship between all measurements ($p<0.05$).

Table 3. Distribution of the relationship between mechanical ventilation time, ıntensive care unit stay, and nitric oxide onset time

		Duration of MV	Duration of stay in the ICU	NO initiation time
Duration of MV	Correlation coefficient	1.000	0.855	0.542
	p-value	-	<0.001	<0.001
Duration of stay in the ICU	Correlation coefficient	0.855	1.000	0.655
	p-value	<0.001	-	<0.001
NO initiation time	Correlation coefficient	0.542	0.655	1.000
	p-value	<0.001	<0.001	-

The distribution of the relationship between the intubation status of the patients before or after iNO usage and their final status was given in **Table 4**. When the table was examined, 81.8% (n=18) of the patients intubated before iNO died, while 95.5% (n=21) were intubated after iNO deceased. This relationship between the two groups was not statistically significant ($p<0.05$).

Table 4. Distribution of the relationship between mechanical ventilation time, ıntensive care unit stay, and nitric oxide onset time

		Mortality		p-value
		Survived	Deaceased	
Intubation Time	Before	4 (18.2)	18 (81.8)	0.345
	After	1 (4.5)	21 (95.5)	

Table 2. Distribution of laboratory parameters of the patients before and after nitric oxide

Laboratory Parameters	Before NO	NO (Day 1)	NO (Day 2)	NO (Day 3)	p-value
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
IL-6	27,2 (4,6-5500)	28.2 (3.1-5500)	22.5 (2.8-5500)	18.9 (2,2-374)	0.362
CRP	0,09 (0,01-0,33)	0.07 (0.01-0.38)	0.08 (0.01-0.21)	0.07 (0.01-0.34)	0.208
Lymphocyte	0,5 (0,1-2,1)	0.5 (0.1-2.4)	0.5 (0.1-3.6)	0.5 (0.1-2.2)	0.241
Ferritin	865,5 (47-11371)	903.5 (66-14278)	805 (47-43078)	750.5 (62-7255)	0.740
Procalcitonin	0,2 (0-23,9)	0.3 (0-229.9)	0.2 (0-40)	0.3 (0-18.9)	0.508
D-Dimer	4 (0,3-36,6)	4.2 (0.5-55)	4 (0.2-35.2)	3.3 (0.3-35.2)	0.806
pO_2	52,4 (47-70)	68.1 (50-101)	71 (50-98)	68.6 (49-87.1)	<0.001
sO_2	80 (66-92)	91 (76-98)	92 (78-98)	91 (73-99)	<0.001
pCO_2	45,2 (22-119)	47 (26.9-284)	48.1 (25-98)	49.3 (29.8-103)	0.979
Ph	7,4 (6,8-7,5)	7.4 (6.9-7.6)	7.4 (7.1-7449)	7.4 (6.9-7.6)	0.975
Laktat	1,7 (0,6-12,1)	2 (0.6-11.8)	1.7 (0.8-12.4)	1.7 (0.8-16.4)	0.546
LDH	567,5 (205-8291)	600.5 (246-8291)	531 (2-3088)	533 (223-3384)	0.850
AST	44,5 (17-9265)	48.5 (16-9265)	46 (14-1321)	49 (14-1828)	0.065
ALT	49 (8-3168)	50 (11-3168)	46 (8-601)	47.5 (12-1577)	0.523
PaO_2/FiO_2	52 (50-71)	75.5 (50-118)	90 (55-120)	79 (50-156)	<0.001

DISCUSSION

In 1987, it was understood that nitric oxide (NO) was the substance known as an "endothelium-derived relaxing factor," which was known to be present in exhaust gas and cigarette smoke and has been positioned as an element of air pollution.[15] NO is a highly lipophilic molecule that can easily cross membranes. As a result of a series of reactions catalyzed by nitric oxide synthesizing enzyme (NOS), L-arginine is converted to L-citrulline, and NO.[16] NOS-II or iNOS is present in the respiratory epithelium and various other cells.[17-19]

The most important finding of ARDS is severe hypoxemia resulting from physiological shunt and ventilation/perfusion (V/Q) imbalance. Inhaled vasodilators such as iNO and prostacyclin increase oxygenation by vasodilation, especially in well-ventilated vessels, and improve V/Q imbalance. iNO is consistently used in the treatment of hypoxic respiratory failure at doses of 1.25-40 ppm.[20] However, in case of interruption or abrupt discontinuation of treatment, it causes serious deterioration in oxygenation and a sudden increase in pulmonary artery pressure. This research found a statistically significant difference in PaO_2, SpO_2, and FiO_2/PaO_2 ratios obtained before and after iNO utilization ($p<0.05$).

The most important features of iNO are selective pulmonary vasodilation, correcting hypoxia, and reducing elevated pulmonary artery pressure by reducing pulmonary vascular resistance. Thanks to its selective vasodilation feature, iNO is a valuable treatment method as a salvage treatment method in all types of pulmonary hypertension and in cases of severe respiratory failure with resistant hypoxia, which is difficult to treat with conventional treatments. Anti-viral drugs currently available to clinicians have little or no effect on mortality, length of hospital stay, need for mechanical ventilation, or long-term effects.[18] In our study, there is not enough data on this subject, since we did not have our patients undergo echocardiography due to pandemic conditions.

The high mortality rates in COVID-19 patients requiring mechanical ventilation are prompting clinicians and scientists to seek new technologies and pharmacological interventions that can improve outcomes. Researchers and clinicians consider iNO therapy promising for patients with COVID-19 and respiratory failure,[21] 13 supported by in vitro research from Akaberi et al.[22] In 2003, during the SARS epidemic in China, a small observational study of patients with SARS pneumonia receiving non-invasive support, biphasic positive airway pressure (BiPAP), were treated with iNO improved oxygenation, accelerated the resolution of chest X-ray infiltrates, reduced the need for intubation, and led to a more rapid and sustained ARDS resolution and improved overall clinical outcomes.[23]

About 30% of patients with severe CARDS in healthcare practice have received iNO as a life-saving therapy.[24,25] However, the results of published randomized trials and clinical observations are highly controversial. Small cohort studies have not significantly improved oxygenation and

clinical outcomes with iNO therapy.[26] On the other hand, the frequency of responders ranges from 25%to 40% with a tendency of a more pronounced effect on gas exchange in patients with right ventricular dysfunction. The percentage of iNO responders is much lower than in patients with non-CARDS.[27] Our observations were that oxygenation improved in patients using iNO.

A retrospective observational study showed that iNO was useful in improving oxygenation in spontaneously breathing patients with COVID-19 pneumonia.[14] High-dose iNO (160 ppm) was safely administered to pregnant women with severe COVID-19 pneumonia and as a rescue therapy to spontaneously breathing patients with COVID-19 and hypoxemic respiratory failure.[28,29] A recent trial of non-invasively treating patients with moderate COVID-19 hypoxia demonstrated that iNO-therapy produced an acute improvement of systemic oxygenation in hypoxemic patients and reduced the respiratory rate.[30] In our study, 20 ppm iNO was used.

Preliminary data support the iNO-mediated improvement of oxygenation in mechanically ventilated patients and spontaneously breathing patients with COVID-19.[31] Another strategy for iNO administration in COVID-19 involves the potential for selective pulmonary vasodilation to optimize V/Q matching by reducing pulmonary vascular resistance and decreasing alveolar dead space. In this research, we found a statistically significant relationship as positively correlated in all parameters when we examined the relationship between the duration of mechanical ventilation, the duration of stay in the ICU, and the onset of iNO. The reason for this relationship is due to the known hypoxemia correction mechanism of iNO and less complications related to hypoxemia.

The study of iNO treatment in spontaneously breathing COVID-19 patients demonstrates not only an increase in oxygenation in all patients, but also provides evidence that iNO therapy may have a role in preventing the progression of hypoxemic respiratory failure.[14] In this study, when the living and deceased patients were examined, it was seen that whether ino was given before and after intubation did not affect mortality in both groups.

iNO has been suggested as an alternative rescue method before invasive treatment, especially for the relief of hypoxemia. However, according to recent clinical trials in Italy, iNO appears unable to reverse oxygenation in patients with extensive mechanical ventilation who have developed persistent hypoxemia.[26,27] Therapeutic doses for COVID-19 patients range from 20 to 300 ppm. Only a few studies have examined the safety and efficacy of 80, 150, and 160 ppm iNO. The results of these studies have yet to be published.[25]

CONCLUSION

iNO can be used as a rescue therapy in patients who develop severe respiratory failure due to COVID-19 and do not respond to all recommended treatments, since it corrects hypoxemia. The therapeutic effects of iNO in COVID-19 and the safe and effective dose for iNO are still unclear. This therapy could pave the way for better management of COVID-19 before the onset of disease-related complications. However, due to the small number of patients in our study, it is not possible to reach a definite conclusion. Due to this situation, which led to the limitation of our study, it should be supported by studies with large sample size.

Abbreviations

ACE: Angiotension converting enzyme, ARDS: Acute Respiratory distress syndrome, ARF: Acute renal failure, BMI: Body mass index, CAD: Coronary artery disease, COPD: Chronic pulmonary obstructive disease, DM: Diabetes mellitus, ECMO: Extracorporeal membrane oxygenation, ICU: Intensive care unit, iNO: Inhaled nitric oxide, LDH: Lactate dehydrogenase, MV: Mechanical ventilation, NO: Nitric oxide, RT-PCR: Real Time polymerase chain reaction, SPSS: Statistical package for the social sciences

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 17.08.2022 Decision No: E1-22-2747).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Surgically treated pneumonic-type lung adenocarcinoma with long survival characteristics

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ABSTRACT

Aims: Pneumonic-type lung adenocarcinoma is defined as a pneumonia-like area of infiltration or consolidation involving a region of the lung. These carcinomas, which are suitable for curative treatment options and have a long survival when detected at an early stage, may resemble infectious or inflammatory lung diseases due to their radiological appearance and clinical findings, may lead to potential delays or difficulties in diagnosis, and this may cause progression in patients who are suitable for curative treatment options.

Methods: A total of 41 patients which were surgically treated between 2011-2020 and diagnosed pathologically with pneumonic-type adenocarcinoma. The patents' TTF-1, type of operation, pathological stages, overall/progression-free survival, as well as overall/progression-free survival according to the type of operation and radiological appearance, were also evaluated.

Results: The study included 41 patients. Although overall survival times were long, there was no statistically significant defference between wedge resection and lobectomy group in overall survival by operation type. Although progression-free survival times were long, there was no statistically significant defference between wedge resection and lobectomy group in progression-free survival by operation type. There was no statistically significant difference between solid and consolidated groups in terms of overall survival and progression-free survival according to radiological appearances. There was no statistically significant difference between TTF-1 positive and negative groups in overall survival.

Conclusion: Pneumonic-type lung adenocarcinomas respond to curative treatments when diagnosed at an early stage. The optimal treatment method for operable patients is surgery, which is associated with prolonged survival.

Keywords: Lung cancer, pneumonic-type adenocarcinoma, surgical treatment

INTRODUCTION

Non-small cell lung cancers account for 85% of all lung cancers, the most common subtype of which, especially in non-smokers, is adenocarcinoma.[1,2] The 2015 WHO classification categorizes lung adenocarcinomas into adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA) and invasive adenocarcinoma.[3]

Pneumonic-type lung adenocarcinoma is defined as a pneumonia-like area of infiltration or consolidation involving a region of the lung (**Image 1**).[4] Pneumonic-type lung adenocarcinomas can be confused with infectious pneumonias because of their appearance.[5] Histologically, it manifests with a lepidic predominant growth pattern with mucin or tumor cells in the alveolar spaces.[1] It is referred to as pneumonic-type lung cancer due to its radiological resemblance to pneumonia. Demographically, this type of cancer occurs in those aged 41-66 years, but with variations associated with gender, age or geographical region.[6,7] The 2015 WHO classification states that invasive mucinous adenocarcinomas account for most of the pneumonic-type adenocarcinomas.[3] Histologically, it is defined predominantly as mucinous, although there are also mucinosis, non-mucinosis and mixed types.[1] There is no radiological difference between the mucinosis and non-mucinosis types.[3] Pneumonic-type adenocarcinomas typically do not show lymph node metastasis or distantmetastasis, despite diffuse pulmonary involvement.[1,8] With its radiological appearance and clinical findings, pneumonic-type adenocarcinomas can mimic infectious or inflammatory pulmonary diseases that can bring about a delay or difficulty in diagnosis, resulting in disease progression

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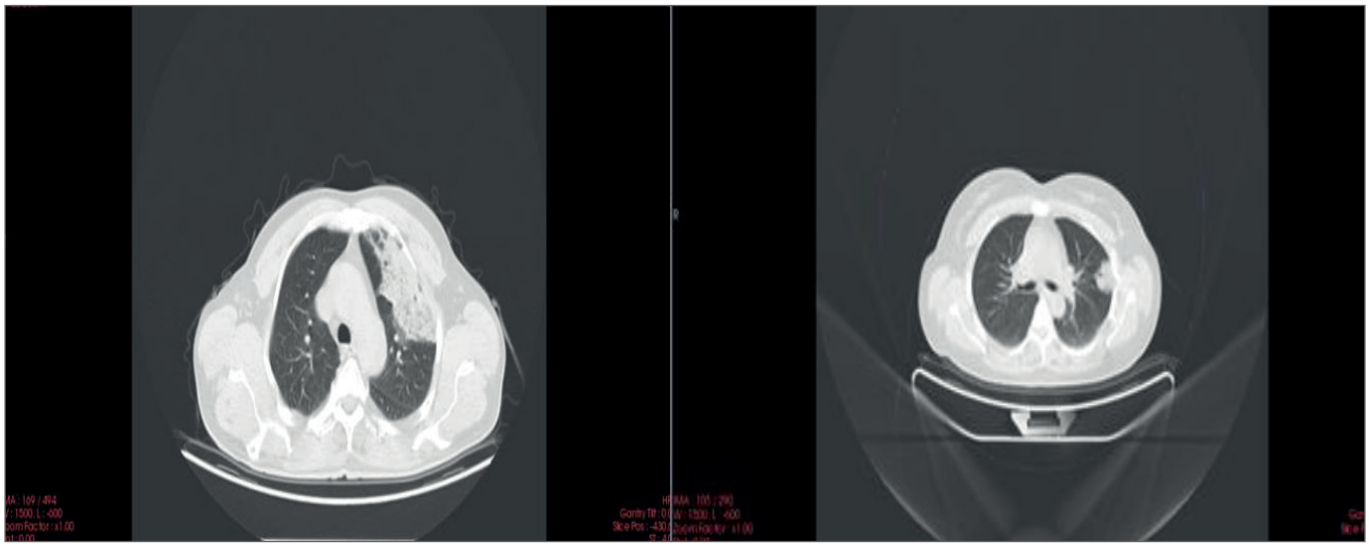

Image 1. Pneumonia-like area of infiltration or consolidation involving a region of the lung. 61-year-old male patient was diagnosed with invasive adenocarcinoma by transthoracic biopsy in the consolidated lesion in the upper lobe of the left lung, and left upper lobectomy was performed. Image-2: 52-year-old male patient underwent lobectomy for the consolidated lesion on thoracic computed tomography

that may result in respiratory failure prior to diagnosis. Pneumonic-type adenocarcinomas, which are suitable for curative treatment options and have a long survival when detected at an early stage, may resemble infectious or inflammatory lung diseases due to their radiological appearance and clinical findings, may lead to potential delays or difficulties in diagnosis, and this may cause progression in patients who are suitable for curative treatment options.

METHODS

The study was carried out with the permission of Ankara Atatürk Sanatorium Training and Research Hospital Clinical Researches Ethics Committee (Date: 12.07.2023, Decision No: 2012-KAEK-15/2739). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

'Involved in the study were 41 patients who underwent surgery with a diagnosis of pneumonic-type lung cancer in our hospital between 2011 and 2020, whose reports were reviewed retrospectively. The demographic data of the patients, including age, gender, comorbidities, smoking, dates of diagnosis and progression, and pathological data were examined after being retrieved from the system, and the TTF-1, type of operation and pathological stages were recorded. The patients were restaged according to the 8th TNM classification by scanning the hospital system.

The pathology reports of the patients were categorized according to the 2015 WHO classification and those whose pathology report was diagnosed as invasive adenocarcinoma were included in the study. The pathological staging of the patients was repeated by examining the pathology reports. A total of 59 patients who were diagnosed with pneumonic type adenocarcinoma and operated on in the system screening were reached, and only 41 patients with T1a (Stage 1a) tumors were included in the study.

Patients with regular follow-up were included in the study, and total and progression-free survival status of the patients was obtained by scanning the last visit and death notification system. The type of operation was decided by the staging of the patients with PET_CT before the operation and the staging during the surgery. The primary endpoint of the study was to determine the survival rates according to the type of surgical operation. The patients operated with thoracotomy were divided into two groups according to type of operation, being the lobectomy and wedge resection groups, and were evaluated for progression-free survival and overall survival. The patients were divided into three groups: solid, consolidated and solid-consolidated, based on the radiological appearance, and were evaluated for progression-free survival and overall survival. secondary endpoint of the study was progression-free/overall survival according to radiological appearance.

Statistical Analysis

Descriptive statistics were used to present the demographic data of the study patients. The survival analysis was carried out using the Kaplan-Meier (Log-rank) method. IBM SPSS Statistics (Version 27.0. Armonk, NY: IBM Corp.) was used for statistical analysis. A p value of <0.05 was considered statistically significant.

RESULTS

The study included 41 patients, of which 21 (51.2%) were female, and 20 (48.8%) were male. The mean age of the patients was 60.8±12.6 years. Of the total, 20 of the patients had no comorbidity, while the most common comorbidity was chronic obstructive pulmonary disease (COPD) (n=7) in the remainder (**Table 1**).

Table 1. Characteristics of study population

Characteristics	Number (%)	Mean±SD
Gender		
Female	21 (51.2 %)	
Male	20 (48.8 %)	
Age±SD		60.8±12.6
Comorbidities		
COPD	7 (21.9%)	
HT	3 (9.4%)	
Asthma	1 (3.1%)	
DM	1 (3.1%)	
Smoking		
Yes	16 (50%)	
No	16 (50%)	
Diagnostic method		
Surgical	22 (53.6%)	
TTBX	17 (41.5%)	
FOB	1 (2.4%)	
Transbronchial	1(2.4%)	
TTF-1		
Positive	9 (22%)	
Negative	8 (19.5%)	
Not evaluated	24 (58.5%)	
Radiology		
Consolidation	16 (39.0%)	
Solid	15 (36.6%)	
Solid-consolidation	5 (12.2%)	
Surgical type		
Lobectomy	30 (73.2%)	
Wedge	8 (19.5%)	
Pneumonectomy	3 (7.3%)	
Progression Status		
Yes	7 (17.1%)	
No	34 (82.9%)	
Mortality		
Alive	38 (92.7%)	
Exitus	3 (7.3%)	

COPD: Chronic obstructive pulmonary disease, DM: Diabetes mellitus, HT: Hypertension, TTBX: Transthoracic biopsy, FOB: Fiberoptic bronchoscopy

Overall survival was 474±19.8 weeks and progression-free survival was 212±60.6 weeks. The patients underwent three types of surgical resection: lobectomy (n=30), pneumonectomy (n=3) and wedge resection (n=8). Overall survival according to the type of operation was 237.6±135.3 weeks in the lobectomy group and 230.5±110.7 weeks in the wedge resection group, with no statistically significant difference between the groups (p=0.75). Progression-free survival according to the type of operation was 231.5±66.8 weeks in the lobectomy group and 78±0.0 weeks in the wedge resection group, with no statistically significant difference between the groups (p=0.53) (**Figure 1**). Only three patients died during follow-up; there were no mortalities in the pneumonectomy group.

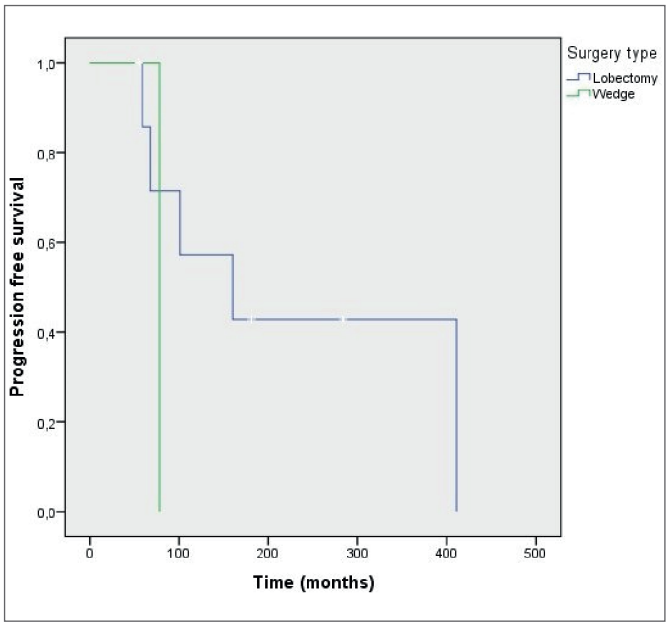

Figure 1. Progression-free survival according to the type of operation

Of the total, 17 patients were diagnosed by transthoracic biopsy and 21 by the surgical method, while other diagnostic methods included fiberoptic bronchoscopy, transbronchial biopsy and wedge resection.

Radiologically, the patients had three types of appearance: solid (n=15), consolidation (n=16) and mixed (solid + consolidation) (n=5). The patients underwent three types of surgical resection: lobectomy (n=30), pneumonectomy (n=3) and wedge resection (n=8).

According to the radiological appearance, overall survival was 392.8±26.2 weeks in the solid group, 482±26.7 weeks in the consolidated group, and 402.7±79 weeks in the solid+consolidated group, with no statistically significant difference between the groups (p=0.58). No progression was identified during the follow-up of the patients in the solid group according to their radiological appearance. According to the radiological appearance, progression-free survival was 305±36 weeks in the consolidated group, 394±24 weeks in the solid group and 313±81 weeks in the solid+consolidated group, with no statistically significant difference between the groups (p=0.17) (**Figure 2**).

Pathological examinations revealed eight TTF-1 negative patients and nine TTF-1 positive patients. No TTF-1 evaluation could be made for the other patients. Overall survival was 141.4 weeks in the TTF-1 positive group and 247 weeks in theTTF-1 negative group, with no statistically significant difference between the groups (p=0.628).

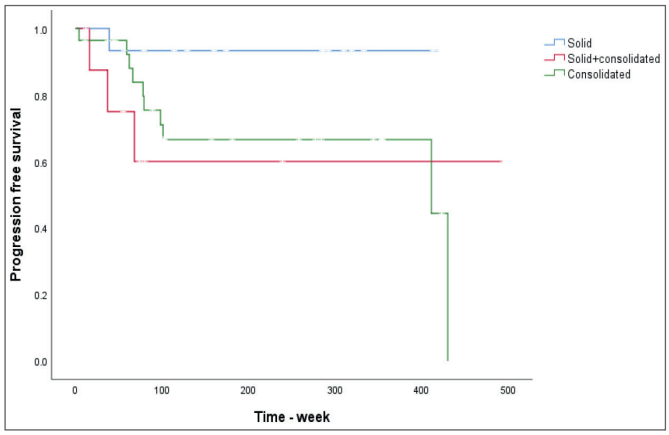

Figure 2. Progression-free survival according to the radiological appearance

DISCUSSION

Pneumonic-type lung adenocarcinoma can be easily confused with pneumonia, tuberculosis or other interstitial lung diseases due to its usually non-specific symptoms, and diagnosis is sometimes delayed, especially in developing countries, resulting in diagnosis at an advanced stage.[9-11] Pneumonic type lung adenocarcinoma has a poor prognosis when diagnosed in locally advanced and advanced stages.[12] In this patient group, even increased T stage and seperate nodules adversely affect prognosis and survival.[13] Pneumonic-type lung cancer, when diagnosed at an early stage, is eligible for curative treatment options, with the most effective treatment method being surgical resection. Patients eligible for surgical resection have a high survival rate, with lobectomy being the best option when possible.[14] In the present study, concurring with literature, patients undergoing surgery had prolonged overall and progression-free survival. Although progression-free survival was longer in the lobectomy arm than in the wedge resection group, the difference was not statistically significant. There was no mortality in the pneumonectomy group.

Previous studies have identified the most effective biopsy method for this patient group as surgical biopsy or transthoracic biopsy, and this method was identified as the most common also in the present study.[15]

Previous studies have established a correlation between radiological appearance and histological subtype, reporting a consolidative pattern incidence of 33-75% in mucinous tumors.[16] On the other hand, pneumonic type adenocarcinomas present a histological appearance in a mucinous pattern in approximately 45%, a non-mucinosis pattern in 40%, and a mixed pattern in 15% of cases.[1] In the present study, in line with literature, the histological appearance of all pneumonic-type adenocarcinoma patients undergoing surgery was in the mucinous pattern.

A previous study reported a consolidative appearance incidence in 83% of pneumonic-type adenocarcinomas.[14,17] Likewise, the consolidation and mixed (solid-consolidated) groups were more common radiologically in the present study, which is consistent with the literature.

In the same study, it was shown that overall survival was better in stage 1 and 2 invasive mucinous adenocarcinomas than invasive non-mucinous adenocarcinomas, but overall survival was similar in stages 3 and 4.[18] In a study in patients with a diagnosis of invasive mucinous adenocarcinoma, it was found that spiculation, increased density, emphysema were poor prognostic factors, and pneumonic-appearing tumors had a worse prognosis than solid-appearing tumors.[19] In the present study, postoperative overall according to radiological appearance was longer in the consolidative group than in the solid group, but progression-free survival according to radiological appearance was longer in the solid group than in the consolidative group among the pneumonic-type lung cancer patients. While the difference was not statistically significant, this may be associated with the small number of cases. TTF-1 positivity is a good prognostic factor in adenocarcinoma. Previous studies have shown TTF-1 to be negative in some pneumonic-type adenocarcinoma, and to be associated with a poor prognosis.[20] In the present study, survival was longer in the TTF-1-negative patients, although there was no significant difference in survival between the two groups, which may be associated with the small number of TTF-1 cases evaluated.

CONCLUSION

Pneumonic-type lung adenocarcinoma responds to curative treatment when diagnosed at an early stage. Since its radiology can be confused with pneumonia, early diagnosis by biopsy is important in suspected cases to prevent any delay in the start of treatment. The optimal treatment approach in operable patients is the surgical method, being associated with prolonged survival.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Atatürk Sanatorium Training and Research Hospital Clinical Researches Ethics Committee (Date: 12.07.2023, Decision No: 2012-KAEK-15/2739).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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

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Original Article

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The relationship between oral and dental health and appendicitis

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ABSTRACT

Aims: In this study, we aimed to evaluate the relationship between the scores of the "Simplified Oral Hygiene Index" (OHI-S) indicating poor oral hygiene and the "Decayed, Missing and Filled Teeth" (DMFT) index indicating oral health impairment, and acute appendicitis (AA).

Methods: Eighty four patients who were operated on with the diagnosis of acute appendicitis between April 2022 and May 2023 were included. The control group included 32 individuals without a history of appendectomy. In our study, oral health was evaluated using the DMFT and OHI-S indices. The DMFT index is one of the indices quantifying dental health status based on the number of cavities. OHI-S is an index used to evaluate oral hygiene.

Results: The OHI-S index scores of the patients with AA were significantly higher than those of the control group (3.53±1.43, 2.56±1.24, $p<0.05$, respectively). DMFT index scores of the patients with AA were significantly higher than the DMFT scores of the control group (12.09±5.51, 8.4±4.73, $p<0.05$, respectively). Among all individuals who participated in the study, OHI-S and DMFT index values of those who never or occasionally brushed their teeth were significantly higher than those who brushed their teeth at least once a day ($p<0.05$).

Conclusion: In this study, the OHI-S index scores indicating poor oral hygiene, and the DMFT index scores indicating poor oral health status were found to be higher in patients operated for AA; It suggests that poor oral health may increase the risk of appendicitis.

Keywords: Appendicitis, oral health, decayed missing and filled teeth, simplified oral hygiene index

INTRODUCTION

Acute appendicitis (AA) is the most common cause of acute abdomen in all age groups and one of the leading pathologies of emergency abdominal surgery.[1-3] Although approximately 7% of the entire population is diagnosed with acute appendicitis at some point in their lives, AA is most common between the ages of 10 and 30.[4] Delayed diagnosis may result in complications such as abscess, plastron, perforation or peritonitis and may lead to mortality in complicated cases. Even though the cause of acute appendicitis is not exactly clear, the most common causes include fecaliths, lymphoid hyperplasia, parasites, malignant and benign tumors.[5-7] Furthermore, it was also reported that appendicitis is a polymicrobial process and both aerobic and anaerobic bacteria play a role in cases of both acute and complicated appendicitis.[8,9]

Oral health is defined as the absence of periodontal (gum and surrounding tissues) disease, dental cavities and tooth loss, and psychosocial well-being without any biting, chewing, smiling and speaking difficulties.[10] There is a powerful and complex relationship between oral health and systemic health. Periodontitis, a chronic inflammatory disease, is one of the common chronic infections. If left untreated, it can lead to gum problems and tooth loss. Periodontitis may sometimes be asymptomatic and thus remain untreated for years. It has been reported that circulating proinflammatory bacterial constituents are significantly increased in patients with periodontal disease compared to those with healthy gums.[11] In recent studies, poor oral health has been associated with a number of systemic diseases including cardiovascular disease, pneumonia, diabetes, obesity, rheumatoid arthritis and kidney diseases.[12]

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This study aims to address the relationship between oral and dental health and appendicitis.

METHODS

The study was carried out with the permission of Siirt University Non-invasive Clinical Researches Ethics Committee (Date: 18.05.2023, Decision No: 2023/05/01/01). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Eighty four patients who underwent surgery with the diagnosis of AA which was confirmed by postoperative histopathologic examination between April 2022 and May 2023 were included in the study. The patients' demographic characteristics, complete blood count parameters at admission, radiologic results, operative findings and postoperative pathology data were recorded. The control group consisted of 32 subjects with no known systemic diseases or history of appendectomy who were admitted to the dental hospital for treatment and who consented to participate in the study. Patients younger than 18 years of age, patients with a known systemic disease, patients who underwent periodontal treatment within the last six months and patients with neoplastic lesions, parasites or foreign bodies causing appendicitis were excluded.

After consent forms were signed by the patients who agreed to participate in the study, oral examinations were performed by an experienced dentist. In our study, "Decayed, Missing and Filled Teeth" (DMFT) and "Simplified Oral Hygiene Index" (OHI-S) indices were used to evaluate oral health.[13-15] The DMFT index is an index evaluating all teeth to establish the cavity rate. The DMFT index is widely used worldwide to detect dental cavities in epidemiologic studies. The DMFT score is the sum of the number of decayed, missing and filled or crowned teeth. If a tooth has both decay and a filling, only one is scored.

OHI-S index is an index system used in the assessment of oral hygiene. In this index system, 3 regions in the upper and lower jaw, namely the right-posterior region, left-posterior region and anterior region, are assessed. A total of 12 measurements are made on 6 teeth in the mouth in terms of both dental plaque and calculus. In our study, buccal surfaces of upper first molars, lingual surfaces of lower first molars, and labial surfaces of upper right and lower left incisors were examined. As a result of the examination, the dental plaque and calculus indices were calculated separately and the total score obtained was accepted as the oral hygiene index score.

Statistical Analysis

SPSS for Windows Version 18.0 software was used for the statistical evaluation of our trial data. Mean ± standard deviation (SD) was used to describe the data related to quantitative variables; numbers (n) and percentages (%) were used to describe the data related to qualitative variables.

The Shapiro Wilk normality test was used to establish whether the quantitative variables were normally distributed. According to the results of the normality test, Student's t-test and Mann-Whitney-U test were used to compare the quantitative data of the two groups, and Pearson Correlation Analysis was used to test whether there was a correlation between the quantitative variables.

Categorical values were compared with Pearson's chi-square test. The results were evaluated at 95% confidence interval while significance was assessed at $p<0.05$ level.

RESULTS

A total of 116 patients, including 84 patients who underwent surgery due to AA with histopathological confirmation and 32 patients for control group, were included in our study. The control group was named Group 1 and Group 2 included the patients with AA. Demographic characteristics such as age, gender and OHI-S and DMFT index scores of both groups were compared (**Table 1**).

Table 1. Comparison of demographic characteristics, OHI-S and DMFT scores between groups

	Group 1 (n=32)	Group 2 (n=84)	p
Age ±SD	27.1±6.4	29.8±9.1	0.134
Female ±SD	25.5±5.5	29.5±10	0.179
Male ±SD	28.9±7.1	29.7±8,5	0.726
OHI-S ±SD	2.56±1.24	3.53±1.43	<0.05
DMFT ±SD	8.4±4.73	12.09±5.51	<0.05

OHI-S: Simplified Oral Hygiene Index, DMFT: Decayed, Missing and Filled Teeth, SD: Standard deviation

The mean age of subjects in group 1 and group 2 were was 27.1±6.4 and 29.8±9.1 years, respectively, and no statistically significant difference was found between the two groups (p=0.134).

Of the study participants, 65.5% brushed their teeth either never or occasionally, while 34.5% brushed their teeth at least once a day. Of the study participants, OHI-S scores of those who brushed their teeth either never or occasionally were significantly higher than those who brushed their teeth at least once a day (4±1, 1.8±0.9 respectively, $p<0.05$). DMFT scores of those who brushed their teeth either never or occasionally were significantly higher than those who brushed their teeth at least once a day (13.8±4, 5.8±4 respectively, $p<0.05$) (**Table 2**).

Table 2. Comparison of OHI-S and DMFT index values according to tooth brushing habits of all individuals participating in the study

	Regular (n=40)	Irregular (n=76)	p
OHI-S ±SD	1.8±0.9	4±1	p<0.05
DMFT±SD	5.8±4	13.8±4	p<0.05

Regular: brushes teeth at least once a day, Irregular: never or occasionally brushes teeth, OHI-S: Simplified Oral Hygiene Index, DMFT: Decayed, Missing and Filled Teeth, SD: Standard deviation

The comparison of the OHI-S index scores between the groups revealed that the OHI-S index scores of patients with AA were statistically significantly higher than those in the control group (3.53±1.43, 2.56±1.24 respectively, p<0.05). DMFT index scores of patients with AA were statistically significantly higher than those in control group (12.09±5.51, 8.4±4.73 respectively, p<0.05) (**Figure**).

DISCUSSION

Although appendicitis is one of the common causes of acute abdomen, its etiology remains unclear in most cases.[16] Despite the fact that luminal obstruction is considered to be the most significant factor in the etiology, some evidence suggests that obstruction is not the main cause in the majority of cases.[17,18] In some studies, it was even suggested that the obstruction is not a cause but a result.[19,20] Andreou et al.[21] reported that although fecaliths were observed in histopathologic examinations of appendicitis specimens, they were rare. Arnbjörnsson et al.[22] found that intraoperative intraluminal pressure measurements were normal in cases of appendicitis and elevated only in advanced stages. The submucosa of the appendix contains abundant lymphoid follicles and acute mucosal and submucosal inflammation were suggested as the primary cause of appendicitis.[23] Infections are thought to cause appendicitis by stimulating lymphoid hyperplasia, which obstructs the appendix by leading to luminal obstruction.[24] This view is supported by the fact that appendicitis occurs between the ages of 10 and 30, i.e., the period when lymphoid tissue is most dense.

According to the data obtained by molecular biology methods, the oral cavity, which hosts more than 700 microorganisms, is a region containing more microorganisms than other parts of the body. Oral cavity microorganisms or their products can cause infections in different parts of the body. Metastatic infection due to bacterial displacement, metastatic damage caused by microbial toxins, and metastatic inflammation due to weak immune system can play a role in their pathogenesis.[25] Blod et al.[26] reported that they detected several oral bacterial pathogens in the appendix lumens and suggested that the oral cavity may be a reservoir for AA. Aiyoshi et al.[27] stated in their study that ectopic colonization of the appendix by oral *Fusobacterium* species may play an important role in the pathogenesis of AA. In addition, oral bacteria such as *Parvimonas*, *Alloprevotella*, *Streptococcus*, *Prevotella*, *Peptostreptococcus* and *Porphyromonas* have been isolated in the appendix lumen of AA patients.[28] Among them, *Parvimonas*, which has the highest rate, is among the pathogens that cause periodontal disease.[29] Microorganisms such as *Fusobacterium* and *Parvimonas* in the appendix, may form a biofilm that causes mucosal inflammation.[26]

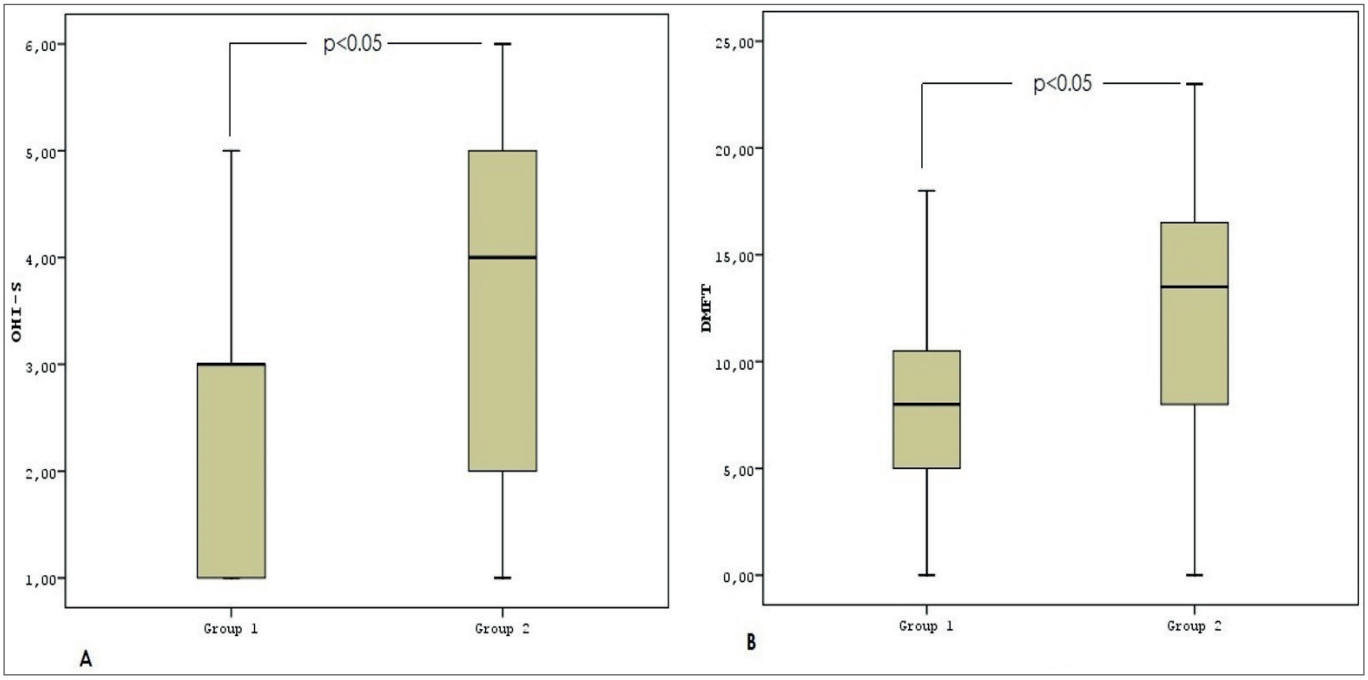

Figure. Box plots A and B showing OHI-S and DMFT index values for group 1 (control group) and group 2 (subjects with appendicitis). The horizontal lines inside each box represent the mean values while the bottom and top rows of each box represent the minimum and maximum values respectively.

In some studies, bacteria belonging to *Fusobacterium* genus, which are abundant in the oral cavity, were isolated in bacteriologic studies conducted by using microbiologic culture techniques on appendectomy specimens.[30,31] Swidsinski et al.[32] found that Fusobacteria spp. were an important constituent of mucosal and submucosal inflammation in 62% of appendectomy specimens and correlated positively with the severity of appendicitis. In addition, *Fusobacterium* spp. were isolated in most cases of suppurative appendicitis in recent studies.[32,33] In a study conducted by Guinane et al.[28] it was observed that oral pathogens, especially *Parvimonas* and *Gemella* spp., were found abundantly in appendix samples. However, it is unknown whether these microorganisms are primarily accountable for the etiology of appendicitis or whether they proliferate secondary to inflammation. In our study, indicators of oral hygiene, namely OHI-S and DMFT index values, were significantly higher in appendectomy cases compared to the control group, suggesting that these microorganisms may be involved in the etiology of appendicitis.

Some authors have argued that diet and hygiene play an important role in the etiology of acute appendicitis.[34-37] According to this view, a diet poor in fiber but rich in meat and sugar causes an increase in the incidence of AA. It has been reported in some studies that the incidence of appendicitis is significantly lower in third world countries where people consume a diet that mainly consist of cereals, legumes, vegetables and high-fiber foods.[38,39] Low fiber intake may cause the colonic transit time to shorten and the fecal reservoir in the lumen to increase, and thus cause appendicitis.[40] Studies showing that unhygienic environments may lead to an increase in the incidence of AA are available.[41,42] Furthermore, an increase in the prevalence of appendicitis has been demonstrated in societies with poor personal hygiene care.[43-45]

Oral health is one of the most prominent indicators reflecting personal hygiene. Attin et al.[46] stated that brushing teeth at least once a day protects oral health and prevents caries and periodontal diseases. In our study, we found that OHI-S and DMFT index values, which are oral health indicators, were higher in individuals who never or occasionally brushed their teeth compared to individuals who brushed their teeth at least once a day. While diet may cause oral health problems, poor oral health may lead to nutritional issues.[47] Moreover, adverse changes in nutrition and the digestive system arising out of inadequate chewing, which may be due to poor oral and dental health, may cause fecaliths to form.

In this study, OHI-S that indicate a lack of oral hygiene, and DMFT index values that indicate poor oral health were found to be higher in patients who underwent surgery for appendicitis, which in turn led us to correlate appendicitis with oral health. If these findings are confirmed, measures against and information about poor oral health may help reduce the incidence of appendicitis.

There were several limiting factors in our study. Firstly, due to the small number of patients, our findings need to be supported by studies with a larger patient population. Secondly, the control group consisted of subjects who applied to the dental hospital for treatment, which resulted in a difference in the study groups.

CONCLUSION

Our findings suggest that poor oral health may increase the risk of appendicitis. From this standpoint, it may be useful to consider oral health in the etiology of appendicitis in future studies. Furthermore, to the best of our knowledge, this is the first ever study to investigate the relationship between appendicitis and oral health.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Siirt University Non-invasive Clinical Researches Ethics Committee (Date: 18.05.2023, Decision No: 2023/05/01/01).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Predialysis education program and early vascular access: a single center experience

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ABSTRACT

Aims: The main objective of this study is to investigate the impact of a pre-dialysis education program on the selection of vascular access during the initiation of maintenance hemodialysis therapy and the short-term impact of this education on patient outcomes.

Methods: The data were collected from two different times in the past from patients under maintenance hemodialysis: the first group consisted of a group of patients who received a predialysis education program (intervention group) from a dedicated nephrologist, and the second group included those who did not undergo a control program (control group). Predialysis education program involved six modules addressing understanding kidney disease, diet, and nutrition, treatment options for end-stage kidney disease, dialysis procedures, medication management, and self-care/independence. Patients aged ≥18 years were enrolled in the study. The patients were compared according to their clinical status during the onset of maintenance hemodialysis, including vascular access type, serum potassium level, previous hospitalization, and urgent hemodialysis need. Additionally, the hospitalization and infection rates within 6 months following the onset of maintenance hemodialysis were compared. P<0.05 was assigned as significant.

Results: A total of 203 hemodialysis patients, 129 patients in the intervention group and 74 patients in the control group, were assessed. The mean age was 65.06±13.14 for the intervention versus 65.66±9.13 for the control group (p=0.729). 51.9% (n=67) in the intervention group versus 55.4% (n=41) were females (p=0.371). The control group had more hospitalization (p<0.001), intervention need of vascular access problems (p<0.001), vascular access infection (<0.001), exposure to an intermittent catheter (<0.001), 127 of 129 patients in the intervention group started hemodialysis with an arteriovenous fistula. The control group started hemodialysis with a catheter Potassium, phosphorus, and bicarbonate levels were higher in the control group during the first hemodialysis session (5.25±1.12 vs 4.59±0.79, p<0.001, 6.60±1.18 vs 4.25±1.04, p<0.001, and 21.77±3.96 vs 14.18±2.83, p<0.001, respectively).

Conclusion: This study demonstrated that a well-organized education program can lower the burden of morbidity in end-stage kidney disease and support the patient and healthcare providers with a favorable transmission from a non-hemodialysis period to a hemodialysis period.

Keywords: End-stage kidney disease, pre-dialysis education, maintenance hemodialysis, morbidity, vascular access

INTRODUCTION

End-stage kidney disease (ESKD) poses a significant global health challenge, imposing a substantial burden on both patients and healthcare systems.[1,2] Maintenance hemodialysis (MHD) therapy is a critical intervention for patients with ESKD as it sustains life. The choice of vascular access during the initiation of HD plays a crucial role in ensuring successful treatment outcomes and enhancing patients' overall quality of life.[3,4]

In recent years, pre-dialysis education programs have gained recognition as an essential component in the care of patients with ESKD.[5-8] These programs aim to provide patients with comprehensive information about kidney disease, treatment options, and self-management strategies to optimize their health and decision-making during the transition to maintenance HD as well as other choices.[9-11] By empowering patients with knowledge and awareness, pre-dialysis education programs have the potential to improve patients' active involvement in their care and enable them to make informed choices, particularly regarding vascular access options. However, clinical evidence is lacking regarding the question, "What are the most effective educational methods?" It is also unclear how to provide the staff's competencies to develop such an education.[7,12]

The current study aims to investigate the effects of a pre-dialysis education program on the choice of vascular access during the initiation of maintenance HD therapy and the impact of this program on the 6-month outcomes of the patients.

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METHODS

The study was carried out with the permission of the Sakarya University Faculty Non-invasive Researches Ethics Committee (Date: 02.05.2023, Decision No: 107). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Participant

This study is a retrospective case-control study that was conducted at our State Hospital between January 1, 2020, and December 31, 2022. Because of being retrospective, informed consent was not obtained. The participants were divided into two groups: the intervention group, consisting of ESKD patients who received a pre-dialysis education program (intervention group), and ESKD patients who received urgent dialysis without contributing to a pre-dialysis education program (control group), comprising patients who did not undergo any specific education program. 64 out of 74 ptaients in the control group were chosen from another centre.

Inclusion Criteria

Age ≥18 years, surviving within 6 months following initiating maintenance HD, CKD stage 3-5.

Exclusion Criteria

Deaths, incomplete data, switching peritoneal dialysis or transplantation, malignancy, hospitalization due to decompensated heart failure, and infections unrelated to vascular access.

Data Collection

The data for this study was collected retrospectively from patient records maintained during the study duration. Information related to the clinical status of patients during the onset of maintenance HD was gathered, and 6-month outcomes were compared between the two groups, including the type of vascular access chosen (arteriovenous fistula or catheter), serum potassium, phosphorus, bicarbonate levels, hospitalization and infection rates, the need for urgent HD, and mortality rates following the onset of maintenance HD. Additionally, hepatitis B virus surface antigen (HbsAg), antibody of HbsAg (AntiHBs), antibody of hepatitis C virus, and antibody of human immunodeficiency virus (Anti-HIV) tests were performed.

Pre-Dialysis Education Program

The pre-dialysis education program involved six modules delivered to the intervention group face-to-face by a dedicated nephrologist. The modules covered crucial topics related to kidney disease, including understanding the disease process, dietary and nutritional considerations, available treatment options for end-stage kidney disease, dialysis procedures, effective medication management, and promotion of self-care and independence (**Table 1**).

Table 1. Modules of pre-dialysis education program

	Content of the modules
Module-1	Understanding Kidney Disease: Focus on educating patients about the anatomy and function of the kidneys, as well as the causes and progression of kidney disease.
Module-2	Diet and Nutrition: Provide patients with guidance on maintaining a healthy diet and nutrition plan, including guidelines for fluid and salt intake.
Module-3	Treatment Options: Provide an overview of the different types of dialysis and other treatment options available for ESKD, including peritoneal dialysis, hemodialysis, and transplantation.
Module-4	Dialysis Procedures: Focus on educating patients about the procedures involved in dialysis, including preparation for dialysis, catheter care, infection control, and monitoring for potential complications.
Module-5	Medication Management: Focus on the medications commonly used to treat kidney disease and the importance of adherence to medication regimens.
Module-6	Self-Care and Independence: Focus on empowering patients to take an active role in their own care, including self-monitoring, self-care techniques, and communication with healthcare providers.

RESULTS

A total of 203 participants with a mean age of 65.28±11.82 years were enrolled in the study. The two groups were similar in terms of age (p=0.729). 51.9% (n=67) in the intervention group versus 55.4% (n=41) were females (p=0.371). In the intervention group, the mean duration from AVF creation to initiating HD was 147.23±7.22 days. The clinical and laboratory features of the participants are given in **Table 2**. All 74 patients in the control group needing dialysis underwent their first dialysis through a temporary non-tunneled catheter (25 of 74 were exposed to a femoral intervention). Among the control group, 50 out of 74 patients still had a catheter at the end of month 6 after the initiation of dialysis.

Biochemical Comparison

The intervention group showed significantly lower phosphorus (p<0.001) and potassium (p<0.001) levels and significantly higher bicarbonate (p<0.001) levels during their first HD session compared to the control group (**Table 3**).

Clinical Outcomes

The data demonstrated that the intervention group, which had AVF as the primary vascular access, experienced fewer hospitalizations (<0.001), shorter hospitalization durations (<0.001), and fewer vascular access-related complications (<0.001) compared to the control group, which relied solely on catheter access (**Table 4**). Only two patients in the intervention groups required a temporary catheter within 6 months of follow-up after starting HD.

Table 2. The clinical and laboratory features of the participants at the entry into the study

Characteristics, n=203			**Serology, yes/no, %**
Age (years)		65.28±11.82	HBsAg positive; 4/193 Anti Hbs positive; 70/133 Anti-HCV positive; 2/201 Anti-HIV positive; 0/2023
Gender, Female, no, %		108/95(53.2/46.8)	
BMI (kg/m²)		27.02±6.93	
eGFR at the entry of the intervention group		16.42±6.57	
eGFR at the first dialysis in the intervention group		11.76±4.54	
Active smoker		19(11.3)	
Comorbidity During Dialysis; yes/no, n, %			**Drugs, yes/no, n***
DM HT CAD	80/123 (39.4/60.6) 132/65 (62/35) 52/151 (25.6/74.4)	ACEi ARB Loop diuretics Spironolactone Aspirin Apixaban Warfarin	34/95 28/101 110/19 10/119 82/121 5/124 6/147 *Note: there are missing data

Abbreviations: ACEi: Angiotensin-converting enzyme inhibitor, ARB: Aldosterone antagonist, BMI: Body mass index, CAD: Coronary artery disease DM: Diabetes mellitus, HT: Hypertension, eGFR: Estimated glomerular filtration rate, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus.

Table 3. The comparison of the two groups for the stage, phosphorus, potassium, and bicarbonate at the beginning of the study

		Intervention group n=129	**Control group n=74**	**P value**
Age, year		65.06±13.14	65.66±9.13	0.729
BMI, kg/m²		27.18±6.97	26.46±6.82	0.575
Gender, male/female, n		62/67	33/41	0.371
CKD stage * n (%) Stage 3 Stage 4 Stage 5		8 (6.2) 60 (46.6) 61 (47.2)	0 0 74 (100)	NA
First HD	Phosphorus, mg/dl	4.59±0.79	5.32±0.93	<0.001
	Potassium, mEq/L	5.05±0.97	5.67±0.67	<0.001
	Bicarbonate, mmol/L	21.77±3.96	14.18±2.83	<0.001

Abbreviations: BMI: Body mass index, *eGFR wrere calculated according to CKD-EPI 2021

Table 4. The comparison of the two groups regarding vascular access and hospitalization

After initiating maintenance HD, yes/no, n (within 6 months)	**Intervention group n=129**	**Control group n=74**	**P value**
Hospitalization	32/97	53/20	<0.001
Total Hospitalization number	0.5±0.41	1.21±0.55	<0.001
Vascular access, AVF/catheter	127/2	0/74	<0.001
Vascular access-related hospitalization	7/122	56/18	<0.001
Vascular access-related infection (catheter infection, thrombophlebitis, cellulitis, etc.)	1/128	1/73	<0.001
Need for temporary catheter insertion	0/129	2/72	<0.001
Hospitalization duration at the HD initiation, days	2.48±2.02	4.68±2.03	<0.001

Abbreviations: HD: Hemodialysis

Pre-dialysis education program improved the serology of HBV. Pre-education, 77 of 129 participants were negative for HbsAb; however, following the education program, the count of negative individuals reduced to 42 during the first HD session. In the control group, only 16 patients were positive for HbsAb.

DISCUSSION

This study aimed to investigate the impact of a pre-dialysis education program on the choice of vascular access and its subsequent effects on the 6-month outcomes of ESKD patients undergoing maintenance HD. The results revealed that the intervention group, which received a specialized pre-dialysis education program, experienced significant improvements in various clinical parameters (better potassium, phosphorus, and bicarbonate levels, lower hospitalization rates and duration, better vaccination rates, and lower intermittent catheterization rate) compared to the control group, which did not undergo any specific education program. In a study of 302 patients with stage 3-5 CKD, a comprehensive 8-module multidimensional pre-dialysis training program demonstrated a potential delay in CKD progression. Inspired by these findings, we developed a modified multidimensional program in a similar manner, creating specific training topics and modules.[13]

Maintenance hemodialysis (MHD) therapy is crucial for sustaining the lives of ESKD patients. One essential aspect of ensuring high quality of life for patients in the management of ESKD patients is the choice of vascular access during the initiation of HD. Moreover, pre-dialysis education programs have emerged as an essential component in the care of ESKD patients, aiming to provide comprehensive information about kidney disease, treatment options, and self-management strategies.[7] Predialysis education provides closer clinical and laboratory monitoring, which leads to a mild transmission from a non-hemodialysis period to a maintenance HD period. This approach gives the opportunity to regulate fluid and electrolyte imbalances, create desirable vascular access, prevent urgent dialysis sessions, and select an individualized treatment modality.[8] Moreover, Previous studies demonstrated a

direct association between the number and consistency of care provided by a nephrologist in 3 months or more of the 6 months before dialysis and a superior prognosis.5 In our study, a specialized pre-dialysis education program provided clear benefits such as better serum potassium, phosphorus, and bicarbonate levels at the time of first HD sessions and a low catheter incidence as vascular access. This education program significantly increased the likelihood of initiating maintenance HD therapy with an AVF, reaching almost 99%.

A pre-dialysis education can improve the contribution of the patients to self-care and increase adherence to medicines and lifestyle change, a lower hospitalization rate can be expected as they become more aware of expected scenarios.[16,17] These education programs offer substantial benefits to patients, both during the initiation of dialysis and throughout their treatment course, fostering improved treatment compliance and empowering patients to find incorporate with healthcare providers regarding hemodialysis-related problems.[18,19] This study found a significant reduction in hospitalizations and hospitalization duration in the intervention group following a face-to-face pre-dialysis education program. Programmed initiation is better compared to emergent, unplanned dialysis.[20] Patients with AVF as their primary vascular access had fewer hospitalizations related to vascular access complications, such as catheter infection, thrombophlebitis, and cellulitis. The reduced need for temporary catheter insertion in the intervention group further highlights the positive impact of AVF as the preferred vascular access. Furthermore, In our study, we managed many of our patients as outpatients taking discrete sessions of dialysis without admission to the ward. Our patients were mostly supported and prevented from unnecessary emergency department applications. even admitted patients needed less follow-up duration. Our vascular access gave an option to maximize the diuretic and antihypertensive therapy as needed with more confidence with less fear from adverse effects like deterioration in renal function and electrolyte imbalance.

Moreover, the pre-dialysis education program led to improvements in serological markers related to HBV. The program significantly increased the number of participants testing negative for HBV surface antibody during their first HD session. This result is encouraging, as it indicates that education and awareness efforts can positively influence serology and potentially reduce the risk of infectious complications among ESKD patients.

Limitations of the Study

Retrospective study design: The study's retrospective nature could introduce inherent biases and limit our ability to establish causality. The lack of randomization might lead to potential confounding factors affecting the observed outcomes.

Single-center setting: The study was conducted at a single center, which may limit the generalizability of the findings to other healthcare settings with different patient populations and care practices.

Missing data: Incomplete data for some participants may have affected certain analyses, potentially introducing bias or limiting the accuracy of the results.

Selection bias: The inclusion and exclusion criteria might have introduced selection bias, as patients with specific characteristics or conditions could have been excluded, potentially influencing the study outcomes.

Short-term follow-up: The study's 6-month follow-up period may not capture long-term outcomes, and the effects of the pre-dialysis education program beyond this duration remain unknown.

Pre-dialysis education variability: The study did not account for potential variations in the delivery or depth of pre-dialysis education among participants, which might impact the outcomes.

Non-randomized allocation to AVF: The higher proportion of AVF use in the intervention group might also be influenced by other unmeasured patient characteristics such as education, high sociocultural status, and incomes or preferences that were not controlled for in the analysis.

Calculation of costs: Emergent unplanned was shown to have a negative effect on costs and hospitalization, but we did not calculate the costs in our study.

CONCLUSION

The positive outcomes of this pre-dialysis education program underscore its value as an essential component of ESKD patient care. Integrating such programs into routine clinical practice can empower patients, optimize their treatment journey, and improve their long-term quality of life. By emphasizing AVF as the primary vascular access and promoting informed decision-making, pre-dialysis education holds promise for enhancing patient outcomes and reducing healthcare burdens.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Sakarya University Faculty of Medicine Non-invasive Researches Ethics Committee (Date: 02.05.2023, Decision No: 107).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Turkish children myopia progression in the urban area, a retrospective evaluation

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ABSTRACT

Aims: To investigate myopia trends and progression in urban school-aged myopic children in Turkey.

Methods: This retrospective study included myopic children aged 6-18 years attending the ophthalmology clinic for regular eye and refractive examinations between 2003 and 2021. Myopia progression was calculated as the difference between the baseline and the last visit spherical equivalent refractive (SER) values. Individuals were further categorized to determine the age-specific myopia progression as 6-11, 12-16, and 17-18 age groups based on the school periods of the country. According to the SER values, individuals were classified into mild, moderate, and high myopic groups.

Results: A total of 602 eyes of 301 children (191 female, 110 male) with a mean age of 11.64±2.81 (6-18) years were included in the study. The mean follow-up time of patients was 37.51±19.18 (6-98) months. The baseline mean SER value was -1.5±1.07 D (range: -0.50 and -5.62) and -2.55±1.50 at the final visit. The overall mean myopia progression was -0.35±0.37 D (range: +0.35 D and -3.75 D/year. There were 46 children between 6-11 years, 173 children between 12-16 years, 82 children between 17-18 years, and the annual SER changes were -0.46±0.40 D; -0.37±0.39 D and -0.26±0.29 D in the groups, respectively ($p < 0.001$). Baseline, final, and annual myopia progression were greater in females. Although there was no statistical significance, myopia progression was faster in moderate myopes (-0.39±0.33 D/a year), followed by mild (-0.35±0.37 D/a year) and high myopes (-0.21±0.20 D/a year) (p=0.37).

Conclusion: The progression of myopia in school-aged Turkish children from the Western Black Sea Region is comparable to the world. Our study revealed the greater myopia progression in the youngest children, moderate myopia group, and females. Myopia prevention recommendations should be carefully advised to the youngest female ones to reduce myopia progression.

Keywords: Myopia, progression of myopia, urban area, myopia in children

INTRODUCTION

Myopia is a common refractive error causing vision loss and is becoming a public health problem due to its increasing prevalence all over the world. The worldwide prevalence of myopia and high myopia is expected to be 52% (almost 5 billion) and 10% (almost 1 billion) by 2050. Myopia may develop in early childhood, late teens, or adulthood.[1,2] Early onset of myopia has been reported to lead to more myopic refractive error or high myopia later in life.[3] Donovan et al.[4] indicated that the mean annual myopia progression in children was about half-a-diopter in Europeans (-0.55 D) and a higher progression rate in Asians (-0.82 D). In our previous study aiming to investigate the relationship between increased digital screen time and the development and progression of myopia during the COVID-19 pandemic, we found the mean annual change in spherical equivalent refractive error (SER) as -0.97±0.66 D in urban area school-aged children in Turkey.[5] The Northern Indian Myopia study that involved 10000 school children aged 5 to 15 years from Delhi reported an annual myopia progression of -0.27±0.42 D.[6]

Both genetic and environmental factors influence myopia.[7] Given the potential role of geographic location on myopia progression, information on the pattern of progression of myopic refractive error across different age groups in Turkish children could help clinicians choose appropriate myopia prevention strategies. In this study, the data on refractive error and the variability between ages were obtained from urban school-aged children living in the Western Black Sea Region of Turkey.

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METHODS

This retrospective study was conducted in accordance with the Declaration of Helsinki with written permission from Zonguldak Bülent Ecevit University Faculty of Medicine Clinical Researches Ethics Committee (Date: 06.04.2022, Decision No:2022/07). The study was carried out in Devrek State Hospital, Zonguldak, Turkey. 301 individuals aged 6 to 18 years and only with the diagnosis of 'myopic refractive error' at their first visit (taken as a baseline) were included in the study. Patients examined at least twice with six months intervals between 2003-2021 years were evaluated. Children were categorized into three groups according to their refractive error. Mild myopia was defined between -0.50 to -2.99 D SER value, moderate myopia as SER between -3.00 to -4.99 D, and high myopia as SER of at least -5.00 D. Based on the World Health Organization (WHO) definition; we defined high myopia as ≤-5.00 D.[1] To determine the age-specific myopia progression, individuals were further categorized as 6-11, 12-16, 17-18 age groups for the school periods in the country. Myopia progression was calculated as the difference between SER at baseline and at the last visit. The best corrected visual acuity was 1.0 for all patients under the correction of refractive status. Patients with other ocular diseases like uveitis, trauma, strabismus, and retinal diseases were excluded from this study.

Statistical Analysis

Descriptive and statistical analyzes were performed using IBM SPSS Statistics 21. Demographic characteristics and clinical data were expressed as mean, standard deviation, frequency, or percentage. The Kolmogorov-Smirnov test evaluated the normal distribution test of continuous data. Since the data were suitable for normal distribution, the independent T-test was used for independent groups. One-way ANOVA test was used for multiple categorical data. P value of 0.05 or less was considered statistically significant.

RESULTS

A total of 602 eyes 301 children with a mean age of 11.64±2.81 (6-18) years were included in the study. 191 (63.5%) patients were female with a mean age of 12.15±2.5 (6-18), and 110 (36.5%) patients were male with a mean age of 10.77±2.98 (6-18) years. The data are summarized in **Table 1**.

The mean follow-up time of patients was 37.51±19.18 (6-98) months. The baseline mean SER value was -1.5±1.07 D (range: -0.50 and -5.62) and -2.55±1.50 D (range: -0.50 and -8.50) at the final visit (p<0.001). The overall mean myopia progression was -0.35±0.37 D/year (range: +0.35 D and -3.75 D). When the patients were evaluated according to age groups, there were 46

Table 1. Follow-up period, baseline and final mean spherical refractive equivalent (SER) values and myopia progression based on age, gender and severity of myopia

	N	Follow-up period (months)	Baseline SER values	Final SER values	P value	Annual SER progression
Total	301	37.51±19.18 (6 - 98)	-1.5±1.07 (Range: -0.50 and -5.62)	-2.55±1.50 (Range: -0.50 and -8.50)	<0.001	-0.35±0.37 (Range +0.37 and -3.75)
Age groups						
6-11	46 (15.3%)	39.17±17.91 (6.23 - 76.53)	-1.61±1.12 (Range: -0.50 and -5.25)	-2.95±1.63 (Range: -0.50 and -7.75)	<0.001	-0.46±0.40 (Range +0.30 and -1.93)
12-16	173 (57.5%)	42.57±19.73 (6 - 98)	-1.43±1.02 (Range: -0.50 and -5.5)	-2.64±1.57 (Range: -0.50 and -8.50)	<0.001	-0.37±0.39 (Range +0.47 and -3.75)
17-18	82 (27.2%)	25.92± 12.77 (6 - 61.87)	-1.57±1.13 (Range: -0.50 and -5.62)	-2.15±1.30 (Range: -0.50 and -6.00)	<0.001	-0.26±0.29 (Range: +0.16 and -1.49)
p value		0.001	0.212	<0.001		<0.001
Gender						
Females	191 (63.5%)	37.36±18.94 (6 - 98)	-1.54±1.11 (Range: -0.5 and -5.62)	-2.67±1.59 (Range: -0.50 and -8.50)	<0.001	-0.37±0.39 (Range: +0.47 and -3.75)
Males	110 (36.5%)	37.77±19.63 (6.23 - 91)	-1.42±1.0 (Range: -0.5 and -5.25)	-2.35±1.43 (Range: -0.50 and -7.50)	<0.001	-0.31±0.33 (Range: +0.3 and -2.06)
p value		0.94	0.149	0.015		0.024
Severity of myopia						
Mild	269	37.17±18.87 (6 - 98)	-1.2±0.64 (Range: -0.5 and -2.87)	-2.24±1.24 (Range: -0.50 and -7.25)	<0.001	-0.35±0.37 (Range: +0.47 and -3.75)
Moderate	27	40.02±22.34 (6 - 79.8)	-3.76±0.59 (Range: -3,0 and -4.875)	-5.03±1.26 (Range: -3.00 and -8.125)	<0.001	-0.39±0.33 (Range: +0.16 and -1.27)
High	5	42.44±18.44 (19.4 - 61.17)	-5.17±0.22 (Range: -5.0 and -5.62)	-6.13±1.04 (Range: -5.00 and -8.50)	=0.018	-0.21±0.20 (Range: 0 and -0.67)
p value		0.42				0.37

Results indicate as mean±standard deviation.

children between 6-11 years with a mean age of 9±0.96, 173 children between 12-16 years with a mean age of 14.29±1.35, 82 children between 17-18 years with a mean age 17,44±0.26. The follow-up period was 39.17±17.19, 42.57±19.73, and 25.92±12.77 months for the groups, respectively. The follow-up period of the last group was significantly lower ($p<0.001$, one-way ANOVA, $F(2,598)=48.99$). There was no significant difference between the baseline refractive values of the groups ($p=0.212$, one-way ANOVA, $F(2,598)=1.556$). However, there was a statistical significance between the final SER values ($p<0.001$, one-way ANOVA, $F(2,598)=9.496$). The annual SER changes were -0.46±0.40 D, -0.37±0.39 D, and -0.26±0.29 D in the groups, respectively. The progression of myopia between the groups was statistically significant ($p < 0.001$, one-way ANOVA, $F(2,598)=8,677$), and the greatest myopia progression was detected in the youngest group.

According to the severity of myopia, while there were 269 patients in the mild myopic group (-0,5 D to -2,99 D), there were 27 patients in the moderate myopic group (-3 D to -4,99 D) and 5 patients in the high myopic group (-5D and above). Although there was no statistical significance, myopia progression was faster in moderate myopes (-0.39±0.33 D/a year), followed by mild (-0.35±0.37 D/a year) and high myopes (-0.21±0.20 D/a year) ($p=0.37$, one-way ANOVA, $F(2.598)=0.995$). The post hoc analysis of the groups revealed no significant difference between the moderate myopic group showing the fastest progression, and the high myopic group showing the slowest progression ($p=0.118$, Mann Whitney U).

Baseline, final SER values, and annual myopia progression were greater in females. They were -1.54±1.1 D, -2.67±1.59 D, -0.26±0.29 D/year in females and -1.42±1.0 D, -2.35±1.43 D, -0.31±0.33 D/year in males, respectively. A statistical significance was observed between the gender when the final SER values and annual change of SER value were examined ($p=0.015$ and $p=0.024$, respectively).

DISCUSSION

The results from this study indicate that the annual myopia progression in school-aged children in urban areas varied with age, the age of onset, the severity of myopia, and gender. The mean myopia progression was about -0.35±0.37 D/a year overall, but this value was -0.46±0.40 D/a year in the 6-11 years old children.

Myopia tends to increase as children grow up. The annual progression values are similar to those in Caucasian children (aged 6 to 15 years) living in Australia (-0.31 to -0.41D), Europe (-0.55D), the UK, and the USA (-0.34 D to -0.50D) and in East Asian countries like China and Singapore (-0.31 to -1.2 D).[4,8-10] In a meta-analysis including 2194 participants in total, children wearing single-vision spectacles with an average age of 9.3 had a progression of -0.52 D (%95 CI -0.39 to -0.72 D) myopia per a year in Europe and -0.82 D (%95 CI -0.71 to -0.93 D) per a year in Asia.[4] The median progression rate of myopia was found to be -0.16 D/year, and 62% of children with myopia progressed in London, UK.[11] Further, 400 children aged 6-12 years with spherical equivalents of -1.00 and -6.00 D were followed for two years. -1.20±0.69 D/2 years myopic change was detected in the non-treated atropine placebo group (n=200) in the ATOM1 study.[12] In the present study, annual change in SER was found to be similar to East Asian countries and higher than in London. The variations in the myopia progression among different countries could be explained by the location, lifestyle, and ethnicity variations among different population groups.

In our study, individuals with a moderate degree of myopia had greater progression than those with a mild and high degree of myopia. Verkicharla PK et al. found different outcomes from our study. They indicated a faster myopia progression in the patients with higher degrees at baseline. A similar result was also reported in Taiwanese, Chinese, and Singaporean school children.[14-17] The conflicting results of our study may be explained by the small number of our high-myopic patient group. In the study conducted in London, the progression was found to be higher in the moderate myopia group than in the mild myopia group ($p<0.001$, -0.54, and -0.37 D/year, respectively), which matches our results.[11] The exact mechanism of why moderate myopes progress at a faster rate compared to that mild myopes needs to be clarified. The moderate myopia population might have a different causal relationship with myopiogenesis, unlike physiological myopia, and influence genes in myopiogenesis and progression.[18]

There is a conflict of gender dominance in myopia. Studies show that myopia is more common but progresses more slowly in school-age girls, and the frequency is higher in boys at advanced ages.[19,20] In our study, myopia was more common in girls, consistent with the literature. However, there was a significant difference between the genders regarding myopia progression. Girls were more prone to have progression of myopia.

The strength of the present study is the inclusion of extensive data for determining the annual myopia progression in 301 patients. Also, being the first study conducted in an urban area in the Western Black Sea Region of Turkey is another feature of this study.

There are several limitations of our study. Being a citywide population study, it may only reflect some of the country, especially due to the known difference in myopia between urban and rural regions. In addition, the retrospective nature and non-cycloplegic refractive measurements may lead to bias. Beyond that, this study did not evaluate the other potential factors, such as time spent outdoors, parenteral myopia, and time spent near work. Further studies involving separate data from rural and urban areas, with data related to various factors such as exposure to light levels/time outdoors, are required in our country.

CONCLUSION

The progression of myopia in school-aged Turkish children from the Western Black Sea Region is comparable to the world. This finding of the greater progression in 'moderate myopes' compared to that of the mild myopes and the tendency of the younger age group emphasizes the need for regular follow-ups with short intervals and the application of anti-myopia strategies to control myopia progression.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Zonguldak Bülent Ecevit University Faculty of Medicine Clinical Researches Ethics Committee (Date: 06.04.2022, Decision No: 2022/07).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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The diagnostic accuracy of coronary angiography to detect left anterior descending artery myocardial bridging in coronary artery bypass grafting: a retrospective single-center study

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ABSTRACT

Aims: The left anterior descending artery is the most involved vessel in the myocardial bridging of the coronary arteries. Revascularization of the left anterior descending artery (LAD) is considered an essential component of coronary artery bypass grafting (CABG) procedures. This study aims to evaluate the correlation between angiographic views of the coronary artery and intraoperative findings of the left anterior descending artery myocardial bridge (LADMB).

Methods: The records of patients who underwent the CABG procedure between January 2015 and October 2022 were reviewed retrospectively. A total of 349 patients who had LADMB on coronary angiography (CAG) images and/or intraoperatively were evaluated. Patients were divided into two groups. The CAG group (n=50) consisted of patients with angiographic LADMB, and the CABG group (n=40) consisted of patients with LADMB that was detected intraoperatively. The correlation between myocardial bridge signs of the LAD in CAG and intraoperative observations was investigated.

Results: In the coronary angiography group, 50 patients had signs of depression on coronary angiography, of whom 35 had LADMB intraoperatively. In the CABG group, 40 patients were found to have a myocardial bridge intraoperatively, and 5 had normal CAG images. The prevalence of LADMB was 11.5%. The sensitivity of CAG was 87.5%, the specificity was 95.15%, the positive predictive value was 70%, and the negative predictive value was 98.32%.

Conclusion: The myocardial bridge signs of the LAD on CAG correlate with intraoperative observations with high sensitivity and specificity.

Keywords: Coronary artery bypass, coronary angiography, myocardial bridging, prevalence

INTRODUCTION

The main coronary arteries run subepicardially at the cardiac surface and penetrate the myocardium almost at the terminal segment.[1] However, in some cases, intramyocardial coronary arteries can be observed in proximal and middle segments or throughout the entire course.[2,3] The intramyocardial course of coronary arteries has always been a challenge during coronary artery bypass graft (CABG) procedures because of the difficulties in exposing the distal anastomosis segment, which may lead to inadequate coronary artery flow and intraoperative complications such as prolonged ischemic period, ventricular perforation, coronary artery injury, and intraoperative hemorrhage.[1,4,5] Coronary angiography (CAG) is the gold standard for the diagnosis of coronary artery disease (CAD).[1-4] Images of coronary arteries often demonstrate characteristic anatomical variation. For example, a depression sign of the left anterior descending artery (LAD) would indicate an intramyocardial course, particularly in the right anterior oblique view of CAG. The purpose of this study was to investigate the correlation between the characteristic image of the LAD myocardial bridge (LADMB) on CAG and the intraoperative finding of the course of the LAD. Many studies in the literature investigate the intramyocardial course of the LAD and possible complications during surgical procedures.[6-12] However, this is the first study to investigate the sensitivity and specificity of CAG in predicting the progression of LADMB.

METHODS

The study was carried out with the permission of Bozok University Hospital Ethics Committee (Date: 17.02.2023, Decision No: 2017-KAEK-189_2023.02.17_6). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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Study Design and Population

This observational retrospective study was conducted in the Department of Cardiovascular Surgery of Bozok University Hospital. A total of 468 patients who underwent CABG procedures between January 2015 and October 2022 were analyzed.

Coronary angiographies were performed by an interventional cardiologist with a transfemoral approach and standard Judkins' technique. All patients received 2500 to 5000 units of unfractionated heparin. A total of 100-200 µg of nitroglycerin was administered depending on blood pressure. A biplane cine-angiography system was used to obtain standard angiography images.

Identification of LADMB

The presence of LADMB in CAG images was identified visually by our interventional cardiologists who were blinded to the intraoperative findings and was based on the following findings: (1) systolic compression or milking effect, which is defined as a diameter narrowing limited to a restricted vessel segment with contrast agent extraction that is not interpretable by normal coronary artery flow. (2) A wide-U-shaped image or the step-down-step-up phenomenon, which is described as a localized change in vessel course into the ventricle.

Other measurements, such as intramyocardial segment length and depth, or other imaging modalities were not performed.

Intraoperative LADMB was identified as not being visible on the cardiac surface in any part of its overall course.

According to that definition, patients without LADMB were excluded (n=119). A total of 349 patients who had LADMB in CAG images and/or intraoperatively were assessed separately. First, we evaluated all CAG images, and reports of those patients and individuals with LADMB were identified. Then, we reviewed the operative notes that were reported by a single surgeon who performed the procedures, and patients who were reported to have LAMB were identified. The studied population was divided into two groups: the CAG group (n=50), consisting of patients with angiographic LADMB, and the CABG group (n=40), consisting of patients with LADMB that was detected intraoperatively. The standard for reporting diagnostic accuracy (STARD) flow chart of the cases enrolled in the study is shown in **Figure 1**.

Data Collection and Endpoints

Data regarding baseline characteristics such as sex, age, EuroSCORE II, patient comorbidities (diabetes mellitus, dyslipidemia, systemic hypertension, smoking), echocardiography features, and clinical indications for coronary angiography were obtained from the computerized database and patient files. Our endpoint was to estimate the diagnostic value of CAG by evaluating the correlation between the sign of depression of the left anterior descending artery on coronary angiography and intraoperative observations, along with the prevalence of intraoperative LADMB.

Statistical Analysis

Descriptive statistics are presented as the mean with SD or median with İQR for numerical variables, while frequencies and percentages are used for the categorical variables. The distribution of variables was assessed by Kolmogorov–Smirnov and Shapiro–Wilk tests. For analytical statistics, the Mann–Whitney test was used to compare two numerical variables based on the normality assumption, while the Pearson chi-square test was used to compare two categorical variables. Diagnostic test evaluation parameters for CAG were calculated. The data were analyzed using IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. and MedCalc Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium). A P value <0.05 was considered to indicate statistical significance.

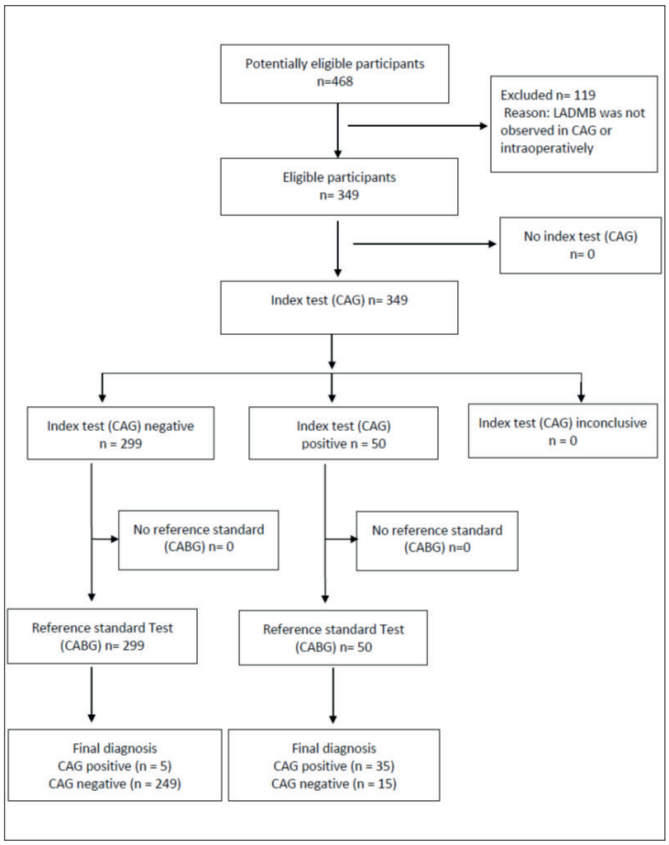

Figure 1. Standard for Reporting Diagnostic Accuracy (STARD) flow chart of the cases enrolled in the study. CAG: coronary angiography, CABG: coronary artery bypass grafting, LADMB: LAD myocardial bridge

RESULTS

Table 1 presents the baseline characteristics and clinical data of all patients included in the study. A total of 349 patients had LADMB on either coronary angiography or detected intraoperatively. The patients' median age was 61.64 years, and the majority were male (55%). The mean Euroscore II was 1.2%. The median body mass index (BMI) was 27,7 kg/m². Systemic hypertension, diabetes mellitus, and dyslipidemia were the most common comorbidities, with prevalence rates of 38%, 36%, and 27.5%, respectively. The clinical indication for CAG included acute coronary syndrome (ACS) in 39.5% of cases, angina in 50%, arrhythmia in 2.3%, and ventricular dysfunction in 7.7%. Echocardiography features showed a mean ejection fraction (EF) of 50%±15.

Table 1. Baseline characteristics and clinical data of the patients

	LADMB in CAG and/or CABG n=349
Age (y), Median (İQR)	61.64 (12.2)
Sex, n (%)	
Male	193 (55)
Female	156 (44.6)
Euroscore II (%), Mean (±SD):	1.2 (0.5)
BMI (kg/m2), Median (İQR):	27.7 (4.9)
Comorbidities, n (%):	
Systemic hypertension	136 (38)
Diabetes milletus	127 (36)
Dyslipidemia	96 (27.5)
Pulmonary disease	36 (10)
Smoking	105 (30)
Carotid stenosis	18 (5)
Clinical indication for CAG:	
ACS, n (%)	138 (39.5)
Angina, n (%)	176 (50)
Arrhythmia, n (%)	8 (2.3)
Ventricular dysfunction, n (%)	27 (7.7)
Echocardiography features, Mean (±SD):	
EF (%)	50 (15)
EDD, mm	46 (9)
ESD, mm	28 (11)
Extent of coronary lesions, n (%)	
1 vessel	11 (3)
2 vessels	91 (26)
3 vesseles	162 (46)
> 3 vessels	85 (24)
LMCA, n (%)	7 (2)
Type of emergency, n (%)	
Elective	259 (74)
Urgent	68 (19.5)
Emergent	22 (6)

LADMB: Left anterior descending myocardial bridge BMİ: Body mass index, ACS: Acute cronary sendrome, EF: Ejection fraction, EDD: End diastolic diameter, ESD: End systolic diameter, LMCA: Left main coronary artery.

According to intraoperative or CAG image observations regarding LADMB, the patients were divided into two groups. A total of 349 patients were included in the study. LADMB was detected in CAG images in 50 patients (50/349, 14%); 66% of patients were males, and the median age was 67.5 years. Forty patients were confirmed to have LADMB during intraoperative assessment (LADMB-CABG) (40/349, 11.5%). The median age was 67.5 years. 70% males, Table 2. Of these 40 patients, 35 were correctly identified with LADMB during coronary angiography (LADMB-CAG), while 5 were missed. Additionally, among the 309 patients without LADMB during the intraoperative assessment, 294 were correctly identified as negative for LADMB in the coronary angiography images, while 15 were false-positive Table 3.

Table 2. Demographic characteristics of the study groups

	Total n=90	CAG group n=50	CABG group n=40	P value
Age (y), Median (IQR)	67 (12)	65.5 (13)	67.5 (12)	>0.999*
Sex, n (%)				
Male	61 (68)	33 (66)	28 (70)	0.688**
Female	29 (32)	17 (34)	12 (30)	

CAG: coronary angiography, CABG: coronary artery bypass graft. * Mann-Whitney U test, ** Pearson Chi-Square test.

Table 3. Contingency table of the data used to calculate sensitivity and specificity*.

LADMB (CAG)	LADMB (CABG)		
	Yes	No	Total
Yes	35	15	50
No	5	294	299
Total	40	309	349

LADMB: left anterior descending artery myocardial bridge, CAG: coronary angiography, CABG: coronary artery bypass graft. * Sensitivity=35/40=0.875, Specificity=294/309=0.951.

The sensitivity of coronary angiography in detecting LADMB was calculated as 35/40, resulting in a sensitivity of 87.5% (95% CI, 73.20-95.81). The specificity of coronary angiography was calculated as 294/309, yielding a specificity of 95.1% (95% CI, 92.12-97.26). Tables 3 and 4. The prevalence of LADMB in patients who underwent CABG procedures was 11.5%. The positive predictive value (PPV) and negative predictive value (NPV) of CAG were 70% and 98.32%, respectively (Table 4, Figure 2).

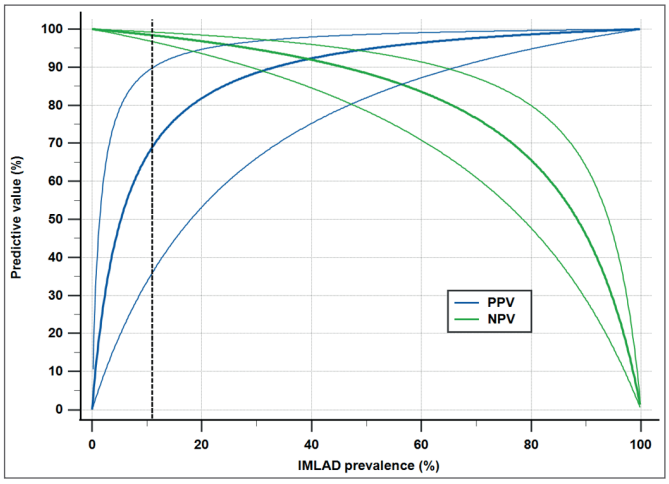

Figure 2. Plot versus prevalence graph for PPVs and NPVs with confidence intervals; dashed line represents the prevalence, PPV: positive predictive value, NPV: negative predictive value.

Table 4. Summary estimates of diagnostic values of LADMB in CAG.

	Value	95% CI (lower limit-upper limit)
Sensitivity	87.5%	73.20%-95.81%
Specificity	95.15%	92.12%-97.26%
AUC	0.913	0.88-0.94
PPV	70.08%	58.51%-79.55%
NPV	98.32%	96.27%-99.255
Prevalence	11.46%	8.31%-15.27%
+LR	18.03	10.85-29.94
-LR	0.13	0.06-0.30
Accuracy	94.27%	91.28%-96.46%

CI: confidence interval, AUC: area under the curve, PPV: positive predictive value, NPV: negative predictive value, +LR: positive likelihood ratio, -LR: negative likelihood ratio

In CAG images, the milking sign of LADMB was observed in 13 patients (13/50, 26%), while the wide-U-depression image was observed in 37 patients (37/50, 74%) (**Figure 3**).

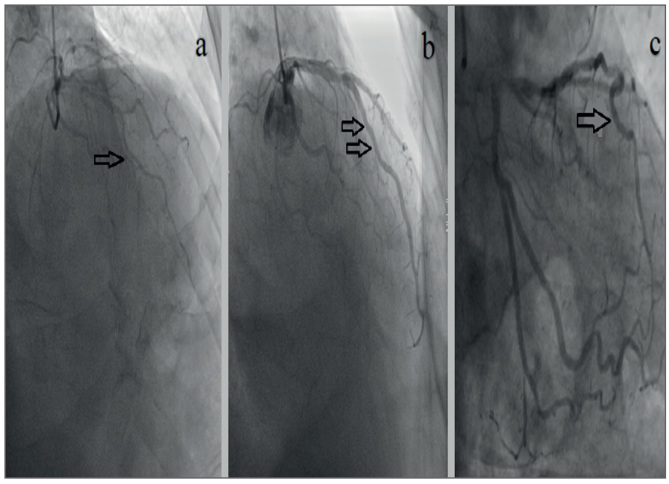

Figure 3. CAG images in different patients with LADMB. a, b: LAD depression sign, c: Right anterior oblique view demonstrates a typical wide-U shaped image. The black arrows indicate intramyocardial LAD location.

Twenty-five (62.5%) of the 40 LADMB-CABG cases had a superficial course, while 15 (37.5%) had a deeper progression inside the interventricular septum. In 19 cases, the LAD was grafted distally in the visible segment of the LAD. In 21 cases, the LAD was not visible in the distal portion. The great cardiac vein was used as a landmark to identify LADMB. There were no intraoperative or postoperative complications in any of the cases.

DISCUSSION

The primary objective of this study was to assess the diagnostic accuracy of CAG in detecting LADMB during CABG procedures. Our findings revealed a significant correlation between the characteristic image of LADMB on CAG and intraoperative observations with a high sensitivity value of 87.5% and a high specificity value of 95.1%.

The myocardial bridge (MB) is considered a congenital coronary anomaly and is described as a segment of a major epicardial coronary artery that passes deeply into the myocardium.[13,14] Although myocardial bridges can occur in any epicardial artery, the LAD is the most commonly involved (70% to 98%).[15] The prevalence of intramyocardial course ranges from 5% to 86% in autopsy series[8,16], and from 0.5% to 12% in coronary angiographic series.[17] Vanker et al.[1] identified LADMB in 293 out of 1349 CABG patients and reported a prevalence of 21.7%. In this study, the prevalence of LADMB in CABG patients was 11.5% (40/349). In our CAG series, the prevalence was 14% (50/349), while other studies reported lower prevalence rates ranging between 1-2% in CAG.[18,19] It has been demonstrated that the extent and frequency of MB can vary depending on imaging modalities. Lu et al.[20] in their study on the same population, found that the frequency of MB with CAG was 6% and 30% with computed tomography coronary angiography (CTCA). Furthermore, the prevalence of MBs was reported to differ according to the method used during CAG. For example, Şenöz et al.[21] Demonstrated that the detection rate of MBs by the transradial approach to CAG was significantly higher than that of the transfemoral approach (10.2% vs 1.8%). The main reason for this variation is the diversity in the sensitivity of CAG procedures in detecting MB. Additionally, the size and ethnicity of the study population may influence the prevalence of MBs.[21] In studies conducted in Turkish population higher than the size of the current study, the prevalence of MB was reported to be approximately 1%.[21-23] Moreover, provocative tests by nitroglycerin and diltiazem might increase the detection rate of MB.[17,21] Other factors that may affect the variations in the reported prevalence of MBs are the depths of the bridging segment. It was assumed that the systolic compression sign might be absent in superficial MB but prominent in deep MB,[24] which to some extent depends on the observer's experience.[25] Leschka et al.[26] reported that the percentage of systolic compression correlated with MB depth, whereas bridged segment length did not correlate with the degree of systolic compression. Furthermore, conventional angiography missed more than 50% of the MBs, suggesting that diagnosis of MBs by visual assessment in CAG can only be performed for segments with more than 20% systolic compression. These findings revealed that conventional CAG is not sufficiently sensitive to detect LADMB, particularly the mild or superficial type.[27] However, the detection rate of LADMB in the present study was higher than that in previously reported studies. In addition, the high sensitivity in our study indicates that CAG correctly identified a substantial proportion of cases with LADMB. In essence, CAG effectively minimizes the risk of false negatives,

ensuring that a large majority of patients with LADMB are correctly identified. The observed high sensitivity value along with PPV increases confidence that a positive test result is reliable and likely corresponds to the true presence of LADMB. Moreover, the high specificity and NPV indicated that CAG is capable of differentiating patients without LADMB, minimizing the occurrence of false positives. This is crucial in avoiding unnecessary interventions during surgery for patients who do not have LADMB, thus enhancing the precision of the diagnostic method. The possible explanation for these results might be evaluating angiographic images with the specific goal of locating the MB and the frequent use of nitroglycerin, particularly in hypertensive patients. In addition, although cardiologists were blinded to surgical reports, including patients with documented evidence of MB in these reports instead of randomly selected patients might result in selection bias and increase the true positive cases. Hence, these results should be interpreted with caution.

In the context of diagnosing LADMB, various imaging modalities present distinct advantages and limitations. While our research primarily focused on the diagnostic accuracy of coronary angiography, it is crucial to acknowledge the strengths and weaknesses of alternative diagnostic techniques. Although CAG continues to be the gold standard among imaging modalities for the diagnosis of coronary artery disease due to its advantages in revealing the characteristics of obstructive coronary artery lesions,[28] it may pose some challenges in terms of diagnosing MBs. CAG can provide both an anatomic and dynamic assessment. However, the tunneled segment cannot be functionally evaluated.[29] In addition, observers have to rely on indirect signs during vessel assessment, which may result in underestimation of MB prevalence, particularly in the shallow type.[17,26,30] Intracoronary imaging methods, such as intravascular ultrasound (IVUS) 2, optical coherence tomography (OCT),[31] and fractional flow reserve (FFR) measurement,[32] as well as emerging modalities such as coronary computed tomography angiography (CCTA) and magnetic resonance imaging (MRI), play pivotal roles in comprehensively assessing the complex anatomical and functional aspects of LADMB.[29]

IVUS offers high-resolution cross-sectional images of coronary arteries and reveals the systolic compression of the bridge segments (half-moon phenomenon) and atherosclerosis, providing valuable insights into plaque morphology, vessel dimensions, and intraluminal structures. IVUS enables accurate assessment of the degree of intramyocardial penetration of the LAD and provides real-time information during interventions. However, the invasive nature of IVUS and potential procedural complications may limit its widespread adoption.[29] A previous study revealed that IVUS could identify bridging in 23% of patients, while angiographic systolic compression was only seen in 3%.[33] OCT provides even higher resolution images, facilitating detailed visualization of coronary artery walls and luminal structures, and can precisely determine the extent of intramyocardial course and offer insights into plaque composition. Nevertheless, the invasive nature and technical complexity could potentially hinder its routine clinical use. OCT has been investigated in previously published reports regarding the diagnosis of MBs, which concluded that MBs were longer, but the diameter stenosis was lower than with angiography-based measures.[31] Using the FFR technique is debatable since MB is a dynamic stenosis that depends on the degree of extravascular compression and intramyocardial tension and can be revealed by provocative pharmacologic tests.[32,34,35] CCTA offers noninvasive three-dimensional images of coronary anatomy, enabling visualization of the course of LADMB with exceptional spatial resolution 8. Moreover, CCTT imaging of myocardial bridging has found intramyocardial segments at substantially higher rates than conventional angiography.[36-38] Conversely, cardiac MRI often employs techniques such as late gadolinium enhancement and contributes to identifying myocardial bridges and assessing their functional implications, providing valuable insights into ischemia. However, due to spatial resolution limitations and technological issues, it cannot provide accurate and strong insight into the LAD's intramyocardial depth.[29] Moreover, functional imaging techniques, including myocardial perfusion imaging through single-photon emission computed tomography (SPECT) and stress echocardiography, provide an assessment of the functional significance of LADMB by evaluating induced ischemia. Integrating both anatomical and functional evaluations enhances diagnostic accuracy and informs clinical decision-making.[39] In the present study, we could not perform other imaging methods to compare our findings in terms of CAG diagnostic accuracy with other imaging modalities, which limits the evaluation of our findings.

Surgical myotomy and CABG are two surgical procedures for myocardial bridging refractory to medical therapy.[17] Myotomy involves the dissection of the overlying muscle fibers. However, perforation of the right ventricle, particularly with deep MBs, may occur during dissection. None of the patients in our sample required a myotomy since all of them, including those with one vascular disease, were operated on due to severe lesions proximal to the MB rather than symptoms related to the MB itself. CABG, which commonly involves anastomosis of the left internal mammary artery to the LAD, has also been recommended as an effective treatment for MB.

particularly for patients with long (>25 mm), deep (>5 mm) MBs, or patients with accompanied severe coronary artery disease.[17] Nevertheless, there may be compelling situations for the surgeon in the case of LADMB as a target artery for distal anastomosis. There are several methods to expose the LADMB. One method is "blind dissection", which involves dissection of the myocardium in the anterior interventricular groove. This technique can lead to severe damage to the myocardium, resulting in ventricle perforation.[12,40] Another option is to use the great cardiac vein as a guide point, which usually runs in the epicardial adipose tissue and is more superficial than the artery. Another method is to insert a coronary probe from the distal visible portion of the artery. However, the risk of coronary artery perforation can cause serious intraoperative morbidities.[11] Other less invasive but more expensive techniques to expose the intraoperative LADMB include Doppler ultrasound with a color Doppler microprobe, intraoperative fluorescein angiography, and cine angiography.[4,41-43]

Study Limitations

The main limitations of this study are the small number of patients for statistical analysis and the single-center retrospective nature of the study. which might impact the generalizability of the findings. Additionally, the reliance on visual assessment of CAG images for diagnosing LADMB could introduce subjectivity and potential observer bias. The absence of other imaging techniques, such as IVUS or CCTA, to confirm the LADMB diagnosis might also be seen as a limitation. Last, the study primarily focuses on CABG patients, potentially limiting the applicability of the findings to a broader population of patients with different clinical profiles. Therefore, patients will continue to be enrolled, and we plan to produce an annual report of results and statistics every 12 months.

CONCLUSION

Our study highlights the diagnostic accuracy of CAG in detecting LADMB during CABG procedures. The high sensitivity and specificity of LADMB imaging on CAG underscore the potential of CAG as a valuable invasive method for the preoperative diagnosis of LADMB, facilitating surgical planning and potentially reducing intraoperative complications. However, the study also highlights the importance of considering the limitations of CAG, such as potential false-negative results. Further investigation is needed to explore the diagnostic accuracy of various imaging modalities, which could shed light on the most successful diagnostic approach. Furthermore, research on the interaction between anatomical and functional assessments may lead to more complete diagnostic techniques for LADMB detection.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Bozok University Hospital Ethics Committee (Date: 17.02.2023, Decision No: 2017-KAEK-189_2023.02.17_6).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Inquiring about the link between urotensin-II and coronary collateral development in coronary artery patients with and without diabetes

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ABSTRACT

Aims: Coronary collateral circulation consists of vascular channels activated to maintain perfusion in major epicardial coronary arteries in severe stenosis or occlusion. Yet, coronary collateral development (CCD) in diabetic patients was previously proven to be poor. Urotesin-II (U-II) is famous for being the most potent vasoconstrictor agent, and plasma levels are known to elevate in diabetic patients and play an important role in diabetic complications. In this study, we inquired about the link between U-II levels and the development of coronary collaterals between diabetic and non-diabetic patients with coronary artery disease (CAD).

Methods: We recruited 31 diabetic and 30 non-diabetic patients with 95% or more coronary artery stenosis or occlusion and considered Rentrop's classification for grading collaterals. In this sense, while Rentrop grades 0-1 are regarded as poor CCD, Rentrop grades 2-3 correspond to well-developed collaterals. Moreover, we compared the patients' serum levels of U-II by the degree of CCD.

Results: The findings revealed that demographic characteristics did not significantly differ between the groups ($p > 0.05$). Although CCD seemed worse in diabetic patients than those without diabetes (DM), the finding was not statistically significant. However, the diabetic patients had significantly higher U-II levels than non-diabetic patients (388.1±314.2 vs. 229.8±216.9, $p=0.026$). Despite not being significant, U-II levels were higher in patients with poor CCD than those with well-developed collaterals in the non-diabetic group (370.6±298; 178.6±158.3, $p=0.2$). In the diabetic group, on the other hand, U-II levels were significantly higher in patients with poor CCD and significantly lower in patients with good CCD (582.7±316.4 and 180.4±121.6, respectively; $p < 0.0001$ for both).

Conclusion: Overall, our findings demonstrated a significant association between U-II levels and the development of coronary collateral circulation in patients with DM. We also determined that U-II levels were low in diabetic patients with good CCD, while those with poor CCD had higher levels of U-II.

Keywords: Diabetes, coronary collateral circulation, urotensin II

INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality in many countries.[1] The human body hosts numerous collateral vessels connecting the major coronary arteries, and these coronary collaterals are potential channels in the human heart. In the case of a narrowing in the coronary arteries, these channels expand depending on the pressure gradient and provide an alternative flow path.[2]

A functional endothelium is essential in the development of collateral vascular networks that varies among individuals. Besides, a plethora of research demonstrated the impacts of diabetes mellitus (DM) on endothelial functions. For example, it was shown that increased glucose levels impair the structure and proliferation of endothelial cells and cause delays in various stages of the endothelial cell cycle.[3] Moreover, it was discovered that coronary collateral development (CCD) is poor in diabetic patients.[4]

Urotensin-II (U-II) is a peptide vasoactive substance similar to somatostatin that yields a more potent effect than endothelin-1, a noteworthy vasoconstrictor. It is known to be expressed in the brain, spinal cord, kidneys, and skeletal muscle and is often found in the myocardium, atrium, ventricles, and vascular endothelial/smooth muscle cells

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in the cardiovascular system. U-II was also discovered to contribute to endothelial cell permeability and to induce endothelial cell proliferation.[5] Moreover, plasma U-II levels were found to be high in renal failure, congestive heart failure, DM, hypertension (HT), and portal HT.[6]

In this study, we inquired about the relationship between U-II levels and CCD in diabetic and non-diabetic patients with CAD.

METHODS

The study was carried out with the permission of İnönü University Clinical Researches Ethics Committee (Date: 2011, Decision No: 128). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We conducted this study with patients who underwent coronary angiography in the cardiology department between August 2011 and February 2012 and were found to have 95% stenosis or complete occlusion in their coronary arteries.

Exclusion Criteria

a. Pre-diagnosed rheumatic valve disease,
b. Moderate and severe stenosis/failure of other valves,
c. Undergoing percutaneous coronary intervention in the last 30 days,
d. Renal and hepatic failure,
e. Less than 95% stenosis in the relevant coronary artery,
f. Undergoing coronary artery bypass surgery,
g. Having an acute or chronic infectious disease.

Initially, we obtained anamnesis from all patients, performed their physical examinations, and noted down their risk factors for CAD (e.g., DM, HT, smoking, age, and sex) and antihypertensive, antidiabetic, and statin drugs they used. We next inquired about their history of myocardial infarction (MI), coronary bypass operation, and percutaneous coronary intervention and calculated their body mass indices (BMI). Then, we performed electrocardiograms and echocardiograms on the patients. While defining the presence of DM as a fasting blood glucose level ≥ 126 mg/dl, a spot blood glucose level ≥ 200 mg/dl, and/or the use of oral antidiabetic and/or insulin, we considered HT in the case of systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg, or the use of blood pressure-lowering medication. In addition, while patients smoking for the past six months or more were defined as active smokers, those having smoked in the past were regarded as quitters. Finally, hyperlipidemia was accepted as positive in the case of measurement of total cholesterol > 200 mg/dl or low-density lipoprotein (LDL) cholesterol > 100 mg/dl or the use of lipid-lowering medication.

Coronary Angiographic Evaluation

All patients underwent selective coronary angiography via the right or left femoral artery using a 6F diagnostic catheter using the Judkins technique. While preferring Philips Integris 5000 as the angiography device, we utilized Iopromide (Ultravist-370) or Iohexol (Omnipaque 350 mg/ml) as opaque material. Coronary arteries were visualized in the right and left oblique positions through cranial and caudal angulations. We took the measurements of all patients at the end-diastole, in the position where the coronary lesion was best seen and the lumen narrowed the most. Finally, we performed the angiographic grading of collaterals providing blood flow to the occluded coronary artery by Rentrop's classification.7

- **Rentrop Grade 0:** No visible filling of collaterals.
- **Rentrop Grade 1:** Very weak collateral flow without any epicardial filling of the target artery.
- **Rentrop Grade 2:** Partial epicardial filling of the target artery by opaque material through collaterals.
- **Rentrop Grade 3:** Complete epicardial filling of the target artery by opaque material through collaterals.

To measure U-II levels, we centrifuged the blood samples of the patients in 10 mL vacuum sterile K3-EDTA tubes at 5000 rpm for 10 minutes and then separated serums and plasmas. Plasma samples were collected into 1.5 mL Eppendorf tubes, stored in a deep freezer at -40 °C, and thawed on the research day. Then, we studied U-II kits (Uscn Life Science Inc. Wuhan, China, E90868Hu, 1120125000) by ELISA.

Statistical Analysis

While we present continuous variables as means and standard deviations, categorical variables are given as percentages. The pair-wise comparisons of the categorical variables were performed using Pearson's chi-square test or Fisher's exact test, and we compared continuous variables between the groups with independent samples t-test. While the relationship between U-II levels and CCD was sought using the Mann-Whitney U test and independent samples t-test in the non-diabetic and diabetic groups, respectively. All analyses were performed on SPSS 17.0 (SPSS Inc, Chicago, USA), and a p-value of <0.05 was considered statistically significant.

RESULTS

We recruited 31 diabetic patients with a mean age of 65.4±6.9 years and 30 non-diabetic patients with a mean age of 61.4±10.5 years. Of the patients, 46 (75.4%) were males, and 15 (24.6%) were females. The findings revealed no significant differences between the groups by sex, smoking, HT, MI, hyperlipidemia (including family history), and the use of beta-blockers,

angiotensin-converting-enzyme inhibitors (ACEI), statin, acetylsalicylic acid (ASA), and nitrate. In addition, no significant difference was observed between the two groups in echocardiographic measurements.(50.9±8.5 vs. 54.8±7.3; p=0.880) (**Table 1**).

Table 1. Clinical and demographic characteristics of groups

	Diabetes mellitus (+) (n=31)	Diabetes mellitus (-) (n=30)	p
Age, years,	65.4±6.9	61.4±10.5	0.873
Female Sex, n (%)	9 (29.0)	6 (20)	0.276
Hypertension, n (%)	16 (51.3)	13 (43.3)	0.912
Smoking, n (%)	12 (38.7)	15 (50)	0.597
History of myocardial infarction, n (%)	16 (51.6)	11 (36.7)	0.546
Systolic BP (mmHg)	133.8±17.4	131.5±21.9	0.347
Diastolic BP (mmHg)	82.8±10.4	80.6±12.2	0.076
CRP (mg/dl)	0.7±1.2	1.7±2.7	0.065
Serum creatinine (mg/dl)	0.91±0.5	0.88±0.4	0.865
Total cholesterol (mg/dl)	190±48	190±43	0.212
Low density lipoprotein cholesterol (mg/dl)	116±40	121±39	0.345
High density lipoprotein cholesterol (mg/dl)	35±8.5	37±9.6	0.923
Triglyceride (mg/dl)	190±165	159±106	0.413
Aspirin, n (%)	23 (74.2)	16 (53.3)	0.990
ACE-inhibitors/ARB, n (%)	10 (32.3)	6 (20.7)	0.665
Statins, n (%)	10 (32.3)	5 (16.7)	0.973
CCB, n (%)	11 (8.2)	12 (4.5)	0.298
Beta-Blocker, n (%)	13 (41.9)	15 (50)	0.869
Ejection Fraction, (%)	50.9±8.5	54.8±7.3	0.880

ACE; angiotensin converting enzyme, ARB; angiotensin receptor blocker, BP; blood pressure, CCB; calcium channel blocker;, CRP; C-reactive protein

In the diabetic group, while we detected poor CCD in 16 patients and good CCD in 15, they were discovered in 8 and 22 patients, respectively, in the non-diabetic group; however, the difference was not statistically significant (p=0.067). Moreover, the levels of CCD in the patients did not significantly differ by their CRP, uric acid, total, LDL, and high-density lipoprotein (HDL) cholesterol, and triglyceride levels, which was also the case between the diabetic and non-diabetic groups (**Table 2**). On the other hand, U-II levels were found to be significantly higher in the diabetic patients compared to non-diabetics (388.1±314.2 vs. 229.8±216.9; p=0.026; **Figure 1**). In the non-diabetic group, although we found the U-II levels of those with poor CCD (370.6±298) to be higher when compared to the patients with well-developed collaterals (178.6±158.3), the difference was not statistically significant (p=0.2). However, in the diabetic group, the U-II levels of the patients with poorly-developed collaterals (582.7±316.4) were found to be significantly higher than when compared to those with good CCD (180.4±121.6; p <0.0001; **Figure 2**).

Table 2. Demographic and clinical features according to collateral development

	Well-developed CCD (n=37)	Poor CCD (n=24)	p
Age, years,	62.1±9.8	65.5±7.2	0.512
Female Sex, n(%)	9 (24.3)	6 (25)	0.454
Hypertension, n(%)	17 (45.9)	12(50)	0.456
Smoking, n (%)	15 (40.5)	12(50)	0.675
Total cholesterol (mg/dl)	197±37	180±54	0.237
Low density lipoprotein cholesterol (mg/dl)	125±37	108±41	0.345
High density lipoprotein cholesterol (mg/dl)	36±8.8	36±9.4	0.923
Triglyceride (mg/dl)	175±152	175±120	0.413
Body mass index (kg/m²)	26.1±3.6	25.5±3.1	0.267

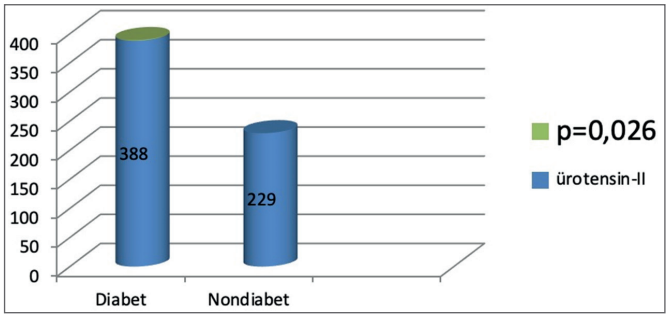

Figure 1. U-II levels were found to be significantly higher in the diabetic patients compared to non-diabetics.

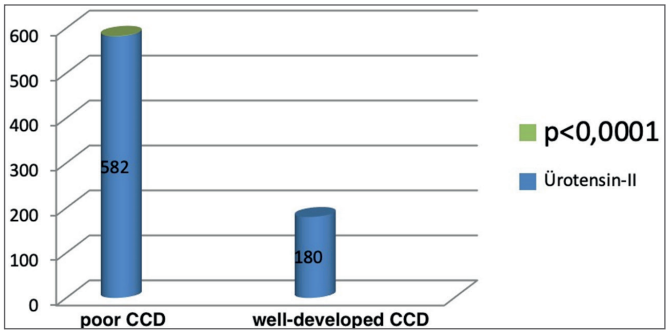

Figure 2. In the diabetic group, the U-II levels of patients with insufficient collaterals were found to be significantly higher than those with good CCD.

DISCUSSION

In this study, we investigated the relationship between U-II levels and CCD between diabetic and non-diabetic patients with CAD. While our findings showed a significant negative difference between U-II levels and CCD in diabetic patients, this difference was not statistically significant in the non-diabetic group.

CAD is the leading cause of mortality in many countries.1 Thus, the pre-detection and elimination of modifiable risk factors (e.g., DM, dyslipidemia, hypertension, obesity, and smoking) are known to reduce the risk of CAD significantly. On the other hand, severe stenosis or occlusion in the epicardial coronary arteries due to atherosclerotic or non-atherosclerotic causes results in loss of function or cell death in the myocardial

tissue supplied by the diseased coronary artery. In such situations, coronary collaterals become involved to be an alternative means of maintaining perfusion. In addition to preventing ischemia, coronary collaterals bear many beneficial effects, such as reduction of infarct area, prevention of left ventricular aneurysm development, improvement of left ventricular functions after infarction, reduction of coronary mortality, and prolongation of long-term survival. In a study by Williams et al., patients with well-developed collateral circulation had a higher ejection fraction, a lower left ventricular end-diastolic pressure, and a more limited wall motion disorder in the ischemic region.[8] The development of collateral vascular networks varies among individuals. Although the progression time and severity of coronary artery stenosis may be the most potent factors in this difference, it is also affected by DM, HT, hyperlipidemia, age, sex, drugs used, and endogenous mediators. A functional endothelium is essential in the process of CCD.

While DM is often shown to be among the significant risk factors for CAD, CAD is then the most apparent cause of morbidity and mortality in diabetic patients. Epidemiological research documented that at least 50% of deaths in diabetic patients can be ascribed to CAD.[9] It was also shown that increased glucose levels impair the structure and proliferation of endothelial cells and cause delays in various stages of the endothelial cell cycle.[10] Another factor contributing to the risk in diabetic patients is poor CCD. Endothelial dysfunction in diabetic patients stimulates negative remodeling in response to atherosclerosis. Then, the vasodilator response of endothelin to cytokines is impaired, and neovascularization and CCD remain insufficient in response to ischemia. It is also thought that Urotensin 2 may have an effect on coronary collateral by affecting angiogenesis through cell proliferation, migration and invasion, especially in diabetic patients. In many studies delving into collateral circulation, CCD was shown to be poor in diabetic patients. For example, Abaci et al. compared CCD in diabetic and non-diabetic patients and discovered lower collateral scores in the diabetic group.[11] Moreover, Islam et al.[12] compared 36 patients with DM and 50 patients without DM by CDD and found that the diabetic group demonstrated poorer CCD.4 A similar finding was reported in the study by Tatlı et al. where CCD was compared between the patients following acute MI. In our study, we found poorer CCD in the diabetic group compared to the non-diabetic group, but the difference was not statistically significant, which may be due to the small sample size in our study.

U-II is a peptide vasoactive substance with a more potent effect than endothelin-1, an important vasoconstrictor. Various studies previously showed that it bears vasoconstrictor, vasodilator, and neutral effects on vascular beds.[13] In general, while having a vasoconstrictor impact in coronary and radial arteries, it brings a vasodilator effect to pulmonary and abdominal arteries. U-II was previously shown to be expressed in the brain, spinal cord, kidneys, and skeletal muscle. It is often found in the myocardium, atrium, ventricles, and vascular endothelial/smooth muscle cells in the cardiovascular system. Plasma U-II levels correlate with congestive heart failure, essential hypertension, CAD, DM, and metabolic syndrome. The previous research consistently reported invcreased plasma levels of U-II in diabetic patients.[14] In fact, this increase occurs independently of plasma fasting glucose and Hemoglobin A1c. Totsune et al.[15] also showed increased U-II diabetic patients with normal renal functions compared to healthy individuals.

U-II expression is often found to be increased in atherosclerotic lesions in the coronary and carotid arteries and aorta.[16] Accordingly, it was suggested that elevated expression of U-II leads to vascular smooth muscle proliferation, accelerating the development of atherosclerotic plaque. In addition, it was shown that locally released U-II leads to coronary vasoconstriction and myocardial ischemia.[17] In a study, the researchers attained a positive correlation between carotid atherosclerosis and U-II levels in essential hypertensives compared to normotensives. The same study also reported positive relationships between plasma U-II levels and systolic blood pressure, carotid intima-media thickness, and plaque score.[18] In another study, patients with acute coronary syndrome had lower U-II levels than those with stable CAD and healthy controls.[19]

U-II boosts the expression of molecules, such as vascular cell adhesion protein-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractive protein-1 (MCP-1), which are involved in the development and progression of diabetic atherosclerosis and contributes to vascular complications by promoting the expression of molecules (e.g., transforming growth factor-β (TGF-β)) that influence cell proliferation, differentiation, migration, and development.[20] Similarly, we concluded that U-II had an adverse effect on CCD in the diabetic group.

Limitations

The present study is not free of a few limitations. One of these may be related to our small sample size, and the other can be shown as the evaluation of CCD only by coronary angiography. Most collateral vessels are often 100 micrometers in diameter, but they must be above 100 micrometers to become angiographically visible. Collaterals with smaller diameters cannot be observed angiographically.

CONCLUSION

In a nutshell, we concluded a significant link between CCD and U-II levels in diabetic patients. Accordingly, while the diabetic patients with good CCD had low U-II levels, it was vice versa in those with poor CCD. U-II levels were found to be statistically significant in diabetic patients compared to the non-diabetic group. On the other hand, although non-diabetic patients with poor CCD had higher U-II levels than those with good CCD, the difference was not statistically significant. Our findings still need to be confirmed by prospective, longitudinal follow-up research with large sample sizes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İnönü University Clinical Researches Ethics Committee (Date: 2011, Decision No: 128).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Anti-Müllerian hormone and HOMA-IR in different phenotypes of polycystic ovary syndrome on insulin resistance

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ABSTRACT

Aims: To examine the link between serum anti-mullerian hormone (AMH) levels and homeostatic model assessment of insulin resistance (HOMA-IR) in different phenotypes of polycystic ovary syndrome (PCOS).

Methods: This retrospective study included 120 patients aged 18-30 who visited our polyclinics between June 2021 and December 2022. Patients were divided into four groups based on the Rotterdam criteria for PCOS phenotypes. A control group of 24 individuals was also included. Clinical data, hormonal profiles, and metabolic parameters were obtained from medical records.

Results: There were significant differences in AMH, follicle stimulating hormone (FSH), luteinizing hormone (LH), and high-density lipoprotein (HDL) levels among the PCOS phenotypes and control group. AMH levels were highest in phenotype 1 (oligo/anovulation + hyperandrogenism + polycystic ovaries) and lowest in the control group. FSH were highest in phenotype 4 (oligo/anovulation + polycystic ovaries) and lowest in the control group. LH were highest in phenotype 2 (oligo/anovulation + hyperandrogenism). HOMA-IR was highest in phenotype 1. However, there were no significant differences in AMH or HOMA-IR levels among the PCOS phenotypes.

Conclusion: Our study found hormone level differences among PCOS phenotypes but no significant differences in AMH or HOMA-IR. This suggests AMH may not distinguish between phenotypes and insulin resistance may not differ significantly among phenotypes.

Keywords: PCOS phenotypes, anti-mullerian hormone, insulin resistance, HOMA-IR, biomarkers

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is an endocrine disorder that is frequently encountered and affects approximately 5-10% of women of reproductive age worldwide.[1] It is characterized by a heterogeneous collection of signs and symptoms, including menstrual irregularities, hyperandrogenism, and polycystic ovaries.[2] Insulin resistance (IR) is a crucial feature of PCOS, with up to 70% of affected women exhibiting this metabolic abnormality.[1] There is a correlation between IR in PCOS and a heightened risk of developing type 2 diabetes, cardiovascular disease, and other adverse health outcomes in the long run.[3]

The homodimeric glycoprotein known as anti-mullerian hormone (AMH) is a member of the transforming growth factor-β family and is expressed in the granulosa cells of secondary, preantral, and small antral follicles that have a diameter of 4 mm or less.[4] AMH has been implicated in the regulation of ovarian function and folliculogenesis, with elevated serum AMH levels observed in women with PCOS compared to age- and body mass index (BMI)-matched controls.[5] However, the effectiveness of AMH as a diagnostic criterion for PCOS is a topic that is still under debate.[4]

The homeostasis model assessment of insulin resistance (HOMA-IR) is a commonly employed instrument to evaluate IR in clinical and research settings.[6] The assessment is based on the measurement of fasting glucose and insulin levels and has been shown to correlate well with more invasive measures of IR, such as the hyperinsulinemic-euglycemic clamp.[7] HOMA-IR has been used to investigate the relationship between IR and various clinical features of PCOS, including hyperandrogenism and menstrual irregularities.[6,7]

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Despite the well-established associations between PCOS, IR, and elevated AMH levels, few studies have explored the link between AMH and HOMA-IR in various PCOS phenotypes. This represents a significant gap in the current literature, as understanding the interplay between these factors may provide valuable insights into the underlying pathophysiology of PCOS and inform the development of more targeted therapeutic interventions.

The primary objective of this study is to investigate the relationship between serum AMH levels and HOMA-IR in women with different phenotypes of PCOS. Specifically, we aimed to determine whether AMH and HOMA-IR are independently associated with specific PCOS phenotypes and whether their combined assessment improves the prediction of these phenotypes.

METHODS

The study was carried out with the permission of Bezmialem Vakıf University Non-interventional Clinical Researchs Ethics Committee (Date: 14.06.2023, Decision No: 2023/191). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This retrospective study included 120 patients aged 18-30 who visited our polyclinics between June 2021 and December 2022. The study population consisted of patients aged 18-30 who visited our polyclinics with menstrual irregularity and a desire to have children. A total of 120 patients were divided into four groups according to their PCOS phenotypes, as determined by the study criteria. Additionally, 24 individuals were included as a control group. The study's eligibility requirements were determined by evaluating the patient's medical history, physical exam, and ultrasonography results., which were obtained from the outpatient clinic records during the specified dates. The diagnosis of PCOS was made if the patients met at least two of the 2003 Rotterdam Consensus criteria.

Based on a specific set of inclusion and exclusion criteria, 120 participants were selected for the study. The study included only patients who were between the ages of 18 and 30 and unable to conceive despite wanting a child for at least one year. They also needed to be diagnosed with PCOS, meeting at least two of the 2003 Rotterdam Consensus criteria.8 In this consensus it was stated that for the diagnosis of PCOS, the patient should have at least two of the three major criteria. 1. Oligo/anovulation 2. Hyperandrogenism (clinical or biochemical findings) 3. Polycystic ovaries (determined by ultrasound) and other androgen excess disorders should be excluded. The presence of at least two of these three findings makes the diagnosis of PCOS after excluding Cushing's syndrome, congenital adrenal hyperplasia, hyperprolactinemia, and androgen-secreting tumors.

Thus, PCOS patients can be categorized into four distinct phenotypes:[8]

- "Phenotype 1: Oligo/anovulation + hyperandrogenism + polycystic ovaries
- Phenotype 2: Oligo/anovulation + hyperandrogenism
- Phenotype 3: Hyperandrogenism + polycystic ovaries
- Phenotype 4: Oligo/anovulation + polycystic ovaries"

We divided our participants into four groups according to this classification.

Participants were required to undergo an oral glucose tolerance test (OGTT) to assess glucose tolerance. Participants with a fasting glucose level ≥126 mg/dl or a 2-hour glucose level ≥200 mg/dl were excluded from the study due to the presence of diabetes. Participants with a fasting glucose level between 100-125 mg/dl or a 2-hour glucose level between 140-199 mg/dl were excluded due to the presence of impaired glucose tolerance. In addition, patients with a history of thyroid dysfunction, hyperprolactinemia, and hypercortisolism were excluded. Patients could not have taken oral contraceptives or any medication known to alter hormone, lipid, or insulin metabolism within three months before the study and had to be non-smokers. Patients with PCOS needed to have similar mean age and body mass index (BMI) across their phenotypes, while individuals with a BMI of 23-25 were also included.

Exclusion criteria for the study were those who did not agree to participate in the study, smokers, individuals diagnosed with hypertension or diabetes, and those with any endocrinopathy. Patients who had taken oral contraceptives in the last three months, used insulin-sensitizing medications, or medications for hyperlipidemia were also excluded. Patients with vitamin B6 or B12 deficiency, or those who had taken vitamin supplements to treat these deficiencies within the last six months, were also excluded, as these may influence homocysteine metabolism. We also considered 24 healthy participants for the control group.

On the third day of the patients' menstrual cycle, several measurements were recorded in the case report form, including their age, BMI, Luteinizing Hormone (LH), Estradiol (E2), levels of Follicle Stimulating Hormone (FSH), Free T4, Prolactin, Thyroid Stimulating Hormone (TSH), Hemogram, Biochemistry (total cholesterol, LDL, HDL, Triglyceride), AMH, fasting insulin, fasting blood sugar (FBS), and HOMA-IR values. The study aimed to evaluate the relationship between AMH and HOMA-IR among the four different phenotypes of PCOS patients and the control group (healthy) concerning IR.

Statistical Analysis

To examine the study results, the SPSS statistical package program for Windows (version 22.0, SPSS Inc., Chicago, Illinois, USA) was used. Numeric variables were reported

as either mean±standard deviation or median (minimum-maximum), while categorical variables were expressed as the number and/or percentage of patients in the form of descriptive statistics. The mean±standard deviation was used to report continuous data. For comparative analysis of continuous data, the Mann-Whitney U test was used.

RESULTS

Table 1 represents the baseline clinical and hormonal profile of the study population. The study parameters include age, BMI, hormone levels like AMH, FSH, LH, E2, prolactin, TSH, FT4, lipid profile including triglycerides, total cholesterol, HDL, LDL, fasting blood sugar, fasting insulin, HOMA-IR, CRP, liver function tests, complete blood count, renal function tests, and platelet indices.

Table 1. Baseline clinical and hormonal profile

Study parameters	Median (Min.-Max.) mean±standart deviation
Age	24(18-30) 24.41±2.75
BMI	25(23-25) 24.28±0.83
AMH	4(2-8) 3.76±1.4
FSH	6.59(3.96-12) 6.85±1.48
LH	7.23(2.75-15.2) 7.48±2.63
E2	32(14.04-73) 35.5±11.35
PRL	13(5-28.6) 13.39±4.99
TSH	1.93(0.01-4.83) 1.81±0.72
FT4	1(0.31-2.66) 1.08±0.28
Total cholesterol	221(123-352) 225.31±52.95
Trıglycerıdes	120(48-228) 123.09±31.61
LDL	135(46-191) 123.21±38.95
HDL	60(38.6-89) 59.29±11.86
FBS	84(71-99) 85.71±8.28
Fastıng Insulın	15(7.2-36.8) 15.98±5.21
HOMA-IR	3.15(1.58-9.08) 3.53±1.43
CRP	1(0.01-6.3) 1.5±1.19
ALT	14(9-23) 14.51±3.36
AST	14(9-30) 14.52±4.02
BUN	20(4.6-36) 18.39±5.69
Creatınıne	0.78(0.42-1) 0.76±0.1
Leukocyte	6.73(3.54-18.45) 7.7±2.57
Neutrophil	3.86(0.62-15.93) 4.74±2.59
Lymphocyte	2.12(0.04-30.03) 2.42±2.21
Monocyte	0.54(0.01-6.7) 0.58±0.49
Basophil	0.06(0.01-1.83) 0.12±0.27
Hemoglobin	12.9(10-14.8) 12.67±1.23
RDWSD	33.6(30.8-49.1) 34.37±3
Platelet	247000(136000-434000) 249093.26±57339.37
MPV	9.8(0-13.1) 9.43±1.49
PDW	12(0-22.5) 13.63±4.03

BMI;body mass ındex, AMH; anti-mullerian hormone, FSH; follicle-stimulating hormone, LH; luteinizing hormone, E2 ; estradiol, PRL; prolactin, TSH; thyroid-stimulating hormone, FT4; free thyroxine, LDL; low-density lipoprotein, HDL; high-density lipoprotein, FBS; fasting blood sugar, HOMA-IR; Homeostatic Model Assessment of Insulin Resistance, CRP; C-Reactive Protein, ALT; alanine aminotransferase, AST; aspartate aminotransferase, BUN; blood urea nitrogen, RDWSD; Red Cell Distribution Width-Standard Deviation, MPV; Mean Platelet Volume, PDW; Platelet Distribution Width.

The first set of parameters listed in the table relates to demographics, including age and BMI. The study population had a median age of 24 years, with a range between 18 and 30 years, with a mean value of 24.41±2.75. The median BMI was 25 kg/m², with a range of 23-25 kg/m², indicating that the study participants had normal weight or were slightly overweight. The mean BMI was 24.28±0.83, which falls within the normal range.

Next, the table shows the hormone levels of the study participants, which include AMH, FSH, LH, E2, and prolactin. The median AMH value was 4 ng/ml, with a range of 2-8 ng/ml, showing the ovarian reserve of the participants. The mean FSH level was 6.85±1.48 mIU/ml, and the mean LH level was 7.48±2.63 mIU/ml. The median E2 level was 32 pg/ml, with a range of 14.04-73 pg/ml, and the median prolactin level was 13 ng/ml, with a range of 5-28.6 ng/ml. These hormone levels fall within the normal ranges for premenopausal women.

The lipid profile of the study population is also included in the table, including total cholesterol, triglycerides, LDL, and HDL. The median total cholesterol level was 221 mg/dl, with a range of 123-352 mg/dl, and the mean value was 225.31±52.95 mg/dl. The median triglyceride level was 120 mg/dl, ranging from 48-228 mg/dl, with a mean value of 123.09±31.61 mg/dl. The median LDL value was 135 mg/dl, ranging from 46-191 mg/dl, with a mean value of 123.21±38.95 mg/dl. The median HDL level was 60 mg/dl, with a range of 38.6-89 mg/dl, and the mean value was 59.29±11.86 mg/dl.

The fasting blood sugar level of the participants was measured, with a median value of 84 mg/dl, ranging from 71-99 mg/dl, and a mean value of 85.71±8.28 mg/dl. The median fasting insulin level was 15 µIU/ml, with a range of 7.2-36.8 µIU/ml, and a mean value of 15.98±5.21 µIU/ml. The HOMA-IR value, which assesses IR, had a median of 3.15, ranging from 1.58-9.08, and a mean value of 3.53±1.43. These values suggest that the study participants had some degree of IR.

The liver function tests, including ALT and AST levels, were also measured. The median ALT level was 14 U/L, with a range of 9-23 U/L, and a mean value of 14.51±3.36 U/L. The median AST level was 14 U/L, ranging from 9-30 U/L, with a mean value of 14.52±4.02 U/L. The renal function tests, including BUN and creatinine levels, had median values of 20 mg/dl and 0.78 mg/dl, respectively. The complete blood count and platelet indices, including leukocyte, neutrophil, lymphocyte, monocyte, basophil, hemoglobin, RDWSD, platelet, MPV, and PDW, were also measured.

Table 2 presents a comparison of various study parameters among different phenotypes of PCOS patients and a control group. The table shows the mean values and standard deviation of each parameter for four different phenotypes of PCOS patients, as well as a control group.

The analysis of the table reveals that the age and BMI of PCOS patients are similar across all four phenotypes and the control group. As expected, the levels of AMH, FSH, LH, and HOMA-IR differ significantly among the four phenotypes of PCOS patients compared to the control group. In particular, AMH levels are highest in phenotype 1 and lowest in the control group, while FSH levels are highest in phenotype 4 and lowest in the control group. LH levels are highest in phenotype 2, and HOMA-IR is highest in phenotype 1.

The table also shows that HDL levels are significantly lower in phenotype 4 compared to the control group, while fasting blood sugar (FBS), platelet count, mean platelet volume (MPV), and red cell distribution width-standard deviation (RDWSD) are significantly higher in this phenotype than in the control group. Moreover, phenotype 4 has a higher hemoglobin level than the other phenotypes, and phenotype 1 has a higher neutrophil count than the control group.

However, there were no significant differences in AMH levels among different phenotypes of PCOS patients. This suggests that AMH may not be a useful biomarker for distinguishing between different phenotypes of PCOS. Furthermore, the results found no significant differences in HOMA-IR levels among the different phenotypes of PCOS patients. This implies that insulin resistance may not differ significantly among the different phenotypes of PCOS patients. However, the study did find that fasting blood sugar levels were significantly higher in Phenotype 1 (oligo/anovulation + hyperandrogenism + polycystic ovaries) compared to other phenotypes. This finding may suggest that Phenotype 1 is associated with a higher fasting blood sugar and greater risk of developing diabetes. The difference of AMH and HOMA-IR in different PCOS phenotypes in this study are shown in **Figure 1.**

Table 2. Comparison of Study Parameters among Different Phenotypes in Polycystic Ovary Syndrome Patients and Control Group

Study parameters	Phenotype 1	Phenotype 2	Phenotype 3	Phenotype 4	p value	Control	p value
Age	24.32±2.74	24.46±1.81	24.42±2.78	24.56±2.73	0.949	24.41±2.75	0.991
BMI	24.28±0.86	24.31±0.85	24.25±0.87	24.38±0.79	0.967	24.26±0.84	0.978
AMH	5.06±1.13	4.77±0.83	4.67±0.78	4.44±0.56	0.121	2.47±0.50	<0.001
FSH	6.37±1.27	6.42±1.84	6.44±1	6.5±1.39	0.768	7.37±1.48	<0.001
LH	7.57±2.57	9.03±3.01	8.03±3.05	6.3±1.99	0.017	7.56±2.64	0.044
E2	35.27±12.45	34.95±9.07	35.33±6.76	35.33±12.99	0.986	35.79±11.06	0.999
Prolactin	13.49±4.88	13.65±6	13.25±4.24	13.26±3.42	0.981	13.36±5.55	0.999
TSH	1.84±0.89	1.81±0.6	1.79±0.65	1.84±0.7	0.999	1.78±0.65	1.000
FT4	1.07±0.32	1.08±0.17	1.06±0.2	1.05±0.17	0.967	1.09±0.33	0.988
Total Cholesterol	224.38±59.67	224.15±69.86	226.33±34.1	226.88±29.74	0.910	225.30±55.82	0.952
Trıglycerides	126.8±25.91	123.85±26.06	124±17.45	124.38±14.95	0.916	120.22±40.44	0.765
LDL	127.4±41.15	124.92±22.11	124.58±24.84	124.84±32.11	0.847	119.71±43.61	0.887
HDL	55.79±10.89	57.38±10.25	57.75±5.75	56.22±9.43	0.786	62.97±13.14	0.006
FBS	92.8±4.01	89.38±7.68	86±8.33	87.03±8.49	0.007	80.50±6.50	<0.001
Fasting Insulin	17.81±7.23	15.03±7.6	14.48±4.03	14.94±3.54	0.223	15.65±3.65	0.204
HOMA-IR	4.09±1.6	3.97±1.85	3.75±1.17	3.5±1.11	0.561	3.12±1.28	0.006
CRP	1.62±1.46	1.54±0.69	1.58±0.51	1.53±0.76	0.592	1.41±1.29	0.195
ALT	14.7±3.46	14.23±3.7	14.5±2.68	14.59±3.85	0.961	14.42±3.22	0.984
AST	14.14±3.48	14.31±3.84	14.25±3.72	14.81±3.68	0.820	14.70±4.52	0.946
BUN	18.55±6.8	18.68±6.41	18.62±3.52	18.56±4.67	0.859	18.15±5.58	0.957
Creatinine	0.76±0.11	0.73±0.12	0.77±0.16	0.73±0.11	0.223	0.77±0.08	0.103
Leukocyte	7.73±2.67	6.24±1.34	5.91±0.81	8±2.75	0.018	8.04±2.61	0.005
Neutrophil	5.01±2.92	3.83±1.09	3.19±0.59	4.97±2.62	0.076	4.85±2.65	0.082
Lymphocyte	2.44±1.03	2.14±0.8	1.9±0.64	2.28±0.97	0.276	2.59±3.14	0.390
Monocyte	0.55±0.22	0.53±0.15	0.49±0.14	0.74±1.11	0.888	0.56±0.24	0.657
Basophil	0.1±0.17	0.09±0.18	0.11±0.19	0.06±0.05	0.638	0.17±0.36	0.027
Hemoglobin	12.11±1.31	12.67±1.5	12.5±1.16	12.51±1.18	0.402	13.08±1.04	0.001
RDWSD	34.92±2.63	36.67±2.23	36.53±2.01	33.88±2.32	<0.001	33.58±2.32	<0.001
Platelet	245300±53437	211692±20673	208000±35411	258062±55436	0.001	25934±6196	0.001
MPV	9.48±1.59	9.7±1.41	9.98±1.08	9.49±1.25	0.173	9.27±1.58	0.048
PDW	13.66±3.58	13.3±3.28	12.8±2.8	11.84±2.73	0.054	14.44±4.70	0.070

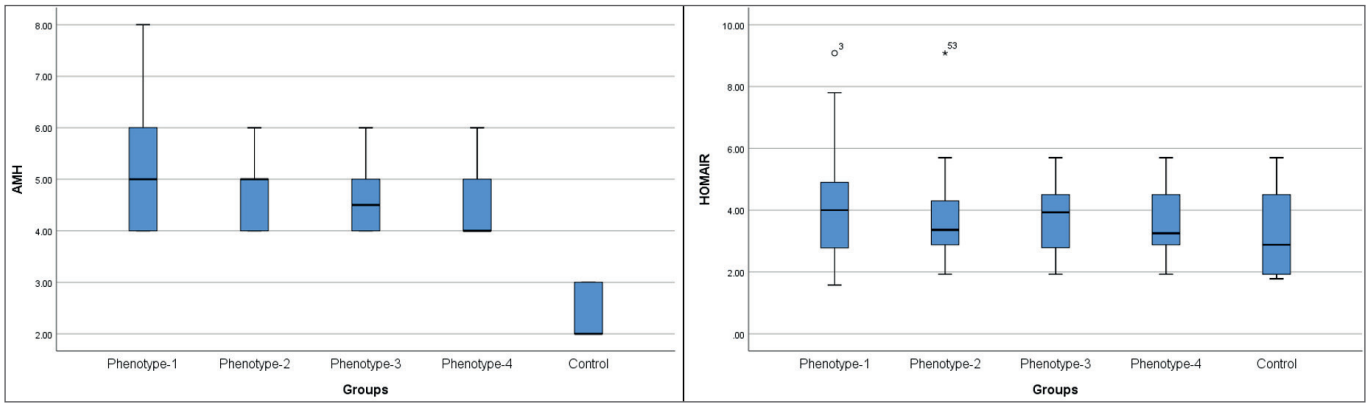

Figure 1. The difference of AMH and HOMA-IR in different PCOS phenotypes

DISCUSSION

The present study investigates whether AMH and HOMA-IR are independently associated with specific phenotypes of PCOS and whether their combined assessment improves the prediction of these phenotypes. Understanding the associations between PCOS phenotypes, AMH, and HOMA-IR is crucial for better diagnosis and management of this complex endocrine disorder. PCOS is a condition that frequently affects women during their reproductive years, which is associated with various symptoms, including irregular menstrual cycles, hirsutism, and infertility.[6] Identifying the underlying hormonal and metabolic factors that play a role in the onset and advancement of PCOS is essential for the development of targeted therapeutic interventions.[9]

Our study found significant differences in the levels of AMH, FSH, LH, and HDL among the four phenotypes of PCOS patients compared to the control group. Phenotype 1 had the highest AMH levels, while the control group had the lowest. FSH levels were highest in phenotype 4 and lowest in the control group. LH levels were highest in phenotype 2, and HOMA-IR was highest in phenotype 1. These results align with prior research studies that have reported varying hormone levels among different PCOS phenotypes.[9-12] The observed differences in hormone levels among the phenotypes may provide insights into the underlying pathophysiology of PCOS and help clinicians better understand the heterogeneity of this disorder. Furthermore, these differences may have implications for the development of targeted treatment strategies for each phenotype.

Despite previous research suggesting insulin resistance is associated with PCOS,[13,15] our study found no significant differences in HOMA-IR levels among the different PCOS phenotypes. However, we did observe that in comparison to other phenotypes, Phenotype 1 had significantly higher fasting blood sugar levels. This finding aligns with a previous study that reported higher HOMA-IR levels in more severe PCOS phenotypes.[14-16] The lack of significant differences in HOMA-IR levels among the phenotypes may indicate that IR is a common feature of PCOS, regardless of the specific phenotype. Alternatively, it may suggest that other factors, such as obesity or genetic predisposition, play a more significant role in the development of IR in PCOS patients. Further research is needed to elucidate the relationship between IR and PCOS phenotypes.

The finding that AMH levels are highest in phenotype 1 is consistent with previous studies indicating that AMH levels are elevated in PCOS patients,[17,18] particularly those with hyperandrogenism and polycystic ovaries. AMH is produced by small follicles present in the ovaries, and its levels are indicative of ovarian reserve and follicular activity. In PCOS patients with hyperandrogenism and polycystic ovaries, there is an increase in the number of small follicles and a reduction in the growth and maturation of larger follicles, leading to elevated AMH levels.[18,19]

The finding that HOMA-IR is highest in phenotype 1 is also consistent with previous studies showing that IR is more pronounced in PCOS patients with hyperandrogenism and polycystic ovaries.[20,21] Insulin resistance is a hallmark of PCOS and is associated with hyperinsulinemia, which in turn, contributes to hyperandrogenism and ovulatory dysfunction. The primary cause of insulin resistance in PCOS is thought to be related to deficiencies in insulin signaling pathways, defects in glucose transport, and increased lipolysis in adipose tissue.[20,22]

However, the difference in AMH levels among different phenotypes of PCOS patients was not significant, suggesting that AMH may not be a useful biomarker for distinguishing between phenotypes. This finding is in line with previous research that reported a lack of correlation between AMH and PCOS phenotypes.[23,24] The inability of AMH to differentiate between PCOS

phenotypes may be because AMH levels are influenced by various factors, such as age, body mass index, and ovarian reserve. Additionally, AMH levels may not accurately reflect the severity of PCOS symptoms or the presence of specific phenotypic features. As a result, clinicians may need to rely on a combination of clinical, hormonal, and metabolic markers to accurately diagnose and classify PCOS phenotypes.

This study's conclusions could be useful in the clinical setting for the diagnosis and management of PCOS. Understanding the associations between hormone levels, IR, and PCOS phenotypes can help clinicians better tailor treatment plans for patients. However, our study has limitations, such as a relatively small sample size and a retrospective design. Future research should focus on larger, longitudinal studies to further explore the relationships between AMH, HOMA-IR, and PCOS phenotypes, as well as the potential utility of other biomarkers for distinguishing between phenotypes.

CONCLUSION

Our study found significant differences in hormone levels among PCOS phenotypes, but no significant differences in AMH or HOMA-IR levels among the different phenotypes. These findings suggest that AMH may not be a useful biomarker for distinguishing between PCOS phenotypes, and insulin resistance may not differ significantly among phenotypes. Further investigation is required to improve our understanding of the associations between hormone levels, insulin resistance, and PCOS phenotypes, as well as to identify potential biomarkers for improved diagnosis and management of this complex endocrine disorder.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Bezmialem Vakıf University Non-interventional Clinical Researchs Ethics Committee (Date: 14.06.2023, Decision No: 2023/191).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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The role of hormonal status, morphological subtypes and proliferative marker Ki-67 labeling index on long-term outcomes in patients with acromegaly: a single tertiary center's experience

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ABSTRACT

Aims: Acromegaly is a rare disorder resulting from benign growth hormone secreting pituitary adenomas. Many factors affect long-term outcomes in acromegaly. In this study we aimed to investigate effects of hormonal status, morphological subtypes, immunohistochemical expression of pituitary hormones and Ki-67 labeling index on long-term outcomes in patients with acromegaly.

Methods:. We collected the medical and pathological records of sixty-four patients who underwent surgery for growth hormone (GH) secreting somatotroph tumors between 2005-2017.

Results: The remission rate after surgery was 48% (31/64) in all patients (33% for macroadenomas, 94% for microadenomas; p <0.001) with a median follow up of 48 months (12-198). There was no significant relationship between Ki-67 labeling index and remission status (p=0.140). The remission group were significantly older than the nonremission group [47 (21-67) vs 36 (18-56); p=0.012]. We found a statistically significant positive correlation between insulin-like growth factor 1 (IGF-1) levels and Ki-67 labeling index (r=+0.382, p=0.004). Also, there was a significant positive correlation between tumor size and GH (r=+0.368, p=0.027). There was no difference between densely and sparsely granulated adenomas in terms of surgical remission (p=0.866). In multivariate regression analysis, tumor size ≥ 10 mm (macroadenoma) was significant independent variable in predicting remission [95% CI [16.95 (1.92-142)]; p=0.011]. The baseline cortisol levels was correlated negatively with the Ki-67 labeling index (r=+0.293, p=0.02).

Conclusion: The Ki-67 labeling index was not associated with surgical remission in patients with acromegaly. However, the Ki-67 labeling index was higher in younger patients and those with larger adenomas.

Keywords: Acromegaly, Ki-67 labeling index, growth hormone, remission

INTRODUCTION

Acromegaly is a chronic disorder caused by growth hormon (GH) hypersecretion. Surgery is the primary therapeutic option for acromegalic patients, but in the vast majority of patients cure is not achieved by surgical treatment.[1,2] In transsphenoidal surgeries performed by experienced pituitary surgeons, remission is greater than 85% for microadenomas and 40-66% for macroadenomas.[3] In previous studies, higher initial GH levels, larger tumor size, histologic evidence of a sparsely granulated adenoma and local invasion findings have been associated with lower probability of biochemical remission after surgery.[4,5] Even if they have similar characteristics, different disease outcomes may be seen in patients with acromegaly. This has prompted clinicians to investigate other predictive factors that may affect long-term outcomes of acromegaly patients.

Ki-67 antigen is a nuclear protein expressed in all phases of cell cycle except G0 phase. The presence of Ki-67 antigen is measured by a monoclonal antibody called MIB-1.[6] Many studies showed that the fraction of Ki-67 positive tumor cells (Ki-67 labeling index) is often correlated with cellular proliferation and more aggressive behaviour in pituitary tumors.[7-10] However it is still controversial whether Ki-67 index is a definitive

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marker of prognosis in acromegaly patients. In this study, we aimed to evaluate the relationship between immunohistochemical expression of pituitary hormones and Ki-67 labeling index with remission.

METHODS

The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 11.04.2016, Decision No: 07-296-16). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Our cohort was composed of 115 patients with acromegaly and among them 64 patients who underwent surgery between 2005 and 2017 and reevaluated pathological specimens were included in this study. The patients who had not undergone surgery for various reasons, the patients with insufficient laboratory data, and the patients who had lost in follow-up were excluded from the study. The biochemical diagnosis of acromegaly was based on the current criteria.[11] Patients were divided into microadenoma (<10 mm) and macroadenoma (≥10 mm) groups according to adenoma size. Transsphenoidal surgery was the preferred approach for all patients. The biochemical reevaluation was performed after 6-12 week by oral glucose tolerance test (OGTT) and serum insulin-like growth factor 1 (IGF-I) levels in all subjects. Remission criteria determined according to 2010 consensus report (Insulin like growth factor levels normal for age and sex, a postoperative random GH level of <1 ng/ml or GH level of <0.4 ng/ml after an oral glucose tolerance test). Demographic, clinical, laboratory and imaging data of all patients were collected. All tissue specimens were examined for anterior pituitary hormones (GH, prolactin, follicle stimulating hormone, luteinizing hormone, tyrotrophin stimulating hormone, adrenocorticotropic hormone) via immunohistochemistry. MIB-1 antibody was used to identify Ki-67 antigen. According to the World Health Organization (WHO) classification, adenomas were classified as sparsely or densely granulated adenomas.[12] Two exprienced pathologists (SK, EE) re-evaluated hematoxylin and eosin (H&E)-stained slides of the adenoma samples. For each case, blocks were determined to use for immunohistochemical evaluation. The Ki-67 labeling indexes of adenomas were performed (clone SP6, Cell Marque, 1:200 dilution) and the rate of Ki-67 were measured as the maximum percentage of positive nuclei by counting at least 1000 adenoma cells.

SPSS software version 20 was used for statistical analysis. Simple descriptive statistics were expressed as mean±standard deviation for normally distribution variables, and as median values for non-normally distributed variables. The frequency distribution of categorical variables between subgroups was compared by the chi-square test. Numerical variables were compared by unpaired t tests or Mann Whitney U-test. Statistically significant results obtained from univariate analysis were submitted to multivariate logistic regression. Spearman correlation analysis was performed to evaluate the relationship between variables. P<0.05 was accepted as significant.

RESULTS

Patients' Characteristics

Sixty -four patients [Male:26 (41%); Female: 38 (59%)] who underwent surgery due to GH-secreting pituitary tumor were included in the study with a median follow up of 48 months (13-198). The median age of the patients at diagnosis was 44 (18-67) (**Table 1**).

The remission rate after surgery was 48% (31/64). Disease control was achieved with second-line treatments in 21 of 33 patients who were not in remission. Remission rates in patients with macroadenoma were lower than those with microadenoma (33% vs. 94%, p <0.001). There was no significant association between initial GH and insulin like growth factor (IGF-1) levels and remission (p=0.109 and p= 0.177). We also did not found a significant relationship between gender and remission status (46% for males and 50% for females; p=0.760) (**Table 2**). The age at diagnosis was significantly lower in patients with macroadenomas than with microadenomas [41 (18-67) vs 53 (23-64); p=0.012]. IGF-1 levels at diagnosis were significantly

Table 1. Baseline characteristics of patients

	Total (n=64)	Male (n=26)	Female (n=38)	P value
Follow-up time (months)	48(13-198)	48 (12-198)	48 (12-132)	0.544
Age at diagnosis (years)	44(18-67)	44 (23-67)	44 (18-63)	0.614
Macroadenoma	48 (75%)	19 (73%)	29 (76%)	0.821
Maximal diameter of adenoma (mm)	18 (6-42)	18 (7-28)	17 (6-42)	0.410
Initial IGF-1 (ng/ml)	949(244-4000)	1027 (435-4000)	882(244-2300)	0.031
Initial GH (ng/ml)	7 (0.30-58)	7.2 (0.60-58)	6.3 (0.30-49)	0.796
Ki-67 Labeling Index (%)	3.23±2.75	3.21±3.24	3.26±2.43	0.980
Prolactin immunostaining	21/64 (32%)	9/26 (34%)	12/38(31%)	0.712
Surgical Remission	31/64 (48%)	12/26 (46%)	19/38 (50%)	0.760

higher in male patients than in female [1027 (435-4000) ng/ml vs 882 (244-2300) ng/ml; p=0.031). IGF-1 level was significantly higher in patients with macroadenomas than in patients with microadenomas [1027 (244-4000) ng/ml vs 810 (579-1162) ng/ml; p=0.001).

Age at diagnosis was higher in the remission group than in the non-remission group, and the maximal adenoma diameter was smaller in the remission group [47 (21-64) vs 36 (18-56); p=0.012 and 11.62±5.14 mm vs 21.33±8.12 mm; p=0.002, respectively] (**Table 2**). In multivariate regression analysis, patients with tumor size lower than 10 mm (microadenoma) was found to be significant in predicting remission [95% CI [16.91 (1.92-142)]; p=0.011].

When the patients were divided into groups as those below 40 years old and those over 40 years old; the remission rate was significantly lower in the group of patients below 40 years of age (32% vs 63%; p=0.013). The incidence of macroadenomas was higher in the group below 40 years of age (91% vs 33%; p=0.022). We also found that the level of IGF-1 was significantly higher in the group of below 40 years of age [1338 (470-4000) ng/ml vs 849 (244-2699) ng/ml; p=0.008].

The most common symptom at diagnosis was acral enlargement (91%; 58/64). Other common symptoms were headache (32%), excessive sweating (27%), loss of vision (27%). There were no correlation between remission status and presentation signs such as acral enlargement, amenorhea, visual disturbances, headache, perspiration, libido loss. Panhypopituitarism was seen in 16% of patients postsurgically. The most common isolated hormone deficiency was gonadotropin deficiency (17%).

Relationship between Hormonal Status, Tumoral Characteristics and Out-Comes

Although not statistically significant the patients with remission had lower Ki-67 index than in patients without remission (2.69±2.22 vs 3.58 ±3.22; p=0.140). When the patients were classified according to their Ki-67 index as Ki-67<3 (Group 1) and Ki-67 ≥3 (Group 2), remission rate was higher in group 1 (58% vs 35%; p= 0.072) but this result did not reach statistical significance. Ki-67 index was almost the same between female and male groups (3.26±2.43 vs 3.21±3.24; p=0.980) (**Table 3**).

Table 3. Ki-67 labeling index according to clinical and laboratory parameters in patients.

	Ki-67 < 3 (n=36)	Ki-67 ≥ 3 (n=28)	P value
Sex (male/female)	16/20	10/18	0.480
Age at diagnosis	46 (22-67)	41 (18-60)	0.041
Macroadenoma	23 (63%)	25 (89%)	0.023
Cortisol (mcg/dl)	13.51±6.42	9.49±3.75	0.004

Macroadenomas were more common in group 2 than group 1 (89% vs 63%; p=0.023) and Ki-67 index was significantly higher in patient with macroadenoma (3.56±3 vs 2.07±1.65; p=0.024). When the granulation patterns were evaluated, 62% of the patients had dense granulated adenoma and 38% had sparsely granulated adenoma. No difference was found between densely and sparsely granulated adenomas in terms of Ki-67 labeling index values (3.23±2.56 vs 3.38±2; p=0.632). Also, we did not find any significant difference in remission status between dense or sparsely granulated adenomas (P=0.866).

Immunohistochemical examination showed that 27 patients (42%) had GH staining alone (monohormonal), 20 patients had plurihormonal staining (40%). We did not found any relationship between remission status and prolactin, adrenocorticotropic hormone (ACTH), Follicle stimulating hormone (FSH), Luteinizing hormone (LH) expression or multihormonal expression in our patients. No significant difference was found in terms of Ki-67 labeling index and remission status in mixed GH/Prolactin tumors.

Table 2. Comparison of clinical and laboratory characteristics of patients according to remission status.

	Remission (n=31)	Non-remission (n=33)	P value
Age at diagnosis	47 (21-67)	36 (18-56)	0.012
Sex (Female/Male)	19/12	19/14	0.760
Macroadenoma	16 (51%)	32 (96%)	<0.001
Adenoma diameter (mm)	11.62±5.14	21.33±8.12	0.002
Ki-67 labeling index (%)	2.69 ± 2.22	3.58 ± 3.22	0.140
Ki-67<3 / Ki-67≥3	21/10	15/18	0.072
IGF-1 (ng/ml)	850 (435-2699)	1050 (244-4000)	0.109
GH (ng/ml)	6.60 (0.6-49)	12.4 (0.3-59)	0.177
LH (mIU/ml)	12.20 (7.40-17.10)	4.75 (2.6-6.9)	0.005
FSH (mIU/ml)	10 (0.10-113)	5.7 (0.50-81)	0.019
ACTH (pg/ml)	29.5 (12-101)	46 (11-107)	0.085
Prolactin (ng/ml)	9.1 (2.4-48)	13 (2-100)	0.548
TSH (mIU/L)	1.34 ± 1.1	1.24 ± 1.01	0.791
Cortisol (mcg/dl)	12 ± 5.3	11.8 ± 6.1	0.909

The median IGF-1 level at diagnosis in group 1 was significantly lower than group 2 [817 (244-2699) ng/ml vs 1239 (540-4000) ng/ml; p=0.002]. The age at diagnosis in group 1 was significantly higher than group 2 [46 (22-67) vs 41 (18-60); p=0.041)]. In correlation analysis, there was a statistically significant positive correlation between IGF-1 levels and Ki-67 labeling index (r=0.382, p=0.004). Also, there was a significant positive correlation between tumor size and GH (r=0.368, p=0.027). There was no correlation between Ki-67 labeling index and tumor size (p=0.109). Furthermore basal cortisol level was significantly higher in group 1 than those group 2 (13.51±6.42 vs 9.49±3.75; p=0.004) (**Table 3**). There was also an inverse correlation between cortisol level and absolute Ki-67 values (r=-0.301, p=0.017). In multivariate regression analysis, younger age at diagnosis and lower cortisol levels were found as independent variables predicting the patients with Ki 67≥ 3 (p=0.019 and p=0.019, respectively) (**Table 4**).

Table 4. Predicting factors for Ki67 labeling index ≥3

	P	Odds ratio	95% confidence interval	
			Lower	Upper
Lower cortisol	0.019	1.16	1.02	1.32
Younger age	0.019	1.06	1.01	1.12

DISCUSSION

In this study, 48% of the patients with acromegaly achieved remission and 51% of them had macroadenomas. The Ki-67 labeling index was not associated with remission status. The younger age at diagnosis, the lower baseline cortisol value and the larger tumor size were significantly associated with Ki-67 labeling index in patients with acromegaly. We also found that smaller tumor size, the older age at diagnosis were associated with surgical remission in patients with acromegaly. In the literature the remission rate after surgery alone is reported to be 27 and 80%. Our results were compatible with the literature.[13]

Whether the Ki-67 labeling index is a factor in surgical remission is controversial. To the best of our knowledge there were very limited studies showing that high Ki-67 labeling index values affect surgical remission. Fusco et al.[5] reported that Ki-67 labeling index is significantly higher in surgically not cured patients. They claimed that Ki-67 labeling index is associated with a better clinical outcome in patients with acromegaly. However, some other studies, like ours did not find any significant association.[14,15]

Jaffrain et al.[16] (2002) reported that Ki-67 labeling index decreased with increasing age and tumor volume but other studies claim the opposite of this. Similar to our study, Mohseni et al.[17] found higher Ki-67 levels in younger patients. According to Pinto et al.,[18] Fusco et al.[5] and Mastronardi et al.[19] Ki-67 expression was independent from the tumor size. In a recent study by Alimohamadi et al.[6] Ki-67 level was not significantly different according to tumor size. In our study, Ki-67 labeling index values were significantly higher in the macroadenoma group (p=0.027).

The relationship between histological subtype and postsurgical surgical cure is a controversial topic in the literature. While some studies have demonstrated lower surgical remission rates for sparsely granulated adenomas compared to densely granulated adenomas, in others remission rates did not differ by histological subtypes.[14,20-22] The frequency of histologic subtypes of adenomas in our cohort (62% densely ; 38% sparsely) is almost consistent with the range of frequencies reported in the literature.[15,23,24] Also, no difference was found in terms of remission status according to histological subtypes.

In our study low cortisol levels were associated with higher Ki-67 labeling index. Such a relationship has not been shown in previous studies. In a recent study evaluating the postoperative outcomes of 348 functional pituitary tumors, higher MIB-1/Ki-67 labeling index and preoperative low cortisol axis were shown to be associated with suboptimal outcomes.[25] This finding in our study can be attributed to the inhibitory effect of cortisol on cell proliferation, but this relationship cannot be clearly stated with the current study findings.[26,27]

According to previous studies younger age at diagnosis, higher pretreatment IGF-1 and GH levels and larger tumor size are associated with a lower probability of biochemical remission after surgery.[14] In accordance with previos studies, in our study, younger age at diagnosis and larger tumor size were associated with lower remission rates. Fernandez Rodriguez et al.[28] identified the main factors determining the prognosis of acromegaly and proposed risk score for each factors. In line with this scoring system we found that larger tumor size was independent risk factor in determining remission. Preoperative GH and IGF-1 levels have been associated with surgical remission in many studies but we did not find any association with remission as in the studies of Yıldırım et al.[29] and Shirvani et al.[30]

There are several limitations of this study. First, our study was retrospective in nature. Second, our study had small sample size therefore some results may not have reached statistical significance. Third, the pathological examination of immunohistochemical parameters such as mitotic index, p53 positivity other

than Ki-67 index could increase the strength of the study. More detailed classification of invasiveness according to radiologic apperance such as Knosp classicifation would have allowed to assess the parasellar invasion status. Also it would be better to evaluate the low cortisol levels with ACTH test for corticotroph function.

CONCLUSION

This study suggest tumor size is an important parameter for predicting remission. Younger age and lower cortisol level are associated with higher proliferation index in GH-secreting pituitary tumors. There is no significant relationship between Ki-67 labeling index and surgical remission.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 11.04.2016, Decision No: 07-296-16).

Informed Consent: Written informed consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Evaluation of immunochromatography method in the diagnosis of cystic echinococcosis

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ABSTRACT

Aims: *Echinococcus granulosus* is the causative agent of hydatid cyst, or cystic echinococcosis (CE), with its current name. *Echinococcus granulosus* is a zoonotic cestode; commonly found in humans and farm animals. In cystic echinococcosis infection, transmission occurs by oral ingestion of parasite eggs excreted in infected dog feces. The larval form is responsible for the formation of slowly growing cysts in the organs and tissues of mammals such as humans, sheep, goats and cattle. In this study, it was aimed to compare indirect hemagglutination (IHA) and immunochromatographic (ICT) methods from the sera of patients with suspected CE and to evaluate serological tests based on imaging and clinical diagnosis.

Methods: Between 31 October 2022 and 31 January 2023, blood samples of 95 patients with suspected CE from different units of our hospital and for whom IHA was routinely requested were included in the study prospectively. VIRAPID® Hydatidosis (Vircell, Granada, Spain) test using the immunochromatographic method and ELI.H.A. *Echinococcus* (ELITech Microbio, France) test was studied in accordance with the manufacturer's instructions.

Results: Based on clinical and imaging methods of 95 patients included in our study, 64 (63.1%) were diagnosed with hydatid cyst. Based on imaging and clinical diagnosis; sensitivity, specificity, positive predictive value and negative predictive values were calculated as 81.3%, 96.8%, 98.1%, 71.4% for the IHA test, and were calculated as 75.0%, 93.5%, 96.6%, 64.4% for the ICT test, respectively. Good agreement was found between the two tests (percent agreement=68.0%; kappa value=0.682; p<0.001). Sensitivity (IHA: 87.2%; ICT: 94.9%) and specificity (IHA: 96.8%; ICT: 93.5%) of IHA (positive titer of 1/160 and above) and ICT methods in active cysts with cyst stage (CE) 1-2-3) values were found to be compatible, The sensitivity of the ICT method in inactive cysts with CE 4-5 (IHA: 72%, ICA: 44%) was found to be statistically significantly lower than the IHA method (p<0.001).

Conclusion: Rapid diagnostic tests generally stand out as they do not require personnel training in health institutions and are easy to apply. Especially in the active period of the cysts, the tests show a very good and harmonious performance, and it significantly supports the clinical and radiological findings in the early diagnosis of the disease and in the treatment follow-up, however, they need to be developed in order to be used in the differential diagnosis of inactive cyst stages, especially in cases in between, and performance studies in larger patient groups are required.

Keywords: Cystic echinococcosis, *Echinococcus granulosus*, immunochromatography, indirect hemagglutination

INTRODUCTION

Cystic echinococcosis (CE) is a disease caused by *Echinococcus granulosus* belonging to the Taeniidae family.[1,2] It is a ubiquitous zoonotic agent with worldwide distribution except Antarctica.[3,4] CE is one of the most common parasitic diseases threatening human and animal health in the world and in Turkey.[4,3] Especially the Mediterranean basin is known to be endemic.[5,6] Within the scope of the HERACLES project supported by the 7th Framework Program of the European Union, abdominal CE was detected with ultrasonography (USG) at a rate of 0.6% in Turkey. The Turkey leg of the study was carried out in six provinces (Ankara, Aksaray, Balıkesir, Bitlis, Edirne, Şanlıurfa) in different geographical regions and the prevalence was determined as 6%. According to the data of USG-based CE field studies reported from different provinces in Turkey, the prevalence varies between 0.15% and 1.05%.[7] According to the evaluations of the World Health Organization (WHO), CE was listed as one of the 17 neglected tropical diseases of animal origin and recorded among the most serious parasitic diseases in humans.[6] Infection can occur by ingestion of parasite eggs, which are excreted in the feces of the last host dog, by natural intermediate hosts such as humans, sheep, goats, cattle, through digestion and respiration.[21] Infectious cysts are most commonly located in the liver; It is also seen in various organs and tissues such as lung, spleen and kidney.

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The clinic is usually asymptomatic; It occurs depending on the size and localization of the cysts.[4]

The diagnosis of cystic echinococcosis is mainly made by imaging methods. While computed tomography and magnetic resonance are used in almost all organ locations, USG has been the first choice for liver cysts and direct radiography for lung cysts.[1] It is recommended to use radiological diagnosis methods together with serological diagnosis methods in cases such as differential diagnosis of the cyst with other space-occupying lesions, determination of postoperative recurrences and the absence of a clear clinical picture. In addition, serological tests are used not only in the diagnosis of CE, but also in determining its prevalence in the community and in identifying asymptomatic individuals. In the serological diagnosis, indirect hemagglutination (IHA), enzyme-linked immunosorbent (ELISA), latex agglutination, indirect fluorescent antibody (IFA) and immunoblotting (IB) methods, in which specific immunoglobulin G antibodies are detected, are frequently used.[5,8]

Recently, the use of the immunochromatographic (ICT) method, which gives rapid results for the diagnosis of CE, has become quite common. This study, it was aimed to compare IHA and ICT methods from sera of patients with suspected CE and to evaluate serological tests based on clinical and imaging diagnoses.

METHODS

The study was carried out with the permission of Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date: 24.11.2022, Decision No: 21/14). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Between 31 October 2022 and 31 January 2023, blood samples of 95 patients with suspected CE from different units of our hospital and whose IHA tests were routinely requested were included in the study. The clinical diagnoses and radiological reports of the patients were obtained through the hospital information system (LIS) and epicrisis. in our study, cyst stages (CE) were determined by imaging methods according to the criteria of the WHO Echinococcosis Informal Study Group (WHO-IWGE).9 Accordingly, cyst stages are defined in three groups: "active" cysts that are usually viable, unilocular (CE-1) and multivesicular (CE-2) with daughter vesicles, "transitional" with separation of endocyst (CE-3a), and predominantly with daughter vesicles (CE-3a) solid cysts (CE-3b) and "inactive", nonviable, solid and calcified cysts (CE-4 and CE-5). While evaluating the results of our study, CE-1-2-3 stages were classified as active cysts and CE-4-5 stages were classified as inactive cysts.

Test procedures

Serum samples were studied simultaneously with the *Echinococcus* assay (ELI.H.A.; ELITech Microbio, France) with IHA method and the VIRAPID® Hydatidosis (Vircell, Granada, Spain) immunochromatographic assay with the ICT method. By the manufacturer's recommendations, sera were diluted in eight-well microplates for the IHA method. Serum dilutions with antigen erythrocyte suspension (reagent) added were evaluated after 2 hours of incubation. Ringing at the bottom of the well was considered negative, a red/brown cloudy image was considered positive, and the last well dilution seen was recorded.

In the kit package insert, serums with a titration value below 1/160 are indicated as the possible absence of hydatid disease and the need to repeat the test after 2-3 weeks. Suspected infection for serum samples with a titration value of 1/160; Titrations of 1/320 and above were indicated as an important reaction in favor of progressive hydatid cyst. For the ICT method, the serum sample and developer solution were dropped into each cassette and incubated for 30 minutes. The results were evaluated visually according to the interpretation chart in **Figure 1A**. According to the test procedure, samples with a control line but not a test line and a density value less than 0.5 were evaluated as negative. Those with a test line intensity value of 0.5 and above were considered positive and 0.5, 1, 2, and 3 according to their intensity. Images of some patient samples are shared in Picture 1B. It is stated in the package insert that the internal quality control studies were carried out by the manufacturer before the kit was put on the market, and the sensitivity and specificity were reported as 94.74% and 99.5%, respectively. The VIRAPID® Hydatidosis test interpretation chart and ICT images of some patient samples (patients 4 and 5 were evaluated as negative, other patients as positive) were presented in **Figure 1**.

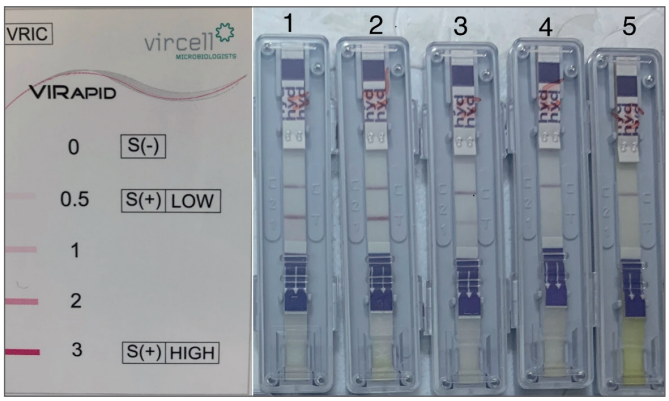

Figure 1. A. VIRAPID® Hydatidosis test interpretation chart B. ICT images of some patient samples (patients 4 and 5 were evaluated as negative, other patients as positive).

Statistical analysis

SPSS (Statistical Packages for the Social Sciences) software version 22.0 (SPSS Inc., Chicago, USA) program was used for statistical analysis of the study. The results obtained with two different tests were recorded as categorical variables. The agreement between these results was analyzed by calculating Cohen's kappa value and percent agreement. Kappa value: <0.20 poor, 0.21-0.40 near medium, 0.41-0.60 moderate, 0.61-0.80 good, and 0.81-1.00 almost perfect fit. Shapiro-Wilk test was applied to check the normality assumption of continuous variables such as age. Kruskal-wallis analysis of variance was used to determine whether there was a significant difference in age distribution between the different groups. Clinical diagnosis and imaging methods were accepted as the gold standard; Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of IHA and ICT methods were calculated using the Chi-Square test. With the Receiver Operating Characteristic (ROC) analysis, the threshold values at which IHA and ICT methods gave high sensitivity and specificity positivity were calculated according to the method accepted as the gold standard method (separately as cyst active and cyst inactive). Statistically significant p value was determined as <0.05.

RESULTS

Blood samples of 95 patients aged between 10 and 83 (mean: 50.16), 59 (62.1%) female, 36 (37.9%) male, suspected of CE and routinely requested IHA test were included in the study. Of the patients (n:64) who were positive according to imaging and clinical diagnosis, 5 (7.8%) were aged 16 years or younger, 18 (28.2%) were aged 16-44, and 41 (64.0%) were aged 44 years or older determined. Considering the distribution in age groups, a statistically significant difference was found between patients aged 44 years and older and other age groups (p<0.001).

Of the patient samples, 51 (53.7%) were in general surgery, 17 (17.9%) in gastroenterology, 6 (6.3%) in infectious diseases, 6 (6.3%) in internal medicine, 6 (6.3%) in pediatrics, 2 (2.1%) were sent from intensive care units and 7 (7.4%) were sent from different clinical branches.

Liver in 56 (87.5%), lung in 3 (4.6%), kidney in 2 (3.2%), and spleen and liver in 2 (3.2%) of patients (n:64) who were positive according to imaging and clinical diagnosis However, it was determined that 1 (1.5%) had intra-articular localization.

All of the patients (n:64) who were positive according to imaging and clinical diagnosis were found positive at 1/80 titer in the IHA method, and 53 (82.8%) were positive at 1/160 and higher titer. Fifty (52.6%) of these patients were found to be positive with an intensity value of 0.5 and above on the test line by the ICT method. The IHA and ICT results of the patients who were positive according to imaging and clinical diagnosis are presented in **Table 1**. Based on imaging and clinical diagnosis, the sensitivity, specificity, PPV, and NPV were 81.3%, 96.8%, 98.1%, and 71.4% for the IHA method, and 75.0, 93.5%, 96.6%, and 64.4% for the ICT method, respectively. One (4.2%) of 24 samples found to be negative by indirect hemagglutination method was found to be 0.5 positive by ICT. 9 (16.9%) of the 53 samples found to be 1/160 or more positive with IHA were found to be negative with ICT. The number of samples with positive results with both tests was calculated as 44 (68.7%) and the number of samples with negative results as 36 (56.2%), and a good level of agreement was found between the two tests (percent agreement=68.0%, kappa value=0.682, p <0.001). **Table 2** shows the results of IHA and ICT methods according to titer and test line intensities.

Table 1. IHA and ICT results by imaging and clinical diagnosis

	Imaging and Clinical Diagnosis				
		Positive			
	Negative (n: 31)	Active CE-1-2-3 (n: 39)	Inactive (CE-4-5) (n: 25)	Total CE-1-2-3-4-5 (n: 64)	Total (n: 95)
IHA (1/160)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
positive	1 (3.2)	34 (87.2)	18 (72.0)	52 (81.2)	53 (55.7)
negative	30 (96.8)	5 (12.8)	7 (28.0)	12 (18.8)	42 (44.3)
ICT	n (%)	n (%)	n (%)	n (%)	n (%)
positive	2 (6.5)	37 (94.9)	11 (44.0)	48 (75.0)	50 (52.6)
negative	29 (93.5)	2 (5.1)	14 (56.0)	16 (25.0)	45 (47.4)

Table 2. Results of IHA and ICT methods according to titer and test line intensities.

	VIRAPID® Hydatidosis Test Results					
	3+ n (%)	2+ n (%)	1+ n (%)	0.5+ n (%)	Negative n (%)	Total n (%)
ELI.H.A. *Echinococcus* Test Results						
1/1280 titer	11 (30.6)	8 (22.2)	10 (27.8)	4 (11.1)	3 (8.3)	36 (100)
1/640 titer	- (0)	1 (25)	1 (25)	1 (25)	1 (25)	4 (100)
1/320 titer	- (0)	- (0)	1 (25)	1 (25)	2 (50)	4 (100)
1/160 titer	- (0)	- (0)	- (0)	6 (67)	3 (33)	9 (100)
1/80 titer	- (0)	- (0)	- (0)	5 (27.8)	13 (72.2)	18 (100)
Negative	- (0)	- (0)	- (0)	1 (4.2)	23 (95.8)	24 (100)
Total	11 (11.6)	9 (9.5)	12 (12.6)	18 (18.9)	45 (47.4)	95 (100)

When patients who are positive according to imaging and clinical diagnosis are divided into active and inactive cysts; The sensitivity, specificity, PPV and NPV of the IHA method (1/160 titer and above) in cyst active patients (CE 1-2-3) were; as 87.2%, 96.8%, 97.1% and 85.7%; If the cyst is in inactive patients (CE 4-5); It was calculated as 72%, 96.8%, 94.7% and 81.1%.

When patients who are positive according to imaging and clinical diagnosis are divided into active and inactive cysts; When the ICT method (0.5 line intensity and above line intensity) is evaluated, sensitivity, specificity, PPV and NPV in cyst active patients (CE 1-2-3); 94.9%, 93.5%, 94.9%, 93.5% and in inactive patients (CE 4-5); It was calculated as 44%, 93.5%, 84.6% and 67.4%.

ROC analysis and comparison results of ICT and IHA method results of cyst active and inactive patients are shown in **Table 3** and **Figure 2**.

Table 3. ROC analysis and comparative results between methods in cyst active and inactive patients

	AUC			
	ICT		IHA	
Cyst active	0.964 (0.918-1.000)	p<0.001	0.981 (0.958-1.000)	p<0.001
Cyst inactive	0.695 (0.551-0.840)	p=0.013	0.963 (0.922-1.000)	p<0.001

AUC=Area Under Curve [95% CI].

DISCUSSION

Diagnosis of cystic echinococcosis usually requires the use of imaging methods. However, the radiological diagnosis should be supported by laboratory diagnostic methods to make the differential diagnosis of cyst with other space-occupying lesions such as tumor, abscess, and simple cyst, and to evaluate recurrences after surgery in a healthier way.[1,10,11] In addition, since laboratory test results are required in the follow-up of the treatment for CE, it is extremely important to know the sensitivity and specificity values of these laboratory tests and the factors affecting the test results. For this purpose, standard diagnostic tests with high sensitivity and specificity are still being investigated in the diagnosis of CE.

Immunological methods used for screening and follow-up of CE are ELISA, ICT, and IB test which is commonly used as the confirmatory method due to its higher sensitivity-specificity values. Other less frequently used methods can be listed as IFA, IHA and

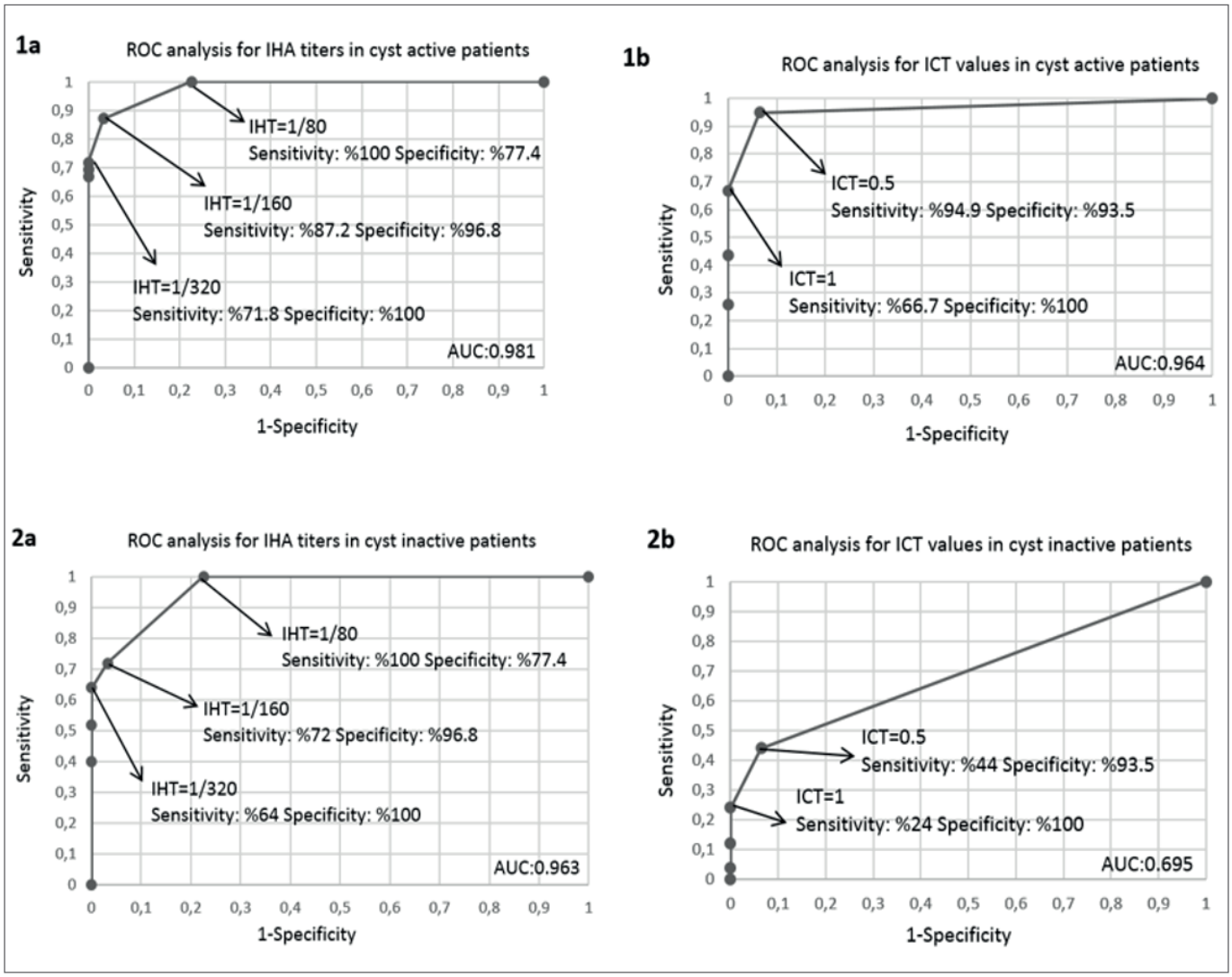

Figure 2. 1a-1b ROC curves of IHA and ICT methods in cyst active patients.2a-2b ROC curves of IHA and ICT methods in cyst inactive patients. AUC: Area Under Curve

point immunogold filtration (DIGFA).[12] Among the serological methods, IHA methods provide advantages such as low cost and high specificity and sensitivity.[6] The IHA method, which is routinely used for diagnostic purposes in our laboratory, has been reported to have a sensitivity of 65-90% and a specificity of 97.5%-100% in different studies.[3] In two studies reported from our country; The specificity and sensitivity of the hemagglutination method were determined as 94.59% and 88.76% by Akgün et al.[10] and 97.0% and 86.2% by Zait et al.[11] respectively.

VIRAPID® Hydatidosis, a commercially available rapid diagnostic test for the diagnosis of cystic echinococcosis using the ICT method, is widely used in laboratories and is being investigated for ease of use, specificity and sensitivity. Ertuğ et al. studied the VIRAPID® Hydatidosis test using the ICT method on 50 clinically and pathologically positive samples and reported the specificity and sensitivity as 100% and 96%, respectively. As a result of their studies, they predicted the rapid diagnostic test as practical and easily applicable in the diagnosis of CE.[8]

In the study conducted by Tamer et al.[5] the specificity and sensitivity of the VIRAPID® Hydatidosis (Vircell, Granada, Spain) ICT test was 87.5% and 96.8%, respectively, according to clinical and radiological diagnosis. They showed advantages such as low cost, long shelf life, fast results, no special equipment, easy readability and usability by non-experts.

Tamarozzi et al.[13] compared three rapid identification tests with a commercial ELISA test that they routinely use in their laboratories, and reported that the VIRAPID® Hydatidosis rapid diagnostic test showed the best diagnostic accuracy but the sensitivities of these three tests were lower than the ELISA (R-Biopharm, Darmstadt, Germany) test. However, they found that the sensitivities of all three tests were lower than the ELISA (R-Biopharm, Darmstadt, Germany) test. They found the specificity and sensitivity of the VIRAPID® Hydatidosis test to be 74% and 96%, respectively, and predicted that this test could be used in environments where there are insufficient resources to complete USG diagnosis in patients with suspected hydatid cysts.

At a veterinary faculty in Italy, Peruzzu et al.[12] evaluated the diagnostic performance of four commercial test kits in the serum of 259 patients with positive (n:74) and negative (n:185) CE. They specified the IB test method as the best in terms of sensitivity-specificity and diagnostic performance, and the VIRAPID® Hydatidosis test as the second ICT method. They stated that in endemic areas, these tests can be considered as support for clinical evaluation.

In our study, IHA and ICT methods were compared using ELI.H.A. *Echinococcus* (ELITech Microbio, France) test and VIRAPID® Hydatidosis (Vircell, Granada, Spain) test to detect E. granulosus antibody in 95 serum samples. In our study, based on imaging and clinical diagnosis, the sensitivity and specificity of the IHA method at titrations of 1/160 and above were calculated as 81.3% and 96.8%, respectively, and the sensitivity and specificity of the ICT method at 0.5 and above were determined as 75% and 93.5%, respectively. Of 64 patients diagnosed by imaging methods and clinically, 53 (55.7%) were found to be positive with IHA method and 50 (52.6%) with ICT method, and good agreement between the two methods (percent agreement=68%, kappa value= 0.682, $p<0.001$).

Studies report that the specificity and sensitivity of the tests used in serological diagnosis may vary depending on the characteristics of the antigen, the organ where the cyst is localized, and the host immune response.[14] Similar to our results in studies conducted in our country, it was found that the most common liver-localized CE cases were, and the rate of seropositivity between liver and other organ involvements was not found statistically significant in many studies.[6,10,12] In our study, hepatic involvement of CE was detected in 56 (87.5%) of 64 patients based on imaging methods and clinic. The number of cases with extrahepatic involvement is not sufficient to statistically compare organ involvement in terms of seropositivity. Extra-hepatic CE cysts should be evaluated with a larger cohort in terms of the use of serological diagnostic methods.

Some researchers may also recommend testing the same serum with more than one method in order to increase the sensitivity and specificity of laboratory diagnosis and to obtain the most reliable results.[1,15,16] However, in recent years, studies have been started to determine the diagnostic efficacy of rapid diagnostic tests in different stages of the disease instead of working with all laboratory and imaging methods in each patient.[12,17] Although clinical and imaging methods are used in the diagnosis, laboratory tests should have a confirmatory role in cases where the diagnosis is in between. However, in the test performance studies conducted by Tamarozzi et al.[17] serological test results were generally evaluated as variable and insufficient for the diagnosis of inactive CE4 and CE5 stages. In our study, cyst stages were evaluated according to the criteria determined by the World Health Organization (WHO) Echinococcosis Informal Study Group (WHO-IWGE), and CE1-2-3 stages were classified as active cysts and CE4-5 stages as inactive cysts.[9] In active cysts (CE 1-2-3), the sensitivity (IHA: 87.2%; ICT: 94.9%) and specificity (IHA: 96.8%; ICT: 93.5%) were found to be compatible with the IHA

test (at a titer of 1/160 and above), while inactive The sensitivity of the ICT method in cysts (IHA: 72%, ICT: 44%) was statistically significantly lower than the IHA method.

Our results show that IHA and ICT methods have overall comparable performance based on clinical and radiological diagnoses. However, especially the VIRAPID® Hydatidosis test shows poor sensitivity in the presence of inactive (CE-4-5) cysts with cyst stages, which may cause important problems in differential diagnosis. Although it is a common approach by clinicians to use rapid diagnostic tests and imaging methods together to monitor and confirm each other, determining their effectiveness in diagnosis in different stages of the disease and choosing the right method will be a cost-effective and rational diagnostic strategy for patients and health institutions in terms of reducing workload and saving time.

CONCLUSION

In the present study, it can be predicted that rapid diagnostic tests, which we have evaluated in general, do not require personnel training in health institutions where opportunities are limited, they are easy to apply and give fast results, as well as their low cost and long shelf life. Especially in the active phase of the cysts, rapid diagnostic tests show a very good and harmonious performance, support the clinical and radiological findings in the early diagnosis of the disease and in the treatment follow-up, however, they need to be developed and performance studies in larger patient groups are needed in order to be used in differential diagnosis, especially in the inactive cyst stages.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date 24.11.2022, Decision No: 21/14).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Comparison of anti-HCV seroprevalence of patients who underwent cataract surgery and other ophthalmic procedures

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ABSTRACT

Aims: The aim of this study is to evaluate the Anti-HCV seroprevalence in patients who underwent cataract surgery and to compare the prevalence with other ophthalmic surgery procedures.

Methods: Patients who underwent ophthalmic surgeries between January 2017 and January 2023 and were preoperatively screened for anti-HCV by ELISA test were included in this study. Patients who underwent cataract surgery formed the study group and other patients were compared as the control group. All data were obtained from the database of the institute and were retrospectively evaluated.

Results: A total of 15799 cases were included in the study, and 69.9% had cataracts whereas 30.1% had non-cataract surgery. The mean age of the patients was 63.24±16.04 years. The rate of Anti-HCV seropositive patients was 0.48% (n=53) in the cataract surgery group, and 0.42% (n=20) in those who had non-cataract surgery. However, the difference was not statistically significant (p=0.696). The overall rate of Anti-HCV seropositive cases was %0.46.

Conclusion: Even though there was no significant difference between the two groups, the Anti-HCV was positive for almost 1 in 200 ophthalmic procedures. We strongly recommend preoperative screening due to the severity of HCV infection and the risk of surgical transmission.

Keywords: Cataract, cataract surgery, opthalmic surgery, anti-HCV seroprevalence

INTRODUCTION

Cataract surgery is one of the most common surgical procedures all over the world. The incidence of senile cataracts is 17.2% in the world and the rate is exponentially increasing with the aging population.[1] Hepatitis C virus (HCV) is one of the leading causes of liver cancer and the number of people infected with HCV is approximately 180 million worldwide.[2] Senile cataract is also one of the extrahepatic findings of Hepatitis C disease. Patients with senile cataracts have significantly higher HCV seropositivity than the general population of the same age.[3] Hence, HCV infection may have a role in the etiology and/or prognosis of lens opacification. Although several studies in the literature evaluated the relationship between cataract formation and HCV infection, there is no study so far comparing this relationship with other eye surgeries. This study aimed to retrospectively investigate Anti-HCV seropositivity in patients who underwent cataract surgery among all eye surgeries.

METHODS

This retrospective study was carried out with the permission of Samsun University Clinical Researches Ethics Committee (Date: 07.06.2023 Decision No: 2023/11/10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was carried out in the ophthalmology clinic of Samsun Training and Research Hospital in Samsun. All patients who underwent eye surgery between January 2017 and January 2023 and were preoperatively screened for anti-HCV by ELISA were included in the study. Cataract patients formed the study group and were compared with other ophthalmic surgery patients as the control group. All data were obtained from the hospital database and were analyzed retrospectively. SPSS v22.0 (Statistical Package for the Social Sciences, IBM, NY, USA) program was used for data analysis. A p value <0.05 was set as statistically significant.

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RESULTS

Of the 15799 cases included in the study, 69.9% of the patients had cataracts and 30.1% had non-cataract surgery. The mean age of the patients was 63.24±16.04 years (0.0-105.0). The mean age of cataract patients was 68.25±10.62 years (0.0-105.0), and the mean age of those with non-cataract surgery was 51.62±19.98 (0.0-93.0) and the difference was statistically significant (p<0.001). The distribution and demographic data of all patients are shown in **Table 1**.

Tablo 1. The distribution and demographic data of all patients

	Cataract surgery n (%)	Other ophthalmic procedures n (%)	Total n (%)	P
Number of patients	11040 (69.9)	4759 (30.1)	15799(100)	
Age	68.25±10.62	51.62±19.98	63.24±16.04	<0.001
Gender				<0.001
Male	5558 (50.3)	1939 (40.7)	7497 (47.5)	
Female	5482 (49.7)	2820 (59.3)	8302 (52.5)	
Anti-HCV result				0.696
Anti-HCV (+)	53 (0.48)	20 (0.42)	73 (0.46)	
Anti-HCV (-)	10987 (99.52)	4739 (99.58)	15726 (99.54)	

Of those included in the study, 47.5% (n: 7497) were male and 52.5% (n: 8302) were female. The rate of males who had cataract surgery (50.3%) was significantly higher than that of males (40.7%) who had non-cataract surgery (p<0.001). The number of anti-HCV-positive patients was 73 (0.46%). The rate of Anti-HCV positives in those who had cataract surgery was 0.48% (n=53), which was higher than the rate of Anti-HCV positives (0.42%; n=20) in those who had non-cataract surgery. However, this difference was not statistically significant (p=0.696) (**Table 1**).

Cataract surgery patients were also evaluated in terms of gender. However, there was no significant difference between genders in terms of Anti-HCV seropositivity. On the other hand, male patients had cataract surgery at an earlier age in the study group (**Table 2**). There was a similar distribution in terms of Anti-HCV seropositivity between the genders in the control group, as well. However, females had other ophthalmic procedures at an earlier age (**Table 3**). There was no statistically significant difference between seropositive and seronegative cataract patients in terms of age and gender (p=0.082, p=0.595, respectively) (**Table 4**).

Tablo 2. Distribution of patients who underwent cataract surgery by gender

	Male	Female	Totale	P
Number of patients	5558 (50.3%)	5482 (49.7%)	11040 (100%)	>0.05
Anti HCV +	33(0.59%)	20 (0.36%)	53 (0.5%)	0.098
Age	67.63±10.51	68.89±10.68	68.25±10.62	<0.001

Tablo 3. The gender distrubition of patients who underwent other ophthalmic prosedure

	Male	Female	Totale	P
Number of patients	1939 (40.7%)	2820 (59,3%)	4759 (100%)	>0.05
Anti HCV +	8 (0.41%)	12 (0.42%)	20 (0.4%)	0.946
Age	52,45±21.31	51.05±18.99	51,62±19,98	<0.017

Tablo 4. Evaluation of Cataract Patients Based on Anti-HCV Result

	Anti-HCV negative n(%)	Anti-HCV positive n(%)	Total n(%)	P
Gender				0.082*
Male	5525 (50.3)	33 (62.3)	5558 (50.3)	
Female	5462 (49.7)	20 (37.7)	5482 (49.7)	
Age	68.25±10.62	68.94±9.27	68.25±10.62	<0.595**

*; Pearson chi squared test, **; Mann Whitney U test

DISCUSSION

Cataract surgeries are among the most frequently performed surgeries all over the world.[4,5] As in all body fluids, HCV RNA can be detected in humoral aqueous and tear fluid, as well.[6-8] In this study, approximately two-thirds of the ophthalmic procedures were cataract surgeries and Anti-HCV seropositivity was found in 0.46% of all patients and 0.42% of cataract surgeries. This rate is lower than the prevalence of Anti-HCV in general Turkish population. A meta-analysis examining a total of 246 articles revealed the prevalence of HCV infection in Turkey as 1.6%.[9] Yoshida et al.[3] reported the prevalence of HCV in cataract patients significantly higher than the healthy individuals (p<0.01). The rates were 18.3% and 7.1% in the 60-69 age subgroup; 6.6% and 17.8% in the 70-79 age subgroup; and 3.7% and 15.1% in the 80-90 age subgroup, respectively. However, they found no significant difference in HCV seropositive and seronegative groups in terms of hepatitis B virus prevalence in the cataract group (p=0.548).

In a recent study investigating 6858 patients in the United States, it was reported that anti-HCV positivity was 1.86%. The mean age at surgery was 63.4 years for HCV-positive patients, while it was 69.1 years for HCV-negative patients. Patients with HCV infection were significantly more likely to experience complications during cataract surgery than those without HCV disease (2.9% vs. 1.2% OR 2.27, 95% CI 1.03-5.01, p=0.0415). The main reason for the complication in these patients was associated with high alanine transaminase levels.[10]

A study evaluating 240 patients who underwent cataract surgery in Pakistan determined the anti-HCV positivity and the rate was found as 12.13%.[11] The same authors reported the rate as 11.1% in another study with 377 patients.[12] The anti-HCV seropositivity of cataract patients in Pakistan was reported as 14.29% (45 of 315 patients).[13] The prevalence of Anti-HCV positivity was found to be 12.4% in Egypt which included 3067 patients who applied for elective eye surgery.[14-16] In a report from India; Anti-

HCV seropositivity was found in 11 patients (0.1%) in the preoperative screenings of 7316 patients before elective cataract surgery.[17] These rates from the middle-east region are similar to the values we obtained in our study.

In the Sustainable Development Goals published by WHO (World Health Organization) in May 2016; it is aimed to eliminate viral hepatitis from being a public health threat by 2030. To this end, it is recommended to raise awareness and mobilize screening activities.[18-20] Ophthalmologists can contribute to this goal of WHO by performing hepatitis screening with the ELISA test before their frequent cataract surgeries. Referring positive cases to the Infectious Diseases department for further examination and treatment will be a beneficial step for both patient and public health.

CONCLUSION

We evaluated the prevalence of Anti-HCV in patients who underwent cataract surgery and compared it with the prevalence of Anti-HCV in other ophthalmic procedures. There was no significant difference between the two groups and lower anti-HCV seropositivity was found compared to the general population. We strongly recommend preoperative screening due to the severity of HCV infection and the risk of surgical transmission.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Samsun University Clinical Researches Ethics Committee (Date: 07.06.2023, Decision No: 2023/11/10).

Informed Consent: Because the study was designed retrospectively, no written informed consent was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Pneumococcal and influenza vaccine awareness in individuals over 65 years

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ABSTRACT

Aims: Vaccination is of great importance due to the increased risk of infection as a result of changes in the immune system with aging and the weak immune response against these infections. In this study, it was aimed to determine the pneumococcal and influenza vaccination awareness and vaccination rates of individuals aged 65 years and over, to direct the unvaccinated individuals to be vaccinated and to protect them from possible infections and complications.

Methods: A questionnaire form was filled in by asking demographic information, awareness of influenza and pneumococcal vaccines and the status of vaccination of individuals aged 65 years and over who applied to the clinic between June and September 2020. Unvaccinated individuals were referred to the vaccination unit.

Results: A total of 553 elderly individuals aged 65 years and over, 338 women and 215 men, were included in the study. Among the study participants, the rate of patients with awareness of influenza/pneumococcal vaccine was 48.5%. Although the vaccination awareness rate was higher in males, non-smokers, those with higher education level, those with at least one of the comorbidities such as hypertension, coronary artery disease, hyperlipidemia, chronic renal failure, vaccination rates were lower in patients with these comorbidities and additionally diabetes mellitus. The majority of individuals with awareness were informed by healthcare workers. The rate of vaccination among the individuals participating in the study was 18.4%. Vaccination was higher in individuals with awareness. While 5.3% of those who were not vaccinated were hospitalized for pneumonia, those who were vaccinated did not have pneumonia severe enough to require hospitalization.

Conclusion: In order to fight with the increasing vaccine hesitancy in our society, healthcare workers should be educated, the media should be supported, and vaccination should be encouraged in every clinic application.

Keywords: Aging, pneumococcus, influenza, vaccine awareness

INTRODUCTION

Aging is a biological process resulting from the accumulation of various molecular and cellular damage over time. As a result, a gradual decrease in physical and mental capacity, an increased risk of disease and finally death occurs. According to the World Health Organization (WHO) 2021 report, individuals over the age of 65 years were considered elderly. As the average life expectancy is prolonged, the population aged 65 years and over is increasing worldwide and in our country. For this reason, it is necessary to manage the health problems that may be encountered in this age group well.[1]

In the 2021 report of the Turkish Statistical Institute (TUIK), the population aged 65 years and over in our country increased by 22.5% compared to 2015 and reached 7 million 953 thousand 555 in 2020. The proportion of the elderly in the total population is increased by 1.3% compared to 2015, and reached 9.5% in 2020. According to the expectations, the proportion of the elderly in the total population is going to reach 11.0% in 2025, 12.9% in 2030, 16.3% in 2040, 22.6% in 2060 and 25.6% in 2080.[2]

Diabetes mellitus (DM), coronary artery diseases (CAD), congestive heart failure (CHF), asthma, chronic obstructive pulmonary disease (COPD), chronic renal failure (CRF), hypertension (HT), cerebrovascular events (CVO) and the risk of infection have been increased due to changes in the immune system that occur with aging.[3]

Changes that occur in the immune system with aging are called immunosense.[4] Aging of the immune system is associated with decreased immune protection, in part due to failed lymphopoiesis (immunosense). Although the changes in the adaptive immune system are more pronounced during the aging process, the innate immune system is also affected. In this process, increased inflammatory responses that occur through the activation of innate immunity (inflammation)

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also play an important role. Acquired immune system changes, on the other hand, can be counted as a decrease in B lymphocyte function with decreased immunoglobulin production and a decrease in T lymphocyte function with weakening of vaccine responses.[3,5]

An increase in autoantibody production may occur as a result of the increase in the number of memory T and B cells with immunosense associated with aging.[4] As a result of these changes, clinically increased risk of autoimmune diseases, malignancies, and infections may be observed.[6] Therefore, vaccination is of great importance due to the increased risk of infection and the weak immune response against these infections in elderly individuals.

Effective vaccination is important in preventing possible complications with infectious diseases and reducing morbidity and mortality. In our country, vaccination against influenza (seasonal flu), tetanus-diphtheria, chickenpox, and herpes zoster is recommended for individuals aged 60 years and over, and vaccination against pneumococcal infections for individuals aged 65 years and over.[7]

Influenza has been reported as the 4th most common cause of death in lower respiratory tract infections according to WHO 2019 data.[8] Influenza-induced respiratory failure is seen 10-30 times more frequently in elderly individuals. Although the mortality rate is less than 1/1000, deaths are mostly seen in individuals aged 65 years and over. Since the incidence and mortality of pneumococcal disease, which is another common vaccine-preventable disease in elderly individuals, increase significantly over 65 years of age, pneumococcal vaccination is recommended especially for this population.[3] Almost 90% of deaths due to influenza and pneumonia occur in individuals aged 65 years and over. The effect of the vaccine in preventing the disease is 40%-60% in all age groups.[9]

Vaccination rates of patients aged 65 years and over, which is a fragile population, may be low due to insufficient knowledge, misconceptions, deficient guidance, or bad experiences about vaccines. In this study, it was aimed to determine the pneumococcal and influenza vaccination awareness and rates of individuals aged 65 years and over, to direct unvaccinated individuals to get vaccinated and to protect them from possible infections and related complications.

METHODS

The study was carried out with the permission of Kartal Dr. Lütfi Kırdar Training and Research Hospital Clinical Researches Ethics Committee (Date: 27.05.2020, Decision No: 2020/514/178/15). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written informed consent was obtained from the patients included in the study.

Study Design

Individuals at aged 65 years and over who applied to the internal medicine clinic for any reason, between June and September 2020, were included in the study. Demographic information, chronic diseases, reasons for applying to the clinic, knowledge of influenza and pneumococcal vaccines of the patients, whether they had been vaccinated before, and if not, the reasons, were filled in a questionnaire. Volunteers over the age of 65 years, without severe neurological or psychiatric disorders, without terminal malignancies, and who could give their consent, were included in the study. Individuals who did not meet these criteria were not included in the study. Elderly individuals who participated in the study and were found to be unvaccinated were directed to the vaccination unit in the hospital.

Statistical Analysis

The data obtained were evaluated by descriptive statistics (number, percentage distribution, mean, median, standard deviation, etc.), continuous numerical variables, t-test in independent groups, one-way analysis of variance. Categorical variables were evaluated using the Pearson chi-square test, and Fisher's exact test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Demographic Data

A total of 553 elderly individuals [338 women (61.1%) and 215 men (38.9%)] were included in the study. The mean age of the individuals participating in the study was 71.2±5.3 years and the mean body mass index (BMI) was 29.3±5.5 kg/m². When the educational status of the patients was examined, 26.6% were illiterate (n:147), 14.5% were literate (n:80), 48.3% were primary school graduates (n:267), 6.7% of them were high school graduates (n:37), 2.5% of them were university graduates (n:14) and 1.4% of them were master graduates (n:8). 75.4% of the patients were married (n:417), 8% of them were smoking (n:44) and 1.6% were using alcohol (n:9). In the study, 90.1% (n:498) of the individuals had at least one comorbidity. When examined in terms of comorbidities, HT (n:352) was the first with 63.7%, followed by DM (n:254) with 45.9%, hyperlipidemia (HL) (n:116) with 21%, CAD (n:101) with 18.3%, thyroid diseases and COPD (n:63) with 11.4%, CRF (n: 37) with 6.7%, CHF, CVO, and rheumatological diseases (n: 20) with 3.6%, depression and malignancies (n: 14) with 2.5%, inflammatory bowel disease (IBD) and cirrhosis (n:4) with 0.7% (**Table 1**).

Table 1. Participant characteristics and the reasons for not having vaccinated

	N
Gender	
Female	338 (61.1%)
Male	215 (38.9%)
Age, year (SD)	71.2 ±5.3
BMI, kg/m² (SD)	29.3 ±5.5
Education	
Illiterate	147 (26.6%)
Literate	80 (14.5%)
Primary School	267 (48.3%)
High School	37 (6.7%)
University	14 (2.5%)
Master	8 (1.4%)
Marrital Status	
Married	417 (75.4%)
Single	136 (24.6%)
Comorbidity	
+	498 (90.1%)
-	55 (9.9%)
Comorbidities	
HT	352 (63.7%)
DM	254 (45.9%)
HL	116 (21%)
CAD	101 (18.3%)
Thyroid disease	63 (11.4%)
COPD	63 (%11.4)
CRF	37 (6.7%)
CHF	20 (3.6%)
CVO	20 (3.6%)
Rheumatological disease	20 (3.6%)
Depression	14 (2.5%)
Malignancy	14 (2.5%)
IBD	4 (0.7%)
Cirrhosis	4 (0.7%)
Reasons for not having vaccinated	
Thinking that it was unnecessary	54 (32.53%)
Out of reach of the vaccine	35 (21.08%)
Lacking physician's recommendation	28 (16.9%)
Thinking that it was harmful	19 (11.44%)
Neglection	18 (10.84%)
Unwillingness	7 (0.42%)
Afraid of needle	4 (0.24%)
History of severe infection after the previous vaccination	1 (0.06%)

CAD: Coronary artery diseases, CHF: Congestive heart failure, DM: Diabetes mellitus, HL: Hyperlipidemia, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure, CVO: Cerebrovascular events, IBD: Inflamatory bowel disease

Individuals applied to the clinic for general examination by 70.4% (n:386), DM control by 12.4% (n:68), pain in various parts of the body by 6.2% (n:34), dyspeptic complaints by 6% (n:33), HT control by 2.6% (n:14), respiratory symptoms by 1.3% (n:7) and complaint of weakness by 1.1% (n:6).

Vaccine Awareness

The rate of patients who were aware of influenza/pneumococcal vaccination was recommended among individuals aged 65 years and over who participated in the study was 48.5% (n:268). The mean age of individuals with vaccine awareness (70.6±4.9 years) was younger than individuals without awareness of vaccination (71.8±5.6 years) (p:0.005).

In the study, the rate of vaccine awareness was higher in males (n: 120) by 55.8% than in females (n: 148) by 43.8% (p:0.006). It was observed that patients with higher education levels had awareness of vaccination (p:0.001). While 50.4% of individuals with comorbidities were aware of the recommendation for vaccination in individuals aged 65 years and over. The vaccination awareness level was 30.9% in individuals without comorbidities (p:0.006). Vaccine awareness was significantly higher in patients with HT (51.98% vs. 48.02%; p:0.028), CAD (60.4% vs. 39.6%; p:0.008), (61.21% vs. 38.79%; p: 0.002), CRF (64.87% 35.13%; p:0.039) comorbidities. On the other hand, there was no difference in vaccine awareness among those with CHF, DM, COPD, IBD, CVO, depression, malignancy, thyroid disease, rheumatological disease, and cirrhosis (p>0.05). Demographic characteristics and comorbidities of individuals aged 65 years and over, participating in the study according to their vaccination awareness were given in **Table 2**.

Table 2. Vaccine awareness in individuals aged 65 years and over.

	Unaware of vaccination (n:285)		Aware of vaccination (n:268)		p
Age, year (SD)	71.8	(±5.6)	70.6	(±4.9)	0.005*
BMI, kg/m² (SD)	29.4	(±5.9)	29.2	(±4.9)	0.778*
Gender					0.006+
Female	190	(56.2%)	148	(43.8%)	
Male	95	(44.2%)	120	(55.8%)	
Education					0.001++
Illiterate	104	(36.5%)	43	(16.0%)	
Literate	49	(17.2%)	31	(11.6%)	
Primary School	111	(38.9%)	156	(58.2%)	
High School	12	(4.2%)	25	(9.3%)	
University	7	(2.5%)	7	(2.6%)	
Master	2	(0.7%)	6	(2.2%)	
Comorbidity +	247	(86.7%)	251	(93.7%)	0.006+
Comorbidities					
HT	169	(48.02%)	183	(51.98%)	0.028+
CAD	40	(39.60%)	61	(60.40%)	0.008+
CHF	11	(55.00%)	9	(45.00%)	0.752+
DM	127	(50.00%)	127	(50.00%)	0.505+
HL	45	(38.79%)	71	(61.21%)	0.002+
COPD/Asthma	26	(41.26%)	37	(58.74%)	0.083+
Cirrhosis	0	(0.0%)	4	(100%)	0.055++
CRF	13	(35.13%)	24	(64.87%)	0.039+
CVO	9	(45.00%)	11	(55.00%)	0.551+
Depression	4	(28.57%)	10	(71.43%)	0.082++
Malignancy	6	(42.85%)	8	(57.15%)	0.510+
IBD	2	(50%)	2	(50%)	0.665++
Rheumatological disease	9	(45.00%)	11	(55.00%)	0.551+
Thyroid disease	27	(42.85%)	36	(57.15%)	0.143+

*: Student's t-test; +: Pearson Chi-Square; ++: Fisher's Exact Test; p<0.05; HT: Hypertension, CAD: Coronary artery diseases, CHF: Congestive heart failure, DM: Diabetes mellitus, HL: Hyperlipidemia, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure, CVO: Cerebrovascular events, IBD: Inflamatory bowel disease

In the study, 73.1% (n:196) of individuals who had the awareness that vaccination is recommended for elderly individuals stated that they learned this information from healthcare workers, 18.7% from the media (n:50), and 8.2% (n:22) from their friends. While the rate of awareness was 47.4% (n: 93) in individuals who had the information from healthcare workers, 8% (n: 4) in individuals who had the information from the media, and 22.7% (n:5) in individuals who were informed through friends. Healthcare workers were more effective in the vaccine awareness of individuals aged 65 years and over than other referrals (p:0.001).

Vaccination Situations

While the rate of vaccination among individuals participating in the study was 18.4% (n:102), the rate of vaccination among individuals with awareness of vaccination was 38.1% (n:102). All of the individuals who had the vaccine had awareness of the vaccine. Of those who were aware of the vaccine, 16.66% (n: 17) had only the pneumococcal vaccine, 47.07% (n: 48) had only the influenza vaccine, while 36.27% (n: 37) had both pneumococcal and influenza vaccines. Among the individuals participating in the study, the rate of those who were aware of the vaccine but did not get vaccinated was 61.9% (n:166). When the reasons for vaccine refusal were examined; 32.53% (n:54) considered the vaccine unnecessary, 21.08% (n:35) could not reach the vaccine, 16.9% (n:28) were not recommended by the physician, 11.44% (n:19) thought it was harmful, 10.84% (n:18) neglected it, 0.42% (n:7) simply did not want it, 0.24% (n:4) had a fear of needle and 0.06% (n:1) had a history of severe infection after the previous vaccination (**Table 1**).

While the mean age of the vaccinated individuals was 70.67±4.6 years, the mean age of the non-vaccinated individuals was 71.36±5.4 years, and there was no difference between the two groups (p:0.238). When the distribution of vaccinated individuals by gender was examined, the rate of vaccination was 21.86% (n:47) for males, 16.27% (n:55) for females, and there was no difference in terms of gender (p:0.12). When vaccinated individuals were examined in terms of their educational status, 24.5% were illiterate (n:25), 9.80% were literate (n:10), 53.90% were primary school graduates (n:55), 7.80% were high school graduates (n:8), 2.90% were university graduates (n:3), and 1% were master graduates (n:1). There was no significant relationship between education status and vaccination rates (p:0.352) (**Table 3**).

Of the participants with at least one comorbidity, 20.12% were vaccinated (n:100) in the last year, while 79.87% were unvaccinated (n:397). The majority of individuals with at least one comorbidity were unvaccinated (p:0.003). When the status of getting vaccinated in terms of comorbidities was examined, the vaccination rate was low in patients with HT, CAD, DM, HL, and CRF, and there was a difference when compared to those who were not vaccinated (respectively; p:0.011, p:0.001, p:0.002, p:0.001; p:0.007). There was no difference in individuals with CHF, COPD/asthma, cirrhosis, CVO, depression, malignancy, IBD, rheumotologic, and thyroid diseases (p>0.05) (**Table 3**).

Table 3. Results of patients according to vaccination status in the last year.

	Not vaccinated (n:451)		Vaccinated (n:102)		p
Age, year (SD)	71.36	(±5.4)	70.67	(±4.6)	0.238*
BMI, kg/m² (SD)	29.4	(±5.5)	29.0	(±5.4)	0.559*
Gender					0.102+
Female	283	(83.73%)	55	(16.27%)	
Male	168	(78.14%)	47	(21.86%)	
Education					0.352++
Illiterate	122	(27.10%)	25	(24.50%)	
Literate	70	(15.50%)	10	(9.80%)	
Primary School	212	(47.00%)	55	(53.90%)	
High School	29	(6.40%)	8	(7.80%)	
University	11	(2.40%)	3	(2.90%)	
Master	7	(1.60%)	1	(1.00%)	
Comorbidity +	397	(79.87%)	100	(20.12%)	0.003+
Comorbidities					
HT	276	(78.40%)	76	(21.60%)	0.011+
CAD	67	(66.33%)	34	(33.67%)	0.001+
CHF	15	(75.00%)	5	(25.00%)	0.392+
DM	193	(75.98%)	61	(24.02%)	0.002+
HL	49	(77.77%)	14	(22.23%)	0.001+
COPD/Asthma	3	(75.00%)	1	(25.00%)	0.377++
Cirrhosis	24	(64.86%)	13	(35.14%)	0.559+
CRF	17	(85.00%)	3	(15.00%)	0.007++
CVO	12	(85.71%)	2	(14.29%)	0.478++
Depression	13	(92.85%)	1	(7.15%)	0.505++
Malignancy	2	(50.00%)	2	(50.00%)	0.484++
IBD	16	(80.00%)	4	(20.00%)	0.157++
Rheumatological disease	51	(8.95%)	12	(19.05%)	0.774+
Thyroid disease	81	(69.82%)	35	(30.18%)	0.508+

*: Student's t-test; +: Pearson Chi-Square; ++: Fisher's Exact Test; p<0.05; HT: Hypertension, CAD: Coronary artery diseases, CHF: Congestive heart failure, DM: Diabetes mellitus, HL: Hyperlipidemia, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure, CVO: Cerebrovascular events, IBD: Inflamatory bowel disease

In individuals who did not get vaccinated, the rate of influenza/pneumonia disease, severe enough to require consulting a physician in the last year, was 29.6% (n:133), while it was 21.6% (n:80) in individuals who had been vaccinated, and there was no difference between the groups (p:0.105). While 5.3% of the unvaccinated individuals were hospitalized due to pneumonia, the vaccinated individuals did not develop pneumonia severe enough to require hospitalization (p: 0.594).

DISCUSSION

In this study, it was aimed to determine the pneumococcal and influenza vaccination awareness and vaccination rates of elderly individuals aged 65 years and over and to direct the unvaccinated elderly individuals to be vaccinated and to protect them from possible infections and complications. Among the individuals participating in the study, the rate of patients with influenza/pneumonia vaccine awareness was 48.5%. Vaccine awareness rate was higher in males, those with higher education levels, and those with at least one of the comorbidities such as HT, CAD, HL, CRF, compared to those without. It was determined that the majority of individuals with awareness were informed by healthcare workers. While the rate of vaccination among the individuals participating in the study was 18.4%, the rate of vaccination in individuals with awareness of vaccination was 38.1%. In a study conducted with a total of 14308 elderly people, it was seen that 44.7% of the elderly individuals were aware of the vaccines recommended at elderly.[10] In the same study, it was determined that 64.5% of the elderly did not receive information about vaccination, and 45.1% of those who had information obtained the information from doctors, nurses, and other healthcare workers. In another study investigating the source of information about vaccination requirements and the factors affecting vaccination, it was recommended that the source and effective factors were their doctors by 76.93% and 70.41% respectively.[11] In another study, it was shown that individuals learned about the pneumococcal vaccine from social media at a rate of 46.5% and from a family physician at a rate of 54.5%.12 Similar to the information obtained from the literature, 48.5% of the individuals participating in this study stated that they knew that individuals over the age of 65 years were recommended to have the influenza/pneumonia vaccine. In our study, it was determined that the main source of vaccination awareness was healthcare workers. It is visible that healthcare workers have important duties to increase the vaccination rate since they are more effective in patients' decisions in terms of gaining community immunity. Within the scope of preventive medicine practices, adequate information training should be given to individuals in the risk group, especially those aged 65 years and over, about vaccines, the importance of vaccination should be explained, and seminars, meetings, public service announcements, etc. should be held regularly every year. It should also be reminded frequently with activities. In addition, the importance of getting vaccinated should be emphasized through social media and public service announcements, and vaccine hesitancy should be fought. In this study, the vaccination awareness rate of males was 55.8% and females was 43.8%. The mean age of patients with awareness was lower than those without. It was observed that patients with higher education levels had awareness of vaccination. However, it was observed that vaccination rates did not change according to education level. Similar to our study, Akman et al.'s[13] study showed that as the education level of the participants increased, the awareness of vaccination increased, but the rate of vaccination also increased in their study unlike our study. Similarly, in another study, it was found that both influenza and pneumococcal vaccination rates and awareness of the participants were increased with increasing levels of education.[14] As a result of the increase in education level, it can be thought that the increase in the confidence of individuals in positive science by moving away from cultural prejudices and superstitions may be effective in the emergence of this result. While 50.4% of individuals with comorbidities were aware of the recommendation for vaccination in the elderly aged 65 years and over, this awareness level was only 30.9% in those without comorbidities. When the vaccination awareness status of individuals with comorbidities was examined, it was found that vaccination awareness was higher in those with HT (51.98%), CAD (60.4%), HL (61.21%), and CRF (64.87%). While 98% of those who were vaccinated in the last year had at least one comorbidity, 88.2% of those who were not vaccinated had at least one comorbidity. In our study, 20.12% of those with at least one comorbidity were vaccinated with influenza and/or pneumococcal vaccine within the last year, while 79.87% were not. When the status of being vaccinated in terms of comorbidities was examined, the vaccination rate was lower in patients with HT (78.40%), CAD (66.33%), DM (75.98%), HL (77.77%) and CRF (85.00%). There was no difference in individuals with CHF, COPD/asthma, cirrhosis, CVO, depression, malignancy, IBD, rheumatologic and thyroid diseases. In this study, although the vaccination awareness of patients with HT, CAD, HL, and CRF was higher, the vaccination rates were low. False beliefs, vaccine hesitancy, psychological status, and the patient's desire not to wait in line in the vaccination units or difficulty in transportation, etc. thought to be the causes of low vaccination rates. Similarly, in the presence of a comorbidity that requires regular drug use, influenza vaccination had been found to decrease significantly.[13] As a result, it could be expected that individuals with comorbidities have a higher risk of infectious diseases such as pneumonia and influenza compared to the other population, so it could be expected that these individuals would have a higher vaccination rate but, as seen in the literature and this study, although the vaccination awareness rate of individuals with comorbidities was high, vaccination rates were low. There is a need for studies that examine both psychological and social aspects of why patients are not vaccinated despite their awareness of vaccination.

When the vaccination status of the patients in the last year was examined, it was found that 18.4% of all patients and only 38.1% of those who were aware of vaccination were vaccinated. All of the individuals who had the vaccine had awareness of the vaccine. It was determined that 16.66% of those who were aware of the vaccine had only the pneumococcal vaccine, 47.07% had only the influenza vaccine, and 36.27% had both pneumococcal and influenza vaccines together. Among the individuals participating in the study, the rate of those who were aware of the vaccine but did not get vaccinated was 61.9%. In a study conducted with 147 patients aged 65 years and over, it was reported that the rate of getting at least one of influenza, pneumococcal, herpes zoster, and tetanus vaccines was 53.7%.[15] The high rate of vaccination may be due to the small population and the fact that research has been investigated for 4 vaccines. Similar to our study, in the study of Erdoğdu et al.[16] investigating people aged 65 years and over, the percentage of getting at least one of influenza, pneumococcal, and herpes zoster vaccines was 12.5%. According to a study conducted in the USA, the rate of influenza vaccination of people aged 65 years and over was 67% and the rate of pneumococcal vaccination was 60%, and these rates were interpreted as low.[17] According to the results of this study, influenza and pneumococcal vaccination rates in Turkey are lower than the USA data.

Among the individuals participating in the study, the rate of those who had awareness of vaccination but not vaccinated was 61.9%. When the reasons for not having vaccinated were examined; thinking that it was unnecessary (32.53%), out of reach of the vaccine (21.08%), lacking physician's recommendation (16.9%), thinking that it was harmful (11.44%), neglection (10.84%), unwillingness (0.42%), afraid of needle (0.24%), and a severe infection after the previous vaccination (0.06%). When the literature was examined, in a study, the reasons for not having vaccinated for the individuals aged 65 years and over were respectively; lack of knowledge about vaccines, unwillingness, thinking that vaccines are not protective, thinking that vaccines have side effects.[10] Similarly, in another study, 60.3% of the participants reported that they did not get vaccinated because they did not receive information about vaccination from a health institution or doctor.[15] In our study, the rate of influenza/pneumonia infection severe enough to require consulting a physician within the last year was 29.6% (n:133) in individuals who did not get the vaccine, while it was 21.6% (n:80) in individuals who had been vaccinated, and there was no difference between the groups. Pneumonia that required hospitalization did not occur in individuals who were vaccinated while 5.3% of the individuals who did not been vaccinated were hospitalized for pneumonia. It can be thought that vaccination in the elderly population prevents hospitalizations due to these infections. As a result of a study conducted on 2918 patients with an average age of 62.1, the vaccination rates for influenza and pneumococci were found to be very low. In this study, the influenza vaccination rate was 12.3%, while the pneumococcal vaccination rate was 3%. Only 2.8% of the individuals participating in the study had both vaccines together. While 95.3% of the patients declared that they were not aware of both influenza or pneumococcal vaccines, 83.2% of them declared that the doctors did not recommend the influenza vaccine and 96% of them declared that the doctors did not recommend pneumococcal vaccines. Low vaccination rates have been associated with these conditions.[18] Compared to this study, it was observed that the vaccination rates in our study were higher. The reasons for not getting vaccinated were similar in both studies. In a study conducted on 642 people aged 65 years and over in Spain, the rate of influenza vaccination was found to be 68%.[19] In another study evaluating the vaccination status of individuals over the age of 65 years, the rate of influenza vaccine, which was the most administered vaccine, was 26.5%. In the same study, the vaccination rates for influenza and pneumococci in males were 31.3% and 3.7%, respectively, while the rates were 22.3% and 3.7% in females, respectively. Similarly in our study, the rate of males having influenza and/or pneumococcal vaccines was 21.86%; while 16.27% of females were vaccinated. The vaccination rate of males was found to be high in both studies.[13] The reasons for high rates of male vaccination may be associated with that, males were more likely to perceive infectious diseases as very dangerous than females, and they mostly trusted scientists, they had a lesser tendency to believe in conspiracies from vaccines compared to females who relied more on social media platforms.[20,21]

The limitations of this study was single-centered, the small sample size, the research was conducted for only two vaccines, and the psychological aspects of the patients were not examined.

CONCLUSION

In recent years, with the effect of social and visual media, vaccine hesitancy has emerged. People who have insufficient knowledge about vaccines believe that vaccines can be harmful to health due to some chemical substances in their contents. For this reason, vaccine indecision as well as vaccine hesitancy is increasingly taking place in society.

In the fight against these factors, the communication of health professionals and physicians with individuals and the trust they give to them, as well as the Ministry of Health, are of great importance. For this reason, training

programs should be organized to increase the knowledge of healthcare professionals about the vaccination of individuals aged 65 years and over. With the support of social and visual media organizations, vaccine hesitancy and indecision should be combated. In all applications of individuals in the risk group to health institutions, vaccination should be encouraged by informing them.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kartal Dr. Lütfi Kırdar Training and Research Hospital Clinical Researches Ethics Committee (Date: 27.05.2020, Decision No: 2020/514/178/15).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Evaluation of gynecology and gynecologic oncology cases who received massive blood transfusion: a tertiary center experience

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ABSTRACT

Aims: To examine the frequency, indications and results of massive blood transfusion in gynecology and gynecological oncology cases.

Methods: The data of 56 cases who were underwent massive blood transfusion and operated on for benign/ malignant pathology indications in the gynecology and gynecological oncology clinics between October 1, 2022 and August 1, 2023, within a period of 10 months, were retrospectively analyzed. Demographic data of the cases (age, gravida, parity, body mass index), indications for hospitalization, vital signs during hospitalization, hemoglobin (Hb), hematocrit (Htc), platelet and INR values, massive transfusion indications, transfused blood products (erythrocyte suspansion, fresh frozen plasma (FFP), pooled platelet suspension, cryoprecipitate, fibrinogen) and the length of stay in the intensive care unit and hospitalization were retrospectively screened and analyzed statistically. The statistical significance level was accepted as $p<0.05$.

Results: 56 (1.8%) of 3146 patients were received massive blood transfusion. Massive blood transfusion was given to 30 (1.4%) of 2093 inpatients in the gynecology clinic, while this rate was found to be 2.5% (26/1053) in gynecologic oncology patients. The time between the decision to start transfusion and total transfusion times were similar between the groups ($p>0.05$). However, when the decision for transfusion was made, the INR value was statistically significantly higher in gynecological oncology cases ($p=0.001$). While the amounts of erythrocyte suspension given were similar between the two patient groups (5.1±1.4 vs. 6.3±3.5 U, $p= 0.082$), FFP amounts were higher in the gynecologic oncology group (3.3±2.0 vs. 6.2±3.7 U, $p=0.001$). When the blood groups of the cases were examined, it was seen that the most common blood groups were O (+) (n= 18, 32.1%) and A (+) (n=16, 28.6%). The duration of stay in the intensive care unit and hospitalization of gynecological oncology cases was significantly longer in gynecological cases. While 1 of 56 patients who underwent massive blood transfusion died (gynecological oncology case), 55 patients were discharged.

Conclusion: Timely transfusion decision is safe and life-saving in massive hemorrhages.

Keywords: Gynecology, gynecologic oncology, massive blood transfusion

INTRODUCTION

Massive hemorrhage has been described in many different ways in the literature. A few of these are >10 units over 24 h; total blood volume replaced within 24 h; 50% of total blood volume replaced within 3 h; four units of red blood cells (RBCs) transfused within 4 h with active major bleeding of more than 150 ml/min; three units RBCs administered over 60 min.[1] The replacement of these amounts is considered as massive blood transfusion. In the Patient Blood Management Guide published by the Ministry of Health, in Turkey, massive transfusion for adults is considered if more than half of the blood volume is transfused within 4 hours or more than the total blood volume (approximately 70 ml/kg of blood volume in adults) within 24 hours.[2]

In this study, our aim is to examine the frequency and indications of massive blood transfusion in gynecology and gynecological oncology cases in a tertiary center, as well as to investigate whether there is a difference between this group in terms of demographic and clinical characteristics.

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METHODS

The study was carried out with the permission of Ankara Etlik City Hospital No: 1 Clinical Researches Ethics Committee (Date: 16.08.2023, AEŞH-EK1-2023-482). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In our study, which was designed as a observational retrospective study, the data of 56 cases who were operated on for benign and malignant pathology indications and underwent massive blood transfusion in the gynecology and gynecological oncology clinics between October 1, 2022 and August 1, 2023 were retrospectively analyzed.

In this study, the criteria for massive blood transfusion were >10 U within 24 hours or 4 U of Erythrocyte suspension (ES) replacement within 4 hours massive hemorrhage. Demographic data of cases (age, gravida, parity, body mass index), indications for hospitalization, vital signs during hospitalization, hemoglobin (Hb), hematocrit (Htc), platelet and INR values, and massive transfusion indications from the hospital database and medical files, transfused blood products (erythrocyte suspension, fresh frozen plasma, pooled platelet suspension, cryoprecipitate, fibrinogen), and length of stay in the intensive care unit and hospital stay were recorded. After evaluating the data of all cases who underwent massive transfusion, the cases were divided into 2 groups as gynecological cases and gynecological oncology cases. Data were also compared between the 2 groups.

SPSS (Statistical Package for Social Sciences) for Windows version 22.0 software was used for the statistical analysis of the data obtained in our study Descriptive and categorical data were expressed as numbers (n) and percentage (%). The results of the continuous data were given as mean±SD, median, and minimum-maximum values. The mean values of the data according to the groups were made using the Independent Sample -T test. A p value of <0.05 was considered statistically significant.

RESULTS

In our study, in which the gynecology and gynecological oncology data of our hospital were examined retrospectively, it was observed that a total of 3146 patients were hospitalized in an 8-month period. 56 of these patients received massive blood transfusion and the rate was calculated as 1.78%. When we separate cases in terms of clinics, massive blood transfusion was adminestered to 30 (1.4%) of 2093 patients in the gynecology clinic, while this rate was found to be 2.5% (26/1053) in gynecologic oncology patients.

The mean age of the gynecological oncology cases was significantly higher than the gynecological cases (p=0.001). Gravida, parity, and body mass index (BMI) were similar between the two groups (p>0.05) (**Table 1**). The most common indication for hospitilazition was severe anemia (Hb<7 g/dl) (n=14, 46.7%) in gynecological cases due to severe abnormal uterine bleeding, which constitutes almost half of the cases. In gynecological oncology cases, the most common indication was ovarian cancer (n=14, 53.8%) (**Table 2**).

Hemoglobin (Hb) and hematocrit (Htc) levels at hospitalization were found to be significantly lower in gynecological cases (respectively 8.1±3.4 g/dl vs. 10.7±2.2 g/dl, p=0.002; 26.5%±9.5 vs 33.7±6.6%, p=0.002). In 30 (53.6%) of 56 cases who underwent massive blood transfusion, the Hb value was below 10 g/dl at the time of hospitalization. In 16 of them (28.6%), emergency transfusion was initiated due to the presence of severe anemia (Hb<7 g/dl) at the time of hospitalization without surgery-related bleeding. 14 of 16 cases were admitted to the gynecology clinic due to severe abnormal uterine bleeding. The remaining 2 cases were diagnosed with ovarian cancer and had severe anemia. The hospitalization Hb value of 16 patients with severe anemia was 5.3±1.4 (median: 5.7; min: 2.4- max: 7.0) gr/dl. Indications requiring massive blood transfusion in the remaining 40 cases are shown in **Figure**.

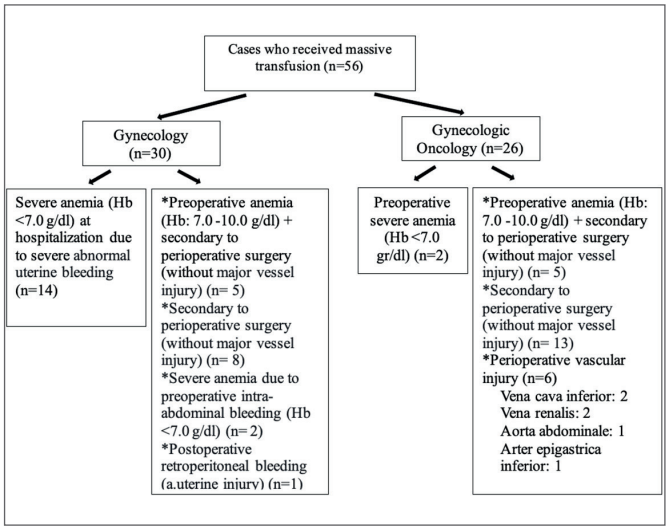

Figure. Indications for massive transfusion

Vital signs and shock index values were similar between clinics when deciding on transfusion due to surgery perioperatively (p>0.05). Likewise, the time between the decision to start transfusion and the total transfusion times were similar between the groups (p>0.05). However, the INR value was statistically significantly higher in gynecological oncology cases at the time of transfusion decision (p=0.001) (**Table 1**).

Table 1. Distribution of demographic, clinical and laboratory characteristics of cases

	All cases (n=56)	Gynecological cases (n= 30)	Gynecological oncology cases (n=26)	p
	Mean±SD (Median; minimum-maximum)	Mean±SD (min-max)		
Age	49.1±11.0 (49.0; 16 -73)	44.6±10.4 (25-69)	54.3±9.4 (38 -73)	0.001
Gravity (n)	3.0±2.5 (3; 0-13)	3.2±2.9 (0-13)	2.8±1.8 (0-8)	0.480
Parity (n)	2.7±1.4 (2; 0-7)	2.7±1.3 (0-6)	2.7±1.5 (0-7)	0.944
BMI (kg/m2)	28.3±5.9 (27.3; 18.7-43.0)	27.6±6.3 (18.7-43.0)	29.2±5.6 (20.0-42.5)	0.292
On admission to hospital				
Hb (g/dl)	9.3±3.2 (9.4; 2.4-14.7)	8.1±3.4 (2.4 -14.7)	10.7±2.2 (6.4-14.5)	0.002
HTC (g/dl)	29.9±9.0 (31.2; 10.5-47.0)	26.5±9.5 (10.5-45.8)	33.7±6.6 (19.6-47.0)	0.002
Platelets (x10 3)	317±145 (293; 71-767)	294±99 (78-641)	343±183 (71-767)	0.206
INR	1.1±0.2 (1.0; 0.9-1.8)	1.1±0.2 (0.9-1.8)	1.1±0.1 (1.0 -1.4)	0.219
Time between the decision of perioperative transfusion and the start (min)	58±20 (61; 15-95)	61±10 (47-75)	56±24 (15-95)	0.512
Total transfusion time (hours)	5.3±1.0 (5.6; 1.8-6.0)	5.1±1.1 (1.9-6.0)	5.4±0.9 (1.8-6.0)	0.214
When the transfusion is started				
BP (mmHg)				
systolic	108±15 (106; 70-138)	105±14 (70-129)	110±16 (75 -138)	0.211
diastolic	65±10 (66.5; 40-86)	65±10 (40-80)	65±10 (44-86)	0.877
heart rate (/ min)	87±14 (87; 60-131)	86±14 (62-130)	88±15 (60-114)	0.672
shock index	0.8±0.2 (0.8; 0.5-1.4)	0.8±0.2 (0.6-1.4)	0.8±0.2 (0.5 -1.2)	0.605
Hb (g/dl)	8.7±2.5 (9.2; 2.4-13.8)	7.7±2.8 (2.4 -13.8)	9.7±1.5 (6.2-11.9)	0.002
HTC (%)	27.7±7.0 (28.1; 10.5-39.6)	25.0±7.7 (10.5-39.6)	30.7±4.6 (19.7-38.9)	0.002
Platelets (x10 3)	262±115 (233; 78-767)	237±80 (78-474)	291±143 (108-767)	0.077
INR	1.1±0.2 (1.1; 0.9-1.8)	1.0±0.1 (0.9-1.3)	1.2±0.2 (1.0-1.5)	0.001
During hospital stay				
lowest Hb (g/dl)	7.1±20. (7.0; 2.4-10.9)	6.6±2.1 (2.4 -10.8)	7.7±1.7 (5.5 -10.9)	0.047
lowest HTC (%)	22.5±5.0 (22.9; 10.5-33.6)	21.7±5.8 (10.5-33.6)	23.3±3.8 (17.2-29.9)	0.256
lowest Platelet ($x10^3$)	192±115 (173; 23-764)	188±74 (39-464)	196±150 (23 -764)	0.794
highest INR	1.3±0.5 (1.1; 0.9-4.3)	1.1±0.2 (0.9-2.0)	1.4±0.6 (1.0-1.7)	0.045
On discharge				
Hb (g/dl)	10.1±1.1 (10.2; 7.9-13.3)	9.8±0.9 (7.9-11.7)	10.3±1.3 (8.0-13.3)	0.135
HTC (%)	31.8±3.4 (31.5; 24.8-42.6)	31.1±2.5 (25.0-36.9)	32.5±4.1 (24.8-42.6)	0.106
Platelets (x10 3)	327±177 (256; 114-877)	253±95 (134 -510)	415±211 (114-877)	0.001
INR	1.1±0.1 (1.0; 0.9-1.6)	1.0±0.1 (0.9-1.3)	1.1±0.1 (0.9-1.6)	0.031
Length of stay in intensive care (days)	2.4±2.2 (1; 1-7)	1.1±0. 4 (1-2)	2.9±2.4 (1-7)	0.006
Length of stay in hospital (days)	9.7±6.7 (8; 2-29)	6.4±4.4 (2-20)	13.5±7.0 (4-29)	<0.001

Table 2. Distrubition of diagnoses (n=56)

Hospitalization diagnoses	n
Gynecology cases (n=30)	
Severe anemia (Hemoglobine <7 g/dl) + gynecological pathology (abnormal uterine bleeding; myoma uteri)	14
Myoma uteri	8
Adnexal mass	4
Intraabdominal bleeding (ectopic pregnancy rupture, ovarian cyst rupture)	2
Postmenopausal bleeding	one
Uterine prolapse	one
Gynecological oncology cases (n=26)	
Ovarian cancer	14
Endometrial cancer	5
Recurrent gynecological malignancy	3
Uterine sarcoma	2
Suspected adnexal mass	2

The most common blood groups were O (+) (n=18, 32.1%) and A (+) (n=16, 28.6%) among cases. (**Table 3**). While the amounts of erythrocyte suspension given were similar between the two patient groups (5.1±1.4 vs. 6.3±3.5 U, p=0.082), FFP amounts were higher in the gynecologic oncology group (3.3±2.0 vs. 6.2±3.7 U, p=0.001). Pooled platelet transfusion was performed in only 2 of 56 cases (1 U in 1 case, 2 U in 1 case) and cryoprecipitate (40 U) in one patient. The amount of fibrinogen is also significantly higher in gynecological oncology cases (p=0.037) (**Table 4**).

Table 3. Distribution of cases in terms of blood group

ABO	Rh / rh Antigen	n	%
HE	- +	3 18	5.4 32.1
A	- +	3 16	5.4 28.6
B	- +	one 10	1.8 17.9
EU	- +	- 5	8.9

Table 4. Amounts of blood products transfused

	All cases (n=56)	Gynecology cases (n= 30)	Gynecological oncology cases (n=26)	p
	Mean±SD (Median; min-max)	Mean±SD (min-max)		
Erythrocyte suspension (U)	5.6±2.7 (5; 4-17)	5.1±1.4 (4-9)	6.3±3.5 (4-17)	0.082
Fresh frozen plasma (U)	4.7±3.2 (4; 1 -15)	3.3±2.0 (1-8)	6.2±3.7 (2-15)	0.001
Fibrinogen (gr)	2.4±1.0 (2; 1-6)	2.1±0.8 (1-4)	2.8±1.1 (1-6)	0.037

When the allergic reaction and transfusion-related complications in the cases who received massive blood transfusion are examined, febrile nonhemolytic transfusion reaction (high fever; >38°C) that was controlled with medication in 5 patients at the time of transfusion, acute hemolytic transfusion reaction in 2 cases (fever, chills chest and back/ low back pain) and an allergic reaction (dyspnea, urticaria) was detected in 1 case. In these cases, transfusion was temporarily suspended and continued after medication. In one case, the transfusion was terminated due to uncontrollable fever. Anaphylactic reaction (angioedema, hypotension and wheezing) was detected during transfusion in 1 case, the reaction was terminated and medical treatment was started. In 1 case, the diagnosis of Transfusion-related acute lung injury (TRALI) based on the examination performed on the development of respiratory distress, hypoxia, and hypotension during transfusion and the findings of abnormal chest X-ray, and in 1 case, dyspnea, respiratory distress, and development of hypoxia in the lungs after the end of the transfusion. A diagnosis and treatment of transfusion-associated circulatory overload (TACO) was made based on the findings on chest X-ray and abnormal chest X-ray. Two patients who developed TRALI and TACO were also gynecological oncology cases. The duration of stay in the intensive care unit and hospitalization of gynecological oncology cases was significantly longer. 1 of 56 patients who underwent massive blood transfusion died (gynecological oncology case).

DISCUSSION

In this study, the frequency of blood transfusion meeting the criteria for massive blood transfusion to patients receiving treatment in gynecological and gynecological oncology clinics in a tertiary center was 1.78%. When the need for blood transfusion is discussed in surgical clinics, it is thought that transfusion is needed because of bleeding secondary to the surgery performed first. However, cases may present with severe anemia (Hb <7 g/dl) due to abnormal bleeding originating from the uterus, which are especially specific to the gynecology clinic, and these cases may require transfusion up to massive transfusion regardless of surgery. In our study, out of a total of 56 patients who underwent massive blood transfusion, 28.6% (n=16) received massive blood transfusion regardless of surgery. Severe anemia was found in 14 of them due to severe abnormal uterine bleeding. Unless these patients have a known malignant disease, they are primarily treated in gynecology clinics due to severe anemia. These cases are also predominantly pre- and perimenopausal women. Thus, the mean age of the cases who underwent massive blood transfusion in the gynecology clinic was found to be significantly lower than the gynecological oncology group (44.6±10.4 vs. 54.3±9.4, p=0.001). In addition, hemoglobin levels at hospitalization were significantly lower in this group (8.1±3.4 vs. 10.7±2.2; p=0.002).

In our study, vital signs, shock indices, and total transfusion times of the cases were found to be similar between the groups during the perioperative decision to transfusion. This proves that there is a standard management among clinics in terms of approach to bleeding and patient blood management in our hospital.

In a study evaluating the knowledge levels of healthcare professionals about the transfusion of blood products, it was found that 60% of the questions about blood product transfusion of healthcare personnel working in our hospital were answered correctly. The highest correct response rate (73%, n: 66) was found in the field of basic transfusion information, while the least (47%, n:45) correct response rate was obtained in the storage of blood products. However, it was found that the level of knowledge about basic information and the storage of blood products did not differ between doctors and nurses or between internal and surgical clinics.[3] Knowledge and awareness about the subject is critical for the prevention of complications. To prevent errors that may arise in determining the suitability of donor components for the recipient; At the same time, it is the responsibility of the doctor and nurse who takes care of the patient to recognize the transfusion reactions and to apply the most appropriate treatment in a short time. It is defined as side effects observed during transfusion or within the

first 24 hours. Acute transfusion complications can be classified as immunological and non-immunological. Immunological transfusion reactions occur when transfused erythrocytes, leukocytes, platelets and plasma proteins stimulate antibody production in the recipient. Non-immunological reactions, on the other hand, occur due to the physical and chemical properties of the transfused blood product.[4] Rapidly transfusing large amounts of blood may cause hyperkalemia in the patient. This is more common in patients with renal failure, acidosis with shock, and hemolysis. Citrate, which is used as an anticoagulant in blood products, is metabolized in the liver. The amount of citrate increases in massive transfusion, hepatic failure and shock. Increased citrate level may also lead to mortal complications due to hypocalcemia.[4,5]

In the applications of massive blood transfusion, the general acceptance is to provide the replacement of blood content at a ratio of 1:1:1 (Erythrocyte suspension (ES): Fresh frozen plasma (FFP): Pooled platelets).[6,7] This concept is critical due to the fact that, coagulation factors and platelets are consumed simultaneously as well as the loss of erythrocyte mass during massive bleeding.[8-10] However, in our study, the ratio of ES / FFP was not 1:1 especially in gynecology cases. The reason is that severe anemia in a significant part of these cases develops as a result of a process due to severe abnormal uterine bleeding without being secondary to surgery, therefore coagulopathy does not develop and it will be sufficient to correct anemia and hypovolemia with ES replacement, even in massive amounts.[11,12] However, in this case, as a complication of massive ES transfusion without FFP administration, the development of dilutional coagulopathy by reducing the existing coagulation factors and platelets, development of hypocalcemia due to citrate toxicity and development of hyperkalemia should be vigilant.[10,13] In gynecological oncology cases, it is noteworthy that 1:1 ratio is achieved in ES/FTP. The reason for this is that replacements are made considering that coagulation factors are consumed along with erythrocyte loss during perioperative surgery.[14] Likewise, fibrinogen supplementation is used more in gynecological oncology cases. Thrombocyte replacement was performed in only 2 cases because the thrombocyte count decreased below 50 thousand during transfusion.[15,16]

Perioperative blood transfusion is associated with increased morbidity and prolonged length of stay in hospital.[17,18] In a study evaluating the incidence of perioperative blood transfusion and association with 30 day postoperative outcomes in gynecologic cancer surgery (n=62 531), the overall incidence of transfusion was found to be 9.4%. On multivariable analysis, blood transfusion was predictive of composite morbidity (adjusted odds ratio (OR) 1.65, 95% confidence interval (CI) 1.48 to 1.85) and length of stay in hospital ≥5 days (adjusted OR 9.02, 95% CI 8.21 to 9.92). Perioperative blood transfusion was the most predictive factor for composite morbidity (adjusted OR 1.67, 95% CI 1.35 to 2.07) and length of stay in hospital ≥7 days (adjusted OR 9.75, 95% CI 7.79 to 12.21).[19] Authors revealed that, preoperative patient optimization and local institutional practices should be reviewed to improve the use of blood bank resources and adherence to restrictive blood transfusion protocols.[19]

In a study comparing differences in blood transfusion and surgical complication rates before and after the implementation of a restrictive blood transfusion protocol in patients undergoing major abdominal surgery by the gynecology and gynecologic oncology services before, and after initiation of the transfusion protocol, a similar number of patients received blood transfusions in both groups (9.3% versus 10.6% p=0.57). However, significantly fewer units of blood were given post-protocol initiation. For every patient who received a transfusion pre-protocol, 2.66 units were administered compared to 1.2 units after the protocol was initiated (p=0.003). They claimed that a restrictive transfusion protocol is effective in decreasing the number of units of blood transfused without affecting postoperative complication rates in gynecologic surgery patients.[5]

CONCLUSION

In our study, especially in in massive hemorrhage secondary to perioperative surgery, the decision of transfusion was made at the stage when the patient's vital signs and coagulation factors could be compensated. In massive hemorrhages, while compensation mechanisms are still in effect, it is life-saving to make a timely decision for transfusion before hypovolemia symptoms and the severity of coagulopathy worsen.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Etlik City Hospital No: 1 Clinical Researches Ethics Committee (Date: 16.08.2023, AEŞH-EK1-2023-482).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Does vitreous galactin-3, copeptin and retina binding protein-4 concentrations change in diabetic retinopathy?

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ABSTRACT

Aims: This study aimed to investigate whether the concentrations of Galactin-3 (G-3), Copeptin (CP) and Retina Binding Protein-4 (RBP-4) are affected in the vitreous humor of patients with diabetic retinopathy (DR).

Methods: Thirty-six patients with diabetes mellitus (DM) were included in the study, consisting of 10 patients without DR and 26 patients with proliferative diabetic retinopathy (PDR). The control group comprised 15 patients who underwent vitrectomy for epiretinal membrane and macular hole surgeries. Vitreous CP, G-3, and RBP-4 concentrations were examined using the enzyme-linked immunosorbent assay (ELISA) method. The groups were compared internally

Results: We did not observe any significant differences in the concentrations of G-3, CP and RBP-4 in the vitreous humor between diabetic patients and the control group (p=0.56, p=0.65 and p=0.11, respectively). When comparing vitreous samples of diabetic subgroups with and without DR findings to the control group, no significant differences were detected (p=0.51, p=0.66, and p=0.19, respectively).

Conclusion: Our results indicate that the concentrations of G-3, CP, and RBP-4 in the vitreous humor remain unchanged in both diabetic patients and those with proliferative diabetic retinopathy (DRP).

Keywords: Copeptin, galactin-3, retina binding protein-4, diabetes mellitus, diabetic retinopathy

INTRODUCTION

Diabetic retinopathy (DR) is one of the most severe chronic microvascular complications of diabetes mellitus (DM) and ranks among the leading causes of vision impairment and irreversible blindness in adults in developed countries.[1] Considering the increasing prevalence of diabetes, longer life expectancies, and the aging population, it is estimated that the number of DR patients could reach up to 191 million by the year 2030. This poses a significant economic burden both on individuals and society as a whole.[2]

Among the most important risk factors in the development of DR are considered to be the duration of diabetes and glycemic control, while the impact of dyslipidemia, hypertension, and obesity on DR has also been demonstrated.[3] It has been shown that regulating serum glucose, hemoglobin A1c, and lipid levels is essential for controlling DR in diabetic patients and reducing the severity of DR progression.[4] However, achieving normoglycemia has not always proven to be effective in preventing DR progression, suggesting that other additional factors may play a role in the development of DR.

One of the key factors in the pathogenesis of DR is chronic low-grade inflammation triggered by glycolytic metabolites. Numerous studies have been conducted on the significant role of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), IL-8, and IL-1β in DR inflammation. These studies have found a substantial correlation between proinflammatory markers and the severity of DR.[5] However, the exact pathogenesis of DR remains not fully understood, and much remains unknown.

Galactin-3 (G-3) is a protein belonging to the lectin family, which binds to galactose. It plays a role in many biological processes such as apoptosis, cellular growth, differentiation, proliferation, cellular adhesion, and tissue preservation.[6] G-3 can promote the secretion

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of other proinflammatory factors such as TNF-α and IL-6 by activating macrophages in a dose-dependent manner.[7] It serves as a potent inflammatory promoter, contributing to the initiation of the inflammatory response associated with acute and chronic inflammation by facilitating chemotaxis in monocytes and macrophages.[8]

Copeptin (CP) represents the C-terminal portion of the precursor of arginine vasopressin (AVP). CP is a reliable and clinically useful biomarker that can be used as a substitute for AVP. Somatic stress plays a significant role in the regulation of CP. CP is recognized as a prognostic marker in various acute diseases, including sepsis, myocardial infarction, pneumonia, or ischemic stroke.[9,10] Due to its positive correlation with disease severity, CP is considered a prognostic factor.

Retinal binding protein-4 (RBP4) is a newly identified adipokine primarily associated with retinol and secreted by white adipose tissue.[11] It can induce CD4 T cell Th1 polarization and trigger inflammation in adipose tissue by activating antigen-presenting cells.

In our study, we aimed to determine the relationship between DM and DR of G-3, CP, and RBP-4, which are associated with inflammation.

METHODS

The study was carried out with the permission of Tokat Gaziosmanpaşa University Clinical Researches Ethics Committee (Date: 02.09.2021, No: 21KAEK-195). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This prospective and randomized study was conducted between January 2022 and May 2023. All patients gave written informed consent before their participation.

The study included thirty-six patients who were diagnosed with Diabetes Mellitus (DM) and followed up with. The control group comprised 15 individuals who had undergone vitrectomy surgery for conditions like epiretinal membrane, macular hole, or retinal detachment. DM patients were divided into two subgroups according to the International Classification of Clinical Diabetes Retinopathy as proliferative DR (PDR) and without DR findings.

The patients preoperative glucose, HbA1c, low density lipoprotein (LDL), and triglyceride values were recorded.

Patients with a history of previous eye diseases such as glaucoma, corneal neovascularization, and uveitis, as well as those who had previously undergone vitrectomy surgery, were excluded from the study.

Vitreous Samples Collection

Undiluted vitreous fluids were collected from patients before undergoing primary pars plana vitrectomy. Vitreous samples were obtained using a three-port 25-gauge transconjunctival suture-less vitrectomy system and were directly suctioned into a 5 ml syringe. The vitreous samples were stored at -80°C for six months for subsequent analysis, ensuring prevention of repeated freeze-thaw cycles.

The concentrations of Vitreous G-3 were measured using a commercially available Human Galectin-3 Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Sun-Red Bio Company, Catalog No. 201-12-1952, Shanghai, China). Enzymatic reactions were measured in an automated microplate photometer (BioTek Instruments Inc. Synergy 4, Serial No. 233513, USA). G-3 concentrations were determined by comparing the optical density of the samples to a standard curve. The inter-assay and intra-assay coefficients of variation for G-3 were <12% and <10%, respectively. The kit's test range was 0.2-60 ng/ml, and the sensitivity of the test was 0.186 ng/ml. All analyses were performed according to the manufacturer's instructions.

The concentrations of Vitreous CP were measured using a commercially available Human CP ELISA Kit (Sun-Red Bio Company, Catalog No. 201-12-5463, Shanghai, China). Enzymatic reactions were measured in an automated microplate photometer. CP concentrations were determined by comparing the optical density of the samples to a standard curve. The inter-assay and intra-assay coefficients of variation for CP were <12% and <10%, respectively. The kit's test range was 0.07-20 ng/ml, and the sensitivity of the test was 0.067 ng/ml. All analyses were performed according to the manufacturer's instructions.

The concentrations of Vitreous RBP-4 were measured using a commercially available Human RBP-4 ELISA Kit (Sun-Red Bio Company, Catalog No. 201-12-1207, Shanghai, China). Enzymatic reactions were measured in an automated microplate photometer. RBP-4 concentrations were determined by comparing the optical density of the samples to a standard curve. The inter-assay and intra-assay coefficients of variation for RBP-4 were <12% and <10%, respectively. The kit's test range was 0.6-180 mg/L, and the sensitivity of the test was 0.518 mg/L. All analyses were performed according to the manufacturer's instructions.

Statistical Analysis

The statistical analysis was performed using SPSS 22.0 software. To assess the normal distribution of variables, visual and analytical methods such as the Kolmogorov-Smirnov and Shapiro-Wilk tests were employed.

Descriptive analyses were presented using means and standard deviations for variables that showed a normal distribution. To compare differences in characteristics between different groups, the chi-square test and Mann-Whitney U-test and Kruskal-Wallis test was used. P value less than 0.05 was defined statistically significant.

RESULTS

In this study, vitreous samples were analyzed from a total of 51 patients, including 36 diagnosed with DM and 15 in the control group. Among the DM patients, 10 showed no evidence of DR, while 26 were diagnosed with proliferative DR. While there was a significant difference in glucose levels between the control and DM groups, there were no significant differences in LDL and triglyceride levels (p=0.024, p=0.94, p=0.85, respectively). When comparing the DR subgroups, although glucose, HbA1c, LDL, and triglyceride levels were generally higher in the proliferative DR group, the differences were not statistically significant (p=0.3, p=0.13, p=0.66, and p=0.09, respectively). The demographic characteristics and laboratory values of the groups are presented in **Table 1**.

There was no significant difference in G-3, CP and RBP-4 concentrations between individuals without DM and those with DM (**Table 1**). In addition, there was no significant difference between the control group and DR subgroups (p=0.51, p=0.66, and p=0.19, respectively).

DISCUSSION

The pathogenesis of DR involves metabolic pathways such as the polyol pathway, advanced glycation end products pathway, hexosamine pathway, and protein kinase C pathway. With the increase in blood glucose levels, these metabolic pathways become activated. Oxidative balance is disrupted, leading to oxidative stress. This, in turn, activates apoptosis in mitochondria and results in neurovascular dysfunction. Additionally, oxidative stress leads to increased inflammation through cytokine upregulation, resulting in hypoxia and an increase in vascular endothelial growth factor.[12] Despite numerous studies on the pathogenesis and treatment of DR, it remains incompletely understood.

The current study was conducted to evaluate the vitreous humor concentrations of G-3, CP, and RBP-4, which are considered proinflammatory biomarkers, and their relationship with DM and DR.

While there have been various studies investigating the association of G-3, CP and RBP-4 with DR development and progression, all of them focused on evaluating serum concentrations. No prior study has examined the relationship between vitreous fluid concentrations of these biomarkers and DR. Thus, we decided to investigate this association.

G-3 is known as a proinflammatory molecule that triggers the inflammatory response and oxidative stress.8 Existing literature has linked serum G-3 concentrations to various diseases, including gastritis, asthma, cancer, heart diseases, kidney diseases, and obesity.[6] G-3's stability as a biomarker, its independence from factors like gender, age, and body mass index, and its lack of circadian variation increase its applicability in disease diagnosis and prognosis.[13,14]

The effect of G-3 on chronic inflammation in DM is not fully understood. Some studies have suggested that G-3 delays the development of diabetes by preventing the chronicization of the inflammatory process, while others indicate that it worsens diabetes progression by increasing inflammation and fibrosis.[6] Experimental studies on mice lacking G-3 showed increased fat accumulation and insulin resistance along with increased inflammation in adipose tissue.[15] Another study found that G-3-deficient mice exhibited higher hyperglycemia and impaired glucose tolerance compared to control wild-type mice, suggesting that G-3 deficiency contributes to diabetes pathogenesis. These studies have argued that G-3 plays a protective role against both obesity and DM, regulating natural susceptibility to overnutrition.[16,17]

Table 1. Basal characteristic of controls and diabetes patients with DR or without DR.

	Control n: 15	Diabetes mellitus n: 36	Retinopathy status		p
			No (n: 10)	Yes (n: 26)	
Age	68.5±7.4	64.3±9.1	71.1±9.3	61.7±7.8	0.12
Male (%)	10 (66.6%)	17 (47.2%)	5 (50%)	12 (46.1%)	0.24
Glucose (mg/dl)	95.5±30	187.7±73[a]	162.5±48	194.9±78	0.015
HbA1c (%)	-	9.44±2.1	9.07±1.9	10.6±2.5	0.13
LDL (mg/dl)	138.6±16.5	140.5±5.6	130.4±16.8	143.1±17.5	0,94
Triglyceride (mg/dl)	226.6 ±93	235.8±130	230.8±124	241.2±156	0.85
Galactine-3 (ng/ml)	10.36±3.0	10.48±3.1	10.88±2.7	10.25±3.1	0.52
Copeptin (ng/ml)	9.65 ±1.67	9.78±2.4	9.84±2.02	9.76±2.64	0.65
Retina binding protein-4 (ng/ml)	81.6±18.1	75.17±22.6	75.29±11.4	75.12±25.8	0.11

LDL=Low Density Lipoprotein, p Mann-Whitney U test compared control and diabetes mellitus group

In a study by Mendonça et al.[18] G-3 deficiency in mice led to reduced inflammation and preserved neuronal, retinal, and optic nerve structures after 8 weeks of diabetes. Higher circulating G-3 levels in DM patients have been associated with microvascular and macrovascular complications.[19]

CP inhibits Na^+-K^+-ATPase activity by binding to receptors on the cell membrane, disrupting vascular functions by inhibiting nitric oxide synthesis. It has been argued that CP participates in the retinal vascular endothelial function and contributes to the DR process.[20] Zhu et al.[21] found that increased CP levels were associated with DR and diabetic nephropathy independently of DM in their study involving 306 patients, suggesting a potential role for CP in the pathophysiology of DM. Additionally, they found correlations between plasma copeptin levels and the severity of insulin resistance and disease duration.

In a study by Li et al.[22] CP levels were found to be higher in DM patients compared to the healthy group, and when comparing DR within the DM group, PDR exhibited higher CP concentrations than nonproliferative DR (NPDR). CP demonstrated different increasing trends as DR clinical stages progressed, suggesting that CP might be a determining factor for DR.

RBP4 is a newly discovered adipocyte-secreted hormone associated with obesity and known to play a role in insulin resistance.[23] Akbay et al.[24] argued that there was no association between RBP-4 and DR in DM patients, but they proposed that serum RBP-4 levels could be influenced by kidney functions. However, Malechka et al.[25] suggested that RBP-4 plays a significant role in DR incidence and progression.

The connection between increased serum RBP-4 levels and DR incidence has been speculated to be related to increased inflammation in human retinal microvascular endothelial cells.[26] Du et al.[27] argued that RBP-4 contributes to DR progression through the upregulation of retinal IL-18 protein expression, leading to proinflammatory mechanisms. Studies with mice treated with RBP4 antagonists reported reduced vascular leakage.[28] Zhang et al.[29] reported that dietary intervention with retinol significantly reduced RBP-4 levels and DR incidence. Sun et al.[30] found higher RBP-4 levels in DR patients with type 1 DM in their study.

Contrary to previous studies, our current study did not find any significant difference in G-3, CP, and RBP-4 levels in vitreous humor between DM and DR patients and the control group. To the best of our knowledge, this study is the first to compare vitreous fluid concentrations of these biomarkers in DM, and thus, no previous study has been conducted on this specific aspect. Previous investigations were limited to either animal experiments or studies involving serum samples. This study, therefore, is the first to evaluate the proportions of these molecules in vitreous humor in the context of DM. It is a well-established fact that molecules can have different functions in various regions of the body and, as a result, may be expressed at different levels in different tissues, such as serum and vitreous. In our other study, there was a difference in serum samples, and the reason why there was no difference in the vitreous sample can be attributed to this.

There are some limitations to our study. First, the sample size was relatively small. Additionally, patients in the control group had other accompanying diseases. Another shortcoming of the study is that serum samples cannot be analyzed and compared with previous studies and vitreous samples. Therefore, further studies with larger sample sizes and more comprehensive control groups are needed for confirmation.

CONCLUSION

Our study found no significant differences in G-3, CP, and RBP-4 vitreous concentrations between DM and DR patients and the control group.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tokat Gaziosmanpaşa University Clinical Researches Ethics Committee (Date: 02.09.2021, No: 21KAEK-195).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

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The new score predicts 1-year poor outcome in patients with successful percutaneous coronary intervention: Naples prognostic score

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ABSTRACT

Aims: This study investigated the relationship between Naples prognostic score (NPS) and 1-year poor clinical outcomes in patients presenting with non-ST-segment elevation myocardial infarction (NSTEMI).

Methods: The study included 121 patients who had NSTEMI and received successful PCI treatment. The researchers calculated NPS using the neutrophil/lymphocyte ratio, lymphocyte/monocyte ratio, serum albumin level, and total cholesterol. The patients were divided into two groups based on their NPS scores: those with scores of 0.1 and 2 and those with scores of 3 and 4. The study compared the occurrence of major cardiovascular events (MACE) such as 1-year all-cause mortality, 1-year nonfatal recurrent MI, and stroke between the two groups.

Results: Patients with high NPS scores were observed to have significantly higher all-cause mortality than those with low NPS scores (23.9% vs. 9.3%, p=0.029). When the MACEs of the patients were compared, significantly higher MACE was observed in the high NPS group (39.1% vs. 18.7%, p=0.013).

In multivariate logistic regression analysis, creatinine (OR:4,914, CI 95%: 1.310-18,433, p=0.018) and NPS 3-4 (OR:2.565, CI 95%: 1.093-6.017, p=0.030) were independent predictors of MACE.

Conclusion: Composite MACEs of non-fatal recurrent MI, cerebrovascular accident, and all-cause death were higher at one year in patients with high NPS who underwent successful percutaneous intervention. High NPS is an indicator of MACE.

Keywords: Naples prognostic score, acute coronary syndrome, mortality

INTRODUCTION

Despite significant progress in diagnosis and treatment, acute coronary syndrome (ACS) remains a leading cause of morbidity and mortality worldwide. Previous studies have shown that inflammation is essential in atherosclerosis.[1] Inflammation and oxidative stress can trigger plaque rupture, leading to ACS.[2] Although mortality and morbidity in patients with acute coronary syndrome have decreased thanks to the development of PCI techniques and medical treatments, it is essential to predict adverse events that will develop during the follow-up of these patients in terms of follow-up and treatment strategy. Many computational tools have been developed to determine the prognosis of patients with ACS. Many of these tools are impractical to use. New scoring systems are needed to avoid wasting time and to obtain accurate prognostic value.

Previous studies have shown that neutrophil/lymphocyte ratio (NLR) and lymphocyte/monocyte ratio (LMR), which are inflammatory markers, together with albumin and total cholesterol, which are used as indicators of nutritional status and inflammation, can be used as prognostic indicators in patients presenting with ACS.[3-5] The Naples prognostic score (NPS) combines NLR, LMR, serum albumin level, and total cholesterol levels. It has previously been used as a valuable prognostic tool in cancer patients.[6] NPS provides nutritional status and inflammation information. The long-term prognostic value of NPS has been previously demonstrated in a study of ST-segment elevation myocardial infarction (STEMI) patients.[7] Our study aimed to investigate the relationship between NPS and MACE within one year in patients with non-ST-segment elevation myocardial infarction (NSTEMI). The NPS score is a simple way of measuring

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inflammation and was intended to explore its potential as a prognostic marker for patients with NSTEMI. Previous studies have not investigated the relationship between NPS and 1-year prognosis in NSTEMI patients undergoing successful PCI. This study aims to fill this gap and emphasize the importance of NPS in patient follow-up.

METHODS

The study was carried out with the permission of Health Sciences Non-interventional Researches Ethics Committee (Date: 21.06.2023, Decision No: 2023-96). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was designed retrospectively and included 121 patients who applied to a Training and Research Hospital with NSTEMI and underwent successful percutaneous intervention with PCI between June 2019 and June 2022. In the study, Patients with severe intolerance or allergy to dual antiplatelet therapy, receiving anticoagulant therapy for mechanical heart valve or atrial fibrillation, acute coronary syndrome in the last 1 year, patients with coronary artery bypass graft operation in the last 1 year, history of hematological disease, chronic kidney disease, severe liver disease, patients using anticoagulants, patients with active infection and patients with a history of infectious disease in the last one month, and malignancy were excluded from the study.

The NPS score is determined by using NLR, LMR, serum albumin level, and total cholesterol. If a person has an albumin level of less than 4 mg/dl, a total cholesterol level of 180 mg/dl or less, an NLR level greater than 2.96, or an LMR level of 4.44 or less, they are assigned one point when calculating the NPS score. However, if a person has an albumin level of greater than or equal to 4 mg/dl, a total cholesterol level greater than 180 mg/dl, an NLR level of 2.96 or less, or an LMR level greater than 4.44, they are assigned a score of 0. To determine the NPS score, all of these scores are added together.[6] In the study, patients were categorized into two groups based on their NPS score: those with a score of 0.1 to 2, and those with a score of 3 to 4. Major cardiovascular event (MACE) was defined as 1-year all-cause mortality, 1-year stroke, nonfatal recurrent MI, stroke, and MACEs were compared between the groups.

We used IBM SPSS Statistics 23 for analysis. Continuous variables were presented as mean±standard deviation if normally distributed, and median [IQR] if not. Student t-test compared normally distributed independent groups, while Mann-Whitney U test compared non-normally distributed. Categorical variables were presented as percentages and compared using chi-square test. A multivariate logistic regression model was created with significant variables from univariate regression. p values below < 0.05 were considered significant.

RESULTS

In a retrospective analysis of 121 patients, the study grouped them into two categories based on their NPS scores. Group 1 included 75 patients who scored 0-1-2, while Group 2 comprised 46 patients who scored 3-4. The average age of patients in Group 1 was 71.2±11.5, while in Group 2, it was 71.3±11 years. Interestingly, there were no significant differences between the two groups in terms of gender, age, presence of diabetes mellitus, hypertension, and previous coronary artery disease (CAD) left ventricular ejection fractions. However, Group 2 displayed significantly higher levels of WBC and neutrophils, and lower levels of lymphocyte, total cholesterol, and serum albumin. **Table 1** provides an overview of the study participants' laboratory, clinical, and demographic data and the drugs they used.

Table 1. Demographic, clinical, and laboratory characteristics of the patients

Variables	All (121)	Grup 1 (75)	Group 2 (46)	p
Age, year	76.3±7.0	71.2±11.5	71.3±11.0	0.951
Male gender, n (%)	64 (52.9)	38 (50.7)	26 (56.5)	0.531
Hypertension, n (%)	97 (80.2)	63 (84.0)	34 (73.9)	0.177
Diabetes mellitus, n (%)	44 (36.4)	27 (36.0)	17 (37.0)	0.915
Smoke, n (%)	31 (25.6)	17 (22.8)	14 (30.4)	0.342
Hyperlipidemia, n (%)	41 (33.9)	24 (32)	17 (37)	0.576
Previous CAD, n (%)	42 (34.7)	24 (32.0)	18 (39.1)	0.424
ASA, n (%)	45 (37.2)	27 (36.0)	18 (39.1)	0.729
Statin, n (%)	28 (23.1)	16 (21.3)	12 (26.1)	0.346
ACEi, n (%)	65 (53.7)	42 (56.0)	23 (50.0)	0.521
Beta-Blocker, n (%)	35 (28.9)	22 (29.3)	13 (28.3)	0.899
OAD, n (%)	37 (30.6)	22 (29.3)	15 (32.6)	0.428
OAD + Insulin, n (%)	17 (14.0)	12 (16.0)	5 (10.9)	0.430
LvEF, %	52.2±8.4	51.3±8.5	53.8±8.1	0.122
WBC, (103/µl)	7.4±2.4	9.3±3.3	11.4±4.9	0.005
Hemoglobin, g/dl	12.3±1.1	12.3±1.2	12.1±1.1	0.255
Lymphocyte, (10^3/µl)	1.6±0.6	2.3±0.9	1.6±0.7	<0.001
Neutrophil, (10^3/µl)	5.0±2.2	6.1±3.0	8.9±4.4	<0.001
Monocyte, (10^3/µl)	0.5±0.2	0.7±0.3	0.9±0.4	0.053
Platelet count, (10^3/µl)	235.0±82.2	223.3±67.8	232.1±71.1	0.496
Creatinine, mg/dl	1.1±0.32	1.07±0.31	1.16±0.33	0.162
Total cholesterol, mg/dl	163.6±42.9	182.1±48.8	142.9±23.8	<0.001
Albumin, (mg/dl)	4.2±0.4	4.2±0.3	4.0±0.5	0.024

CAD, coronary artery disease; ASA, Acetylsalicylic acid; OAD, Oral antidiabetic drug; LvEF, Left ventricular ejection fraction; WBC, White blood cell

Adverse cardiovascular events developed in the 1-year follow-up of the patients were compared and are given in **Table 2**. All-cause mortality in Group 2 was significantly higher than in Group 1 (23.9% vs 9.3%, p=0.029). When MACEs consisting of all-cause mortality, recurrent

MI, and development of cerebrovascular accident were compared, a significantly higher MACE was observed in Group 2 (39.1% vs 18.7%, p=0.013).

Table 2. Clinical outcomes of the groups at 1-year follow-up

Variables	All (121)	Group 1 (75)	Group 2 (46)	p
Composite MACE, n (%)	32 (26.4)	14 (18.7)	18 (39.1)	0.013
All-cause death, n (%)	18 (14.9)	7 (9.3)	11 (23.9)	0.029
Non-fatal recurrent MI, n (%)	10 (8.3)	5 (6.7)	5 (10.9)	0.415
Stroke, n (%)	12 (9.9)	7 (9.3)	5 (10.9)	0.784

MACE, Major cardiovascular event; MI, Myocardial Infarction

In multivariate logistic regression analysis, creatinine (OR:4,914, CI 95%: 1.310-18,433, p=0.018) and NPS 3-4 (OR:2.565, CI 95%: 1.093-6.017, p=0.030) were found to be independent predictors of the development of MACE (**Table 3**).

Table 3. Univariate and multivariate regression analysis for detecting mace

	Univariate	Multivariate
	OR (95% confidence interval)	OR (95% confidence interval)
Age	0.973 (0.938-1.010, p=0.145)	
Hypertension	2.025 (0.636-6.472, p=0.232)	
Diabetes mellitus	1.280 (0.559-2.934, p=0.559)	
LvEF	0.962 (0.918-1.009, p=0.110)	
Creatinine	5.638 (1.516-20.966, p=0.010)	4.914 (1.310-18.433, p=0.018)
NPS (3-4)	2.801 (1.222-6.420, p=0.015)	2.565 (1.093-6.017, p=0.030)

NPS, Naples Prognostic Score

DISCUSSION

Looking at previous studies, no study was observed showing the relationship between NPS and 1-year prognosis in patients who presented with NSTEMI and underwent successful PCI. Our study determined that more MACE was detected with a higher NPS score in patients with successful PCI due to NSTEMI. NPS was an independent predictor of MACE.

Inflammation is known to be the most critical component of the pathogenesis of CAD. White blood cell count has been used as an inflammatory biomarker in many studies to predict outcomes and prognosis in patients with ACS.[8] Although thrombosis leading to the development of ACS is a process different from inflammation, inflammation also causes thrombosis.[9] In addition to thrombosis, inflammatory cells also cause plaque rupture. Especially neutrophils have an active role in all stages of formation, development, and rupture of atheroma plaque in the coronary vessels in ACS patients. It is known that neutrophils use local chemicals that facilitate the rupture of atheromatous plaque and cause cell aggregation, especially leukocytes, and thrombocytes, in the coronary vessel.[10] On the contrary, it has been shown in previous studies that low lymphocyte levels cause an increase in mortality and morbidity in patients with CAD.[11,12] The relative decrease in lymphocyte levels is thought to be caused by endogenous cortisol, which increases due to stress, and inflammation causes lymphocyte apoptosis.

NLR is a marker of inflammation and a prognostic indicator in cancer, infectious diseases, and cardiovascular diseases.[13-15] It has been shown that NLR, which is used as an inflammation marker, can predict mortality in ACS patients.[16] Similarly, in LMR, the myelodysplastic syndrome is used as a prognostic indicator in ovarian cancers but has been associated with the presence and severity of CAD.[17-19] Yılmaz et al.[20] showed that NLR combining these two blood parameters could be a prognostic value in ACS patients.

Albumin is a negative acute phase reactant produced by the liver. In addition to showing nutritional status, serum albumin is associated with chronic and acute inflammatory responses, and increased inflammation reduces serum albumin levels.[21,22]

Albumin is an antioxidant protein. Therefore, the amount of free oxygen radicals and reactive oxygen products increases in cases of hypoalbuminemia, which may have a role in the pathophysiology of ACS.[23] Another essential function is to prevent thrombotic events, ensure the proper functioning of endothelial functions, and ultimately preserve vascular integrity.[24] In previous studies, low serum albumin levels were associated with mortality in patients with ACS.[25]

Although total cholesterol levels are a risk for developing atherosclerotic plaque, previous studies have shown that low cholesterol levels are paradoxically associated with mortality, on the contrary.[26] It is known that it mainly occurs with the rupture of atheroma plaques in the coronary vessels, followed by the settlement of a thrombus. Inflammation is essential in the atheroma plaque's formation, progression, and rupture stages. In one study, it was shown that cytokines can cause a decrease in blood cholesterol levels by changing cholesterol metabolism.[27] Cholesterol level, one of the NPS parameters, may indicate the inflammation status in ACS patients. In our study, no significant difference was found between the groups regarding previous diagnosis of hyperlipidemia, use of statins, and diagnosis of diabetes mellitus, which has prognostic significance in patients with coronary artery disease. Initiation of statins after percutaneous coronary intervention is expected to affect the NPS of these patients in their follow-up, and prospective studies are needed to investigate the effect of this situation.

Since ACS is a vital disease, early diagnosis, and treatment should be planned. These patients present with mild chest pain and life-threatening arrhythmias and shock. Therefore, it is crucial to predict mortality in these patients. The biochemical parameter, troponin, increases approximately 3 hours after symptom onset and does not increase in all ACS. In ACS patients, markers with rapid response and high prognostic value, which can be easily used in any laboratory environment, continue to be investigated. In this sense, NPS is a scoring system that is easy to look at and predicts inflammation and prognosis, forming the basis of ACS pathophysiology. New scoring systems are needed to avoid wasting time and to obtain accurate prognostic value; NPS may be one of them. NPS, first associated with poor prognosis in cancer patients, provides valuable information regarding prognosis in STEMI patients in more recent studies.[28,29] Birdal et al.[30] showed a relationship between NPS and low ejection fraction in patients with STEMI. Karakoyun et al.[31] showed the relationship between NPS and acute kidney injury in patients with STEMI. In a previous study, a significant relationship was found between the severity of coronary artery disease and the systemic immune inflammation index, including NLR and platelet components in patients with stable coronary artery disease, but the relationship between NPS was not significant. The reason for this is that total cholesterol, which is an important substrate in the formation of atherosclerotic plaque, is used in the calculation of NPS. However, since our study is a study showing the prognosis in patients with coronary artery disease, it shows the prognostic value of NPS with inflammation and nutritional status.[32] NPS: It consists of NLR, LMR, serum albumin, and total cholesterol components. High NLR, low LMR, low serum albumin, and low total cholesterol levels have been associated with poor prognosis.[7] NPS, a combination of these, is thought to be a more robust prognostic marker and has been proven by studies. Similar to previous studies, higher composite MACE was observed in the high NPS group in our study.

Our study has limitations, such as being retrospective and being conducted in a small group of patients. The importance of prognostic markers in larger patient groups and prospective studies are needed.

CONCLUSION

As a result, composite MACEs consisting of non-fatal recurrent MI, cerebrovascular accident, and all-cause death were found to be higher in patients with high NPS who underwent successful percutaneous intervention due to NSTEMI at 1-year follow-up. High NPS can be said to be a predictor of MACE. NPS is an easily calculated inflammatory score and can be used as a prognostic marker in patients with NSTEMI.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Health Sciences Non-interventional Researches Ethics Committee (Date: 21.06.2023, Decision No: 2023-96).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Evaluation of variants in maturity onset of diabetes young related genes in Balıkesir region

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ABSTRACT

Aims: Maturity-onset diabetes of the young (MODY) is an early-onset, monogenic diabetes with an autosomal dominant inheritance pattern. Single gene mutations that cause dysfunction in pancreatic beta cells are responsible for MODY etiology. In this study, we investigated the genetic variants involved in the etiopathogenesis of MODY in our region.

Methods: Between May 2018 and April 2023, 40 pediatric patients (n=25 females, n=15 males) with a clinical diagnosis of MODY were evaluated by targeted genome sequencing.

Results: Among the 40 pediatric patients included in this study, variants in MODY-associated genes were detected in 21 patients (52.5%), eight (38.09%), of which were pathogenic (38.09%), five (23.8%) were probable pathogenic, and eight (38.09%), were of uncertain significance.

Conclusion: In this study, genetic diagnostic yield (including pathogenic and likely pathogenic variants) was detected in 32.5% (13/40) patients with MODY using targeted genome sequencing analysis. This rate is consistent with other studies. However, unlike other similar studies, the MODY12 subtype was the second most frequent in our study. In addition, nine novel variants were reported, including *ABCC8* (n=3), *CEL* (n=2), *KLF11* (n=1), *GCK* (n=1), *HNF1A* (n=1), and *HNF1B* (n=1) genes. We have presented clinical findings to improve genotype-phenotype correlation in the literature for novel variants.

Keywords: MODY, Targeted genome sequencing, novel variants, *ABCC8*, *KLF11*

INTRODUCTION

Diabetes is a chronic metabolic disease that occurs mainly on the basis of genetic and environmental factors and results in hyperglycemia.[1-4] Maturity-onset diabetes of the young (MODY) is a form of diabetes characterized by high blood glucose as a result of a defect in the insulin secretion mechanism diagnosed before the age of approximately 25 years.[5] MODY, which accounts for approximately 2% of all diabetes cases, is caused by variants in a single gene.[6] This type of diabetes, which is very rare compared with Type 1 and Type 2 diabetes, has a history of diabetes in two or more generations.[5,6] In this form of diabetes, in which pancreatic beta cells are dysfunctional, molecular genetic tests are very important for appropriate treatment and genetic counseling. Because of its rarity, 90% of MODY patients are mistakenly diagnosed with Type 1 or Type 2 diabetes.[7] MODY should be considered in the differential diagnosis of patients with atypical Type 1 and Type 2 DM with negative autoantibodies.[5]

Advances in high-resolution genome sequencing technologies allow sequencing not only of a specific region of the genome, but also of the entire genome, quickly and with high accuracy. Next generation sequencing technologies (such as targeted panels, medical exome sequencing, whole exome/genome sequencing) have increased the carrier detection rate in diseases with autosomal recessive inheritance patterns. Making the correct molecular diagnosis of patients with diabetes using genetic tests enables the formation of an appropriate treatment option.[8]

The aim of this study was to determine the genetic variants associated with MODY in the Balıkesir region, evaluate the frequency of mutations, and offer appropriate treatment options to pediatric patients.

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Table 1: All variants detected by next generation sequencing

Family	Case	Age	Gender	Gene	Exon	Inheritance	Zygosity	Nucleotide variation	Amino acid variation	Mutation type	ACMG classification	Associated phenotype	Treatment
1	1	5y	F	*GCK* (NM_033507.3)	3	AD	Heterozygous	c.316del (na)	p.His106ThrfsTer11	missense	Likely pathogenic	MODY2	Insulin
2	2	7 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (na)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
3	3	7 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (pat)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
	4	13 y	M										Dietary therapy
4	5	14 y	M	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (mat)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
5	6	18 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (mat)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
6	7	18 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (mat)	p.Ser454Leu	missense	Pathogenic	MODY2	Insulin
7	8	17 y	M	*HNF1A* (NM_000545.8)	3	AD	Heterozygous	c.687_707del (de novo)	p.Glu230_Cys236del	in frame	Likely pathogenic	MODY3	Insulin
8	9	17 y	F	*HNF1B* (NM_000458.4)	4	AD	Heterozygous	c.947A>G (na)	p.Asp316Gly	missense	Likely pathogenic	MODY5	Oral antidiabetic
9	10	14 y	M	*KCNJ11* (NM_000525.4)	1	AR	Homozygous	c.405dup (na)	p.Arg136AlafsTer5	frameshift	Pathogenic	MODY13	Insulin
	11	9 y	M										Dietary therapy
10	12	9 y	F	*ABCC8* (NM_000352.6)	7	AD	Heterozygous	c.1168G>T (na)	p.Ala390Ser	missense	Uncertain significance	MODY12	Dietary therapy
11	13	17 y	F		10	AD	Heterozygous	c.1616A>G (de novo)	p.Tyr539Cys	missense	Uncertain significance		Insulin
12	14	16 y	M		22	AD	Heterozygous	c.2578G>A (na)	p.Asp860Asn	missense	Uncertain significance		Insulin
13	15	16 y	F		39	AD	Heterozygous	c.4673A>T (na)	p.Glu1558Val	missense	Likely pathogenic		Oral antidiabetic
14	16	18 y	F	*CEL* (NM_001807.6)	4	AD	Heterozygous	c.355C>T (pat)	p.Pro119Ser	missense	Uncertain significance	MODY8	Oral antidiabetic
15	17	17 y	M	*CEL* (NM_001807.6)	11	AD	Heterozygous	c.1983del (na)	p.Thr662ArgfsTer42	frameshift	Likely pathogenic	MODY8	Insulin
16	18	14 y	F	*KLF11* (NM_003597.5)	3	AD	Heterozygous	c.1095G>C (na)	p.Lys365Asn	missense	Uncertain significance	MODY7	Insulin
17	19	13 y	F	*PDX1* (NM_000209.4)	1	AD	Heterozygous	c.107T>G (mat)	p.Leu36Arg	missense	Uncertain significance	MODY4	Oral antidiabetic
18	20	13 y	F	*PAX4* (NM_001366111.1)	5	AD	Heterozygous	c.521G>A (na)	p.Arg174Gln	missense	Uncertain significance	MODY9	Insulin
19	21	4 y	F	*INS* (NM_001185097.2)	3	AD	Heterozygous	c.206G>A (na)	p.Gly69Asp	missense	Uncertain significance	MODY10	Dietary therapy

y: years; F: female; M: male; AR: Autosomal recessive; AD: Autosomal dominant; mat: maternally inherited; pat: paternally inherited; na: not available, inheritance not known; bolded novel variant

METHODS

The study was carried out with the permission of Balıkesir Atatürk City Hospital Ethics Committee (Date: 01/06/2023, Decision No: 2023/3/32). The study was evaluated as a research file, and it was decided that it was scientifically and ethically appropriate. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

40 patients from 38 unrelated families who applied for the clinical prediagnosis of MODY between May 2018 and April 2023 were included in this study. The criteria for inclusion in this study were as follows: i) patients of both genders and age of ≤18 years diagnosed with diabetes, ii) diabetes specific autoantibody negative diabetes, and iii) a family history of diabetes in three generations. All patients who underwent targeted genome sequencing (TG-Seq) were retrospectively evaluated. Familial segregation analysis was performed using Sanger sequencing

DNA Isolation

DNA isolation was performed from lymphocytes in 200 µL peripheral venous blood using a QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA).

Targeted Genome Sequencing

Targeted genome sequencing containing 12 genes (*HNF1A*, *HNF1B*, *HNF4A*, *GCK*, *ABCC8*, *PDX1*, *NEUROD1*, *INS*, *KCNJ11*, *BLK*, *KLF11*, *PAX4*) associated with MODY was designed. The genomic DNAs obtained were sequenced with Roche HyperCap DS CES kit and then sequenced with MGI, DNBSeq G400.

Sanger Sequence

Before sequencing, the PCR products were purified using a NucleoFast 96 PCR kit (Macherey-Nagel GmbH, Germany). After completion of the thermal cycle step, the sequence reactions were purified according to the protocol of the ZR-96 DNA Sequencing Clean-up Kit (Zymo Research Corp.) Capillary electrophoresis of the purified sequence products was performed using ABI 3130 (Applied Biosystems Inc.).

Data Analysis

GenomizeSeq (Version 6.13.1) software was used for analysis with an average read depth of 20X and coverage of 94.17%. Exon-intron junction boundaries (±10 base pair) were included in the analysis. Human Genome Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php) and Franklin (https://franklin.genoox.com/clinical-db/home), VarSome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/) novel variants in the databases were checked. The pathogenicity score of new variants was interpreted using the in silico variant prediction program Mutation Taster, CADD (Combined Annotation Dependent Depletion). The data obtained were analyzed according to the American College of Medical Genetics and Genomics (ACMG) criteria.9

RESULTS

A total of 40 cases (n=25 female, n=15 male) from 38 different families were included in this study. Demographic data, variant information, and treatments are summarized in **Table 1**. The current mean age of these patients was 12.5 years (3 years-18 years). The mean age of female patients was 12.2 years, and that of male patients was 13 years. Of the 40 patients, 21 (52.5%) had molecular genetic results consistent with their clinical findings. There were 7 different pathogenic/likely pathogenic variants in thirteen (13/21=63.7%) patients. There were 8 different uncertain significance variants in eight (8/21=38.9%) patients (**Table 1**).

Pathogenic variants were found in the *GCK* (n=6 patients) and *KCNJ11* (n=2 patients) genes. Six patients had the same *GCK* gene: c.1361C>T (p.Ser454Leu) pathogenic variant. Two patients, siblings, had the c.405dup pathogenic variant in the *KCNJ11* gene. The likely pathogenic variants were *GCK* gene: c.316del (p.His106ThrfsTer11), *HNF1A* gene: c.687_707del(p.Glu230_Cys236del), *HNF1B* gene:c.947A>G (p.Asp316Gly), *ABCC8*: c.4673A>T (p.Glu1558Val), and *CEL* gene: c.1983del (p.Thr662ArgfsTer42). All these likely pathogenic variants were novel and have not been previously reported in any public database. In addition, there were a total of eight variants of uncertain significance in *ABCC8* (n=3), *CEL* (n=1), *KLF11* (n=1), *PDX1* (n=1), *PAX4* (n=1) and *INS* (n=1) genes. Among these variants of uncertain significance, the *ABCC8* gene: c.1168G>T (p.Ala390Ser), *ABCC8* gene: c.2578G>A (p.Asp860Asn), *CEL* gene: c.355C>T (p.Pro119Ser), and *KLF11* gene: c.1095G>C (p.Lys365Asn) were novel.

The variants detected in this study were associated with the phenotypes of maturity-onset diabetes of the young, type 2 (MODY2, OMIM # 125851), maturity-onset diabetes of the young, type 3 (MODY3, OMIM # 600496), maturity-onset diabetes of the young, type 5 (MODY5), maturity-onset diabetes of the young, type 13 (MODY13, OMIM # 616329), maturity-onset diabetes of the young, type 12 (MODY12), maturity-onset diabetes of the young, type 8, with exocrine dysfunction (MODY8, OMIM # 609812), maturity-onset diabetes of the young, type 7 (MODY7, OMIM # 610508), maturity-onset diabetes of the young, type 4 (MODY4, OMIM # 606392)), maturity-onset diabetes of the young, type 9 (MODY9, # 612225), and maturity-onset diabetes of the young, type 10 (MODY10, # 613370) phenotypes.

DISCUSSION

In our study presenting the genetic variants of MODY pediatric patients in our region, the yield of molecular genetic diagnosis was 52.5% (n=21 patients; 21/40). In twenty-one patients with positive genetic results by TG-Seq, there were 15 different variants (n=2 pathogenic, n=5 likely pathogenic, n=8 of uncertain significance). Of the 15 variants, 9 (9/15=60%) have not been reported in the relevant scientific literature, such as gnomAD (https://gnomad.broadinstitute.org), Leiden Open Variation Database (LOVD, https://www.lovd.nl/), and ClinVar.

Mutations in the *HNF4A* (MODY, type I), *GCK* (MODY, type II), and *HNF1A* (MODY, type III) genes are responsible for approximately 90% of MODY cases.[10-12] In our study, where we did not detect any pathogenic/likely pathogenic variant in the *HNF4A* gene, this rate was approximately 40%. In our study, the most common *GCK* (MODY, type II) gene variants were observed in seven patients (n=7), including two patients (case 4, case 5) from the same family. Among the *GCK* gene mutations, the most common variant was c.1361C>T (p.Ser454Leu). When evaluated together with our previous study, this mutation was the most common *GCK* variant in all age groups in our region.[13] *ABCC8* gene mutations were the second most common in this study. According to other scientific studies, *ABCC8* gene variants have been implicated in approximately 1% of all MODY.[14,15] Here MODY12 was seen in 19.4% of all MODY. This finding was the most remarkable feature that distinguished our study from other similar literature.[5,14-17]

Homozygous *KCNJ11* gene: NM_000525.4: c.405dup (p.Arg136AlafsTer5) variant was detected in family 9. Two siblings (Case 10, Case11) from family 9 with the same variant exhibited different clinical progressions. Case 10 suffered from hyperinsulinemic hypoglycemia in the neonatal period and required pancreatectomy. Therefore, the patient uses insulin in the treatment. However, Case 11 was controlled with the help of diet therapy, and he had no insulin requirement.

Although mutations in the *BLK*, *PAX4*, and *KLF11* genes have not yet been clearly associated with MODY, these genes are still being screened in studies.[17] In our study, there were patients with variants of uncertain significance in the *KLF11* (MODY7) and *PAX4* (MODY9) genes. All these patients had negative diabetes specific autoantibodies and a low insulin dose requirement. Although we could not make a definitive diagnosis, these findings were consistent with MODY.

Our diagnostic yield of genetic diagnosis was found to be 32.5% (13/40) according to pathogenic/likely pathogenic variants. In previous similar studies, a possible pathogenic/pathogenic variant was found in 6-48% of patients.[17-20] In this study, we obtained results consistent with these studies.

Our study has some limitations. It contains only one province ‘s data. No further genetic testing, such as a whole genome sequencing or medical exome sequencing, was performed in patients with negative test results.

CONCLUSION

MODY is responsible for 2-5% of all diabetes, but a better understanding of the genetic spectrum of MODY is needed. Patients with a family history of diabetes and atypical diabetes manifestations should be screened for mutations in MODY-related genes. These results also suggest that the MODY genotype may have regional differences in our country. In addition, this study reports 9 (9/15=60%) different novel variants that were not previously reported in similar scientific literature.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Balıkesir Atatürk City Hospital Ethics Committee (Date: 01/06/2023, Decision No: 2023/3/32).

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

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

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The effect of direct-acting antivirals (DAA) on confirmed noninvasive fibrous parameters in chronic hepatitis C patients

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ABSTRACT

Aims: Chronic hepatitis C (CHC) is an important public health problem in terms of the number of people it affects worldwide and the diseases it causes. The high sustained virological response (SVR) rates achieved by the use of direct-acting antiviral (DAA) drugs in the recent period have shown that a new era has begun in this disease. It was aimed to evaluate the effect of DAAs on confirmed noninvasive fibrous parameters together with their effectiveness.

Methods: 75 patients who were started on DAA treatment for CHC were included in the study. In addition, laboratory parameters values at the beginning of the treatment, 12 and 24 weeks after the end of the treatment, hepatitis C virus ribonucleic acid (HCV RNA) results and Aminotransferase-to-Platelet Ratio Index (APRI), Fibrosis-4 (FIB-4) scores were compared.

Results: The most common comorbidity in patients is hypertension (HT), and the most common source of transmission is surgical operations. Genotype 1b was the dominant genotype. The SVR rates of all patients 12 and 24 weeks after the end of treatment were 100%. The APRI and FIB-4 scores of the patients decreased significantly at the 12th and 24th weeks at the end of the treatment compared to the beginning of the treatment.

Conclusion: The confirmed noninvasive fibrous parameters used in the treatment of CHC are useful in evaluating the results of the treatments applied.

Keywords: Chronic hepatitis C, direct-acting antiviral agents, sustained virological response, fibrosis-4, aminotransferase-to-platelet ratio index

INTRODUCTION

Hepatitis C virus (HCV) infections are a major public health problem affecting estimated 80 million people worldwide and can result in hepatocellular carcinoma and cirrhosis.[1,2] Chronic infection due to HCV induces the progression of fibrosis in the liver. Elimination of HCV with antiviral treatment, which is defined as a permanent virological response (SVR), prevents the progression of chronic hepatitis and its related complications.[2] Direct-acting antivirals targeting HCV have reformed the treatment of CHC infection by significantly increasing the rates of permanent virological response (SVR) and diminishing the adverse effects of treatment.[3] In Turkey, it has been reported that the rates of SVR increased above 90% with the direct-acting antivirals (DAA) included in the reimbursement scope as of June 2016.[4]

Since liver histology rather than plasma viremia is the most important prognostic factor in patients with CHC infection, it is necessary to determine how virologic clearance is related to histologic recovery.[3] The technique defined as noninvasive fibrosis measurements is the calculation of hepatic fibrosis using non-invasive techniques. The indices called Aminotransferase-to-Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) provide identification of the liver parenchyma, especially in patients at high risk of disease complications, without exposing them to invasive diagnostic methods such as liver biopsy. The use of these methods is also very useful and important in the treatment and post-treatment follow-up of CHC patients without an additional invasive procedure.[5]

The main aim of this study is to evaluate the demographic characteristics and treatment response of CHC patients using DAA followed in our center, and also to reveal the change of proven noninvasive fibrosis parameters with treatment. Thus, it is aimed to bring a modern

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approach to the management of patients with chronic HCV infection by combining the management of HCV infection, which has undergone a great change with the emergence of DAA agents, with proven noninvasive fibrous measurements.

METHODS

The study was carried out with the permission of Van Training and Research Hospital Ethics Committee (Date: 18.02.2021, Decision No: 2021/04). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

75 CHC patients who were followed-up and treated at the infectious diseases outpatient department of a training and research hospital between 1 January 2018 and 31 December 2020 were included in this retrospective cross-sectional analysis.

Patient Selection Criteria

- 18 years or older,
- Having met the criteria for CHC and received DAA treatment,
- Completing DAA treatment and following up regularly in our center (Patients who discontinued the treatment or were out of follow-up were not included in the study).

Definition of Sustained Virological Response and Non-Invasive Tests Used for Liver Fibrosis

- **SVR-12:** Invisible HCV RNA in plasma - 12 weeks following the end of DAA treatment, utilizing a high - sensitivity PCR assay
- **SVR-24:** Invisible HCV RNA in plasma - 24 weeks following the end of DAA treatment, utilizing a high - sensitivity PCR assay

The treatment to be used by the patients was decided by considering the genotype, cirrhosis status, treatment experience, comorbidities and the drugs they used within the framework of the health practice communiqué rules determined by the Republic of Turkiye Ministry of Health.

The treatment methods, genotypes, risk factors for HCV infection, demographic characteristics and laboratory values were all recorded. For these procedures, patient files, forms created for patient follow-up, hospital system and anamnesis taken from patients were used.

The laboratory values of the patients were recorded at the beginning of the treatment, 12 weeks after the end of the treatment, and 24 weeks after the end of the treatment.

APRI score (AST-platelet ratio index): It is a non-invasive serological marker showing the degree of liver fibrosis and was confirmed and suggested by Wai et al.[6] in 2003.

Formula: [AST (IU/L)/AST upper limit of normal (IU/L)]/platelet count (10^9/L)×100.[5]

FIB-4 index: Another confirmed noninvasive serological measurement.

Formula: Age (years)×AST (IU/L)/platelet count (10^9/L)×√ALT (IU/L).[5]

These two scores have been able to reliably identify liver fibrosis in many studies.[5]

Statistical Analysis

The data in the study were evaluated using the SPSS 22.0 statistics package program. The compatibility of the data to normal distribution was evaluated by Kolmogorov-Smirnov analysis. It was seen that the data were suitable for normal distribution. Numerical variables that fit the normal distribution are presented with mean (SD), and categorical variables are presented with percentage (%). In comparison of the differences between groups, the comparison of independent groups proportions of categorical variables was performed by chi-square and FisherExact tests, and comparison of dependent group proportions was performed by McNemar test. In comparison of the means of two groups independent of numerical variables, those that fit the normal distribution were evaluated using Student's t-test. One-way analysis of variance (OneWay: ANOVA) was used to compare more than two independent sample means.

A P value less than 0.05 P was considered statistically significant, and values with p=0.000 were given as P<0.001.

RESULTS

The mean age of 75 patients who received DAA was 51.4 (15.8) years, and 51 (68%) of these patients were male. The most common comorbidity in patients was hypertension (HT) with 27 patients (36%). It was followed by chronic renal failure (22.6%), diabetes mellitus (14.6%), coronary artery disease (10.6%), renal transplantation (6.6%), chronic obstructive pulmonary disease (6.6%), liver cirrhosis (5.3%), HCC (4%), non-HCC malignancies (4%), and chronic hepatitis B (CHB) infection (2.6%).

There was no additional disease in 29 (38.6%) patients. The most common reason among the sources of HCV transmission in the patients was the history of surgical operation. It was detected in 20 (26.7%) patients. The second risk factor was dialysis with 17 (22.7%) patients. Intravenous (IV) drug addiction in 16 (21.3%) patients, dental treatment in 7 (9.3%) patients, being

imprisoned in 4 (5.3%) patients, blood transfusion in 3 (4%) patients, 2 (2.7%) patients tattoo/piercing has been identified other risk factors. No risk factor could be identified in 6 (8%) patients.

Eight (10.7%) patients were genotype 1a, 50 (66.7%) genotype 1b, and 17 (22.7%) genotype 3. Twenty-three of the patients (30.7%) had at least one prior non-DAA treatment experience. Treatment regimens were applied as follows; Paritaprevir + Ritonavir + Ombitasvir + Dasabuvir (ProD) in 30 patients, Ledipasvir/Sofosbuvir (LED/SOF) in 23 patients, Glecaprevir + Pibrentasvir (GLE/PIB) in 16 patients, and Sofosbuvir + Ribavirin (SOF/RIB) in 6 patients. (**Table 1**).

The laboratory median values of the patients at the beginning of the treatment, 12 weeks after the end of the treatment and 24 weeks after the end of the treatment are shown in **Table 2**. Accordingly, the changes in AST, ALT, total bilirubin, ALP, GGT, LDH, AFP, albumin, thrombocyte, white blood cell, INR, PTT values were found to be statistically significantly different when compared to the values 12 and 24 weeks after the end of the treatment, according to the beginning of the treatment. The SVR rates of the patients at 12 and 24 weeks were 100%, and HCV-RNA was not detected in these months. When the patients' APRI and FIB-4 scores at the beginning of the treatment were compared with the values 12 and 24 weeks after the end of the treatment, there was a statistically significant decrease (**Table 3**).

Table 1. Demographic and clinical data of the patients

	n (%)
Age [Mean (SD)]	51.4(15.8)
Gender	
Male	51 (68%)
Female	24 (32%)
Comorbidity	
Metabolic syndrome (HT, DM,CAD)	46 (61.3%)
COPD	5 (6.7%)
Renal comorbidities (CRF, RT)	22 (29.3%)
Hepatic comorbidities (cirrhosis, HCC, CHB)	9 (12%)
Malignite (not HCC)	3 (4%)
None	29 (38.7%)
Treatment Received	
LED/SOF	23 (30.7%)
PrOD	30 (40%)
SOF+RIB	6 (8%)
GLE/PIB	16 (21.3%)
Risk Factor	
Operation	20 (26.7%)
Dialysis	17 (22.7%)
Iv Drugs	16 (21.3%)
Dental treatment	7 (9.3%)
Tattoo/piercing	2 (2.7%)
Prisoner	4 (5.3%)
Blood transfusion	3 (4%)
Unknown	6 (8%)
Genotype	
1a	8 (10.7%)
1b	50 (66.7%)
3	17 (22.6%)
Previous Treatment	
Naive	52 (69.3 %)
Experienced	23 (30.7%)

HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure, RT:Renal Transplantation, HCC: Hepatocellular carcinoma, CHB: Chronic hepatitis b, LED/SOF: Ledipasvir/Sofosbuvir, ProD: Paritaprevir + Ritonavir + Ombitasvir + Dasabuvir, SOF+RIB:Sofosbuvir +Ribavirin, GLE/PIB: Glecaprevir +Pibrentasvir, IV: Intravenous.

Table 2. Changes of laboratory tests at baseline and SVR12, and SVR24

	Baseline Median (min-max)	At 3th month Median (min-max)	At 6th month Median (min-max)	p
ALT (U/L)	45 (13.4-171)	20 (7-130)	15 (2-83)	<.001
AST (U/L)	31 (11.8-110)	20 (7-106)	16 (5-85)	
T. bil (mg/dl)	0.6 (0.2-4)	0.47 (0.15-4.2)	0.4 (0.18-5)	<.001
ALP (U/L)	96 (41-240)	86 (42-380)	82 (38-355)	<.001
GGT (U/L)	42 (6-580)	30 (6-400)	25 (6-350)	<.001
LDH (U/L)	110 (10-325)	110 (20-375)	130 (23-345)	
AFP (U/ml)	3.7 (0.5-76.91)	2.8 (0.5-72)	2.5 (0.3-65)	<.001
Albumin (g/dl)	4.1 (2.9-5)	4.2 (3.2-5.2)	4.3 (3.2-5.3)	<.001
Creatine (mg/dl)	0.85 (0.3-8.5)	0.8 (0.4-7.5)	0.82 (0.45-9.2)	.092
Platelets ($\times10^3$/ml)	185(35-368)	220 (45-400)	235 (75-485)	<.001
Leukocyte ($\times10^3$/ml)	7.1 (2.27-10.7)	7.2 (3.35-11.4)	6.91 (3.6-12.4)	<.001
Hemoglobin (g/dl)	14.3 (8.5-17.4)	14.1 (8-17.1)	14.2 (8.2-17)	.638
INR	1.08 (0.84-1.54)	1.01 (0.8-1.24)	0.95 (0.75-1.12)	<.001
PTT (second)	33.1 (22.9-51.5)	30.8 (26.2-41.4)	29.7 (26.2-41.4)	<.001
HCV-RNA level ($\times10^3$ IU/ml)	359.181(3.38-26502)	0	0	<.001

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, T.Bil: Total bilirubin, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, LDH: Lactate dehydrogenase, AFP: Alpha fetoprotein, INR: "international normalized ratio", PTT: partial thromboplastin time, HCV-RNA: Hepatitis C virus ribonucleic acid.

Table 3. Changes in liver fibrosis scores following anti-viral treatment regimens

	Baseline Median (min-max)	At 3th month Median (min-max)	At 6th month Median (min-max)	p
APRI	0.00045 (0.00014-0.00529)	0.000245665 (0.0000875-0.002722222)	0.00017 (0.00011-0.00152)	<.001
FIB-4	0.00126 (0.00021-0.01442)	0.001043 (0.000219-0.00878)	0.000841(0.000212-0.004732)	<.001

APRI: "aspartate aminotransferase to platelet ratio index", FIB-4: "Fibrosis-4"

DISCUSSION

The primary goal in the treatment of CHC is to avert the development of fibrosis in the liver and to reverse the hepatic and extrahepatic effects of HCV.[7] Unlike previous treatments, direct-acting antivirals have higher efficacy and better safety profile. Similar studies with different genotypes and different regimens have reported a sustained virological response (SVR) of 90% to 100% about direct-acting antivirals. In this study, SVR12 and SVR24 were detected as 100% and were higher than many reported rates.[8,9]

HCV genotype determination is very important in terms of determining the antiviral treatment option, adjusting the treatment dose, determining the treatment time and treatment answer. The predominant genotype in our study was genotype 1b. Also; genotype 3 and genotype 1a were followed up. The findings were similar to other studies conducted in our country.[10,11]

The transmission route of HCV differs according to the development level of the countries. While IV drug use is the most important mode of transmission in developed countries, unsafe injections and medical procedures are the most important transmission route of HCV infection in developing countries.[12] In the study conducted by Karaca et al.[13] with 320 HCV patients, it was reported that 98% of the patients had a history of surgical operation. In this study, it was seen that the commonly risk factor in patients was the past of surgical operation with a rate of 26.6%.

Transmission via blood transfusion was the best defined route in HCV patients and was the most common cause of transmission in previous years. Transfusion-associated HCV infection has decreased due to routine testing of HCV antibodies in blood banks since 1990, and it has become one of the least common transmission routes today.[12] As revealed in our study, one of the least common modes of HCV transmission is a history of transfusion, and it was seen only in 3 patients.

The prevalence of HCV in patients with failured renal function is advanced than the general population (9.5%).[6] HCV can directly lead to diseases that cause kidney damage such as membranoproliferative glomerulonephritis and cryoglobulinemia.[14] In addition, procedures such as dialysis and blood transfusion increase the risk of HCV transmission in patients with CRF.[14] In our study, we attribute CRF being the most common comorbidity after HT to these reasons. In addition, 5 of our patients had renal transplantation.

Histologic changes in the liver affect the prognosis in CHC infection and the relationship between virologic clearance and histologic improvement needs to be investigated.[3] Due to the invasive procedure of liver biopsy, many noninvasive fibrosis markers have been enhanced to assess the stage of liver fibrosis.[15] APRI and FIB-4 are widely used among these and are known to be safe in predicting fibrosis in CHC patients before treatment.[16,17]

Non-invasive measures to assess fibrosis have been widely implemented in CHC patients after successful treatment with DAA, and many studies have shown reduced liver fibrosis and inflammation after treatment with DAA.[18,19] Huang et al.[20] stated that APRI and FIB-4 indices could be used to determine fibrous regression in the liver after DAA. In a study of 392 HCV patients receiving DAA, the median LSM (liver stiffness measure=liver stiffness measure) decreased from 12.7 kPa to 8.6 ($P<0.001$) after SVR, while a significant decrease was also observed in APRI and FIB-4 values.[19] In another study, it was shown that the APRI and FIB-4 indices showed a rapid and continuous decrease at the end of the treatment and at the 12th week after the treatment.[21] Similarly, in this study, there was a significant reduction in APRI and FIB-4 values at 12- and 24-weeks post-treatment in all treatment groups. Upon completion of the therapeutic regimen, it was concluded that the diminution in parameters could be attributed to the accelerated decline in AST and ALT concentrations as a result of amelioration in necrotic inflammation.

In individuals suffering from chronic hepatitis C, platelet count is often influenced by factors such as liver fibrosis, necrotic inflammation of the liver, and insufficiency of thrombopoietin.[22] As platelets move through the liver that has been damaged, they come into contact with the endothelium of the hepatic sinusoids and gather cells and proteins that are involved in the healing process.[23] This interaction leads to a cycle of platelet and white blood cell buildup, which ultimately causes harm to the liver cells.[24] In previous studies, hepatic necroinflammatory activity has been shown to be associated with low platelet counts in CHC patients.[25] This study revealed that the decline in hepatic necroinflammation was associated with increased platelet count at 12- and 24-weeks post-treatment. This result is consistent with some studies reporting an increase in platelet count 12 and 24 weeks after DAA treatment.[26,27] Giannini et al.[28] revealed that the advancing decrease in liver functions in patients with CHC was associated with decreased thrombopoietin. The rise in thrombopoietin production and the subsequent decrease in necrotic inflammation may be the reason for the increased platelet count seen in our study.

In a study comparing biopsies of 40 patients before and 24 weeks after SVR, fibrosis healing was noticed in 38% of patients. However, prominent reductions in LSM, APRI, and FIB-4 were also seen in patients without fibrosis. In 83% of the patients, regression in inflammation was detected.[20] These findings support the conclusion that the rapid decrease in noninvasive measurements observed as early as 24 weeks in our study is mainly due to the improvement in necroinflammation. It has also been confirmed in many other studies that the early decrease in fibrous indices results from the regression in necroinflammation.[21]

In another study comparing long-term liver biopsies, liver biopsies performed 5 years after SVR were compared with APRI and FIB-4 scores. Compared with the first biopsy, significantly lower histological fibrosis was detected from the second biopsy performed 5 years later ($p<0.0001$), confirming the ability of noninvasive indices such as APRI and FIB-4 to predict liver fibrosis.[5] However, this study was conducted with other antiviral treatments used before DAA, and the number of studies showing long-term results of DAAs is extremely limited. The present study leads us to the conclusion that noninvasive fibrous indices calculated in the long term have a stronger correlation with fibrosis and better reflect fibrosis. These results are important in terms of knowing what noninvasive tests reflect at what stage. Because the availability of noninvasive fibrosis measurements together with the high SVR rates developed by the use of DAAs has changed the opinion in the management of CHC infection. In the near future, there will be a dramatic increase in the number of patients reaching SVR, and the need for continuous and non-invasive testing of liver fibrosis status will emerge. Because many studies have reported that the risk of HCC development continues even after HCV eradication and that an important risk factor for HCC development is residual liver fibrosis.[29,30]

Limitations

There are some limitations in the study. Firstly, no study examining the relationship between temporary changes in non-invasive fibrous indicators during and after DAA treatment has been conducted using histological methods. In addition, due to the invasive structure of liver biopsy, there are limitations such as sampling failure, intra-observer and interobserver histological examination diversities, and risk of various complications (pain, bleeding…). Second, this study was managed in only-one center and the number of patients was limited. Our third limitation was that the design of our study was retrospective. Our last limitation was the collection of patient data up to SVR24. Additional research is needed to see the long-period effects of DAAs.

CONCLUSION

In the study, 100% SVR was achieved in patients 12 and 24 weeks after the end of DAA treatment. This rapid response was consistent with the regression of confirmed fibrous scores APRI and FIB-4. The sudden decline in APRI and FIB-4 values may primarily be caused by the decrease in necrotic inflammation. In patients with CHC identified after SVR, more research is required to clarify the link between noninvasive index and fibrosis stage.

In order to see the long-term impacts of DAAs and long-term alterations in liver histology, prospective studies with larger patient numbers are needed many years after SVR. In addition, large-scale and long-term follow-up studies are required to determine the prognosis and management strategies of patients with CHC infection after achieving by effective treatment viral eradication.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Van Training and Research Hospital Ethics Committee (Date: 18.02.2021, Decision No: 2021/04).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Evaluation of distribution and susceptibility of microorganisms isolated from joint fluid cultures: five-year data

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ABSTRACT

Aims: Septic arthritis is joint inflammation. It is an orthopedic emergency that requires prompt diagnosis and treatment. In this study, it was aimed to examine the distribution and antibiotic resistance profiles of microorganisms isolated from joint fluid samples taken from patients who applied to the orthopedic clinic of our hospital in the last five years.

Methods: In our study, 1162 joint fluid samples were sent to the medical microbiology laboratory of our hospital from the orthopedics and traumatology clinic between January 01, 2018 and December 31, 2022. Joint fluid samples taken from clinically appropriate patients under sterile conditions were incubated in a BacT/Alert 3D (Biomerieux, France) culture device. Bacteria isolated from 164 specimens with growth detected, were identified by matrix-mediated laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) based VITEK MS (Biomerieux, France). Antibiotic susceptibility tests were performed on the VITEK 2 Compact (Biomerieux, France) device.

Results: *Staphylococcus aureus* (*S. aureus*) (29.3%) and coagulase negative Staphylococci (CNS) (29.3%) were the most commonly grown microorganisms. Other microorganisms grown were *Streptococcus* spp. (9.1%), *Enterococcus* spp. (6.1%), *Pseudomonas aeruginosa* (*P. aeuruginosa*) (7.3%), *Escherichiae coli* (*E. coli*) (4.3%) and *Klebsiella pneumoniae* (*K. pneumoniae*) (4.3%). When antibiotic susceptibility results were evaluated according to EUCAST restricted reporting criteria, linezolid in Gram-positive strains, amikacin in *Enterobacterales*, colistin and tigecycline in nonfermentative Gram-negative bacteria were found to be the most susceptible antibiotics.

Conclusion: The continuous change in antibiotic susceptibility profiles in joint infections, the long duration of treatment and follow-up, and the increase in polymicrobial infections require regular monitoring of culture and antibiotic susceptibility tests. In our study, the distribution of microorganisms isolated from joint fluid samples of our hospital and the determination of antibiotic resistance profiles will contribute to the clinician in terms of guiding empirical treatment.

Keywords: Joint fluid, antibiotic resistance rates, septic arthritis, *Staphylococcus aureus*

INTRODUCTION

Although bone and joint infections are rare, they are potentially associated with significant mortality and morbidity.[1,2] Septic arthritis is an infection of the joint space and is an orthopedically emergency requiring prompt diagnosis and treatment.[3]

Septic arthritis should be suspected when clinically there is swelling, redness, and pain in one or more joints.[1] The diagnosis of septic arthritis is made by stained microscopic examination and culture of bacteria in synovial fluid.[1] Septic arthritis is caused by bacteria, fungi, mycobacteria or viruses.[3] Although bacterial agents are the most common cause of septic arthritis, fungi, mycobacteria or viruses can also rarely be caused.[3]

In recent years, the incidence of septic arthritis has been increasing gradually.[1] In developed countries, it varies between 2-6/100,000 cases per year in the general population and increases in populations with low socioeconomic status.[1] Although individuals of all ages can be affected, septic arthritis is more common in children and the elderly, and men are affected more often than women.[1] Various factors may have contributed to this increase, including increased infections associated with orthopedic procedures and the increase in the use of immunosuppressive therapy.[1] Risk factors for the development of septic arthritis include rheumatoid arthritis or osteoarthritis, joint replacement, low socioeconomic status, intravenous drug use, alcoholism, diabetes, previous intra-articular corticosteroid injection, and cutaneous ulcers.[4]

In this study, it was aimed to determine the distribution of microorganisms isolated from joint fluid samples and

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antibiotic resistance profiles of patients who applied to the orthopedic clinic in our hospital in the last 5 years. It is predicted that the determination of antibiotic resistance profiles will guide the empirical treatment to be applied and increase the success of the treatment.

METHODS

The study was carried out with the permission of Antalya Training and Research Hospital Ethics Committee (Date: 08.06.2023, Decision No: 8/28). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In our study, the identification and antibiotic susceptibility test results of 164 specimens with growth in culture from 1162 joint fluid samples sent from orthopedics and traumatology clinics to the medical microbiology laboratory during the five-year period between 01 January 2018 and 31 December 2022 were retrospectively analyzed.

Joint fluid samples taken from clinically appropriate patients under sterile conditions were incubated in a BacT/Alert 3D (Biomerieux, France) culture device for 5-7 days. Gram staining was done for each sample in the bottles that gave a growth signal during incubation, and inoculated on 5% sheep blood agar, chocolate agar and Eosin Methylene Blue (EMB) media. It was evaluated as conventional after 24-48 hours incubation at 37°C. Bacteria isolated from specimens with growth were identified by matrix-mediated laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) based VITEK MS (Biomerieux, France). Antimicrobial susceptibility tests were performed on the Vitek 2 Compact (Biomerieux, France) device in line with the manufacturer's recommendations. Antimicrobial susceptibility assessment was performed in line with the recommendations in the EUCAST guidelines of the European Committee on Antimicrobial Susceptibility Testing. Coagulase Negative Staphylococci isolated samples with leukocytes detected in Gram stains and those with high CRP levels was included in the study. Repeated growths were excluded from the study.

RESULTS

Reproduction was detected in 164 of 1162 joint fluid samples sent to the Medical Microbiology Laboratory between 01 January 2018 and 31 December 2022 from patients hospitalized in the orthopedics clinic. While 101 of the patients had a history of joint replacement, 63 patients had natural joint involvement (**Table 1**). Of the patients, 81 (49.4%) were male and 83 (50.6%) were female. The age range of the patients was determined as 6 to 85 years.

When we examined the age distribution of microorganisms grown in joint fluid cultures according to species, it was seen that patients with *S. aureus* growth were mostly (64.6%) in the 18-64 age range. It was determined that 73.3% of the patients with *Streptococcus* spp. growth were between the ages of 18-64, while the patients with gram-negative growths were generally over 64 years (53.3-85.7%) (**Table 2**).

The most frequently isolated microorganisms were *S. aureus* and CNS, and *Staphylococcus epidermidis* was the most isolated among CNS species. Distribution rates of Gram-positive microorganisms are *S. aureus* (29.3%), CNS (29.3%), *Streptococcus* spp. (9.1%), *Enterococcus* spp. (6.1%) and Gram-negative microorganisms are *P. aeuruginosa* (%). 7.3%, *E. coli* (4.3%), *K. pneumoniae* (4.3%). The distribution of isolated microorganisms by years is given in **Table 1**.

Table 1. Distribution of isolated microorganisms by years

Microorganism	2018	2019	2020	2021	2022	Joint prosthesis (%)	Natural joint (%)	Total (%)
S. aureus	5	6	13	13	11	26 (25.8)	22 (34.9)	48 (29.3)
Methicillin susceptible *S. aureus*	4	6	8	7	9	21	13	34 (20.7)
Methicillin resistant *S. aureus*	1	0	5	6	2	5	9	14 (8.6)
CNS	11	15	5	4	13	40 (39.6)	8 (12.7)	48 (29.3)
Methicillin susceptible CNS	0	2	0	1	2	3	2	5 (3.1)
Methicillin resistant CNS	11	13	5	3	11	37	6	43 (26.2)
Streptococcus spp.	2	4	3	3	3	8 (7.9)	7 (11.1)	15 (9.1)
Enterococcus spp.	2	2	1	4	1	7 (6.9)	3 (4.8)	10 (6.1)
Non fermentative bacteria	5	5	2	4	1	8 (7.9)	9 (14.3)	17 (10.4)
Psoudomonas spp.	4	5	2	1	0	4	8	12 (7.3)
Acinetobacter spp.	1	0	0	3	1	4	1	5 (3.1)
Enterobacterales	3	4	4	6	4	11 (10.9)	13 (20.6)	24 (14.6)
E. coli	1	4	0	1	1	4	3	7 (4.3)
K. pneumoniae	1	1	0	3	2	4	3	7 (4.3)
Other *Enterobacterales*	1	2	4	2	1	3	7	10 (6.1)
Yeast	1	1	0	0	0	1 (1)	1 (1.6)	2 (1.2)
Total	29	40	28	34	33	101 (100)	63 (100)	164 (100)

Table 2. Distribution of isolated microorganisms by patient age

Microorganism	0-17 years (%)	18-64 years (%)	Over 64 years old (%)	Total (%)
S. aureus	1 (2.1)	31 (64.6)	16 (33.3)	48 (100)
Methicillin susceptible *S. aureus*	1	19	14	34
Methicillin resistant *S. aureus*	0	12	2	14
CNS	0 (0)	22 (45.8)	26 (54.2)	48 (100)
Methicillin susceptible CNS	0	3	2	5
Methicillin resistant CNS	0	19	24	43
Streptococcus spp.	0 (0)	11 (73.3)	4 (26.7)	15 (100)
Enterococcus spp.	1 (10)	3 (30)	6 (60)	10 (100)
Non fermentative bacteria	0 (0)	6 (35.3)	11 (64.7)	17 (100)
Psoudomonas spp.	0	4	8	12
Acinetobacter spp.	0	2	3	5
Enterobacterales	1 (4.2)	8 (33.3)	15 (62.5)	24 (100)
E. coli	0	1	6	7
K. pneumoniae	0	3	4	7
Other *Enterobacterales*	1	4	5	10
Yeast	0 (0)	0 (0)	2 (100)	2 (100)
Total	3 (1.8)	81 (49.4)	80 (48.8)	164 (100)

Of the *S. aureus* isolates, 14 were Methicillin Resistant *S. aureus* (MRSA) (29.2%) and 34 were Methicillin Susceptible *S. aureus* (MSSA) (70.8%), and antibiotic resistance rates are given in **Table 3**. MRSA strains were found susceptible to vancomycin, teicoplanin, daptomycin, gentamicin, linezolid, tigecycline, and fosfomycin. MSSA strains were found susceptible to vancomycin, teicoplanin, daptomycin, gentamicin, linezolid, tigecycline, trimethoprim/sulfamethoxazole and fosfomycin. High rates of resistance to tetracycline, 64.2%, clindamycin 50.0% and erythromycin 48.2% were detected in MRSA strains. The resistance rates for tetracycline, clindamycin and erythromycin in MSSA strains were lower than MRSA strains as 8.8%, 5.8% and 8.8%, respectively.

Table 3. Antibiotic Resistance Rates of *S. aureus* and CNS Strains Growing in Joint Fluid Cultures

Antibiotic	MSSA n: 34 (%)	MRSA n: 14 (%)	MSCNS n: 5 (%)	MRCNS n: 43 (%)
Trimethoprim/ Sulfamethoxazole	-	2 (14.2)	-	10 (23.2)
Erythromycin	3 (8.8)	6 (48.2)	-	20 (46.5)
Clindamycin	2 (5.8)	7 (50.0)	-	16 (37.2)
Tetracycline	3 (8.8)	9 (64.2)	1 (20.0)	16 (37.2)
Ciprofloxacin	1 (2.9)	2 (14.2)	-	22 (51.0)
Vancomycin	-	-	-	-
Teicoplanin	-	-	-	3 (6.9)
Gentamicin	-	-	1 (20.0)	19 (44.1)
Linezolid	-	-	-	1 (2.3)
Fusidic acid	2 (5.8)	3 (21.4)	2 (40.0)	34 (79.0)
Tigecycline	-	-	-	-
Daptomycin	-	-	-	2 (4.6)
Fosfomycin	-	-	1 (20.0)	13 (30.2)

- No resistant microorganism was detected

Of the 48 isolated CNS strains, 43 (89.6%) were Methicillin Resistant CNS (MRCNS) and 5 (10.4%) were Methicillin Susceptible CNS (MSCNS). While MRCNS strains were found susceptible to vancomycin and tigecycline, MSCNS strains were found to be susceptible to linezolid, vancomycin, teicoplanin, daptomycin, tigecycline, ciprofloxacin, clindamycin, erythromycin, and trimethoprim/sulfamethoxazole. Resistance rates to ciprofloxacin 51.0%, erythromycin 46.5%, gentamicin 44.1%, clindamycin 37.2% and tetracycline 37.2% were detected in MRCNS strains. Tetracycline and gentamicin resistance was found in 20% of MRCNS strains. Antimicrobial resistance profiles of *S. aureus* and CNS growths are given in **Table 4**.

Table 4. Antibiotic resistance rates of other microorganisms grown in joint fluid culture (%)

	Enterobacterales * n: 24 (%)	Non fermentative bacteria** n: 17 (%)
Antibiotics	Resistance rates	Resistance rates
Cefuroxime	14 (58.3)	-
Gentamicin	4 (16.7)	4 (23.5)
Piperacillin/ Tazobactam	8 (33.3)	3 (17.6)
Trimethoprim/ Sulphamethoxazole	9 (37.5)	4 (23.5)
Ceftazidime	12 (50.0)	5 (29.4)
Ceftriaxone	12 (50.0)	-
Amikacin	1 (4.2)	4 (23.5)
Ampicillin	20 (83.3)	-
Ciprofloxacin	10 (41.7)	7 (41.2)
Levofloxacin	-	7 (41.2)
Meropenem	2 (8.3)	4 (23.5)
Ertapenem	6 (25)	-
Tigecycline	2 (8.3)	0 (0)
Cefepim	10 (41.7)	0 (0)
Colistin	4 (16.7)	0 (0)

**E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia marcescens*, *Proteus mirabilis*, *Salmonella enterica* ssp. enterica
** *Pseudomonas aeruginosa*, *Acinetobacter baumannii*
- Antibiotic not tested.

Of the Gram-negative microorganisms isolated from the joint fluid, 24 (14.6%) were *Enterobacterales* and *E. coli* and *K. pneumoniae* were the most isolated species. Among the 17 (10.4%) non-fermentative bacteria isolated in the second frequency, P.aeurginosa was the most isolated. The highest resistance was found against cephalosporin group antibiotics (over 50%) in *Enterobacterales* species. The resistance rates of broad-spectrum antibiotics meropenem, gentamicin and tigecycline were found to be 4.7%, 8.3%, 16.7% and 8.3%, respectively.

Cefepime, colistin and tigecycline resistance were not detected in gram negative nonfermentative bacteria, and the most resistant antibiotics were determined as ciprofloxacin (41.2%) and levofloxacin (41.2%). Antibiotic resistance rates of gram negative bacteria are given in **Table 4**.

DISCUSSION

Bacterial septic arthritis represents joint infection caused by colonization of the joint space by a pathogenic bacteria.[5] Septic arthritis has an incidence of 2-6/100,000 per year and a high mortality rate of 10-15%.[1,2,5] It is an orthopedic emergency that requires early diagnosis and treatment.[5] If left untreated, chronic inflammation and sequelae may ocur.[5] Generally, monoarticular involvement and, less frequently, polyarticular involvement in approximately 22% can be seen clinically. There are physical examination findings such as redness, swelling and pain in the involved joint.[5] The diagnosis of septic arthritis is made by isolating the causative microorganism by Gram staining and culture.[6] Old age, diabetes, cirrhosis, kidney disease, rheumatoid arthritis, osteomyelitis, joint prosthesis, recent joint surgery, concomitant skin infection, intravenous drug use are possible risk factors.[5]

While *Haemophilus influenzae* is the main causative agent in children younger than two years of age, the most common organism in all other age and risk groups is *S. aureus*.[1] It is responsible for 37-56% of cases.[1] Studies have reported methicillin resistance in *S. aureus* isolates at rates ranging from 6 to 22%.5 Other gram-positive bacteria follow, including streptococci. It has been reported that CNSs are isolated from joint fluid cultures at a rate of 30-43%, but surgical interventions applied to the joint area have increased the frequency of identification of these microorganisms as agents in culture.[7] Gram-negative bacilli are the causative agent in 10-20% of bacteria isolated from joint fluid.[1]

As a result of the retrospective evaluation of five-year joint fluid samples in our study, the most frequently isolated microorganisms were *S. aureus* strains and CNS strains and were isolated at a rate of 29.3%. Of the *S. aureus* isolates, 14 (29.2%) were MRSA and 34 (70.8%) were MSSA. Of the *S. aureus* strains, 26 (54.2) were isolated from joint fluids with prosthetic joint involvement and 22 (45.8) with natural joint involvement. Similar to our study, the growth rate for *S. aureus* strains was determined as 46% in synovial fluid cultures taken from natural joints by Flores-Robles et al.[8] The detection rate of *S. aureus* was reported as 35.8% in the post-arthroplasty synovial fluid samples study by Bauer et al.[10] and 48% in the synovial fluid samples taken from both prosthetic and natural joints by El-Ganzoury et al.[9] Although the number and type of samples change when the literature is reviewed, *S. aureus* continues to be reported as the most common agent, as in our study. This is often followed by CNSs. Flores-Robles et al.[8] Bauer et al.[10] and El-Ganzoury I. et al.[9] reported that 6.4%, 26.4% and 30% of CNS strains were isolated, respectively. In our study, 43 (89.6%) of the CNS strains isolated at a rate of 29.3% were MRCNS and 5 (10.4%) were MSCNS. While 40 (83.3%) of CNS strains were detected in prosthetic joints, 8 (16.7%) were isolated from synovial fluids with natural joint involvement. In the study of Hasbek et al.[11] in our country, CNS strains isolated at a rate of 20.4%; 15 (71.4%) were isolated from synovial fluids with natural joint and 6 (28.6%) prosthetic involvement. In the same study, 6 (31.6%) of CNS strains were determined as MSCNS and 9 (10.8%) as MRCNS, and methicillin resistance was found at a lower rate than our study.[11] Differences between CNS growth and methicillin resistance rates generally vary according to the patient group and sample diversity.

Streptococcus spp. are the second most common microorganisms that cause infectious arthritis in adults, after staphylococci, and are often associated with autoimmune disorders, chronic skin infections, and trauma.1 In studies conducted in France, Egypt and Spain, 17%, 13% and 12.7% of *Streptococcus* spp. strains were isolated, respectively.[8-10] In studies conducted with joint fluid samples in Spain and China, *Enterococcus* spp. strains were reported at rates of 1.6% and 7.1%. 8,12 In our study, *Streptococcus* spp. was found at a rate of 9.1% in line with the literature, and it is noteworthy that the growth rate was 53.3% in patients with joint prosthesis. Our rate of *Enterococcus* spp. detected in the presented study was 6.1%, which was compatible with the literature.

When the literature is examined, it has been observed that there are very few studies on antibiotic resistance levels in joint fluid cultures in our country. Studies are generally studies on post-surgical infectious agents. In a study in which Savcı et al.[13] examined postoperative orthopedic surgical wound infections, all *S. aureus* isolates were found to be susceptible to clindamycin,

linezolid, teicoplanin and vancomycin; Over 90% sensitivity was detected against ciprofloxacin, gentamicin, tetracycline, daptomycin, fosfomycin, fusidic acid and trimethoprim/sulfamethoxazole; The highest resistance rate was found against erythromycin with 13.6%.[13] Kurutepe et al.[14] detected vancomycin and teicoplanin as the most susceptible antibiotics in *S. aureus* species isolated from various clinical specimens; They reported resistance to tetracycline 46.8%, erythromycin 42.0%, ciprofloxacin 27.1%, rifampicin 26.1%, clindamycin 23.1%, gentamicin 24.7% and TMP-SXT 18.3%.[14]

While Özel et al.[15] did not detect vancomycin, teicoplanin, daptomycin, linezolid and tigecycline resistance in MRSA and MSSA strains from various clinical samples. They found resistance to erythromycin, clindamycin, TMP-SXT, and tetracycline at a rate of 41.7%, 41.7%, 33.3%, and 33.3%, respectively, in MRSA strains, and 16.4%, 12.7%, 7.3%, and 14.5%, respectively, to erythromycin, clindamycin, TMP-SXT, and tetracycline in MSSA strains.[15] In the studies of Hasbek et al.[11] resistance to vancomycin, teicoplanin, daptomycin, linezolid, and tigecycline was not found in MRSA strains isolated from joint fluids; The highest resistance rates (42.8%) were determined against tetracycline, clindamycin and erythromycin.[11] Resistance to vancomycin, teicoplanin, daptomycin, tigecycline, trimethoprim/sulfamethoxazole and fosfomycin was not detected in MSSA strains; Resistance to gentamicin, erythromycin and clindamycin was found at a rate of 5.6%.[11] In our study, resistance to vancomycin, teicoplanin, daptomycin, gentamicin, linezolid, tigecycline, and fosfomycin was not detected in MRSA strains isolated from joint fluids; the highest resistance was found to tetracycline (64.2%), clindamycin (50%) and erythromycin (48.2%), consistent with the literature data. No resistance was found in MSSA strains to vancomycin, teicoplanin, daptomycin, gentamicin, linezolid, tigecycline, trimethoprim/sulfamethoxazole and fosfomycin. The highest resistance was found with 8.8% against erythromycin and tetracycline. We think that the presented study will contribute to the literature as one of the few studies that reveal susceptibility data from joint fluid.

Şen et al.'s[16] study on various clinical samples and Hasbek et al.'s[11] study of joint fluids found higher levels of antibiotic resistance against erythromycin, clindamycin, ciprofloxacin, levofloxacin, tetracycline, and fusidic acid in MRCNS isolates. In our study, vancomycin and tigecycline resistance was not found in MRCNS strains; Resistance rates were 51.0% to ciprofloxacin, 46.5% to erythromycin, 44.1% to gentamicin, 37.2% to clindamycin and 37.2% to tetracycline. No resistance was found in MSCNS isolates to linezolid, vancomycin, teicoplanin, daptomycin, tigecycline, ciprofloxacin, clindamycin, erythromycin, and trimethoprim/sulfamethoxazole. Tetracycline, fosfomycin and gentamicin resistance was observed in only 1 of the strains with MSCNS growth. The fact that five MSCNS strains were isolated in our study is a limiting situation for evaluating antibiotic resistance rates.

Gram-negative pathogens account for 10-20% of septic arthritis cases. It is more common in patients with long-term implants.[17] In a study conducted; The most susceptible (90%) antibiotics in *E. coli* strains isolated from hip joint fluid were meropenem and ertapenem; Sensitivity rates of 60% to amikacin and levofloxacin and 61% to ciprofloxacin have been reported. Gentamicin 85% and trimethoprim/sulfamethoxazole 75% were resistant. In the same study, all *Klebsiella* strains were reported to be carbapenem resistant but colistin susceptible.[18] In our study, of 41 (25%) Gram-negative microorganisms isolated from joint fluid, 24 (14.6%) were isolated as *Enterobacterales* and 17 (10.4%) were non-fermentative gram-negative bacteria. Of the isolates with Gram-negative agents,[19] were isolated from the prosthetic joint and 22 from the natural joint. In our study, the highest sensitivity rate for the two most frequently isolated microorganisms in *Enterobacterales* species was found in amikacin (95.8%), similar to the studies in the literature. Resistance rates of 8.3%, 16.7% and 11.8% were determined for meropenem, gentamicin and tigecycline, respectively, and resistance rates above 50% were found for ampicillin, cefuroxime, ceftazidime and ceftriaxone. In our study, Gram-negative non-fermentative bacteria were all susceptible to cefepime, colistin and tigecycline; Resistance to ciprofloxacin and levofloxacin was detected at a rate of 41.2%. Arunshankar et al.[18] reported a sensitivity of 100% to colistin, 90% to cefepime, 70% to ciprofloxacin, 70% to meropenem, 60% to gentamicin, 50% to levofloxacin, 50% to amikacin in *P. aeruginosa* strains.

Our study has some limitations. First, this was a retrospective study at a single centre. In addition, the small number of gram-negative microorganisms grown in the synovial fluid cultures included in the study limited our discussion of antibiotic resistance profiles. Secondly, in our study, the microorganisms growing in joint fluid and the antibiotic susceptibilities of these microorganisms were presented retrospectively. Therefore, the clinical significance of the results and treatment follow-up could not be evaluated. However, our study is a guide in terms of conducting studies supported by prospective clinical studies.

CONCLUSION

The continuous change of antibiotic susceptibility profiles in joint infections and the increase in polymicrobial infections require long-term multi-antibiotic therapy. Appropriate antibiotic treatment will be given according to the susceptibility results following the isolation of the agent. However, since this is not always possible, empirical treatment should be planned by evaluating epidemiological data and risk factors. For this reason, multidisciplinary approaches in which culture and antibiotic susceptibility tests are regularly monitored are the main elements of success in treatment planning. Our study is important in that it is one of the few studies conducted in our country in which the distribution of microorganisms isolated from joint fluid samples and their antibiotic resistance profiles were evaluated with five-year data. In the light of regional and epidemiological information, the presented study will contribute to the literature on empirical antimicrobial selection and treatment planning in septic arthritis in terms of agent distribution and resistance profiles, and will guide multidisciplinary studies in our country.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Antalya Training and Research Hospital Ethics Committee (Date: 08.06.2023, Decision No: 8/28).

Informed Consent: Because the study was designed retrospectively, no written informed consent from was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Single-center experience of cubital tunnel syndrome surgery performing transposition or internal neurolysis with external decompression under regional intravenous anesthesia technique

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ABSTRACT

Aims: To share the 7-year experience of a single center in the application of regional intravenous anesthesia (RIVA) for surgical treatment of cubital tunnel syndrome (CTS) caused by compression of the ulnar nerve in the elbow region.

Methods: A total of 100 patients with CTS who were operated with the RIVA technique at a single center between 2012 and 2019 were retrospectively analyzed. In the RIVA technique, after providing venous drainage in the operated side arm, the double cuff tourniquet was inflated in the upper arm, and anesthesia was provided by administering a 30-40 mL solution of 2% lidocaine (3 mg/kg) diluted in 1% saline through the intravenous catheter. The surgical methods applied (transposition or internal neurolysis with external decompression), demographic data, preoperative and postoperative visual analog scale (VAS) scores for pain were compared.

Results: Out of 100 patients, 30 patients underwent surgical transposition (group 1) while internal neurolysis with external decompression was performed in 70 patients (group 2). The mean age of patients in groups 1 and 2 was 66.3±12.1 and 60.6±11.7 years, respectively. Women accounted for 73.3% of patients in group 1 and 87.1% of patients in group 2. The left side was affected in 18 (60%) patients in group 1 and 42 (60%) patients in group 2. In group 1, the mean postoperative 3rd-week VAS score (1.96±0.76) was significantly lower than the mean preoperative VAS score (7.46±0.93; $p<0.001$). Similarly, in group 2, the mean postoperative 3rd-week VAS score (1.84±0.62) was significantly lower than the mean preoperative VAS score (7.45±0.87; $p<0.001$). There was no significant between-group difference with respect to preoperative or postoperative 3rd-week VAS scores.

Conclusion: In the presence of technical infrastructure, the RIVA method can be preferred in the surgical treatment of CTS.

Keywords: Ulnar nerve, decompression, RIVA, VAS

It was presented as "oral presentation" at the 36th Scientific Congress of the Turkish Neurosurgery Society on 16/03/2023.

INTRODUCTION

Due to its peculiar anatomical course, several factors may cause ulnar nerve compression in the upper extremity. Local compression and trauma to the ulnar nerve are most likely to occur at the cubital tunnel level in the elbow region, which is where the nerve is most superficial.[1] Cubital tunnel syndrome (CTS), resulting from compression of the ulnar nerve at this level, is an ulnar nerve neuropathy that causes numbness along the medial part of the forearm, the medial half of the 4th finger, and the complete 5th finger, as well as pain due to overuse of the forearm flexors. Ulnar nerve compression can also occur in the wrist, forearm, and upper arm. Repetitive elbow flexion and extension and injuries to the elbow joint can aggravate damage to the ulnar nerve.[2] In addition, systemic and local factors such as congenital anomalies, iatrogenic injury, synovitis due to rheumatological diseases, osteophytes, ganglion cysts, metabolic diseases, diabetes, and anatomical variations in the path of the nerve are the other causes of CTS.[3,4] After carpal tunnel syndrome, ulnar nerve neuropathy is the second most frequently occurring compression neuropathy in the arm.[5,6] The typical clinical symptoms are numbness and paresthesia in the medial half of the 4th finger and the complete 5th finger. Other accompanying signs and symptoms are decreased hand grip strength, weakness of intrinsic muscles, and decreased dexterity. Symptoms usually

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aggravate when the elbow is in the flexed position. In chronic cases, weakness in the intrinsic muscles may lead to "claw-hand" deformity.[7] The diagnosis can be made clinically. A combination of electromyography and nerve conduction velocity tests are usually performed to confirm the diagnosis and pinpoint the location where the ulnar nerve is being compressed in a pathological manner. However, some patients may have normal nerve conduction during the initial phase of the illness; therefore, it is essential to consider the clinical context when interpreting nerve conduction studies. To eliminate the possibility of bone-related pathologies, such as osteophytes or past fractures that may be causing nerve compression, an X-ray of the elbow joint may be conducted.[8] Ultrasound and MRI are useful in identifying pathologies such as soft tissue swelling, neuroma, ganglia, aneurysms, and alterations to the nerve's structure inside the cubital tunnel.[7]

Conservative treatment is usually the first-line treatment for CTS. Approximately half of all patients respond to conservative treatment.[9] However, surgical treatment should be considered for patients who do not show improvement with non-invasive treatment for 6 to 12 weeks and in patients with progressive paralysis or chronic lesions such as claw hand and muscle atrophy.[10] Surgical treatment options include in situ decompression (decompression in situ), medial epicondylectomy, transposition (anterior subcutaneous, anterior intermuscular, and anterior submuscular), and endoscopic in-situ decompression.[11,12] Most of these procedures require general anesthesia and an operating room environment.[13] Apart from this, procedures can be performed under local anesthesia, brachial plexus block, or even regional intravenous anesthesia (RIVA) (Bier's block).[14] The RIVA method was pioneered by Dr. August Bier (1908) and is referred to as the Bier block.[15] Double lumen tourniquet and lidocaine application by Holmes in 1963 contributed greatly to the widespread use of the method.[16] In this procedure, regional anesthesia is achieved by inflating a tourniquet on the operative extremity close to the injection site, followed by an intravenous (IV) injection of local anesthetic.

Despite the existence of several surgical techniques for managing CTS, there is no clear consensus on the best operative intervention. In addition, there is no clear opinion about the type of anesthesia, and only a few studies have reported the outcomes of the RIVA method.

The aim of this retrospective study was to investigate the effectiveness of the RIVA method in the surgical treatment of CTS and to convey our experience.

METHODS

The study was carried out with the permission of Ankara City Hospital No: 1 Clinical Researches Ethics Committee (Date: 20.04.2022, Decision No: E1/2600/2022). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this study, we retrospectively reviewed patients who underwent unilateral CTS surgery using the RIVA technique between 2012 and 2019 at the Department of Neurosurgery, Ankara Gazi Mustafa Kemal State Hospital (formerly known as "Republic of Turkey State Railways Hospital"; now known as "Ankara Gazi Mustafa Kemal Occupational and Environmental Diseases Hospital"). The patients were categorized into two groups: Group 1 underwent surgical transposition, while Group 2 was characterized by internal neurolysis with external decompression. Details of the surgical methods applied (transposition or internal neurolysis with external decompression), demographic data, and preoperative and postoperative visual analog scale (VAS) scores for pain were compared.

Patient Selection

The inclusion criteria were 1) adult patients (age>18 years) with a clinical and electrophysiological diagnosis of moderate to severe CTS; 2) American Society of Anesthesiologists (ASA) status 1-2; 3) lack of response to conservative treatment for at least 3 months with severe loss of motor power; 4) availability of preoperative and postoperative VAS scores for pain. Patients with a history of operation in the elbow region and patients with a diagnosis of diabetic polyneuropathy were excluded. A total of 100 patients were included in the study. All surgical procedures were performed by two surgeons.

Surgical Procedure

All patients were operated in the operating room under sterile conditions. Previously, RIVA was applied to each patient as a routine anesthesia procedure by the anesthesia team.

The surgical procedure was performed using a RIVA (Bier block) upper arm tourniquet. A single or double-cuffed tourniquet was placed on the proximal upper arm of the surgical extremity in a way that would not prevent ulnar nerve dissection. Venous drainage was then achieved by raising the arm and wrapping it with an Esmarch bandage (**Figure 1**). The proximal cuff was filled to a level of 300 mm Hg, and the Esmarch band was released. The absence of the radial pulse and examination of the hand confirmed the isolation of blood circulation to the arm. Then, 3 mg/kg of 2% lidocaine (® JETMONAL 2% ampoule, Adeka, Turkey) diluted with 0.9% saline (approximately 40 ml solution) was injected through the venous cannula on the dorsum of the hand to provide regional anesthesia (**Figure 1**).

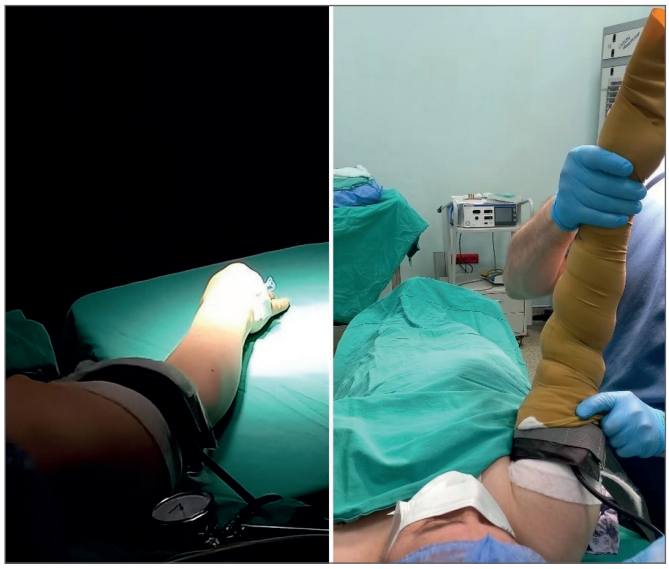

Figure 1. After the application of Esmarch bandage, the proximal cuff is inflated to 300 mmHg and intravenous local anesthetic is administered.

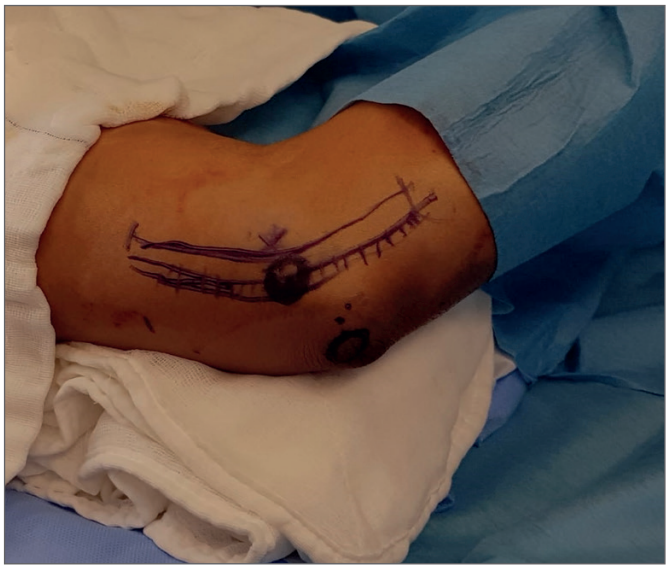

Figure 2. Surgical incision and liberated ulnar nerve image in a patient who underwent transposition under local anesthesia.

After achieving adequate sensory block for the operation, propofol infusion (6 mg/kg/h) was started simultaneously with the surgical incision in order to reduce the patient's operative stress and increase the patient's adaptation to the environment. The infusion was terminated by reducing the dose along with the surgical steps.

Classical Surgery for Simple External Decompression Plus Internal Neurolysis

The arm to be operated on was rotated outward and the elbow was positioned in 60°-90° flexion. At the posterior of the medial epicondyle of the humerus, a 6-8 cm long curved skin incision was made above and below the elbow. Following the skin incision, subcutaneous tissues were cut and the fascia and ulnar nerve were visualized. Subsequently, a distal incision was made to the cubital tunnel retinaculum and flexor carpi ulnaris aponeurosis and the ulnar nerve was decompressed. Subsequently, the nerve sheath was inflated by applying epineural internal neurolysis with saline using a dental tip injector. The subcutaneous tissue and skin were closed with 4/0 sutures. After the procedure, the arm was gently wrapped with an elastic bandage and elevated, and the tourniquet was gradually loosened.

Transposition Surgery

In the surgery performed for transposition, the incision was 8-10 cm long (**Figure 2**). After the ulnar nerve was liberated 360 degrees, the nerve was taken anteriorly from the cubital tunnel and submuscular transposition was performed. After suturing the muscle around the nerve, the subcutaneous and skin were closed with 4/0 sutures. Subsequently, the arm was gently wrapped with an elastic bandage and elevated, and the tourniquet was gradually loosened after the procedure.

Preoperatively, all patients were administered a prophylactic intravenous dose of cefazolin sodium 1 g (® CEZOL 1 g, Deva, Turkey). No antibiotics were prescribed postoperatively. All patients were prescribed analgesics and discharged on the same day after the operation. Postoperatively, an elastic bandage was used and the arm was maintained in 90 degrees of flexion at the elbow for the first 24 hours. The dressing was changed the next day, and the dressing was applied to cover only the incision. Finger mobilization was recommended for all patients in the early postoperative period. Sutures were removed after 2 weeks postoperatively. Since the wound healing control was performed at the 3rd week, the evaluation of the VAS was also recorded in the patient's file.

VAS scores for pain and complications were recorded. We employed a visual pain scale ranging from 1 to 10, where 1 represents lower pain levels and 10 indicates higher levels of pain. Age, sex, preoperative, and postoperative 3rd-week VAS scores were obtained from the medical records (**Table**).

Table. Demographic data and VAS scores among CTS surgical methods performed with RIVA technique

	Transposition (group 1) (n=30)	Internal neurolysis with external decompression (group 2) (n=70)	p
Age (±SD)	66.37±12.19	60.61±11.78	0.677
Gender, female (n; %)	22;73.3	61;87.1	0.094
Affected side, left (n; %)	18; 60	42±60	1.000
VAS preop (mean)	7.46±0.93	7.45±0.87	0.981
VAS post op 3rd-week (mean)	1.96±0.76	1.84±0.62	0.450

VAS: visual analog scale

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences for Windows 23 software. The normality of distribution of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed variables were presented as mean ± standard deviation, while non-normally distributed variables were presented as median (range). Categorical variables were presented as frequency (percentage). Pre- and post-treatment values were evaluated using repeated measures analysis of variance (ANOVA). The results were evaluated at the 95% confidence interval, and p values <0.05 were considered indicative of statistical significance.

RESULTS

Out of the 100 patients operated on during the study reference period, 30 patients (22 women [73.3%]) underwent surgical transposition (Group 1) while 70 patients (61 women [87.1%]) underwent internal neurolysis with external decompression (Group 2). The mean age of patients in Group 1 and Group 2 was 66.3±12.1 year and 60.6±11.7 years, respectively. The left side was affected in 18 (60%) patients in Group 1 and 42 (60%) patients in Group 2. In Group 1, the mean preoperative VAS score was 7.46±0.93 and the mean postoperative 3rd-week VAS score was 1.96±0.76 (p<0.001). In Group 2, the mean preoperative VAS score was 7.45±0.87 and the mean postoperative 3rd-week VAS score was 1.84±0.62 (p<0.001). There was no significant difference between Groups 1 and 2 with respect to preoperative or postoperative 3rd-week VAS scores.

DISCUSSION

In this study, we present the results of internal neurolysis surgery with transposition and external decompression with the RIVA (Bier Block) method performed at a single center in patients with CTS. There was a significant decrease in the pain scores of patients at the end of the 3rd postoperative week. The absence of blood in the tissues facilitated better intraoperative hemostasis, reducing the risk of fibrosis. Moreover, the minimal incision enabled faster healing with less tissue damage. In the transposition procedure using the RIVA method, the incision was slightly longer and the surgical time was longer compared to the external compression with RIVA. However, both surgical techniques had high procedural success and provided adequate analgesia. The significant improvement in the postoperative VAS scores shows that the RIVA method is suitable for CTS surgery.

Direct pressure on the nerve, caused by extended periods of sitting or as a result of occupational tasks, is an important cause of ulnar nerve damage, as the nerve passes behind the medial epicondyle and travels superficially in this region.[17] Studies have showed the effectiveness and safety of forearm and upper arm Bier blocks for hand surgery.[18] The surgeon's experience is also a key determinant of the choice of surgical technique.[19] Various surgical techniques can be used in the treatment of CTS. Simple alleviation of pressure on the ulnar nerve by endoscopic technique has become popular in recent years.[20] Anterior transposition of the ulnar nerve may be appropriate if there are severe changes to the bone or tissue of the elbow. Submuscular transposition may be the favored option if there is scarring, as it offers a nourishing vascular bed for the nerve and provides protection from soft tissue. Possible transposition risks include the nerve becoming bent due to insufficient proximal or distal mobilization, as well as impaired blood flow to the nerve. In these cases, revision surgery is required.[20] A study indicated that various surgical methods are comparable in terms of clinical outcomes.[21] However, transpositional decompression surgery is associated with a higher risk of wound infection compared to simple decompression.[22] Despite a lack of clear consensus in terms of strategies for managing CTS, in-situ ulnar nerve decompression has been shown to be equally effective as anterior transposition, but is associated with fewer complications.[23] Due to the advantages of rapid return to daily life and healing from surgical scars, there is increasing demand for less invasive procedures utilizing the tourniquet-free, awake, and local anesthetic method. In recent years, a method for injecting local anesthetic into the cubital tunnel has been described that involves two stages, provides comfort, allows for clear visualization, and makes it possible to access multiple compression zones with minimal incisions. In this technique, after injecting 3 mL of local anesthetic 3 cm distal to the incision, the cubital tunnel is where the second stage of anesthesia is delivered.[24]

RIVA offers several advantages in upper extremity surgery such as ease of application, fewer complications, low cost, and rapid initiation and ending of anesthesia. This method is frequently preferred because of the safety and effectiveness of anesthesia.[25] Although lidocaine is generally used in the RIVA technique, there is no clear consensus regarding the ideal agent to be used. Various adjuvants can be added to local anesthetics to increase the quality of RIVA and minimize the side effects of local anesthetics. In our study, propofol was used together with lidocaine for sedation.

The complication that causes the most anxiety is the rare occurrence of an ulnar nerve laceration. In comparison to ulnar nerve laceration, damage to the medial antebrachial cutaneous nerve is more commonly observed.[23] However, no significant complications were observed in our series. Studies have found no difference in outcomes between cubital tunnel release under local anesthesia performed in a minor operating setting and that performed in the main operating room.[14] Revision procedures for CTS are less dependable than primary procedures; however, approximately 75% of patients experience improvement in pain and paresthesias. Elderly age and the presence of severe disease during the revision period are associated with worse outcomes; in addition, chronic CTS-related weakness and atrophy can result in extra morbidity and are typically not improved by revision procedures.[6] None of the patients in our study required revision surgery.

Caputo and Watson reported positive outcomes in 15 (75%) of 20 patients who had anterior subcutaneous transposition during revision surgery. Poor outcomes have been reported in older patients and those who have had multiple previous procedures.[26]

The endoscopic approach, which can be performed under local anesthesia without the use of a pneumatic tourniquet, facilitates the examination of the ulnar nerve, enabling the selective release of the tissue compressing the nerve. High rates of improvement in pain and sensory symptoms have been reported.[27] The use of regional anesthesia avoids the potential complications of general anesthesia. In postoperative settings, regional anesthesia reduces the need for analgesics in various cases, avoiding adverse effects associated with postoperative opioids such as respiratory depression, dizziness, and hypotension.[28,29]

Balevi et al.[30] evaluated the outcomes of modified simple decompression (MSD) procedure by performing a postoperative electrophysiological study in 15 patients who underwent decompression with a 4 cm long incision above and below the elbow under regional anesthesia. The results demonstrated that MSD is a technically simple, safe, and effective method with minimal complications. The MSD procedure in their study was performed under regional anesthesia, similar to ours, but the number of patients in our study was much larger.

Ergen et al.[31] reported symptom relief in 89% of the patients who underwent ulnar nerve submuscular anterior transposition surgery for CTS. They reported low recurrence and complication rates. This result is similar to the results of other techniques reported in the literature.[32,33]

Complications are more common in anterior submuscular transfer surgery, which is a more complex surgical technique compared to simple decompression and anterior subcutaneous transfer. Complications of the technique include hematoma at the incision site, medial antebrachial cutaneous neuroma in cutaneous nerve branches, and elbow stiffness due to immobility. None of these complications were encountered in our series. Staples et al.[34] evaluated 78 patients who underwent anterior transposition. The incidence of postoperative hematoma was 15%. Therefore, after the completion of the transposition procedure, the tourniquet should be terminated, hemostasis should be carefully monitored, and compression with an elastic bandage should be applied postoperatively. None of the patients in our study developed a postoperative hematoma. We attribute this to the absence of blood in the tissues in the RIVA method, the short surgical time, and less tissue injury.

Hurwitz et al.[35] showed that ulnar nerve instability can occur in up to 50% of cases after simple decompression and concluded that the nerve should not be dissected more than 4 cm proximal to the medial epicondyle. In contrast, in a cadaver study by Butler et al.[36] decompression was not found to cause instability. The subluxation rate in CTS is approximately 20%. Male sex and young patient age are considered risk factors for post-decompression subluxation.[37] In our study, women accounted for a larger proportion of the study population and the mean age was >65 years. None of the patients in our study showed signs of nerve instability.

Tang et al.[38] reported that ulnar nerve instability after in-situ decompression could be prevented with the "blocking flap technique." This technique entails the injection of a local anesthetic proximal and distal to the incision and the use of an Esmarch bandage to drain the venous blood. In our study, venous drainage was performed with the Esmarch bandage, but the anesthetic agent was administered intravenously afterward. Men are affected more often than women, and the left side is more frequently affected.[39] In our study, while the left side was more frequently affected, the percentage of women was higher. Saeed et al.[40] reported good functional outcomes of internal neurolysis combined with submuscular transposition in patients with McGowan grade II and III late ulnar nerve palsy. In our study, internal neurolysis was combined with simple decompression and a high rate of symptomatic improvement was achieved.

Van Gent et al.[41] reported the clinical outcomes of patients who underwent anterior subcutaneous transposition after neurolysis failure in CTS surgery. Although the majority of patients reported only partial improvement or even worsening of symptoms after CTS surgery, they were generally satisfied. They also identified old age as a risk factor for poor outcomes.

It is important to note some of the limitations of our study. First, owing to the retrospective nature of the study, the influence of selection bias on our results cannot be ruled out and there may be potential inaccuracies in data collection. Second, intraoperative photographs were not obtained. Finally, analysis of long-term follow-up data could not be performed as this study was based on a retrospective review of files and operative notes.

CONCLUSION

Our study suggests that RIVA may offer some advantages in CTS surgery, both transposition and simple decompression, as well as good functional recovery and fewer complications with internal neurolysis. Further studies, including larger randomized controlled trials, are warranted to confirm these findings and provide more definitive guidance on the optimal anesthetic technique for CTS surgery.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No: 1 Clinical Researches Ethics Committee (Date: 20.04.2022, Decision No: E1/2600/2022).

Informed Consent: Because the study was designed retrospectively, no written informed consent from was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Evaluation of hematological parameters in the differentiation of bile reflux gastritis and *Helicobacter pylori* gastritis in children

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ABSTRACT

Aims: Endoscopy is used in the diagnosis of bile reflux gastritis and *Helicobacter pylori (H. pylori)* gastritis. However, endoscopy is an invasive procedure with complications. The study proposes that by analyzing the absolute neutrophil - lymphocyte count ratio and mean platelet volume, it might be possible to distinguish between *H. pylori* gastritis and bile reflux gastritis.

Methods: All patients with endoscopic and histopathological confirmation *H. pylori* gastritis of (Group 1), bile reflux gastritis (Group 2), or both (Group 3) were included in this retrospective study. White blood cells, absolute neutrophil count, absolute lymphocyte count and mean platelet volume were analyzed in all patients. The absolute neutrophil/lymphocyte ratio was calculated.

Results: 218 patients were included in the study. The median absolute neutrophil-lymphocyte ratio was 1.33 in *H. pylori* gastritis, 1.56 in bile reflux gastritis, and 1.47 in both. The mean value of mean platelet volume was 9.97±0.82 in *H. pylori* gastritis, 10.16±0.81 in bile reflux gastritis, and 10.06±0.88 in both. The absolute neutrophil/lymphocyte ratio and mean platelet volume did not differ significantly between the groups.

Conclusion: According to our results, absolute neutrophil/lymphocyte ratio cannot be used as a differential diagnosis marker in children with *H. pylori* gastritis and bile reflux gastritis.

Keywords: Bile reflux gastritis, children, *Helicobacter pylori* gastritis, hematological

INTRODUCTION

Dyspepsia is characterized by various symptoms such as abdominal pain, discomfort in the epigastrium region (upper abdomen), nausea, loss of appetite, weight loss, heartburn, and regurgitation.[1] It's important to note that while the underlying cause of dyspepsia is often functional which there's no apparent structural abnormality, it can also be linked to organic, systemic, or metabolic diseases in some cases.[1-3] In the pediatric population, functional dyspepsia is more common than organic dyspepsia. Organic causes include *H. pylori* gastritis and bile reflux gastritis.[4,5] *H. pylori* is a type of bacteria that commonly infects the stomach. It is known to cause a local inflammatory response in the stomach mucosa, which can lead to gastritis and potentially peptic ulcers. The infection triggers an immune response in the body, leading to the activation of both innate and adaptive immune mechanisms. In particular, the T CD4+ cells are considered the main actors in the establishment of chronic inflammation. The innate immune response involves the activation of immune cells like neutrophils and macrophages, which attempt to eliminate the bacteria.[6] Bile reflux gastritis occurs when the contents of the duodenum, which can disrupt the gastric mucosal barrier with various factors, leak back into the stomach and cause inflammation.[7,8] The majority of cases involve asymptomatic chronic inflammation. Upper gastrointestinal endoscopy is used to differentiate between bile reflux gastritis and *H. pylori* gastritis. The ROMA IV criteria are used in the diagnosis of functional dyspepsia, a common gastrointestinal disorder characterized by recurring or chronic upper abdominal pain or discomfort.[9]

The absolute neutrophil-lymphocyte count ratio (ANC/ALC) is indeed considered to be a marker of subclinical inflammation in exacerbations of many lung diseases, obesity, and many heart diseases.[10,11] This can be an indication of an inflammatory response, as neutrophils are often recruited to sites of infection or inflammation.[12] Studies have indeed shown that elevated mean platelet volume (MPV) values can be associated with various conditions involving low-grade inflammation, such as diabetes and coronary artery disease.[13] The physiological response of circulating white blood cells (WBC) to stress, infection, or inflammation often leads to changes

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in the numbers of different types of leukocytes. This includes an increase in the number of neutrophils and a decrease in the number of lymphocytes. Due to these changes in leukocyte counts during times of stress or inflammation, the ANC/ALC ratio becomes a useful and sensitive marker for assessing the presence of inflammation. The absolute neutrophil-lymphocyte count ratio is derived from a hemogram test and is used as an inflammatory marker of various diseases. These ANC/ALC ratio and MPV in peripheral blood are used as parameters that provide information about the relationship between the inflammatory environment and physiological stress. In this study, our aim is to evaluate whether the hematological parameters are a guide in the differentiation of pediatric patients with bile reflux gastritis and *H. pylori* gastritis.

METHODS

The study was carried out with the permission of Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date:22.06.2022, Decision No: 2022-06/28). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We conducted a retrospective study on 218 children who applied to Sivas Numune Hospital Pediatric Gastroenterology outpatient clinic with dyspeptic symptoms and underwent upper gastrointestinal endoscopy between January 2020 and July 2023. Gender, age, pathological data of endoscopy preparations and complete blood count data were analyzed. Patients with acute infection, chronic/previous (in the last 1 month) drug use, and chronic disease such as Celiac disease, inflammatory bowel disease, familial Mediterranean fever were not included the study.

The patients were divided into 3 groups according to the endoscopy and pathology results. Group 1 were those with *H. pylori* gastritis, Group 2 were those with bile reflux gastritis, and Group 3 were those with both. Sydney classification was used for data on the degree of chronic inflammation and *H. pylori* density.

In the hemogram parameters, WBC, ANC/ALC ratio, and MPV values were examined.

Statistical Analyses

All statistical analyzes were performed using the SPSS version 22.0 statistical software program (SPSS, Chicago, IL). Normality of distribution of numerical variables was evaluated. Numerical data were compared between groups using Kruskal-Wallis test (not normally distributed subjects) and One-Way ANOVA test (normally distributed subjects). Results were considered statistically significant when the P value was less than 0.05.

RESULTS

A total of 218 children were included in the study. Of the patients, 156 were girls and 62 were boys. The mean age of the patients was 14.47±3.07 years. Of the patients, 117 (53.7%) had *H. pylori* gastritis, 47 (21.5%) had bile reflux gastritis, 54 (24.8%) had both bile reflux gastritis and *H. pylori* gastritis.

When 171 patients with *H. pylori* gastritis were evaluated according to the degree of gastritis; *H. pylori* gastritis was mild in 110 patients, moderate in 26 patients, and severe in 35 patients.

Ulcers were detected in 27 (12.4%) patients, 9 of them (4.1%) in the stomach and 18 (8.3%) in the bulb.

The demographic characteristics, laboratory and endoscopic findings of the patients are summarized in **Table 1**.

Table 1. Demographic features, laboratory and endoscopic findings of the study group

n=218	(%)
Age (month) mean±SD	14.47±3.07
Gender	
Female	156 (71.6)
Male	62 (28.4)
Helicobacter pylori gastritis	117 (53.7)
Bilier reflux gastritis	47 (21.5)
Helicobacter pylori gastritis and bilier reflux gastritis	54 (24.8)
Ulcer	27 (12.4)
WBC median (min-max)	6830 (3670-15780)
ANC/ ALC median (min-max)	1.42 (0.28-9.40)
RBC mean±SD	5.0±0.43
HB mean±SD	13.97±1.38
HTC mean±SD	42.08±3.80
MCV mean±SD	84.12±4.33
Platelet mean±SD	294000±66000
MPV mean±SD	10.03±0.83

Variables with normal distribution were shown as mean±SD, and variables not with normal distribution as median (min-max). WBC: White blood cell, ANC/ALC: Absolute neutrophil count/ Absolute lymphocyte count, RBC: Red blood cell, HTC: hematocrit, MCV: Mean corpuscular volume, MPV: Mean platelet volume

When the groups were compared within themselves, no statistically significant difference was found between the rates of WBC, MPV and ANC/ALC ratio (**Table 2**).

Table 2. Comparison of hematological parameters between groups

n=218	*Helicobacter pylori* gastritis (n=117)	Bilier reflux gastritis (n=47)	*Helicobacter pylori* gastritis and Bilier reflux gastritis (n=54)	P value
WBC median (min-max)	6850 (4190-15780)	6520 (3670-13510)	7025 (4110-12600)	0.344
ANC/ALC median (min-max)	1.33 (0.28-8.45)	1.56 (0.54-8.02)	1.47 (0.44-9.40)	0.120
MPV mean±SD	9.97±0.82	10.16±0.81	10.06±0.88	0.376

One way ANOVA was performed for the variables with normal distribution, and Kruskal-Wallis tests were performed for variables not with normal distribution. Variables with normal distribution were shown as mean±SD, and variables not with normal distribution as median (min-max). WBC: White blood cell, ANC/ALC: Absolute neutrophil count/ Absolute lymphocyte count, MPV: Mean platelet volume, Statistically significant at $P \leq 0.05$.

When the patients in the Group 1 were classified as mild, moderate and severe according to the degree of disease, no statistically significant difference was found in hematological parameters (**Table 3**).

Table 3. Comparison of disease degree and hematological parameters of patients in Group 1

***Helicobacter pylori* gastritis (n=117)**	**Mild n=76**	**Moderate n=21**	**Severe n=20**	**P value**
WBC Median (min-max)	6885 (4190-15780)	7180 (4800-9700)	6590 (4680-9880)	0.722
ANC/ALC Median (min-max)	1.29 (0.28-8.45)	1.41 (0.78-2.69)	1.39 (0.49-3.71)	0.479
MPV mean±SD	9.98±0.79	9.80±0.80	10.08±0.96	0.547

One way ANOVA was performed for the variables with normal distribution, and Kruskal-Wallis tests were performed for variables not with normal distribution. WBC: White blood cell, ANC/ALC: Absolute neutrophil count/ Absolute lymphocyte count, MPV: Mean platelet volume, Statistically significant at P ≤0.05.

DISCUSSION

Dyspeptic symptoms in children, which include various gastrointestinal discomforts like pain, bloating, and nausea, are indeed common.[14] While functional issues are a leading cause, there are organic causes such as *H. pylori* infection and bile reflux gastritis that can also contribute. *H. pylori* infection is a bacterial infection that can lead to gastritis and ulcers in the stomach and small intestine.[14,15] It's a significant public health concern, particularly in areas with low socio-economic status and rural regions.[6,16,17] Bile reflux gastritis occurs when bile from the small intestine flows back into the stomach, leading to inflammation and irritation of the stomach lining. Poor eating habits can increase the risk of this condition.[7]

Diagnosing these conditions accurately is important for proper management. Histopathologic examination, which involves analyzing tissue samples under a microscope, is a reliable method for distinguishing between different causes of gastrointestinal diseases. However, this examination usually requires upper gastrointestinal endoscopy. Performing endoscopy in children can be challenging due to their discomfort and potential anxiety.[1-7] In summary, dyspeptic symptoms in children can stem from both functional and organic causes, including *H. pylori* infection and bile reflux gastritis. Accurate diagnosis often involves histopathologic examination through upper gastrointestinal endoscopy, which can be difficult to perform in children. Advances in medical technology and techniques may lead to less invasive methods for diagnosing these conditions in the future.

To our knowledge, this is the first study to evaluate the potential diagnostic value of MPV and ANC/ALC ratio parameters in differentiating pediatric patients with *H. pylori* gastritis and bile reflux gastritis.

Although studies examining the relationship between hematological parameters and *H. pylori* gastritis are rare, we have not found a study evaluating these parameters in patients with bile reflux gastritis. From this point of view, our study contributes to the literature.

In our study, hematological parameters were evaluated in the pediatric patient group who underwent gastroduodenoscopy. There was no statistically significant difference between ANC/ALC ratio and MPV values in all 3 groups. One of the few studies in the literature comparing MPV values in pediatric patients did not show a significant change in MPV values in patients with *H. pylori* gastritis.[16] Similarly, our study did not find a significant change in MPV values in children with gastritis, regardless of etiology.

In a study conducted by Sağlam et al.[17] no statistically significant difference was found between the group with and without *H. pylori* gastritis, between ANC/ALC ratio and MPV values. In a study by Melit et al.[18] no statistically significant difference was found between the ANC/ALC ratio values between the *H. pylori* gastritis group and the control group, similar to our results.

The study conducted by Jafarzadeh et al.[19] aimed to investigate the WBC and ANC/ALC ratio in adult patients with peptic ulcers infected with *Helicobacter pylori* compared to asymptomatic patients, and to analyze if there is a relationship between these parameters. Both *H. pylori* infected peptic ulcer patients and asymptomatic patients had significantly higher WBC compared to the control group. This suggests that the presence of *H. pylori* infection or peptic ulcer might lead to an elevated leukocyte response. In this study, the ANC/ALC ratio was found to be significantly higher in both *H. pylori* infected peptic ulcer patients and asymptomatic patients when compared to the control group. This suggests that the presence of *H. pylori* infection or peptic ulcer is associated with an increased ANC/ALC ratio, indicating higher inflammation levels. In our study, there was no control group, but it was found that WBC was higher in patients with bile reflux gastritis and *H. pylori* gastritis, although it was not statistically significant.

In the study of Şahin et al.[20] no relationship was found between ANC/ALC ratio and MPV values and childhood *H. pylori* infection, severity classification, or pre- and post-treatment status. In our study, no statistically significant difference was found between the ANC/ALC ratio and MPV values between groups.

Limitations of this study include the retrospective nature of the data with all cases collected from a single hospital and the low sample size.

CONCLUSION

ANC/ALC ratio and MPV can be easily calculated from routinely available data. ANC/ALC ratio can be an important measure of systemic inflammation because it is cost-effective and readily available. In this study, we showed that there was no difference between ANC/ALC ratio and MPV values in pediatric patients with bile reflux gastritis, until a new parameter is available, the use of upper gastrointestinal endoscopy, an invasive method to differentiate these diseases, will probably continue.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date:22.06.2022, Decision No: 2022-06/28).

Informed Consent: Because the study was designed retrospectively, no written informed consent from was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

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Prognostic significance of prognostic nutritional index and hemoglobin to red cell distribution width ratio in metastatic colorectal cancer patients

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ABSTRACT

Aims: Malnutrition and systemic inflammation are poor prognostic factors in cancer. Prognostic nutritional index (PNI) and Hemoglobin to red blood cell distribution witdh (HRR) are considered indicators of malnutrition and systemic inflammation. We aimed to investigate the prognostic importance of PNI and HRR on metastatic colorectal cancer survival.

Methods: We retrospectively reviewed all patients diagnosed with metastatic colorectal cancer treated at Kayseri City Training and Research Hospital and Erciyes University Medical School. PNI is calculated as (serum albumin (g/L) +5 x total lymphocyte count (10^9/L)). And HRR was calculated as the ratio of hemoglobin (g/dl) and RDW (%). PNI was divided into two groups based on the cut off points 46.175 as PNI high and low. And we compared these two groups according to general characteristics and overall survival. We performed another comparision between HRR low and high groups based on the cut off points 0.8675 according to general characteristics and overall survival. Kaplan Meier method was used to analyse overall survival and compared survival rates with the log-rank test.

Results: We reviewed 346 metastatic colorectal cancer patients and we included 145 of them who fit to inclusion criteria to the study. Univariate analysis revealed that presence of initially metastatic disease, right located tumor, low HRR, low PNI were independent prognostic markers of poor overall survival. In multivariate analysis, presence of initially metastatic disease and low PNI remain statistically significant independent prognostic markers of poor survival. The median overall survival was statistically longer in HRR and PNI low groups than high groups.

Conclusion: Both PNI and HRR are associated with poor overall survival in metastatic colorectal cancer.

Keywords: Colon cancer, prognostic nutritional index, hemoglobin red cell distribution width ratio

INTRODUCTION

Colorectal cancer is the third most common cancer related death in the World.[1] Overall survival increased by adding biological agents in metastatic colorectal cancer last years.[2] Some molecular markers and clinical characteristics were associated with disease prognosis. Mutational status of KRAS/NRAS, BRAF, microsatellite instability status, tumor sideness were reported as some of the prognostic indicators.[3] Malnutrition and systemic inflammation are also poor prognostic factors in cancer.[4] The prognostic markers are still under investigation.

Prognostic nutritional index is calculated by the serum albumin and peripheral blood lymphocytes. Lymphopenia is related with inadequate cell mediated immune response and malnutrition.[5,6] Poor nutritional status and inflammation decrease production of albumin.[7] PNI is considered as an indicator of nutritional and systemic inflammatory status of cancer patients.[8] It has been studied as a prognostic marker in several cancers.[8-10] HRR is calculated by the hemoglobin and RDW. HRR is an another parameter that reflects nutritional status and systemic inflammation.[11] The knowledge of relationship between metastatic colorectal cancer survival and PNI and HRR is limited in literature. We hypothesized that these two malnutrition and systemic inflammation index could help us to predict prognosis and survival.

We aimed to investigate the prognostic importance of PNI and HRR on metastatic colorectal cancer survival.

METHODS

The study was carried out with the permission of Kayseri City Hospital Clinical Researches Ethics Committee

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(Date: 22.08.2023, Desicion No: 881). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We retrospectively reviewed all patients diagnosed with metastatic colorectal cancer treated at Kayseri City Training and Research Hospital and Erciyes University Medical School between January 2007 and May 2023 with follow up through is August 2023. The patients who had story of chronic disease like chronic cirrhosis and end stage renal disease, antibiotic use for active infection, chronic inflamattory diseases like systemic lupus eritemtaosis, blood transfusion in last 6 months and steroid use were excluded. We also excluded the patients whose laboratory test results were missing.

Patient characteristics, chemotherapy regimens, KRAS mutation status, tumor sideness, presence of lung or liver metastasis, number of metastatic sites, date of death, laboratory datas were examined from hospital patients' records and the patients files.

The counts of serum albumin, total lymphocyte, hemoglobin, RDW before initation of chemotherapy for metastatic disease were saved. PNI is calculated as (serum albumin (g/L) +5 x total lymphocyte count (10^9/L)). And HRR was calculated as the ratio of hemoglobin (g/dl) and RDW (%) before initiation of chemotherapy.

Hemoglobin (Hb) to RDW ratio was divided into two groups based on the cut off point 0.8675 as Hb to RDW high and low (area under the curve: 0.489 (0.395-0.584), sensitivity: 41.9% spesificity: 63.4%, p=0.825). The cut off value of HB to RDW were performed using ROC curve analysis. PNI was divided into two groups based on the cut off point 46.175 as PNI high and low (area under the curve: 0.705 (0.619-0.791), sensitivity: 79.7% spesificity: 56.3%, p<0.001). The cut off value of PNI were performed using ROC curve analysis.

Statistical Analysis

Median, min, max and frequencies were defined for the general characteristics. We performed chi-square and Fisher's exact test for comparision of categorical variables. Mann-Whitney U test were used for comparision of noncategorical variables. We used univariate and multivariate analysis with the use of cox regression analysis to determine association of some variables with overall survival. Kaplan Meier method was used to analyse overall survival and compared survival rates with the log-rank test. OS was defined from the date of chemotherapy initiation to the date of death or last visit. Statistical Package for Social Sciences 22.0 (SPSS Inc., Chicago, IL, USA) software was used in all statistical analyses. A p value of <0.05 was considered statistically significant.

RESULTS

We reviewed 346 metastatic colorectal cancer patients and we included 145 of them who fit inclusion criteria to the study. The median age was 64 (26-87) years old. Sixty patients (41%) were female, 85 of them (59%) were male. Seventy one death were occured (49%). All of general characteristics were showed in **Table 1**.

Table 1. General characteristics

Variables	All patients	PNI low (n=55, 38%)	PNI high (n=90, 62%)	p	HRR low (n=88, 59%)	HRR high (n=57, 41%)	p
Age, year (min-max)	64(26-87)	63 (26-82)	65 (31-87)	0.744	63.5 (26-81)	63 (38-82	0.278
Age							
<65	75 (52)	31 (56)	44 (49)	0.398	42 (48)	33 (58)	0.240
≥65	70 (48)	24 (44)	46 (51)		46 (52)	24 (42)	
Gender							
Female	60 (41)	25 (45.5)	35 (39)	0.489	40 (45.5)	20 (35)	0.232
Male	85 (59)	30 (54.5)	55 (61)		48 (54.5)	37 (65)	
Initially metastatic							
Yes	118 (81)	43 (78)	75 (83)	0.511	77 (87.5)	41 (72)	0.028
No	27 (19)	12 (22)	15 (17)		11 (12.5)	16 (28)	
Tumor site							
Right	31 (21)	14 (25.5)	17 (19)	0.406	21 (24)	10 (17.5)	0.412
Left	114 (79)	41 (74.5)	73 (81)		67 (76)	47 (82.5)	
Liver metastasis							
Yes	99 (68)	35 (64)	64 (71)	0.364	61 (69)	38 (67)	0.855
No	46 (32)	20 (36)	26 (29)		27 (31)	19 (33)	
Lung metastasis							
Yes	54 (37)	18 (33)	36 (40)	0.479	26 (29.5)	28 (41)	0.022
No	91 (63)	37 (67)	54 (60)		62 (70.5)	29 (51)	
Number of metastasis							
1	89 (61)	33 (60)	56 (62)	0.861	56 (64)	33 (58)	0.492
≥2	56 (39)	22 (40)	34 (38)		32 (36)	24 (42)	
KRAS mutation							
Wild	94 (65)	34 (62)	60 (67)	0.334	54 (61)	40 (70)	0.371
Mutant	45 (31)	17 (31)	28 (31)		30 (34)	15 (26)	
Unknown	6 (4)	4 (7)	2 (2)		4 (5)	2 (4)	

PNI High and Low Groups

Fifty five of the patients (38%) were in PNI low group, 90 of them (62%) were in PNI high group. There were no statistically significant difference among the features. All of general characteristics were showed in **Table 1**.

HRR Low and High Groups

Eighty eight of the patients (59%) were in HRR low group, 57 of them (41%) were in HRR high group. The patients were heterogeneous according to initially metastatic disease and lung metastasis. Other variables were homogeneous in the group. All of general characteristics were showed in **Table 1**.

Univariate and Multivariate Analysis

Univariate analysis revealed that presence of initially metastatic disease 0.501(95% CI 0.300-0.836, p=0.008), right located tumor 0.567 (95 0.336-0.957, p=0.034, low HRR 0.591 (95% CI 0.358-0.974,p=0.039), low PNI 0.352 (95% CI 0.219-0.563,p=<001) were correlated with poor overall survival (**Table 2**).

We performed multivariate analysis with statistically significant parameters in univariate analysis. Presence of initially metastatic 0.362 (0.203-0.648, p=0.001) and low PNI 0.393 (0.242-0.637, p=<0.001) remain statistcally significant according to poor survival in multivariate analysis (**Table 2**).

Overall Survival

The median overall survival was 34 (14.69-53.30) months in HRR high group, 28 (23.29-32.70) months in HRR low group (p=0.035). The median overall survival was 44 (33.23-54.76) months in PNI high, 24 (15.14-32.85) months in PNI low group (P<0.001) (**Figure 1**).

DISCUSSION

Numerous studies have been established an association between systemic inflammation and poor cancer related survival.[9,12,13] Also poor nutritional status is related with poor survival.[14] PNI and HRR has been documanted as indicators of both systemic inflammation and nutritional status.[11] We demonstrated low PNI and HRR were independent indicators of poor survival.

Table 2. Univariate and multivariate analysis

Characteristics	Univariate analysis (Hazard ratio (CI 95%))	p	Multivariate analysis (Hazard ratio (CI 95%))	p
Age(<65 or ≥65)	0.937(0.580-.515)	0.791		
Gender (female or male)	1.463 (0.918-2.333)	0.110		
Initially metastatic (no or yes)	0.501 (0.300-0.836)	0.008	0.362 (0.203-0.648)	0.001
Tumor site (right or left)	0.567 (0.336-0.957)	0.034	0.625 (0.366-1.068)	0.086
Liver metastasis (no or yes)	0.737 (0.441-1.230)	0.243		
Lung metastasis (no or yes)	0.779 (0.480-1.263)	0.311		
Number of metastasis (1 or ≥2)	1.016 (0.627-1.647	0.948		
KRAS mutation (wild or mutant)	0.970 (0.573-1.642)	0.911		
HRR (low or high)	0.591 (0.358-0.974)	0.039	0.606 (0.340-1.080)	0.089
PNI (low or high)	0.352 (0.219-0.563)	<0.001	0.393 (0.242-0.637)	<0.001

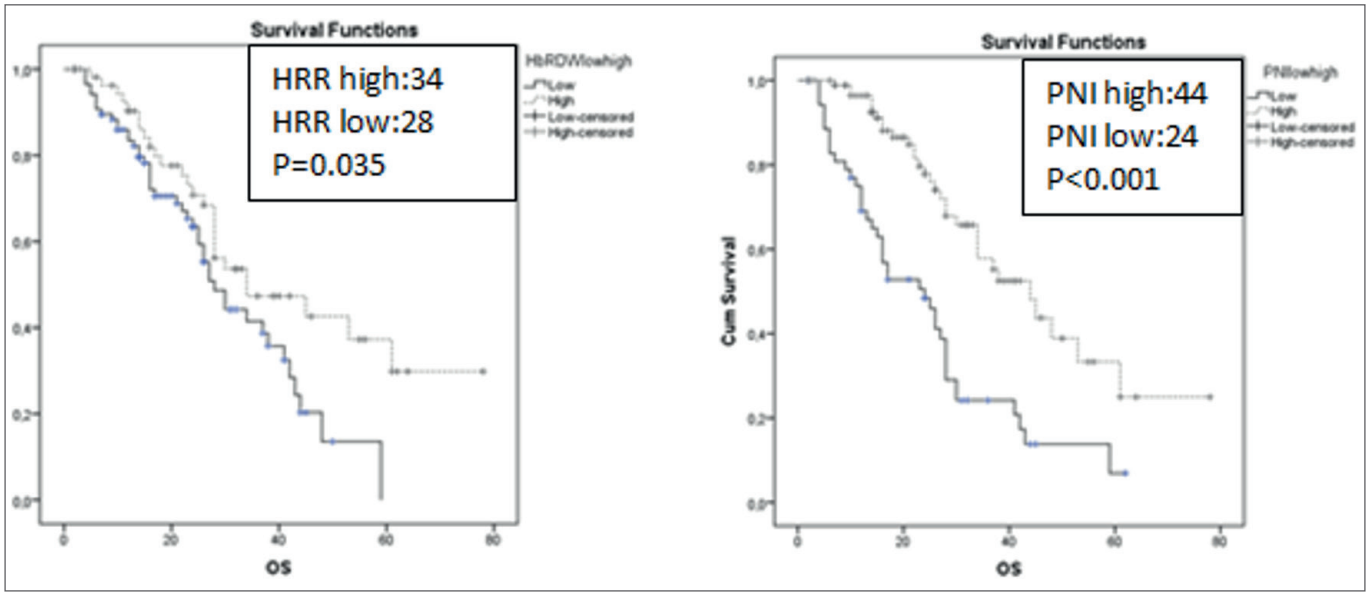

Figure 1. Comparision of overall survival in HRR and PNI low and high groups

PNI is calculated based on albumin and peripheral lymphocytes. Proinflammatory cytokins influence albumin production from hepatocytes.[15] Lymphophenia reflects immun and nutritional status.[16] Takamizawa et al.[17] reported that three nutritional and inflammtory prognostic index. One of them was PNI. They found the median overall survival was 33.8 months in PNI high group and 19.8 months in low PNI group. Our median overall survival was 34 months in PNI high group, 28 months in PNI low grup. In this study the survival difference were much more than ours among PNI low and high groups.[17] In this study right sided tumors were significantly more common in PNI high group. In our study left and right sided tumors were homogenous in both groups. Also in their study there was no knowledge of KRAS mutation. KRAS mutation both prognostic and predictive marker in colorectal cancer.[18] In our study distribution of PNI low and high groups according to KRAS mutation status is similar.

Tumor site is an another prognostic and predictive marker in colorectal cancer. It is suggested that overall survival is longer in left sided than right sided colon tumors.[19] In our study right sided tumor significantly predict poor overal survival in univariate analysis similarly to recent reports. However this prediction didn't remain significant after adjustment of other prognostic variables like prognostic nutritional index.

High levels of RDW is related so many conditions. Some of them were reduced erythropoietin levels due to proinflamatuar cytokins and oxidative stress.[20] These conditions are present in cancer patients. HRR is considered as a marker of poor overall survival.[21] Tuncel et al.[11] showed HRR was a significant predictor of overall survival in rectal cancer patients. They analyzed systemic inflammatory markers in univariate and multivariate analyzed. We found that the HRR was an independent predicor of poor overall survival. We differently added clinical and molecular prognostic characteristics to our multivariate analyse. After adjusted according to clinical characteristic HRR was not statistically significant predictor of overall survival in colorectal cancer.

We found that having initially metastatic disease were another independent prognostic marker. In our study 81% of the patients had initially metastatic disease. We didn't analysed adjuvant or neoadjuvant chemotheapy history in univarite and multivariate analysis. This finding should be researched with the other patients characteristics like adjuvant or neoadjuvant chemotherapy history and in large number of populations.

Our study had some limitations. Firstly retrospective design and small number of patients. Secondly the inclusion of less than half of the patients screened for the study.

CONCLUSION

Both PNI and HRR are associated with poor overall survival in metastatic colorectal cancer. We must also take into account these markers while treating metastatic colorectal patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kayseri City Hospital Clinical Researches Ethics Committee (Date: 22.08.2023, Desicion No: 881).

Informed Consent: Because the study was designed retrospectively, no written informed consent from was obtained from patients.

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Original Article

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Effect of different tea brands on color change of flowable resin composite

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ABSTRACT

Aims: This study aimed to evaluate the color change of a low-viscosity fluid resin composite (FRC) aged in 2 different brands of black tea and a Ceylon tea for three different periods (24 hours, 7 days, and 28 days).

Methods: Twenty-eight Filtek Ultimate FRC samples with a diameter of 10 mm and a thickness of 2 mm were prepared and polymerized using polytetrafluoroethylene molds. All samples were numbered and polished, and initial color measurements were made. Samples were divided into three experimental groups and a control group (Distilled water) (n=7). All samples were kept in solutions for 24 hours, 7 days, and 28 days, and at the end of these periods, color measurements were made with a spectrophotometer. Data were recorded according to the CIE Lab system. ΔE was calculated by dividing the sum of squares of the difference of the last and first color measurement values by two. One-way ANOVA and Tukey test were used in the analysis of the data.

Results: The Yellow Label black tea group caused significantly more color change in 24 hours than the Ceylon tea ($p<0.05$). Significantly more color changes were observed in the Altınbaş black tea group at 28 days compared to 24 hours ($p<0.05$). More color changes were observed in the experimental groups at 7 and 28 days compared to the control group ($p<0.05$).

Conclusion: All the tea solutions made coloration on the FRC. The color change increased as the exposure time to the solution.

Keywords: Flowable resin composite, color stability, spectrophotometer, tea

INTRODUCTION

Resin-based dental composites (RBDC) are widely used in the anterior and posterior regions to design restorations that mimic dental tissue.[1] Various modifications have been made to RBDCs since their introduction in the early 1960s. Composites with different physical properties and clinical performance are available in the dental market, depending on the resin matrix,[1] viscosity,[2,3] filler size, and distribution.[3]

By reducing the amount of filler, the viscosity of RBDCs was reduced, and flowable resin composites (FRC) were obtained.[4] FRCs, initially developed for class 5 cavities, are also used in clinical use in different indications (minimally invasive class 1,2 and 3).[5] The discoloration of RBDC and FRCs due to internal and external factors is one of the main problems of restorations.[6] In addition, with the decrease of the filler content in FRCs, the translucency and polishability increased while the color stability decreased.[7] At the resin matrix and filler interface, the discoloration that occurs over time with the aging of the restoration is intrinsic. External discoloration occurs when substances such as tea, coffee, coloring foods, or cigarette smoke are attached to the restoration surface.[8]

The International Commission on Illumination [Commission Internationale de Liéclairaqe (CIE)] Lab system is a measurement tool that maintains its reliability and validity in dentistry and makes subjective color perception objectively measurable. L* expresses the lightness-darkness or brightness degree, while a*(red-green) and b*(yellow-blue) express the saturation properties of the color. The amount of color difference is expressed as ΔE and calculated by the formula $\Delta E = [(L1^*-L0^*)2 + (a1^*-a0^*)2 + (b1^*-b0^*)2\ ]1/2$. While "0"s in the formula represent the first measurement values, "1"s represent the last measurement values.[9]

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Tea has critical cultural values in the world and Turkey with its features, preparation, presentation, and how it feels when drunk.[10] Regarding tea consumption, Turkey ranks third in the world after China and India. Turkey's annual per capita tea consumption is around three kilograms.[11] While there are many studies on the coloration caused by tea in RBDCs,[6,8,12-15] there are limited studies on this subject in FRCs.[3,16] Therefore, this study aimed to use two different black teas [Yellow Label black tea (Yellow Label, Lipton, Rize, Turkey), and Altınbaş black tea (Altınbaş, Çaykur, Rize, Turkey)] and one Ceylon tea (Istikan, Finlays Colombo FLC, Sri Lanka) to evaluate the color stability of a low-viscosity FRC (Filtek Ultimate Flowable, 3M, USA) aged. The hypotheses of this study can be listed as follows:

H0: There will be no significant difference between the experimental groups regarding coloration at different measurement times.

H1: Experimental groups will cause significantly more coloration than the control group.

METHODS

The study was carried out with the permission of Dicle University Faculty of Dentistry Local Ethics Committee (Date: 29.03.2023, Desicion No: 2013-11). All procedures were carried out in accordance with the ethical rules and the principles.

In the study, twenty-eight A2-colored Filtek Ultimate FRC samples were prepared using polytetrafluoroethylene molds with 12 mm outer diameter, 10 mm inner diameter, and 2 mm thickness. While preparing the samples, transparent tape and a glass coverslip were applied to allow excess material to overflow. The overflowing material was removed from the molds. Then, each sample was polymerized from both surfaces for a total of 20 s with the Elipar S10 (3M Espe, St. Paul, MN, USA) light device with a light intensity of 1200 mW/cm² following the manufacturer's instructions. The samples were kept in an oven at 37°C for 24 hours to absorb water. To ensure surface standardization, Sof-Lex (3M ESPE, St.Paul, MN, USA) polishing discs were applied to all samples for 15 seconds by a single operator at 20.000 rpm, in the order of coarse, medium, fine and superfine discs. The samples were numbered, and the initial color measurements were measured with a spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Germany). Measurements were repeated three times and averaged. The samples were randomly divided into four groups (n=7). All solutions were divided into 3 ml Eppendorf tubes and when the solutions reached 37°C, the samples were immersed in the solutions. The tubes were kept in an oven at 37°C for the measurement period. Solutions were refreshed once a week.

- **Group 1 (Ceylon tea):** 200 ml of 100°C boiling water was used for each sachet to prepare the tea solution. While the tea solution was being prepared, the tea bag was shaken slightly at 0, 2, and 5 minutes, and was removed from the water at 5 minutes.
- **Group 2 (Yellow Label black tea):** It was prepared following the same procedure as Group 1.
- **Group 3 (Altınbaş black tea):** It was prepared following the same procedure as Group 1.
- **Group 4 (Control):** Samples were kept in distilled water at 37°C.

At the end of 24 hours, 7 days, and 28 days, the samples were removed from the incubated solutions and washed in distilled water for 5 minutes each and then dried. Measurements were made in the darkroom only under a fluorescent daylight lamp (Master TL-D 90 Graphica 18W965SLV/10, Philips, Netherland) and on a gray background. Measurements were repeated three times and averaged. The CIE Lab formula calculated the color difference (ΔE) between the initial and final measurements obtained.

Statistical Analysis

The data obtained in the research were analyzed using the SPSS (version 25.0, IBM Corp, Armonk, New York, US) package program. Descriptive statistics (mean, standard deviation) are presented in **Table 1**. The Shapiro-Wilk test showed that the data were normally distributed. One-way ANOVA and Tukey test were used to compare the groups. The significance level was accepted as 0.05.

Table 1. Comparison of color changes according to solution and application times (ΔE)

Exposure time	Control	Ceylon tea	Yellow Label black tea	Altınbaş black tea
24 hours	1.67 ± 1.10^{a}	$3.81\pm1.77^{A,b}$	7.41 ± 2.43^{c}	$4.65\pm2.36^{A,b,c}$
7 days	2.38 ± 0.96^{a}	$8.17\pm3.08^{B,b}$	8.88 ± 2.93^{b}	$7.99\pm2.49^{A,B,b}$
28 days	3.24 ± 1.26^{a}	$10.99\pm2.79^{B,b}$	10.56 ± 2.82^{b}	$8.89\pm3.22^{B,b}$

a-c: Indicates the differences within the same row. A-B: Indicates the differences within the same column. *One way ANOVA

RESULTS

In 24 hours, three groups showed more coloration than the control group ($p<0.05$). In addition, the Yellow Label black tea group caused significantly more coloration than the Ceylon tea group ($p<0.05$). There was no significant difference between Yellow Label black tea and Altınbaş black tea groups and between Altınbaş black tea and Ceylon tea groups ($p>0.05$). At 7 and 28 days, three groups showed more coloration than the control group ($p<0.05$). There was no significant difference between the three experimental groups ($p>0.05$). There was no statistically significant difference in the color measurements made according to the measurement times in the Yellow Label black tea group ($p>0.05$). Significantly

more coloration was observed in the Ceylon tea group at 7 and 28 days compared to 24 hours ($p<0.05$), but no significant difference was found between 7 and 28 days ($p>0.05$). Significantly more coloration was observed in the Altınbaş black tea group in 28 days compared to 24 hours ($p<0.05$), but no significant difference was found between 24 hours and 7 days ($p>0.05$). In addition, no significant difference was found between 7 and 28 days ($p>0.05$).

DISCUSSION

This study investigated the color change caused by three different tea solutions (Ceylon tea, Yellow Label black tea, and Altınbaş black tea) on an FRC (Filtek Ultimate) material. Color change on the 7th and 28th days increased compared to 24 hours in the Ceylon tea and Altınbaş black tea groups at different measurement times. Although the color change increased over time in the Yellow Label black tea group, this change was not significant. Thus, while the H0 hypothesis was rejected for Ceylon tea and Altınbaş black tea groups, it was accepted for Yellow Label black tea. All three tea solutions caused significantly more color change than the control group at all times. Thus, the H1 hypothesis was accepted.

Composite restorations often require renewal primarily due to discoloration observed in the restorations over time.[17] These discolorations can be attributed to internal factors related to the resin's structure and external factors resulting from exposure to various elements, including contamination from blood or saliva. Moreover, external factors include inadequate polymerization, improper finishing and polishing techniques, suboptimal oral hygiene practices, smoking, and dietary habits.[17,18]

The clinical performance of the restoration is directly affected by the surface roughness of the material. Increased surface roughness can cause wear, plaque accumulation, and discoloration.[12] In one study, Sof-Lex discs provided the lowest surface roughness, and samples polished with these discs were relatively less colored.[13] In our study, we used Sof-Lex polishing discs to eliminate the effect of roughness on coloration.

In our study, a spectrophotometer was used to evaluate color measurement objectively. According to the CIE Lab color system, when ΔE is greater than 1, there is a visually perceptible color change in the materials, and 3.3 is an acceptable threshold value. All tea solutions tested in this study and distilled water used as a control solution showed clinically detectable color changes on Filtek Ultimate FRC. The color change caused by the tea solutions was 3.3 above the critical threshold in the literature.

Tea is a beverage consumed a lot in daily life in Turkey.[19] There are many studies in the literature to evaluate the external coloring of composite resin. In these studies, filler particle size, polymerization time, and immersion media were evaluated and found to be effective in color stability.[14,15,20-22] In addition to the immersion environment, another factor to be considered in the color stability of the composite resin is the immersion time.[23] In our study, immersion times are 24 hours, 7 days, and 28 days. Güler et al.[21] examined the effect of polymerization, filler particle type, and dyeing solution on the coloring of the composite resin. They stated that an average cup of beverage was drunk in 15 minutes, and 3.2 cups of coffee per person were drunk and reported that the 24-hour immersion time corresponded to 1 month. In 2016, Turkey consumed an average of 3160 grams of tea per person yearly.[24] Considering that a cup of tea is brewed with 2.5 grams, this corresponds to 1.264 cups per year, 105.33 cups per month, and 3.5 cups per day. Although this rate may seem higher than coffee consumption, the volume of the exposed solution will be lower in a tea glass. Therefore, our study calculated that the 24-hour immersion period corresponds to approximately one month. Therefore, the 7-day immersion period corresponds to 7 months, and the 28-day immersion period corresponds to 2 years and four months.

It has been reported that secondary metabolites such as caffeine, tartaric acid, and phenols are highly effective in the coloration of composites.[25] In the production of tea, the number of secondary metabolites obtained per unit of tea mass as a result of the extraction process increases.[26] In our study, the ΔE value was higher in the Ceylon tea group. However, there was no significant difference between the Yellow Label black tea, Altınbaş black tea, and Ceylon tea groups due to the 7 and 28 days of application. This may be due to the increased amount of secondary metabolites in its content.

Reducing the filler particle size results in a more homogeneous filler distribution in the resin matrix and, thus, smoother surfaces. However, it has been observed that decreasing particle size in nano-filled composite resins increases the coloration potential.[27] One study observed that the micro-filled Durafill composite had significantly more color stability than the nano-filled Filtek Z-350 composite.[28] In a similar study, micro-filled, micro-hybrid, and nano-hybrid composites were compared, and they found that the nano-composite changed color the most in tea on the 7th and 30th days.[27] Similarly, we saw more color changes in the material on our study's 7th and 28th days. In another study, nanohybrid, and nanofilled composites were compared and it was observed that Filtek Ultimate FRC was significantly more colored

than other flowable composites (G-aenial Injectable Flow, Filtek Bulk-Fill Flowable, Estelite Universal Super Low Flow). In addition, when the authors used tea as a coloring solution, they saw that the color change in Filtek Ultimate FRC was above the threshold value and significantly more than other composite resins at the end of 6 days.[29] Our study shows that the coloration potential of a nano-filled FRC is high for all coloring media.

For this reason, if this material is to be preferred, especially in the aesthetic region, patients should be warned about drinking coloring drinks. A study conducted by Türkün et al.[30] determined that the tea and coffee discolorations in composite materials could be removed by bleaching and re-polishing methods. Therefore, regular polishing of restorations in which this material is preferred can restore the discoloration and ensure the sustainability of aesthetics.

Although oral cavity temperature simulation was performed in an oven in this study, the absence of salivary and brushing cycles may have limited the variation of ΔE. Given the ease of application of FRCs, further studies on the color change of these materials are needed.

CONCLUSION

In this study, in which we discussed the problem of color stability of FRCs against stains caused by widely consumed tea brands, clinically unacceptable color change was observed on the material surface for 24 hours or more, regardless of brand.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dicle University Faculty of Dentistry Local Ethics Committee (Date: 29.03.2023, Desicion No: 2013-11).

Informed Consent: Because the study was designed laboratory study, no written informed consent from was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Evaluation of the risk of developing atrial fibrillation with new electrocardiographic parameters in patients with primary hyperparathyroidism

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ABSTRACT

Aims: Primary hyperparathyroidism (PHPT) is a clinical entity characterized by hypercalcemia caused by excessive parathyroid hormone (PTH) secretion from the parathyroid gland and is the most common cause of hypercalcemia in outpatient clinics. Atrial fibrillation (AF) is a common arrhythmia encountered in cardiology practice, the prevalence of which increases with concomitant heart disease and age. P-wave peak time (PWPT) is the time from the onset of the p-wave to its peak and is a recently defined electrocardiographic (ECG) parameter. Recently, studies on the relationship between PWPT and cardiovascular events have been published. In this study, we aimed to evaluate the risk of AF in PHPT patients by detecting PWPT, a new ECG parameter.

Methods: The study included 21 PHPT patients and 20 healthy subjects as a control group. The groups were compared in terms of demographic characteristics, laboratory findings, echocardiography, and ECG findings. D2 and V1 leads were used for PWPT, as recommended in the literature.

Results: When the patient group was compared with the control group, no difference was detected in terms of demographic characteristics and laboratory findings. When compared with the control group, patients with PHPT had significantly longer PWPT (PWPTV1 56.07 msec ± 8.33 s vs. 50.25 msec ± 7.00 s $p<0.05$, PWPTD2 54.57 msec ± 6.28 msec vs. 48.05 msec ± 5.91 msec $p<0.01$).

Conclusion: We observed that PWPT was longer in patients with PHPT compared to controls, and our results suggest that PHPT patients are at risk of AF.

Keywords: Primary hyperparathyroidism, hypercalcemia, atrial fibrillation, P-wave peak time

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a clinical picture characterized by hypercalcemia caused by excessive parathyroid hormone (PTH) secretion from the parathyroid gland and is the most common cause of hypercalcemia in outpatient clinics.[1,2] It has been observed more frequently in the last 40 years due to more frequent measurements of serum calcium levels.[3] As a result, PHPT is becoming a relatively more common endocrine disease, with an incidence of 1/1000.[4] Recently, there has been an increased interest in the cardiac evaluation of patients with PHPT, with the publication of studies showing that PHPT increases cardiac mortality and arrhythmias.[5,6]

Atrial fibrillation (AF), the most common rhythm disorder in clinical practice, is of critical importance due to accompanying hemodynamic disorders and thromboembolic events.[7] Although the mechanisms causing AF are not fully understood, many risk factors, including age, hypertension (HT), coronary artery disease (CAD), cerebrovascular disease (CVD), and diabetes, are thought to play a role in the development of AF.[8]

P-wave peak time (PWPT) is the time from the onset to the peak of the P wave. It is considered a new index of atrial depolarization, and recent studies have shown an association between PWPT and CAD severity, left

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ventricular end-diastolic pressure, absence of coronary reflux, and left atrial volume index.[9-11] This new parameter suggests that it can be used as an indicator of the risk of developing AF.[12,13]

In this article, we aim to determine PWPT times in patients with PHPT and evaluate the risk of AF development in patients with PHPT based on this index.

METHODS

The study was carried out with the permission of Kayseri City Hospital Clinical Researches Ethics Committee (Date: 03.09.2020, Desicion No: 146). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In October 2020, 21 patients who were followed up with a diagnosis of PHPT in our hospital were included in the study and analyzed prospectively. Physical examination findings, medical history, and laboratory results of the patients in the patient group and the control group were recorded from the patient files. The control group consisted of patients of similar age and gender, normal blood PTH levels, and no suspicion of cardiovascular disease based on history, physical examination findings, electrocardiography (ECG), and echocardiography (ECHO).

Exclusion criteria were hypertrophic cardiomyopathy, severe valvular disease, CAD, hypothyroidism and hyperthyroidism, hypokalemia and hyperkalemia, hypomagnesemia and hypermagnesemia, creatinine clearance (CrCl) <60 ml/min, and body mass index (BMI) <30 kg/m². In addition, patients with AF, conduction abnormalities, atrioventricular block, and pacemaker rhythm were excluded from the study. We were obtained from all patients.

Electrocardiogram Analysis

All standard 12-lead ECGs were obtained in the supine position and at rest using an ECG device (Philips brand) standardized to 1 mV/cm and 25 mm/s paper speed. All ECGs were scanned and transferred to personal computers. ECGs were magnified 5-fold and measured using electronic calipers (Cardio Calipers software version 3.3; Iconico.com, Philadelphia, PA, USA) to make the necessary measurements. To reduce erroneous measurements, ECG assessments were performed by two cardiologists blinded to clinical data. The PWPT in lead D2 (PWPTD2) was measured as the time from the onset of the P-wave to its peak in lead D2, and the PWPT in lead V1 (PWPTV1) was defined as the time between the onset of the P-wave and the lower limit of negative deflection in patients with biphasic or pure negative P-wave morphology (**Figure**).[14]

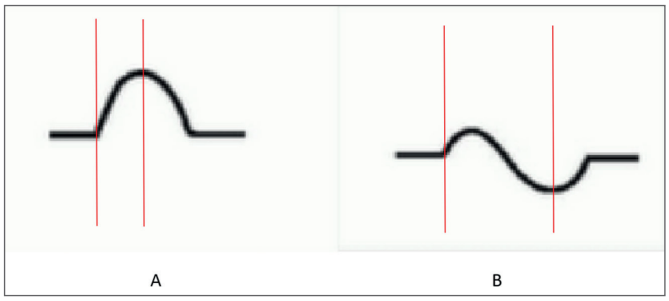

Figure: Measurement of P wave peak time on electrocardiograms
A: Measurement of P wave peak time in the lead D2 (positive wave)
B: Measurement of P wave peak time in the lead V1 (biphasic wave)

Echocardiography Measurements

Patients and healthy volunteers underwent conventional echocardiographic examination with an M4S-RS (1.5-3.6 MHz) cardiac transducer and Vingmed System 5 (General Electronic Horten, Norway) echocardiograph. Left ventricular diastolic (LVIDd) and systolic (LVIDs) diameters, interventricular septum wall (IVSWT), and posterior wall (LVPWT) diastolic thicknesses were measured in the parasternal long axis by M-mode echocardiography according to the standards defined by the American Society of Echocardiography. The ejection fraction was calculated using the Teichholz formula.[15]

Statistical Analysis

Statistical analyses were performed using SPSS Statistics software package version 21.0 (SPSS Inc., Chicago, IL, USA) for Windows. The distribution characteristics of the data were determined using the Kolmogorov-Smirnov test. Independent samples A t-test was used for parametric scale variables. The Mann-Whitney U test was used for nonparametric scale variables. The $\chi 2$ (chi-squared) test was used for univariate analysis of the categorical variables. The variables were reported as means ± SD (standard deviation), whereas the categorical variables were reported as percentages. Correlation analyses were performed using Pearson and Spearman coefficients of correlation. A probability value of $p < 0.05$ was considered significant, and two-tailed p values were used for all statistical analyses.

RESULTS

Baseline clinical and demographic characteristics of the study groups are presented in **Table 1**. There were no statistically significant differences between the patient and control groups in terms of gender, age, smoking status, diabetes, and HT ($p > 0.05$).

The basic laboratory results of the patients are listed in **Table 2**. Total calcium, albumin-corrected calcium, phosphorus, and PTH levels were significantly higher in PHPT patients included in the patient group compared to those included in the control group ($p < 0.01$ for all). Other blood parameters were similar between the

groups. The ECHO parameters of the patient and control groups are shown in **Table 3**. There was no statistically significant difference between the two groups in terms of the ECHO parameters. Heart rate and PR intervals were similar in both groups (77.1±6.1/min vs. 79.2±11.6/min, p=0.442 and 141±14 msec versus 145±16 msec, p=0.891, respectively). PWPTV1 and PWPTD2 were higher in PHPT patients compared to the control group (50.25±7 msec vs. 56.07±8.33 msec p<0.01 and 48.05±5.91 msec vs. 54.57±6.28 msec p<0.01, respectively).

Table 1. Baseline clinical and demographic features of the study groups

Variables	Control group (n=20)	PHPT (n=21)	P value
Age (years)	58.4±10,0	56.7±11.2	.866
Male/female	19/2	18/2	.677
HT	9	8	.925
DM	3	4	.966
Smoke	1	1	.986
SBP (mmHg)	113.5±11	125.2±13.1	.108
DBP (mmHg)	69.7±8.1	72.5±5.9	.606

PHPT; Primary hyperparathyroidism, DM: Diabetes Mellitus, HT: Hypertension,CBP: Sistolic Blood Pressure, DBP: Diastolic Blood Pressure Data are expressed as mean ± standard deviation for normally distributed data and percentage (%) for categorical variables

Table 2. Comparison of baseline laboratory measurements among the study groups

Variables	Control group (N=20)	PHPT (N=21)	P value
Glucose (mg/dl)	98.1±11.2	99.4±12.9	.325
BMI	27.48±2.1	27.05±1.89	.760
Kreatinin (mg/dl)	0.79±0,19	0.86±0.21	,690
AST (U/L)	25.6±7.5	24.8±9.1	.875
ALT (U/L)	22.54±5.9	26.1±8.8	.401
Albumin	3.89±0.89	4.21±0.42	.312
Total calcium (mg/dl)	9.86±0.55	12.01±1.12	.0001
Albumin-corrected calcium (mg/dl)	8.99±0.56	11.01±0.88	.0001
Phosphorus (mg/dl)	3.69±0.52	2.65±0.48	.0001
PTH	38.22±10.1	231.65±165.49	.0001
TSH	2.01±1.15	2.55±1.56	.612
D_Vitamin	21.01±3.2	18.99±9.1	.682
WBC (10^3/μl)	7,78±1,47	7,71±1,73	,847
Hemoglobin (g/L)	13.42±2.1	14.1±.9	.551
Platelet (/mm³)	278.6±72.1	265.6±81.6	.825

Data are expressed as mean ± standard deviation for normally distributed data and percentage (%) for categorical variables. PHPT; Primary hyperparathyroidism, WBC: White Blood Cell, PTH: Parathyroid Hormone, TSH: Thyroid Stimulating Hormone, BMI; Body Mass Index

Table 3. Echocardiography characteristics of the study population

Variables	Control group (N=20)	PHPT (N=21)	P value
LVEDD (cm)	4.34±1.01	4.62±0.86	.266
LVESD (cm)	3.55±.45	3.21±1.23	.655
IVSD (cm)	.99±.55	1.0±0.21	.769
PWD (cm)	.99±0.45	1,01±0.1	.899
LVEF	59.1±4.1	61.1±3.8	.331

Data are expressed as mean ± standard deviation for normally distributed data and percentage (%) for categorical variables. PHPT; Primary hyperparathyroidism, LVEDD: Left Ventricular End Diastole Diameter, LVESD: Left Ventricular End Systole Diameter, IVSD: İnterventricular Septal Diameter, PWD: Posterior Wall Diameter, LVEF; Left Ventricular Ejection Fraction

Table 4. Electrocardiographic characteristics of the study population

Variables	Control group (N=20)	PHPT (N=21)	P value
Heart rate (beat/min)	77.1±6.1	79.2±11.6	.442
PR interval (ms)	141 ± 14	145 ± 16	0.891
PWPTV1 (ms)	50.25 ± 7	56.07 ± 8.33	<.01
PWPTD2 (ms)	48.05 ± 5.91	54.57 ± 6.28	<.01

PHPT; Primary hyperparathyroidism, PWPTD2; P wave peak time obtained from D2 lead, PWPTV1; P wave peak time obtained from V1 lead, Data are expressed as mean ± standard deviation for normally distributed data and percentage (%) for categorical variables.

DISCUSSION

This is the first randomized study to show that PWPT prolongation detected by ECG analysis was higher in PHPT patients included in the patient group than in those included in the control group.

All hypercalcemia, including PHPT-induced hypercalcemia, is a risk factor for cardiac arrhythmias.[16,17] It is traditionally accepted that hypercalcemia developing in PHPT causes shortening of the QT interval, ST segment depression, and mild prolongation of the PR and QRS intervals.[18] Shortening of the refractory period due to QT shortening may cause complex ventricular arrhythmias or sudden death.[18]

Furthermore, recent studies have shown that PHPT patients have impaired LA function.[19] These adverse effects that can be seen in PHPT are also known as risk factors for the development of AF.[20] Studies investigating the effects of PTH and calcium on the heart have shown that both cause changes in both endothelial cells and myocardial cells. PTH may have such effects through calcium; it can also be seen in connection with its effects directly on cells.[21,22]

Clinical observations of conduction disturbances caused by hypercalcemia are surprisingly rare. Case reports have shown a variety of conduction disturbances in patients with PHPT depending on the severity of hypercalcemia, including atrioventricular nodal conduction defects, sinus node disease, and AF; the reason for the prevalence of these disturbances is unknown.[23]

Curione et al.[24] showed that hypercalcemia develops adverse effects on cardiac electrical stability in patients with PHPT. PWPT indicates the time spent for excitation propagating from the sinoatrial node to the maximum sum of positive deflection from the atria. Prolonged PWPT suggests prolonged intra- or inter-atrial conduction time as a consequence of increased intra-atrial pressure.[25] In some previous studies, an association between PWPT and both CAD and cardiac physiologic/pathologic indices had been shown.[9-11] This new parameter has been claimed to be used to predict the development

of AF in recent studies.[11,12] According to our findings, hypercalcemia may cause prolongation of PWPT by affecting atrial conduction pathways. Therefore, it is not unreasonable to think that increased calcium levels may be a predictor for possible AF development.

PTH is vital for calcium hemostasis. However, it is now known that PTH itself causes hypertrophy of cardiac myocytes and vascular smooth muscle, even in the absence of hypercalcemia. Furthermore, parathyroid hormone increases heart rate, an effect mediated by the direct action of PTH on the sinus node and conduction system.[26] PTH also exerts inotropic effects due to increased coronary blood flow, possibly due to the vasodilator effect of PTH on the coronary circulation.[26] Rienstra et al.[27] found that PTH levels were significantly higher in patients with AF. Lee et al.[28] showed that increased PTH levels increased the incidence of AF in their population-based study. In a study by Pepe et al.[29] more frequent atrial extrasystoles were found in 24-hour ECG monitoring of PHPT patients. When evaluated together with the literature, it can be speculated that the prolongation of PWPT found in our study would increase the risk of AF development, which is consistent with the literature.

The present research bears a few limitations. First, we had no information on how long the patients had lived with PHPT and hypercalcemia before the diagnoses. Second, we did not know the threshold values of PTH or calcium levels, which may lead to significant changes in the heart, as well as the duration for how long PTH or calcium levels must remain high for these significant changes to occur. Most of the studies to that effect included patients with symptomatic diseases. Therefore, an intervention in a presymptomatic stage with a lower level of hypercalcemia may alter the disease trajectory. Finally, although values such as PWPT are secondary markers of arrhythmia, we did not perform long-term clinical follow-ups and rhythm Holter follow-ups to detect the development of arrhythmia.

CONCLUSION

The results of the present study suggest that PWPT obtained from ECG, which is a simple, easily measurable, and inexpensive test, can be used as a marker to predict the risk of AF in PHPT patients. More comprehensive and multicenter studies should be performed to better analyze all possible predictors of AF and accordingly make more robust recommendations for the future.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kayseri City Hospital Clinical Researches Ethics Committee (Date: 03.09.2020, Desicion No: 146).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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

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Original Article

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Evaluation of cardiovascular risk factors, prevalence and determinants of coronary artery disease in renal transplant patients: a single center experience

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ABSTRACT

Aims: Cardiovascular disease is the leading cause of morbidity and mortality in renal transplant patients. In our study, we aimed to determine the cardiovascular (CV) risk factors, the prevalence and determinants of coronary artery disease (CAD) in patients who underwent kidney transplantation in our center.

Methods: One hundred sixty nine patients who underwent kidney transplantation in our center were included in the study retrospectively. Demographic and clinical characteristics of the patients, cardiac evaluation findings and further examination results were scanned from the database of our center.

Results: The mean age of the patients was 42.86±12.97 years and 43.19% were female. The most common etiological factors for the development of end-stage renal disease were hypertension (HT) and diabetes mellitus (DM). Ninety seven patients (57.4%) were undergoing dialysis, 4 of whom were on peritoneal dialysis. Renal transplant was performed from a cadaver in two patients and from a living donor in the other patients. CAD was detected in 29 patients (17.15%). The most prevalent CV risk factors were HT and hyperlipidemia (HL). Multivariate logistic regression analysis revealed that age, DM, HL and dialysis history were independent risk factors for the development of CAD. In the postoperative follow-ups, no death or acute coronary syndrome was observed during the hospitalization period.

Conclusion: Prevalence of CV risk factors is high in renal transplant candidates. Our findings support the need for a detailed cardiac evaluation and effective management of CV risk factors in patients preparing for kidney transplantation.

Keywords: Renal transplantation, end-stage renal disease, preoperative cardiac evaluation, cardiovascular risk in renal transplant patients

INTRODUCTION

Kidney transplantation is the most beneficial treatment modality that improves the quality of life and survival of the patients with end-stage renal disease (ESRD).[1] Thousands of patients all over the world are on the kidney transplant waiting list.[2] There are approximately 70,000 ESRD patients in Turkey and approximately 3000 kidney transplants are performed annually, 77% of which are from living donors.[3]

Cardiovascular diseases (CVD) are found in approximately half of the patients with advanced kidney disease and are responsible for 40-50% of all deaths in these patients.[4,5] These rates are higher in dialysis patients.[6] In addition to common risk factors such as hypertension (HT), diabetes mellitus (DM), hyperlipidemia (HL), volume overload and hormonal changes in patients with chronic kidney disease (CKD) also affect cardiac structure and functions and increase cardiac events in these patients.[7,8] Although CV risk decreases after renal replacement, CV complications are responsible for a substantial portion of perioperative morbidity and mortality.[9,10] Therefore, cardiac examination before kidney transplantation becomes more important.

There is no consensus on cardiac evaluation previous to kidney transplantation, between the cardiology and nephrology societies, and European and American guidelines. In the 2022 European Society of Cardiology (ESC) guidelines for CV assessment of non-cardiac surgery, there is no assessment recommendation for patients undergoing kidney transplantation, which is considered a moderate risk surgery. Biomarkers, ECG, and functional capacity assessment are only recommended for patients aged 65 and over who will undergo moderate

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and high risk surgery.[11] The 2020 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline recommends that all transplant candidates be evaluated for CVD, ECG should be taken for all patients, symptomatic patients should be referred to cardiology, and patients with high risk of coronary artery disease (CAD) or low exercise capacity should be screened for CAD with non-invasive tests.[12]

The aim of our study was to determine the demographic characteristics, cardiovascular risk factors, prevalence and predictors of coronary artery disease in kidney recipients in our center.

METHODS

The study was carried out with the permission of Koç University Ethics Committee (Date: 26.08.2019, Decision No: 2019.265.IRB2.087). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This is a retrospective study including 169 patients with ESRD who underwent cardiology evaluation before kidney transplantation in our center between 2018 and 2020. Demographic and clinical characteristics of the patients, cardiac evaluation findings and further examination results were scanned from the database of our center. Hypertension was defined as repeated office systolic blood pressure (SBP) values ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg.[13] Diabetes mellitus was defined as a fasting plasma glucose levels of ≥126 mg/dl or a 2-hour post-load glucose levels of ≥200 mg/dl.[14] Hyperlipidemia was considered as total cholesterol level ≥200 mg/dl or low density lipoprotein (LDL) levels of ≥130 mg/dl or triglyceride levels of ≥150 mg/dl.[15]

The blood samples of the patients were taken in the morning fasting, and in the patients undergoing dialysis, they were taken before dialysis. Estimated glomerular filtration rate (eGFR) were calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for adults.[16] End stage renal disease (ESRD) was defines as eGFR < 15 mL/min.[17]

Cardiac Evaluation

Cardiac examination and non-invasive tests were performed on the same day and on non-dialysis days. The Epiq 7C ultrasound system (Philips, Andover, MA, USA) equipped with a 2.3-3.5 MHz transducer probe was used for transthoracic echocardiographic (TTE) evaluation. All measurements were made in line with the current recommendation guidelines of the American Society of Echocardiography.[18]

Treadmill exercise test was performed on eligible patients. Patients who were unable to perform the test or who were considered to have non-diagnostic test were referred to other non-invasive tests like myocardial perfusion scintigraphy (MPS) or coronary computed tomography (CT) angiography by the evaluating cardiologist. Conventional coronary angiography (CAG) was performed in patients who were found to have ischemia or findings supporting significant CAD in non-invasive tests. A stenosis of 50% or more in the coronary arteries was accepted as obstructive CAD. Revascularization with percutaneous coronary intervention (PCI) or coronary artery by-pass graft surgery (CABG) was performed in patients deemed necessary according to CAG results. Kidney transplantation was performed 3 to 6 months after coronary revascularization.

Statistical Analysis

SPSS 26 program was used to evaluate the data obtained in the study. The normality of the distribution was determined by the Kolmogorov-Smirnov test. Results were expressed as mean ± standard deviation. Normally distributed variables were compared with Student's T test, and non-normally distributed variables were compared with Mann Whitney-U test. Chi-square test was used to compare categorical variables. P value less than 0.05 was considered statistically significant. Pearson analysis was used for continuous variables and Spearman test was used for non-continuous variables in the correlation analysis. The correlation coefficient (r) was calculated. Independent determinants of coronary artery disease were ascertained by univariate and multivariate logistic regression analysis.

RESULTS

The most common etiological factors for the development of ESRD were HT and DM. ESRD was defined as idiopathic in 21 patients (12.42%) whose etiopathogenesis could not be determined clearly. Less common etiologies such as horseshoe kidney, Alport syndrome, renal agenesis, vesicoureteral reflux were classified as others (**Table 1**).

Table 1. Etiologies of end stage renal disease in kidney recipients

Etiology	
Hypertension n (%)	21 (12.42)
Diabetes mellitus type 1 n (%)	4 (2.36)
Diabetes mellitus type 2 n (%)	20 (11.83)
Polycystic kidney disease n (%)	14 (8.28)
Idiopathic n (%)	21 (12.42)
Ig A nephropathy n (%)	15 (8.87)
Focal segmental glomerulosclerosis n (%)	14 (8.28)
Glomerulonephritis n (%)	9 (5.32)
Vasculitis n (%)	12 (7.1)
Amyloidosis n (%)	6 (3.55)
Nephrolithiasis n (%)	6 (3.55)
Others n (%)	27 (15.97)

The mean age of the patients was 42.86±12.97 years and 43.19% were female. Ninety seven patients (57.4%) were undergoing dialysis, 4 of whom were on peritoneal dialysis. Renal transplant was performed from a cadaver in two patients and from a living donor in the other patients. The most common CV risk factors were HT and HL. Calcium channel blockers (CCBs) and beta blockers were the most preferred antihypertensive agents. (**Table 2**). Preoperative laboratory findings of the study group are shown in **Table 3**.

Table 2. Demographic and clinical characteristics of the study group

Parameter	Study group (n=169)
Age	42.86±12.97
Female, % (n)	43.19 (73)
BMI, (kg/m²)	25.67±5.57
Hypertension, % (n)	83.43 (141)
Hyperlipidemia, % (n)	35.50 (60)
Diabetes mellitus, % (n)	14.20 (24)
Smoking, % (n)	30.76 (52)
CAD, % (n)	17.15 (29)
CHF, % (n)	6.50 (11)
AF, % (n)	1.18 (2)
Antiaggregant therapy, % (n)	17.75 (30)
ACEI /ARB, % (n)	15.38 (26)
Beta blockers, % (n)	46.15 (78)
CCBs, % (n)	60.94 (103)
Statin, % (n)	15.38 (26)
Dialysis, % (n)	57.4 (97)

BMI, body mass index; CAD, coronary artery disease; CHF, congestive heart failure; AF, atrial fibrillation; ACEI, angiotensin converting enzyme; ARB, angiotensin receptor blocker; CCBs, calcium channel blockers.

Table 3. Laboratory and echocardiographic characteristics of the patients

Parameter	Study group (n=169)
Glucose mg/dl	117.97±59.01
BUN mg/dl	66.75±25.46
Creatinine, mg/dl	7.30± 2.62
eGFR (ml/min/1.73m²)	8.49±3.95
Sodium (mmol/L)	139.07±4.13
Potassium (mmol/L)	4.96±0.75
Total cholesterol (mg/dl)	195.90±51.14
LDL (mg/dl)	128.37±46.78
HDL (mg/dl)	45.93±17.06
Triglycerides (mg/dl)	171.08±114.19
LV EF (%)	58.22±6.84
sPAP (mmHg)	28.34±7.17
LVH % (n)	40.2 (68)

BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein; HDL, high density lipoprotein; LV EF, Left ventricular ejection fraction. sPAP, systolic pulmonary artery pressure; LVH, left ventricular hypertrophy.

The treadmill exercise test could be performed on 130 patients and 28 patients were evaluated as positive for ischemia. Nineteen patients underwent MPS and ischemia was detected in 5 of them. Coronary CT angiography was performed in 21 patients and obstructive CAD was found in 4 patients. Conventional coronary angiography was performed in 61 patients. Nine of them underwent to CAG without non-invasive tests due to low EF on TTE or a previous history of CAD. It was found to be normal in 32 patients. Stent implantation with PCI was performed in 10 patients, and CABG was performed in 5 patients. Follow-up with medical treatment was recommended for 11 patients. While 2 of them had chronic total occlusion, the others had thin vessels, distal lesions or non-critical (<50%) stenosis. Twenty nine patients of all patients (17.2) had CAD. It was pre-existing in 2 of them (both had CABG), while others were diagnosed in the preoperative evaluation. In 2 patients who had previously undergone CABG, all grafts were found patent and revascularization was not required (**Table 4**).

Table 4. Findings of preoperative non-invasive and invasive tests

Parameter	Study group (n=169)
Treadmill exercise test, (n)	130
Ischemia negative	82
Ischemia positive	28
Non-diagnostic	20
MPS, (n)	19
Ischemia negative, (n)	14
Ischemia positive, (n)	5
Coronary CTA, (n)	21
Non-obstructive CAD, (n)	2
Obstructive CAD, (n)	4
Coronary angiography, (n)	61
Normal coronary arteries, (n)	32
Medical treatment, (n)	12
PCI, (n)	10
CABG, (n)	5
Patients with previous CABG, (n)	2
Coronary artery disease, % (n)	17.15 (29)

MPS, myocardial perfusion scintigraphy: CTA, coronary computed tomography angiography; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CABG, coronary artery by-pass graft.

When patients were compared according to the presence of CAD, patients with CAD were significantly older, had a higher male ratio, more CV risk factors, had higher creatinine levels, dialysis and LVH ratios and lower LV EF (**Table 5**). For the development of CAD, while univariate logistic regression analysis showed that age, DM, HL, dialysis history, LV EF and LVH, were the determining factors, in multivariate logistic regression analysis, age, DM, HL and dialysis history were found as independent risk factors.

In the postoperative follow-ups, no death or acute coronary syndrome was observed during the hospitalization period. Atrial fibrillation developed in two patients, acute pulmonary edema in one patient, and pneumonia in one patient. One patient died 30 days after the operation with complications related to infection.

Table 5. Comparisons of patients according to presence of coronary artery disease

Parameter	Patients with CAD (n=29)	Patient without CAD (n=140)	p value
Age	52±11	41±12.55	<0.001
Male, % (n)	87 (20)	52.1 (76)	0.002
BMI, (kg/m²)	27.44±4.62	25.30±5.69	0.060
Hypertension, % (n)	85.1 (23)	80 (118)	0.021
Hyperlipidemia, % (n)	65.2 (15)	12.3 (18)	<0.001
Diabetes mellitus, % (n)	43.5 (10)	9.6 (14)	<0.001
Smoking, % (n)	56.5 (13)	26.7 (39)	0.004
Dialysis, % (n)	81.4 (22)	52.8 (75)	0.009
eGFR, (ml/min/1.73 m²)	7±3.75	8.7±3.93	0.128
Creatinine, mg/dl	8.6±3.39	7.1±2.37	0.025
LV EF, %	55±8.77	59±6.35	<0.001
sPAP, mmHg	29.3±8.37	28.2±6.94	0.436
LVH, % (n)	62.1 (18)	35.7 (50)	0.008

CAD, coronary artery disease; BSA, body surface area; eGFR, estimated glomerular filtration rate; LV EF, left ventricular ejection fraction; sPAP, systolic pulmonary artery pressure; LVH, left ventricular hypertrophy.

Table 6. Logistic regression analyses for the presence of coronary artery disease

	Univariate analysis		
	p value	OR	95% Confidence interval
Age	<0.001	1.076	1.037-1.118
HT	0.068	0.149	0.019-1.148
DM	<0.001	0.064	0.024-0.173
HL	<0.001	0.073	0.029-0.184
Smoking	0.075	0.475	0.239-1.079
Creatinine	0.052	1.155	0.999-1.326
GFR	0.103	0.897	0.787-1.022
Dialysis	0.004	0.227	0.082-0.629
LV EF	0.015	0.941	0.896-0.988
sPAP	0.296	1.534	0.897-2.623
LVH	0.010	1.028	1.976-1.083
Multivariate analysis			
Age	0.007	1.065	1.018-1.115
DM	0.001	0.122	0.034-0.433
HL	0.001	0.127	0.039-0.410
Dialysis	0.044	0.245	0.062-0.962
LV EF	0.260	0.953	0.877-1.036
LVH	0.448	1.572	0.489-5.049

HT, hypertension; DM; diabetes mellitus; HL, hyperlipidemia; GFR, glomerular filtration rate; LV EF, left ventricular ejection fraction; sPAP, systolic pulmonary artery pressure; LVH, left ventricular hypertrophy.

DISCUSSION

In our study, the leading etiological factors of ESRD were HT and DM, the most prevalent CV risk factors were HT, HL, smoking and DM, respectively. Approximately 17% of the kidney recipients had coronary artery disease. While CV risk factors were significantly higher in patients with CAD, a strong association was found with age, DM, and HL.

When investigating the demographic and epidemiological characteristics of kidney transplant patients, it should be considered that the geographical features, socio-cultural characteristics and organ transplant policies of the countries are important determining factors. In western societies, with the increasing life expectancy, more elderly people need kidney transplantation. In a study by Luxardo et al.[19] comparing the epidemiologic characteristics of patients on renal replacement therapy in Europe and Latin America, 56.2% of patients in Europe were 65 years of age or older, compared to 38.3% in Latin America. In our study, there were 7 patients (4.14%) aged 65 and over, and the mean age of the study group was relatively younger. It may be related to the fact that the research was based on a small-scale single-center data analysis and included a higher proportion of patients with congenital and genetic kidney disease. Despite of younger cohort in our study, age was significantly higher in CAD patients and was found to be an independent risk factor for the development of CAD. Additionally, consistent with the literature, there was a male predominance in the study group and the most common etiologic factors for ESRD were DM and HT.[20,21]

The prevalence of traditional CV risk factors, particularly HT, was high in our study, similar to previous studies.[22,23] Hypertension may occur both as a cause and a consequence of chronic renal diseases.[24] In our study, HT was present at a high rate of 83% of the patients, whereas hypertensive ESRD was defined in only 12% of the patients. Either way, the presence of HT has been shown to be associated with adverse CV events, increased graft failure, and death in kidney transplant recipients.[25] It is essential to keep blood pressure at optimal levels before and after transplantation. Although the prevalence of HT was significantly higher in the patients with CAD than in those without CAD, it was not demonstrated as an independent predictor in the logistic regression analysis.

Diabetes mellitus, HL, and smoking history were also significantly higher in the CAD group, although there were significant associations between these factors and CAD in univariate logistic regression analysis, only age, DM and HL were found to be independent predictors of CAD in multivariate logistic regression analysis. Diabetes mellitus is one of the important CV risk factors in ESRD patients and is associated with increased mortality in patients undergoing kidney transplantation.[26,27] The prevalence of DM in patients awaiting kidney transplantation differs in studies from various countries.[21,28,29] In our study, the prevalence of DM was about 14%, and it was found approximately half of the patients with CAD.

Chronic kidney disease causes dyslipidemia, which is an important risk factor for CV diseases, by causing impairment in lipid metabolism.[30] Statin use has been shown to improve lipid levels and reduce CV events in pre-end stage kidney disease and after transplantation.[31,32]

In the present study, 35% of the patients had HL while the rate of statin use was 15%.

As for smoking, a study by Kasiske et al.[33] in which approximately 24% of the subjects were smokers, demonstrated the association of smoking with increased graft failure, death, and CV events in kidney transplant recipients. It has also been shown that smoking cessation 5 years before transplantation reduces graft failure and mortality, but does not affect the frequency of CV events. In our study, approximately 30% of the patients were smokers, and this rate was 56% in patients with CAD, which was more than twice as high that in patients without CAD, but it was not found to be an independent risk factor for the development of CAD.

Apart from traditional risk factors, it has been shown in various studies that LVH is associated with increased CV risk in patients with chronic kidney disease. It has also been demonstrated that a decrease in LV wall thickness results in better outcomes after kidney transplantation.[34,35] In our study, consistent with the literature, LVH was significantly higher in CAD patients and was associated with the development of CAD.

There is no consensus on preoperative cardiac evaluation guidelines or expert opinions, most of them focuses on presence of symptoms and advanced age for further cardiac evaluation.[36,37] Nevertheless, due to insufficient exercise capacity and comorbidity burden, individuals with CKD may be considered asymptomatic despite significant cardiovascular disease.[38] In our study, although the patients were asymptomatic for angina, most of them had normal LV systolic function and ECG findings, and were relatively young, CAD was detected in approximately one fifth of the patients. Postoperative acute coronary syndrome and cardiac death were not observed after both medical and interventional treatments applied in line with the preoperative assessment.

The main limitation of our study is that it was conducted in a small single-center cohort. Patients are heterogeneous with regard to ESRD etiology. In addition, the study included a small number of elderly patients who had a greater risk of developing cardiovascular disease. The strength of this study is that all patients were examined in terms of cardiovascular diseases, first with noninvasive and then, if necessary, invasive tests.

CONCLUSION

CV risk factors are common in kidney transplant candidates. Our findings support the need for a detailed cardiac evaluation and effective management of CV risk factors in patients preparing for kidney transplantation.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Koç University Ethics Committee (Date: 26.08.2019, Decision No: 2019.265.IRB2.087).

Informed Consent: Because the study was designed retrospectively, no written informed consent d-form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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The prevalence of anomalies in the lumbar spine in the Turkish male population

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ABSTRACT

Aims: It was aimed to determine the prevalence of lumbosacral anomalies in young male population.

Methods: 56.798 male patients, between 18-49 years old, were included in this study during their medical screening in Dışkapı Yıldırım Beyazıt Training and Research Hospital from July 2016-July 2020. The presence of spina bifida occulta (SBO) and transitional vertebrae (TV) anomalies were recorded in the candidates. SBO patients were evaluated according to their S1 and L5 origins, and TV patients were evaluated separately according to sacralization and lumbarization. We identified transitional vertebrae by counting down from the last thoracic vertebra on the AP X-rays, then if necessary looking at the lateral view for confirmation. If hypoplastic ribs were identified, the vertebra immediately beneath would be designated as L1. Castellvi types I, II, III, and IV were included as transitional states.

Results: 56798 patients were evaluated retrospectively. The mean age of the patients was 23.28 (18-49 years). Radiological anomalies were detected in 2577 (4.5%) of 56798 cases. No radiological anomaly was observed in other cases. Spina bifida occulta was detected in 1478 (2,6%) patients. Lumbosacral transitional vertebrae were detected in 1099 cases (1.9%). 745 (1.3%) of these anomalies are sacralization and 354 (0.6%) of them are lumbalization.

Conclusion: In the light of this information, we think that knowing the frequency of lumbosacral anomalies, especially LSVT, in our society should be considered in the evaluation before spinal surgery operations.

Keywords: Lumbar spine, spina bifida occulta, sacralization, lumbalization, transitional vertebra, prevelance

INTRODUCTION

Radiological examination of the lumbosacral region is among the routine examinations in sportsman health screening and before job applications. Spina bifida occulta (SBO) and lumbosacral transitional vertebrae (LSTV) are the most common lumbar vertebral anomalies on routine lumbosacral X-rays.[1]

SBO is a lamina deficiency that does not involve the spinal cord and spinal meninges. The incidence in the population has been reported as 0.6%-25%.[2]

LSTV consists of lumbarization of the uppermost sacral segment or sacralization of the lowest lumbar segment. It includes features of lumbarization of the S1 vertebrae, abnormal articulation, and a squarer appearance of the vertebrae. Sacralization of L5 vertebrae consists of extended longitudinal transverse processes to complete fusion to the sacrum.[3,4]

Young male patients were included in this study because they had medical screening before military service. The presence of lumbosacral anomalies was investigated by routine lumbar X-rays in the candidates. It was aimed to determine the prevalence of lumbosacral anomalies in young male population.[5,6]

METHODS

The study was carried out with the permission of Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Researches Ethics Committee (Date: 08.02.2021, Decision No: 104/15). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The lumbosacral X-rays taken routinely of the patients who applied for a medical board report before military service in our institution were scanned. All patients over the age of 18 years were included in the study where the joint of the last rib with the vertebral body, all transverse processes and sacral wing were seen on X-ray. Patients

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with a history of surgery in the lumbosacral region were excluded from the study. 56.798 male patients, between 18-49 years old, were included in this study during their medical screening in Dışkapı Yıldırım Beyazıt Training and Research Hospital from July 2016- July 2020. Statistical analysis was performed using SPSS for Windows, version 19 (SPSS Inc, Chicago, Illinois).

The presence of spina bifida occulta (SBO) and transitional vertebrae (TV) anomalies were recorded in the candidates. SBO patients were evaluated according to their S1 and L5 origins, and TV patients were evaluated separately according to sacralization and lumbarization.

We identified transitional vertebrae by counting down from the last thoracic vertebra on the AP X-rays, then if necessary looking at the lateral view for confirmation. If hypoplastic ribs were identified, the vertebra immediately beneath would be designated as L1. Castellvi types I, II, III, and IV were included as transitional states. (**Table 1**)

Table 1. Castellvi radiological classification

Type Ia	One-sided TP height from 19 mm greater or equal
Type Ib	Both sided TP height from 19 mm greater or equal
Type IIa	Presence of unilateral articulation between TP and sacrum
Type IIb	Presence of bilateral articulation between TP and sacrum
Type IIIa	Unilateral fusion of the sacrum with TP
Type IIIb	Bilateral fusion of the sacrum with TP
Type IV	Fusion of one side Type II (articulation) and the other side Type IV
**TP	Lowest lumbar transverse process

RESULTS

56798 patients were evaluated retrospectively. The mean age of the patients was 23.28 (18-49 years). Radiological anomalies were detected in 2577 (4.5%) of 56798 cases. No radiological anomaly was observed in other cases. Spina bifida occulta was detected in 1478 (2,6%) patients. Lumbosacral transitional vertebrae were detected in 1099 cases (1.9%). 745 (1.3%) of these anomalies are sacralization and 354 (0.6%) of them are lumbalization (**Table 2**). According to Castellvi classification in cases evaluated radiologically; Type 1a in 279 patients (37.4%), Type 1b in 149 patients (20%), Type 2a in 130 patients (13%), Type 2b in 89 patients 11.9%, 5.3% Sacralization was detected in 40 patients with Type 3a, Type 3b in 46 patients with 6.1%, and Type 4 in 12 patients with 1.6% (**Table 3**).

Table 3. Prevalence of Castellvi classification

	Sacralization	Percentage
Type Ia	279	37.4%
Type Ib	149	20%
Type IIa	130	13%
Type IIb	89	11.9%
Type IIIa	40	5.3%
Type IIIb	46	6.1%
Type IV	12	1.6%
Total	354	100%

DISCUSSION

This study demonstrates that 2.6% of a large, randomly selected adult male population had spina bifida occulta defects involving the lower lumbar spine or sacrum. The wide discrepancies of prevalence rates in the literature may be attributable to regional and racial variations similar to those in spina bifida cystica.[7] The variation between studies may also reflect a lack of general agreement as to what constitutes a spina bifida occulta defect. The 'minimal defect' may not be universally accepted as a form of spina bifida occulta, being dismissed as representing a small bar of unossified cartilage. Its inclusion is justified by the suggestion that tethering of the cord could occur through such a small defect, making it a finding of potential pathological relevance.[8] Similarly, the 'crossover' defect may not be generally accepted as part of the spina bifida spectrum, although it has been included in some reports where a description of the lesion has been given.[7] The differences in prevalence reported in the literature may also reflect differences in the age and sex patterns of the groups studied. Boone et al.[9] while reporting an initial figure of 22%, noted a significantly higher prevalence in the under 40 years' age group which formed the major part of their study group, which, when corrected, gave a prevalence figure of 17.3% for the population as a whole.

According to studies, the frequency of LSVT in the general population varies between 4% and 35.9%.[10] Such wide variation in LSTV prevalence studies is explained by differences in individual diagnostic and classification criteria, errors in assessment, and confounding factors among the population samples studied.[11] Nardo et al. They evaluated 4636 radiographs and found the prevalence of LSTV to be 18.1%.[12] Many authors in the literature have stated that lumbosacral region anomalies may cause pain complaints by causing changes in spine

Table 2. Distribution of lumbar anomalies

Lumbarization	Sacralization	Transitional	Spina Bifida Occulta	Combined	Total
354	745	1099	1478	173	2577
0.6%	1.3%	1.9%	2.6%	0.3%	4.5%

biomechanics over time. In their study, Eren et al.[13] found the rate of congenital anomalies in the lumbar region to be 20.7% and stated that LSVT was the most common (11.4%) and sacralization was the most common one. In our study, we found a 1.9% prevalence of lumbosacral region anomaly.[14]

LSTV anomaly can be detected by lumbosacral anteroposterior and abdominal radiographs. In the case of lumbosacral transitional vertebrae, sacralization of the L5 vertebra is more common than lumbalization of the first sacral segment. The prevalence of sacralization was reported to be between 1.7% and 14%, while the prevalence of lumbalization was found to be between 3% and 7%. Hahn et al. reported in their study that sacralization was seen more frequently than lumbalization. In our study, similar results were obtained, and the prevalence of sacralization and lumbalization was found to be 1.3% and 0.6%, respectively.[15]

Limitations of our study, clinical complaints were not evaluated in the cases included in the study, diagnosis was made with lumbar anteroposterior radiography and other radiographic methods were not used.

CONCLUSION

In the light of this information, we think that knowing the frequency of lumbosacral anomalies, especially LSVT, in our society should be considered in the evaluation before spinal surgery operations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Researches Ethics Committee (Date: 08.02.2021, Decision No: 104/15).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Evaluation of the efficacy of intralesional triamcinolone injection therapy in patients with granulomatous mastitis: a comparative study with oral methylprednisolone

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ABSTRACT

Aims: This study aims to compare the efficacy of oral steroid therapy with the combination of topical and intralesional steroid (TILS) therapy in patients with granulomatous mastitis (GM).

Methods: This is a single-center retrospective longitudinal study. Women with a diagnosis of GM who applied to Medipol Universty Pendik Hospital General Surgery Clinic between January 2020 and April 2022 were included in the study. Participants received TILS or peroral steroid (POS) treatment in sequential order, and each patient received only one of the treatment options. Participants were evaluated by physical examination, USG, and radiography before and after treatment to assess lesion size, side, number of foci, skin characteristics, and the presence of pain. The clinical and radiological findings were compared betwen the groups at the end of the 6-month follow-up and the participants were followed up for the next 12 months to demonstrate the efficacy.

Results: A total of 52 women participated in the study, with 26 in the POS group and 26 in the TILS group. The mean age was 33.33+6.94 years and similar between the two groups (p=0.831). Three patients (11.53%) in the TILS group and 7 patients (26.92%) in the POS group were nonresponders to treatment (p=0.159). Given the nonresponders, GM lesions persisted in one patient (3.84%) in the TILS group and 3 patients (11.53%) in the POS group at the end of the study (p=0.610). At the end of the 12-month follow-up, five patients (19.2%) in the TILS group and 20 patients (76.9%) in the POS group who achieved a complete remission at the posttreatment 6 months experienced disease recurrence (p<0.001). Cox regression analysis revealed that only foci in the breast (p=0.043) and abscess formation (p=0.018) were independent risk factors for GM recurrence. In the TILS group, intervention-related complications were not observed while blood pressure and glucose elevation, weight gain, and edema were found in the POS group (p<0.05).

Conclusion: TILS provides similar efficacy to systemic steroid therapy, with a lower recurrence rate and potential for side effects.

Keywords: Granulomatous mastitis, Intralesional steroid, breast abscess

INTRODUCTION

Granulomatous mastitis (GM) is a rare, chronic, and benign inflammatory disease of the breast. It involves squamous metaplasia of the milk ducts and ectasia of the mammary ducts, leading to non-caseating granulomas located around the breast lobules and ducts, without any nvolvement of traumatic or foreign bodies pathologically.[1-4] GM can be categorized into idiopathic granulomatous mastitis, also known as granulomatous lobular mastitis (GLM), and secondary granulomatous mastitis. The secondary form occurs as a rare complication of various other conditions, such as tuberculosis, fungal infections, sarcoidosis, Wegener's disease, foreign body reactions, and hormonal and metabolic diseases. Importantly, during the differential diagnosis of GM, malignancy should be excluded.[5-7]

The treatment of GM remains challenging, and the literature does not describe any standardized or definitive treatment method. The primary point of distinction between different treatment approaches is the choice between conservative or surgical options. Conservative treatment includes antibiotics, anti-inflammatory drugs (topical or systemic corticosteroids, or non-steroidal anti-inflammatory drugs), and immunosuppressants.[7-9] On the surgical aspect, interventional methods such as wide surgical resection and mastectomy are advocated as primary curative treatments.[8-10]

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The current study aims to compare the efficacy of topical and intralesional steroid (TILS) treatment with peroral steroid (POS) treatment, in patients with GM.

METHODS

The study was carried out with the permission of the Medipol Universty Ethics Committee (Date: 26.07.2022, Decision No: 650). All procedures were conducted in accordance with ethical rules and the principles of the Declaration of Helsinki.

This single-center, retrospective, and longitudinal study was conducted between January 2020 and April 2022 at the General Surgery Clinic of Medipol University Pendik Hospital. Women who were diagnosed with GM were enrolled in the study. The demographic and clinical features of the patients, including the presence of diabetes, thyroid hormone status, prolactin level, and parity, were recorded.

Inclusion and Exclusion Criteria

The study included women over 18 years of age with a histologic diagnosis of GM. Patients with active infections proven by bacterial culture were eligible for inclusion after receiving appropriate antibiotic therapy, while those with abscesses were included in the study following surgical drainage. Patients who were diagnosed with secondary GM (history of breast cancer, previous surgical intervention), who received medical treatment for GM within the previous 3 months, who were pregnant, or who had contraindications for steroid use were excluded from the study. Patients who did not complete the full treatment regimen were also excluded.

Study Groups and Treatment Protocol

The patients were divided into two groups: the systemic peroral steroid (POS) group, which received Prednol tablets (Mustafa Nevzat Ilaclari, İstanbul, Turkey), and the TILS group, which received Hypocort Forte 1% cream (ORVA Ilac, Izmir, Turkey) + Kenacort-A 40 Mg, 1 ml (Deva Holding, İstanbul, Turkey). Depending on the severity of GM, oral methylprednisolone treatment was administered at a dose of 0.5 mg/kg/day (for patients with painful, <5.0 cm, single lesion, and unilateral involvement) or 1 mg/kg/day (for patients experiencing pain, fistula, ulceration, bilateral involvement, or multiple lesions). The treatment was completed over a period of 4 weeks, gradually decreasing the steroid dose. Participants had been informed about potential drug-associated side effects and its administration. Intralesional triamcinolone acetonide (ILS) was applied locally under ultrasonography (USG) guidance. The dosage for ILS was 20 mg 0.5 ml for single foci less than 5 cm or 40 mg 1 ml for multifocal lesions larger than 5 cm. Local anesthesia was not used for injection. In case of intolerance or complications, the treatment was discontinued, and the patient data were excluded from the analysis. Patients in the TILS group were also prescribed hydrocortisone topical treatment to be applied twice daily, covering the entire breast surface.

Outcome Parameters

All patients underwent physical examination, USG, and radiography before and after treatment to assess lesion size, location, number of foci, skin characteristics, and the presence of pain. Post-treatment evaluations were conducted at 1, 3, 6, 12, and 18 months. During these evaluations, the presence of pain, redness, retraction, atrophy, ulceration, fistula formation, and abscesses were recorded. The absence of these findings was considered a cure. Changes in lesion size were assessed radiographically and documented. The findings were then compared between the two treatment groups. Adverse reactions, including weight gain, blood glucose or blood pressure elevations, and psychiatric disturbances, were also noted at each hospital visit.

Measurements

Body mass index (BMI) was calculated using the formula: BMI = weight (kg)/(height (m) x height (m)).

Statistical Analysis

The variables were analyzed using SPSS Version 25.0 for Windows (IBM Corporation, Armonk, New York, United States) and Medcalc 14 (Acacialaan 22, B-8400 Ostend, Belgium). Quantitative variables were presented in tables as mean (standard deviation) and median (minimum/maximum), while categorical variables were expressed as n and (%). The analysis was performed at a 95% confidence level, and a p-value less than 0.05 was considered statistically significant. The distribution of the variables was assessed using the Shapiro-Wilk Francia test, and the homogeneity of variance was evaluated using the Levene test. For comparing parametric continuous variables, the Independent samples t-test was used. For nonparametric continuous variables, the Mann-Whitney U test was employed. Categorical variables were compared using the Pearson Chi-Square test, the Fisher-Freeman-Halton test, and the Fisher exact test Monte Carlo Simulation technique. Cox regression analysis, in combination with the Backward method, was utilized to measure the effects of other prognostic variables on the recovery time of disease recurrence. Statistical significance was assumed at a p-value of less than 0.05.

RESULTS

Sixty patients with a biopsy-proven diagnosis of GM were initially evaluated. However, 2 patients could not continue treatment due to financial problems, and 6 patients were excluded due to non-adherence to treatment. Therefore,

a total of 52 patients (26 in the POS group and 26 in the TILS group) were included in the study. The mean age of the patients was 33.33±6.94 years and was similar between the groups (p=0.831). BMI was between 18.5 and 25 kg/m² in 90.41% of the patients. Fourteen patients (26.9%) had lesions in both breasts, while 50 patients (96.2%) had lesions in more than one focus. Skin lesions were present in 37 (71.2%) patients.

Table 1. A general summary of the findings.

	n (%)
BMI	
10 - 18.5	2 (3.8)
18.5 - 25	47 (90.4)
>25	3 (5.8)
Parity	
No	11 (21.2)
Yes	41 (78.8)
Diabetes Mellitus	
Absent	44 (84.6)
Present	8 (15.4)
Hyperprolactinemia	
Absent	50 (96.2)
Present	2 (3.8)
Hypothyroidism	
Absent	48 (92.3)
Present	4 (7.7)
Place of Lesion	
Single	38 (73.1)
Double	14 (26.9)
Focal in the breast	
Single	2 (3.8)
Multiple	50 (96.2)
Pain	
Absent	1 (1.9)
Present	51 (98.1)
Fistula	
Absent	14 (26.9)
Present	38 (73.1)
Abscess	
Absent	39 (75)
Present	13 (25)
Skin lesion	
Absent	15 (28.8)
Present	37 (71.2)
Size	
1 - 3 cm	23 (44.2)
3 - 5 cm	25 (48.1)
> 5 cm	4 (7.7)
	Mean (SD) median (min/Q1/Q3/max)
Age	33.33 (6.94) 32 (22/28/38/50)
Breastfeeding duration (month)	13.65 (6.33) 12 (3/12/18/24)

SD.:Standard deviation, min:minimum, Q1: Percentile 25, Q3: Percentile 75, max: Maximum

Pretreatment of various breast skin lesions, such as fistula (100%), erythema, swelling, areolar retraction, ulceration, etc., was common in the TILS group (96.2%), while abscess formation (46.2%) was more prevalent in the POS group (p<0.001). The comparison of the two intervention groups is given in **Table 2**.

Table 2. Comparison of findings according to study groups

	TILS, n=26	POS, n=26	P value
Age, yeras	33.12±7.66	33.54±6.29	0.831[t]
Breastfeeding duration (month)	12 (3/24)	12 (3/24)	0.999[u]
BMI, kg/m²			
<18.5	0 (0)	2 (7.7)	
18.5 - 25	24 (92.3)	23 (88.5)	
>25	2 (7.7)	1 (3.8)	
Parity	20 (76.9	21 (80.8)	0.999[c]
Diabetes Mellitus	4 (15.4)	4 (15.4)	0.999[f]
Hyperprolactinemia	1 (3.8)	1 (3.8)	-
Hypothyroidism	1 (3.8)	3 (11.5)	0.610[f]
Bilateral Lesion	10 (38.5)	4 (15.4)	0.116[c]
Multiple lesion	25 (96.2)	25 (96.2)	-
Pain	26 (100)	25 (96.2)	-
Fistula	26 (100)	12 (46.2)	<0.001[c]
Abscess	1 (3.8)	12 (46.2	0.001[c]
Skin lesion	25 (96.2)	12 (46.2)	<0.001[c]
Size			
1 - 3 cm	14 (53.8)	9 (34.6)	0.400[ff]
3 - 5 cm	10 (38.5)	15 (57.7)	
> 5 cm	2 (7.7)	2 (7.7)	

[t]Independent Samples t test (Boostrap), [u]Mann Whitney-U test (Monte Carlo), [c]Pearson Chi-Square test (Monte Carlo), [f]Fisher exact test (Monte Carlo), [ff]Fisher-Freeman-Halton test (Monte Carlo), SD: Standard Deviation), min: Minimum, max: Maximum, BMI; body mass index

At the end of the 6-month follow-up, three patients (11.53%) in the TILS group and seven patients (26.92%) in the POS group did not respond to treatment (p=0.159). These nonresponders received additional treatment regimens with a combination of steroids and TILS. Among the nonresponders, lesions persisted in one patient (3.84%) in the TILS group and three patients (11.53%) in the POS group at the end of the study (p=0.610).

Following a complete remission, the disease recurred in five patients (19.2%) in the TILS group and 20 patients (76.9%) in the POS group (p<0.001). The recurrences occurred with a median interval of 4 (range: 3-12) months, and similar treatment was provided for the recurrent cases. Lesions healed in a median of 2 months (range: 1-12) in the TILS group, which was significantly shorter than that in the POS group (p=0.002).

Additionally, all participants in the POS group received an antibiotic regimen, while only 19.2% of those in the TILS group required antibiotic therapy (p<0.001).

Excision of the lesion was required in one case in the TILS group, while five patients required excision of the lesion, and one patient underwent a mastectomy in the POS group (**Table 3**).

Table 3. Comparison of results between the treatment groups

	TILS (n = 26) n(%)	POS (n = 26) n(%)	p
Recurrence, n (%)	5 (19.2)	20 (76.9)	<0.001[c]
Excision of lesion from the breast, n (%)	1 (3.8)	5 (19.2)	0.191[f]
Mastectomy, n (%)	0 (0)	1 (3.8)	-
Additional antibiotic use, n (%)			<0.001[c]
Absent	21 (80.8)	0 (0)	
Present	5 (19.2)	26 (100)	
	median (min/max)	**median (min/max)**	
Duration of regression of lesions (month)	2 (1/12)	4 (1/12)	0.002[u]

[u]Mann Whitney-U test (Monte Carlo), [c]Pearson Chi-Square test (Monte Carlo), [f]Fisher exact test (Monte Carlo), min: Minimum, max: Maximum

Foci in the breast (p=0.043) and abscess formation (p=0.018) were determined as independent risk factors for GM recurrence among all patients. These patients underwent surgical interventions. Similarly, in the POS group, abscess formation (p=0.030) emerged as an independent risk factor for disease recurrence, while no significant statistical pattern for recurrence was observed in the TILS group (**Table 4**).

Table 4. Regression analysis of possible risk factors for disease recurrence

Independent Variables	B±Sh	P value	Odds Ratio (95% C.I.)
Total (n=52)			
Focal in the breast	2.414±1.194	0.043	11.18 (1.1-116)
Abscess	1.440±0.606	0.018	4.22 (1.3-13.8)
Oral corticosteroid (n=26)			
Abscess	1.107 (0.0511)	0.030	3.03 (1.11-8.23)
G.M topical+intralesional steroid (n=26)			
No significant pattern was obtained			
Dependent variable: relapse			

Cox Regression-Backward Stepwise (Wald) Method, C.I.: Confidence interval B: regression coefficients SE: Standard error

In the TILS group, no adverse events were noted, while in the POS group, three patients experienced blood pressure elevation, three patients had elevated blood glucose levels (all were diabetic), two patients gained weight, and one patient experienced mood changes following steroid use.

DISCUSSION

GM is a heterogeneous disease with variable clinical presentations. Since the etiology is not clear in GM, the treatment strategies are also not uniform and the available treatment options are controversial. The main findings of the study demonstrate faster recovery, lower disease recurrence, and side effects with TILS compared to POS therapy. On the other hand, the number of foci in the breast and the presence of an abscess were independent risk factors for disease recurrence, rather than the treatment modality.

Systemic meta-analyses have revealed that managing IGM with only steroids may be less effective than the combination of steroids and surgery.[5,11] Moreover, surgical management may have a high complete remission rate with a relatively low recurrence rate, with or without steroids.[5] However, surgical treatment is associated with a high risk of wound infection, sinus formation, and cosmetic problems. Therefore, the pharmaceutical approach is considered in the first step, while surgical indications are limited to more specific conditions such as abscesses and resistance to medical treatment.[11,12] Besides it was emphasized that the GM may regress over time and therefore aggressive treatments should be chosen carefully.[13,14] The current literature assumes that there is no significant difference between nonsurgical approaches (systemic and local steroids, and immunosuppressive therapies) and a surgical approach on residual disease or recurrence in the treatment of GM. Given all, there is not yet a widely accepted algorithm based on scientific evidence in the literature to guide the therapeutic management of GM, and the debate on conservative and surgical treatment approaches continues to date.

The literature indicates that GM primarily occurs among women in childbearing age and usually two years after breastfeeding at a median age of 30 years.[4] In this study, the mean age was 33.33±6.94 and the duration from previous breastfeeding to GM development was 13.65±6.33 months. Prolactin is known to facilitate ductal ectasia and milk stagnation, while also exhibiting a proinflammatory effect. The assessment of serum prolactin levels is mostly not performed in patients with GM.[15] In this cohort, hyperprolactinemia prevalence was found 3.8%.

The primary symptom of GM is a painful mass, and as many as 50% of patients experience erythema and swelling as signs of inflammation in the affected breast. Additional symptoms may include hyperemia, areolar retraction, fistula, and ulceration. Approximately 37% of cases present signs of an abscess.[16] 71.1% of patients had one clinical finding of erythema, swelling, areolar retraction, and ulceration and 25.0% of patients had absce formation in our cohort.

Several previous studies reported the efficacy of TILS in GM. Toktas et al.[17] first recommended the treatment of GM.17 DeHerthogh et al.[18] proposed a high-dose corticosteroid therapy involving prednisolone at 30 mg/day for a minimum of 2 months in the treatment of GM. This treatment typically results in a reduction in the lesion's diameter; however, it also brings about various side effects like weight gain, hyperglycemia, and the potential risk of Cushing's syndrome. Similarly, in this study, cases of blood pressure and glucose elevation, weight gain, and mood change were noted in the POS group, while there was no adverse reaction in the TILS group.

Previous studies have also reported corticosteroid treatment success in GM, however, the unfavorable part of this treatment modality is the high recurrence rates after stopping or decreasing the dose of steroids. GM reccurrs in 30-50% of patients who achieve remission following steroid use.[19,20] In this study, 76.9% of patients in the POS and 19.2% of patients in the TILS group developed a recurrence of the disease. However, it should be kept in mind that GM can occur at any time in the future following a successful treatment course. So, we don't claim that TILS has a sustained superior effect against POS in regard to disease recurrence. However, TILS substantially prevented surgical intervention in the following 12 months of the treatment.

TILS as a first-line treatment in patients with GM. Yıldırım et al.[21] compared the TILS injection (ILS, n=17) and POS groups (n=19) in a prospective randomized controlled study in patients with newly diagnosed GM. In this study, they reported that 4 patients (23.5%) in the ILS group and 5 patients (26.3%) in the POS group had required repeated treatment interventions. At the end of the six-month follow-up, 78.9% of patients in the TILS group and 88.2% of patients in the POS group had responded to treatment, but there was no significant difference between the rates. They also reported no statistically significant difference between the two groups regarding side effects and recovery rates.[21]

GM recurrence rates range from 0 to 46% with different treatment approaches within a 10-year follow-up period.[22,23] Systematic meta-analyses indicate that the combination of systemic steroid and surgical treatment is superior to systemic steroid therapy alone or surgery alone for treatment success and disease recurrence.[5,11] The increased risk of recurrence identified in patients with breast fistulae, more than three complaints at the time of diagnosis, erythema nodosum, and multicentric disease may be attributed to a delay in treatment.[24,25] In this study, GM recurrence was 48% and dominantly was seen in the POS group. This may be due to a relatively short treatment duration with oral steroids which was 1 month. It is interesting that at the onset of the study, fistula prevalence was 100% in the TILS group and after treatment, the prevalence was lower than seen in the POS group. Additionally, the treatment response was faster in the TILS group. Together with the different results in similar studies, this study shows that the main factors in disease recurrence are the GM rather than the treatment groups.

Systematic meta-analyses indicate that the combination of systemic steroid and surgical treatment is superior to systemic steroid therapy alone or surgery alone for treatment success and disease recurrence.[5,11] While topical treatments are recommended in addition to other treatments in uncomplicated cases with prominent skin lesions and generally in limited indications, there is insufficient data in the literature regarding TILS applications.[5,10,17] With limited literature information, TILS alone has at least similar efficacy to systemic steroid therapy and is safer in terms of side effects. It can also be combined with other treatment modalities that have demonstrated efficacy, such as surgery or systemic steroids. Nevertheless, TILS can be recommended as an effective and safe treatment in patients, who are not suitable for surgery and/or who are afraid of systemic steroid side effects, such as in the case of pregnant patients.

There is no data regarding the association between the pathological findings of GM and the potential risk of recurrence of the disease. In this study, foci in the breast and abscess formation were independent risk factors for GM recurrence. These results provide support for the notion that delayed treatment or the presence of severe inflammation may be associated with an increased likelihood of GM recurrence.

Limitations of the Study

The study includes a small sample size. Larger sample sizes would increase the statistical power and reliability of the results. The study design is non-randomized, as patients were allocated to treatment groups based on sequential order rather than through random assignment. This can introduce selection bias and impact the validity of the results. The study's follow-up period is only 12 months, which might not be sufficient to capture long-term outcomes, especially in a chronic condition like GM where recurrence can occur over an extended period. The study does not provide detailed information about the treatment history, medical conditions, and other factors that could influence the outcomes. Failure to control for these confounding factors could affect the study's internal validity. A placebo group would have provided a better control for assessing the true efficacy of the interventions. Without a placebo group, it is challenging to attribute treatment effects solely to the interventions being studied. The study was conducted in a single center, which might limit its generalizability to other populations or settings with different patient demographics and healthcare practices. While the study reports no adverse events in the TILS group, the short follow-up period may not be sufficient to assess the long-term safety of ILS injections. The study demonstrates several comparisons with p-values close to the significance threshold ($p<0.05$). With a small sample size, some of these comparisons may lack adequate statistical power to detect significant differences.

CONCLUSION

The findings of this study suggest that TILS offers a comparable level of effectiveness to systemic steroid therapy, with the advantage of a lower recurrence rate and fewer potential side effects. Patients treated with TILS experienced faster recovery, and the therapy proved to be a viable alternative to surgical intervention in preventing disease recurrence.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Medipol University Pendik Hospital Non-interventional Clinical Researches Ethics Committee (Date: 26.07.2022, Decision No: 650).

Informed Consent: A written consent form is not available since the study is retrospective.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: While the author Tuba Mert was carrying out the study, Mehmet Emin Demir, MD provided statistics support and advisory and deserves special thanks in this regard.

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Original Article

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Can artificial intelligence algorithms recognize knee arthroplasty implants from X-ray radiographs?

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ABSTRACT

Aims: This study aimed to investigate the use of a convolutional neural network (CNN) deep learning approach to accurately identify total knee arthroplasty (TKA) implants from X-ray radiographs.

Methods: This retrospective study employed a deep learning CNN system to analyze pre-revision and post-operative knee X-rays from TKA patients. We excluded cases involving unicondylar and revision knee replacements, as well as low-quality or unavailable X-ray images and those with other implants. Ten cruciate-retaining TKA replacement models were assessed from various manufacturers. The training set comprised 69% of the data, with the remaining 31% in the test set, augmented due to limited images. Evaluation metrics included accuracy and F1 score, and we developed the software in Python using the TensorFlow library for the CNN method. A computer scientist with AI expertise managed data processing and testing, calculating specificity, sensitivity, and accuracy to assess CNN performance.

Results: In this study, a total of 282 AP and lateral X-rays from 141 patients were examined, encompassing 10 distinct knee prosthesis models from various manufacturers, each with varying X-ray counts. The CNN technique exhibited flawless accuracy, achieving a 100% identification rate for both the manufacturer and model of TKA across all 10 different models. Furthermore, the CNN method demonstrated exceptional specificity and sensitivity, consistently reaching 100% for each individual implant model.

Conclusion: This study underscores the impressive capacity of deep learning AI algorithms to precisely identify knee arthroplasty implants from X-ray radiographs. It highlights AI's ability to detect subtle changes imperceptible to humans, execute precise computations, and handle extensive data. The accurate recognition of knee replacement implants using AI algorithms prior to revision surgeries promises to enhance procedure efficiency and outcomes.

Keywords: Artroplasty, implant, artificial intelligence, detection

INTRODUCTION

Total knee arthroplasty (TKA) is a highly effective surgical technique for severe knee osteoarthritis, and it is estimated that there will be more than 1.26 million cases per year in the United States alone.[1] However, for some individuals, surgery ends in failure or poor outcomes, requiring revision surgery. As the population of arthroplasty patients continues to grow, so does the volume of patients requiring revision.[2,3] When revision is required, identification of the implant manufacturer and model can be an important step in addressing complications and failures after arthroplasty of the knee. Implants are not recognized preoperatively in patients needing reoperation because of irregularities in the reporting of implant information and difficulties in accessing these records, especially from out-of-hospital systems. As a result, patients wait longer for treatment, are sent to referral facilities more often than required, experience higher rates of perioperative morbidity, and pay more for their medical care. Millions of dollars are wasted annually on identifying arthroplasty implants before revision surgery.[4] In an investigation of arthroplasty surgeons, 88% of the people who participated indicated that implant identification was crucial prior to revision arthroplasty surgery.[5] When patients apply to several facilities, it is not always possible to view their application records. Moreover, it might be challenging to acquire data

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regarding the initial procedure since the switch from paper to electronic recording methods is still relatively recent.

Successful visual processing and automated identification methods using deep learning, a branch of artificial intelligence (AI), have been created, most notably for detecting papilledema from ocular fundus photos. Visual recognition with convolutional neural network (CNN) deep learning algorithms is becoming increasingly popular in many fields, including orthopedics and traumatology.[6-8] Given the challenges of identifying implants among a substantial number of potential manufacturer models, a CNN deep learning algorithm could serve as a promising method to facilitate the instantaneous identification of knee arthroplasty implants, considering the complexities associated with their characterization.

This study aimed to investigate the use of a CNN deep learning approach to accurately identify knee arthroplasty implants from X-ray radiographs. This could improve the efficiency and outcomes of revision surgeries.

METHODS

This retrospective study was carried out with the permission of the Fırat University Non-interventional Researches Ethics Committee (Date: 29.12.2022, Decision No: 16-21). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Using post-operative and pre-revision AP and lateral radiographs of TKA patients, we trained, validated, and tested a CNN deep learning system. AP and lateral knee X-rays of at least 10 patients with the same model and knee prosthesis implantation were examined. Unicondylar knee replacements and revision knee replacement models were excluded from the analysis. Patients whose AP and lateral X-ray images were either unavailable or of low quality were also excluded from the study. The assessment of X-ray quality was conducted by two orthopaedic surgeons who possess experience in arthroplasty. In addition, participants with other implants (plate screws, etc.) in their X-rays were excluded from analysis. The authors of the study assessed the highest possible quantity of knee arthroplasty implant manufacturers and models, taking into account the availability of accurate and reliable information at the respective healthcare facilities. As a result the following 10 different models and manufacturers of TKA replacements were tested: Stryker (Scorpio), Smith and Nephew (Genesis II), Implantcast (ACS), Biomed (Vanguard), Zimmer (NexGen), Hipokrat (2000 CR), Tıpsan (T08), Zimed (V17), Tıpmed (TPM-8), and Neologic (Fortuna). Only cruciate-retaining models of knee arthroplasty implants were evaluated. The main operation operative note was used to identify the implant type, which was then cross-checked with the implant serial number. 69% of the data was used for the training set and the remaining 31% for the test set. To address the limited number of images, we applied image augmentation techniques. Additionally, given the small dataset, we leveraged transfer learning and fine-tuning methods to enhance our model's performance. Fine-tuning was carried out using the images in the training set, and both sets were randomly selected from the complete dataset. For evaluating the classification performance, we employed at least two key metrics: accuracy and F1 score. While accuracy was applied to balanced datasets, the F1 score was used to assess classification performance in situations of data imbalance. Our software development and performance assessments were conducted using the Python programming language, with the implementation of our method facilitated by the TensorFlow library (**Figure 1**). The data processing and testing were expertly overseen by a computer scientist with a specialization in artificial intelligence. We determined accuracy by comparing the predicted implant stem design with the highest degree of confidence to the actual implant design, while also calculating the specificity and sensitivity of our method to provide a comprehensive evaluation of its performance.

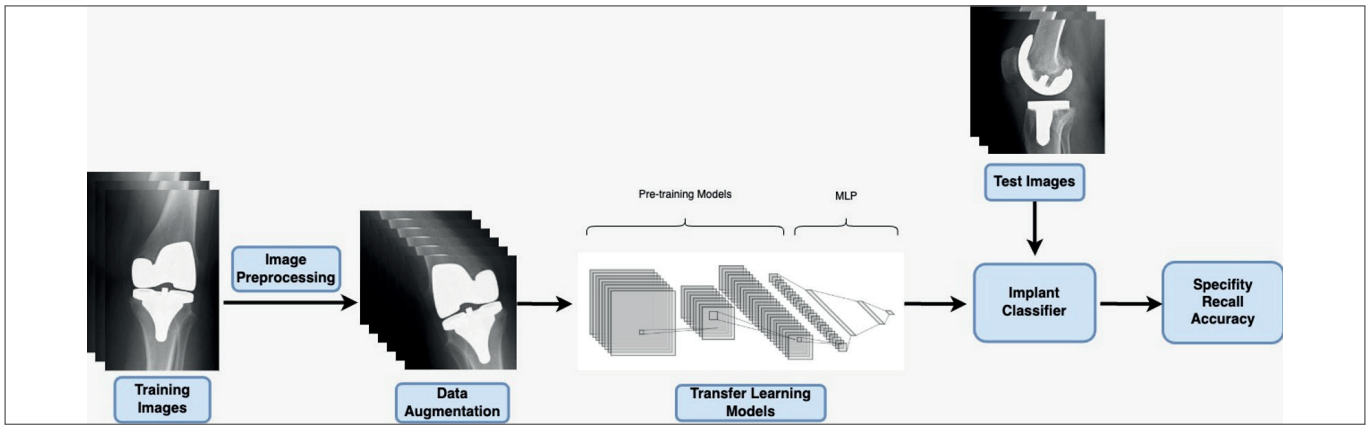

Figure 1. Flowchart of the algorithm model training, left to right: the input images are fed to the convolutional neural network after preprocessing and augmentation (increasing the number of images used for training). Once training is complete, the model is checked against a separate validation set to ensure its accuracy.

RESULTS

In total, 282 AP and lateral X-rays from 141 patients were evaluated. 10 different manufacturers' knee prosthesis models were included with different numbers of X-rays (**Figure 2**). The CNN method demonstrated an identification accuracy of 100% for each individual model in identifying the manufacturer and model of TKA among 10 different models. The specificity and sensitivity of the CNN method were 100% for each implant model separately (**Table 1**).

DISCUSSION

The most important finding of this study is that the deep learning CNN method showed a remarkable identification accuracy of 100% in identifying the manufacturer and model of TKA implants among different models when evaluating AP and lateral X-ray images. Moreover, the CNN method exhibited an extraordinary level of specificity and sensitivity, both set at 100%, for each individual implant model, further underscoring its robustness and reliability in discerning nuanced characteristics among distinct prosthesis brands. Considering that recognizing knee replacement implants prior to revision knee replacement surgeries significantly affects the effectiveness and outcomes of revision surgeries, an AI algorithm that instantly and accurately recognizes knee arthroplasty implants is likely to provide significant benefits.[5,9]

In a prior study related to deep learning CNN method, an evaluation was conducted on nine different manufacturers and models of knee prostheses. The trained AI algorithm achieved an impressive 98.3% success rate. They also found a sensitivity of 94.6% and a specificity of 99.4%.[10] It's important to note that this study exclusively focused on

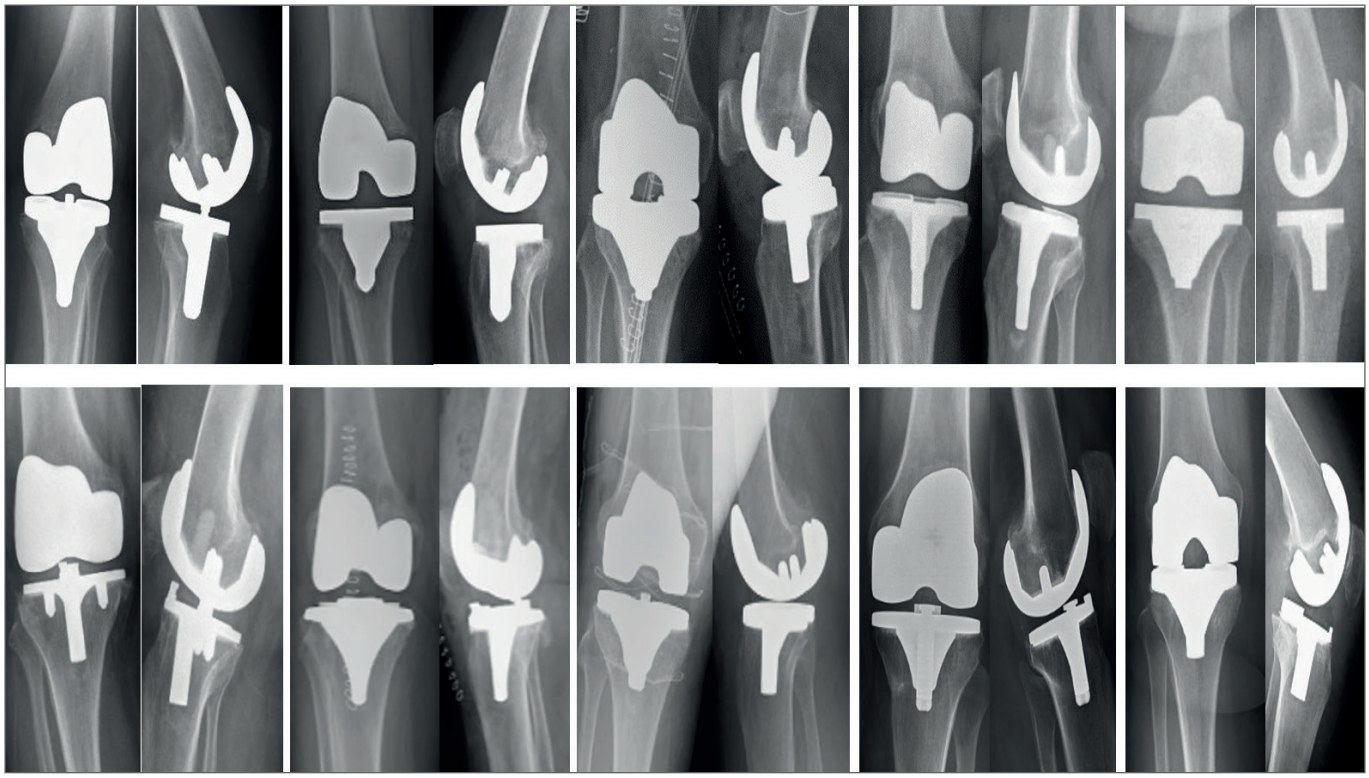

Figure 2. Knee X-rays from ten different manufacturers. From left to right: Tıpsan AP and lateral X-rays, Zimed AP and lateral X-rays, Zimmer Ap and lateral X-rays, Smith and Nephew Ap and lateral X-rays, Stryker AP and lateral X-rays, Neologic AP and lateral X-rays, Biomed AP and lateral X-rays, Hipokrat AP and lateral X-rays, Tıpmed AP and lateral X-

Table 1. Deep learning performans for detection of each knee arthroplasty implant's model and manufacturer

Artroplasty implants	Training X-ray (n)	Testing X-ray (n)	Accuracy (%)	Sensitivity (%)	Specifity (%)
Stryker (Scorpio)	14	6	100%	100%	100%
Smith and Nephew (Genesis II)	16	8	100%	100%	100%
Implantcast (ACS)	16	8	100%	100%	100%
Biomed (Vanguard)	14	6	100%	100%	100%
Zimmer (NexGen)	14	6	100%	100%	100%
Hipokrat (2000 CR)	20	8	100%	100%	100%
Tıpsan (T08)	24	12	100%	100%	100%
Zimed (V17)	24	10	100%	100%	100%
Tıpmed (TPM-8)	26	12	100%	100%	100%
Neologic (Fortuna)	26	12	100%	100%	100%

assessing AP X-rays. The scope of the prosthetic models under examination encompassed both unicondylar and revision knee prostheses. In contrast, our own study may have yielded a higher success rate due to its exclusive analysis of bidirectional radiographs. Furthermore, the inclusion of revision knee prostheses and unicondylar knee prostheses in the evaluation may have created deficiencies in the deep learning CNN method's detection technique, contributing to a lower overall success rate. In similar research, Paul et al.[11] found that AI could reliably discern the difference between two brands of knee prosthesis and discriminate between the presence or absence of knee prosthesis with 100% sensitivity and specificity. Although the scope of this study is smaller than our review, the success power of the CNN method is remarkable, similar to our study.

In recent years, there have been studies documenting the effective application of artificial intelligence in various aspects of arthroplasty and other orthopaedic topics.[12-14] The findings from these studies, including our own, demonstrate the potential success of artificial intelligence in image processing. Furthermore, AI's potential in medical image analysis extends across various domains. For instance, endotracheal tubes and central catheters' positions on X-ray images were successfully identified using the CNN approach in two different experiments.[15,16] The CNN method was also able to identify these medical devices, just like our research indicated they could. Our study adds to this growing body of evidence by demonstrating that the CNN method can identify medical devices like knee prostheses with a high degree of accuracy and precision.

Lakhani and Sundaram investigated the efficacy of a deep learning CNN in detecting features of tuberculosis on chest radiographs.[17] It was verified by a cardiothoracic radiologist that the deep learning CNN correctly detected 100% of cases. From the perspective of researchers, AI has demonstrated remarkable success in various domains, including medical image analysis, owing to several pivotal factors. AI algorithms, particularly those grounded in deep learning, excel at processing vast datasets and discerning intricate patterns within them, enabling the identification of subtle anomalies, variations, or features that may challenge human observers in the realm of medical image analysis.[18,19] Additionally, AI systems exhibit unwavering consistency in their performance, unaffected by factors such as fatigue, distraction, or emotional states, which can significantly impact human accuracy and reliability—especially crucial in medical diagnostics where errors can carry grave consequences.[20] Moreover, AI systems have significant memory capacity, allowing them to store and retrieve extensive knowledge, so they can continually improve their performance over time. This feature can be particularly advantageous in medical fields that rely on accumulated expertise. This success of AI in medical image analysis and healthcare is due to its computational power, pattern recognition ability, and capacity to exploit adaptability.[21-23] However, there are still hesitations to replace healthcare professionals, but rather to emphasize that AI serves as a valuable tool to enhance their ability to deliver superior patient care.

The findings of this study provide a foundation upon which to build more research into AI-assisted identification strategies for knee arthroplasty. Additionally, conducting a longitudinal study to assess the algorithm's performance on a larger and more diverse dataset, including various prosthesis models and manufacturers, would further validate its robustness and reliability in real-world clinical scenarios. As AI technologies continue to evolve, these endeavors will collectively contribute to refining and expanding the capabilities of AI-powered implant identification methods in the realm of knee arthroplasty. CNN may also be employed for more challenging tasks, such as the detection of postoperative problems (such as dislocations and osteolysis). Further progress can be made by increasing research in these areas.

Limitations

One of the limitations of the current study is that the brands of a small number of knee prosthesis manufacturers were evaluated. It's possible that there are other brands and models of knee arthroplasty implants out there that we haven't come across. We cannot predict how the AI program will perform when it encounters more prosthesis brands. The second limitation of the study was the exclusion of posterior cruciate ligament-sacrificing knee prosthesis models. Due to the limited availability of data in our library, we were unable to analyse images of posterior cruciate ligament sacrificing knee arthroplasty implant models. Therefore, our evaluation was restricted to posterior cruciate ligament retainer models of knee arthroplasty implants. When these prosthesis models are included, it may perhaps reduce the detection power of the AI algorithm.

CONCLUSION

This study demonstrates the remarkable potential of deep learning AI algorithms in accurately identifying knee arthroplasty implants from X-ray radiographs. Furthermore, the results of this study underline the enormous potential of artificial intelligence that can recognize subtle changes that may escape human observation, perform precise calculations, and store large amounts of information. Accurate recognition of knee replacement implants with AI algorithms prior to revision

surgeries is likely to improve the efficiency and outcomes of such procedures. While there are some limitations to consider, such as the need for a wider range of prosthesis models and brands, the potential for advancing this technology is clear and promises even more significant contributions to patient care and outcomes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Fırat University Non-interventional Researches Ethics Committee (Date: 29.12.2022, Decision No: 16-21).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Autism spectrum disorder: perspectives from paediatricians

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ABSTRACT

Aims: Since the first medical contact of children and families is mostly with paediatricians, the knowledge and experience of paediatricians on Autism Spectrum Disorder (ASD) is extremely significant. It was aimed to investigate paediatricians' general knowledge on ASD, daily practices in the outpatient clinic and ability to recognize warning signs.

Methods: A 40-item questionnaire prepared by the researchers was completed with 116 specialists or subspecialties working in public and private hospitals of two cities in Türkiye.

Results: While 41.7% of paediatricians received rotation training in residency, 32.8 % reported they have enough experience. While 87% considered ASD screening among their duties, only 12.8% stated they have enough time. The number of patients referred for formal assessment of ASD was low. Gender, type of expertise, duration of experience, and sense of competence were not associated with referral frequency. It's been determined paediatricians have a lack of knowledge about ASD. While not responding to name and difficulty following the instructions were seen as most warning symptoms, other diagnostic criteria and accompanying symptoms were recognized insufficiently.

Conclusions: In addition to increasing the duration and quality of child psychiatry education during residency, regulating the outpatient clinic conditions can overcome an important obstacle in the early diagnosis of ASD.

Keywords: Paediatricians, awareness, autistic disorder, early diagnosis, knowledge

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that first appears in early childhood and is characterized by repetitive behaviours, limited interests and a persistent impairment in social and communication skills. The clinical presentation and severity of symptoms vary between individuals.[1] According to the Centres for Disease Control and Prevention (CDC), the prevalence of ASD was reported to be 1/150, 1/59, and 1/36 in 2000, 2014, and 2020, respectively, in the United States, and there has been a striking increase in the incidence of ASD.[2]

The American Academy of Paediatrics (AAP) advises developmental monitoring at every appointment, formal developmental screening at 9, 18, and 30 months, and autism-specific screening at 18 and 24 months for all typically developing children in order to diagnose ASD early.[3] Similarly, the Public Health Institution of Türkiye recommends that children aged 18-36 months be evaluated for ASD at least once.[4] The most emphasized topic in the literature regarding the prognosis of the disorder is the early diagnosis and the initiation of appropriate educational approaches without delay. Early intervention has been found to result in significant gains in social communication skills such as joint attention, pretend play, imitation, and language use, while reducing stereotypical behaviors.[5,6] In our country, Child Psychiatrists are responsible for the diagnosis and treatment planning of ASD. Families can directly approach these departments for consultations. However, in our country, children's first contact with the health system is usually with their paediatricians or family physicians. Therefore, the knowledge, experience and skills of paediatricians related to ASD are critically important for early diagnosis and intervention.

In studies conducted in different countries, it has been determined that paediatricians do not have enough information about the clinical appearance, etiology, diagnosis and treatment processes of ASD.[7-9] Similar results were obtained in studies conducted in our country.[10-12] In parallel with the theoretical inadequacy regarding the etiology, symptoms, clinical course, and treatment of ASD, problems are also observed in the screening of ASD and its referral to higher units in clinical practice. While it was found that patients screened for ASD were not adequately

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referred to higher units,[13,14] a retrospective study conducted with children diagnosed with ASD in our country found that very few children were referred to child psychiatry despite their previous paediatric clinic applications.[15]

In this study, it was aimed to evaluate paediatricians as follows:

1. Sociodemographic characteristics (gender, title, medical experience, parenting experience)
2. Background related to ASD (trainings received during assistantship and post-expertise, presence of ASD in the family, subjective sense of competence),
3. Clinical practices (routine screening for ASD, referral frequency, referral age range, examination times),
4. Knowledge and beliefs about the clinical appearance, etiology and treatment of ASD,
5. Knowledge about the warning signs for ASD in different age ranges (0-2 years, 2-6 years).

In the literature, there are studies that examine paediatricians' experience, past training, and knowledge levels related to ASD. It is believed that evaluating paediatricians' clinical practices and their knowledge about warning signs for ASD, in addition to these parameters, would contribute to the literature.

METHODS

The study was carried out with the permission of the University of Health Sciences Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date: 12.09.2019, Decision No: 20/16). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All participants were informed verbally or in writing about the purpose and confidentiality of the survey.

This is an observational, descriptive, cross-sectional study. It was conducted between September 2021 and June 2022 in Antalya and Mersin city centers where the authors work as child and adolescent psychiatrists. These two city centres have a population of approximately 1.7 million in total. We aimed to study with paediatric specialists (general paediatricians-paediatric subspecialists) who provide outpatient clinic services in either public or private hospitals. Therefore, paediatric residents and academic faculty members who assessed patients as part of residency training at the university were excluded from the study.

All subspecialists included in the study work in general hospitals similar to specialists (general paediatricians) and provide regular polyclinic service every day. Subspecialists such as neonatal, intensive care, and emergency departments were not included in the study because they did not perform regular outpatient clinics.

Attempts were made to reach all 178 paediatricians who met the criteria, and out of 143 physicians reached, 116 agreed to participate and were included in the study.

A questionnaire consisting of 40 questions created by the researchers was used in the study. The survey questions were arranged in five sections. The section evaluating the socio-demographic characteristics of the participants, their background related to ASD, and their knowledge and beliefs about the clinical appearance, etiology and treatment of ASD was created according to previous studies.[10,12,16] Clinical practice and referral approaches and symptoms seen as stimulant in the clinic, were created based on the outputs of studies related to the field and the main problems we observed in our own outpatient practice. The questionnaire was prepared in the native language of the authors and participants (Turkish).

In the warning signs for physicians, only autism diagnostic criteria were not used and aside from these, clinical conditions frequently seen with ASD were also included. Since they were considered to be restrictive, the questions in this area were not asked as yes-no, but rather graded (not stimulant-mildly stimulant-highly stimulant). Since a scale was not used in our research, the answers given to the questions were not evaluated comparatively, each question was evaluated within itself. Our questionnaire was applied by face-to-face in order to prevent the use of auxiliary information sources, which has been encountered in similar studies. The main purpose of this application was to determine the current situation of the participants regarding ASD more accurately and objectively. Questions that were not understood by the participants during the application were explained when deemed necessary.

Statistical analyses were performed using the SPSS Statistics for Windows, version 26.0. The Kolmogorov-Smirnov test was used to determine whether the variables were normally distributed. Descriptive variables were given as number (N) and percentage (%). The Mann-Whitney U and Kruskal-Wallis tests were used for comparisons between groups. The Pearson Chi-square test or Fisher's Exact test was used to compare categorical variables. A p value less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

A total of 116 paediatricians participated in the study. 53.4% of the participants were male and 46.6% were female. While the rate of specialists (generalists) was 60.9%, the rate of subspecialists was 39.1%. While 30.7% of specialists had less than five years of experience, 52.6% had 10 years or more of experience. These rates were 55.6% and 17.6% for subspecialists. 66.4% of the participants had parenting experience. 66.4% of the participants had parenting

experience. The percentage of those with one child was 29.3%, those with two children was 28.4%, and those with three or more children was 8.6%.

Paediatricians' experiences related to ASD, their past training, and their perceptions of competence in the field are summarized in **Table 1a** and **1b**. 32.8% of the participants self-reported that they had sufficient experience in the field. This subjective sense of competence was not associated with rotation training and post-specialty training.

Table 1a. Physicians' autism-related experience and past training

	Yes (n,%)	No (n,%)
Has anyone in your family been diagnosed with autism?	26 (22.4%)	90 (77.6%)
I received training on autism during my assistantship	48 (41.7%)	67 (58.3%)
I received training on autism after my assistantship	9 (8.3%)	103 (91.7%)
I have sufficient experience in autism.	38 (32.8%)	78 (67.2%)
Child psychiatry rotation should be in the paediatric residency period.	110 (94.8%)	6 (5.2%)

Table 1b. Physicians' autism-related experience and past training

When was the last time you read an article on autism? (n,%)	**In the last year**	**In the last 5 years**	**In the last 10 years**	**Not read**	
	33 (28.4%)	38 (32.8%)	12 (10.3%)	33 (28.4%)	
How long should the rotation period be? (n,%)	**1 month**	**2 months**	**3 months**	**4 months**	**+6 months**
	11 (9.9%)	31 (27.9%)	51 (45.9%)	13 (11.7%)	5 (4.5%)

Table 2 summarizes physicians' clinical approaches to ASD.

Table 2. Physicians' clinical approaches to autism

How often do you refer patients with suspicion of autism? (n,%)	
None	11 (9.7%)
1-2 per day	4 (3.5%)
1-2 per week	22 (19.5%)
1-2 per month	34 (30.1%)
1-2 per year	42 (37.2%)
In which age range do you refer patients with the suspicion of autism?[a,d,e] (n,%)	
0-1 age	0 (0%)
1-2 age	12 (10.5%)
2-3 age	61 (53.5%)
+3 age	41 (36%)
At what age range do you refer children with speech delay for psychiatric evaluation? (n,%)	
1-2 age	19 (16.4%)
2-3 age	49 (42.2%)
+3 age	48 (41.4%)
Do you routinely ask questions about developmental stages in the 0-2 age group?[e] (n,%)	
No	19 (16.4%)
Sometimes	34 (29.3%)
Yes	63 (54.3%)

a: p<0,05 between those children and those without children
b: p<0,05 between experience +10 years and <10 years
c: p<0,05 between specialist and sub-specialists
d: p<0,05 between male and female
e: p<0,05 between those feeling competent vs non- competent

87% of paediatricians believed that there was an increase in the prevalence of ASD. Similarly, the same percentage of physicians considered screening of ASD among their duties, but only 12.8% reported that they have enough time for it. The rate of screening developmental stages in the 0-2 age group was 54.3%, and this rate was significantly higher among those who self-reported having sufficient experience ($p<0.05$).

Table 3 demonstrates physicians' information on the clinical appearance, causes and treatment of ASD.

Table 3. Physicians' knowledge on the clinical appearance, causes and treatment of autism

	Yes (n,%)	No (n,%)
Mental retardation may be co-diagnosed with autism	65 (56.0%)	51 (44.0%)
Autism is a neurodevelopmental disorder	99 (85.3%)	17 (14.7%)
Autism is an autoimmune disorder	24 (21.1%)	90 (78.9%)
Hyperactivity is one of the main symptoms in autism.	67 (59.3%)	46 (40.7%)
Symptoms in autism begin after 3 years of age	12 (10.3%)	104 (89.7%)
The intelligence level of autism cases is normal and above normal.	55 (48.2%)	59 (51.8%)
Excessive TV, telephone and information exposure are causes of autism.	80 (69.6%)	35 (30.4%)
Serious life events such as divorce, accident, loss of a family member are among the causes of autism.	42 (36.8%)	72 (63.2%)
Lack of interest in the family is one of the causes of autism.[c]	70 (61.4%)	44 (38.6%)
Parents' wrong attitudes are among the causes of autism.[c]	63 (55.3%)	51 (44.7%)
Heavy metals are among the causes of autism.	79 (69.3%)	35 (30.7%)
Vaccines are one of the causes of autism.	11 (9.6%)	103 (90.4%)
Autism is a treatable disorder.	75 (67.0%)	37 (33.0%)
Diet is effective in the treatment of autism.[b]	64 (56.6%)	49 (43.4%)
Vitamin-mineral supplements are effective in the treatment of autism.[b]	50 (43.9%)	64 (56.1%)
Hyperbaric oxygen therapy is effective in the treatment of autism.	18 (16.2%)	93 (83.8%)
	n (%)	
Primary treatment in autism	0 (0%)	
Pharmacotherapy	2 (1.7%)	
Diet	0 (0%)	
Special education	79 (68.7%)	
Family interest	34 (29.6%)	

a: p<0,05 between those children and those without children
b: p<0,05 between experience +10 years and <10 years
c: p<0,05 between specialist and sub-specialists
d: p<0,05 between male and female
e: p<0,05 between those feeling competent vs non- competent

Symptoms considered highly stimulating by paediatricians are presented in **Figures 1** and **2**. The rates of symptoms not considered as stimulating in the 0-2 age group are as follows: Breast refusal (64.9%), gastrointestinal system problems (60.2%), absence of

separation anxiety (53.1%), sniffing objects (37.5%). The rates of symptoms not considered as stimulating in the 2-6 age group are as follows: hyperlexia (63.2%), smelling objects (38.6%), excessive playing with water (35.1%), delaying in toilet training (33.9%), inability to learn colours (28.7%) and indifference to pain and heat (27%).

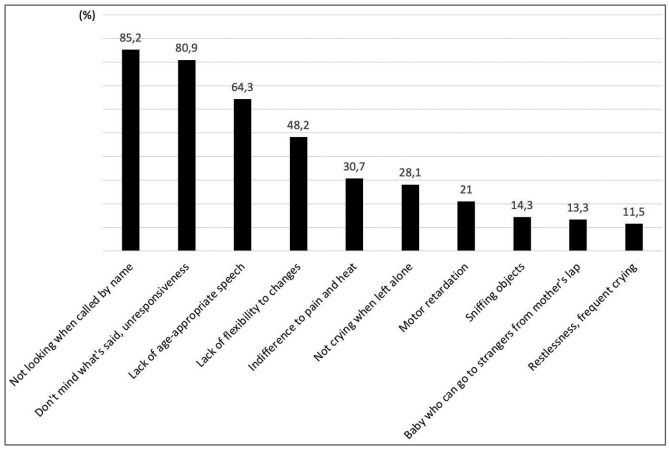

Figure 1. Symptoms highly stimulating for age 0-2

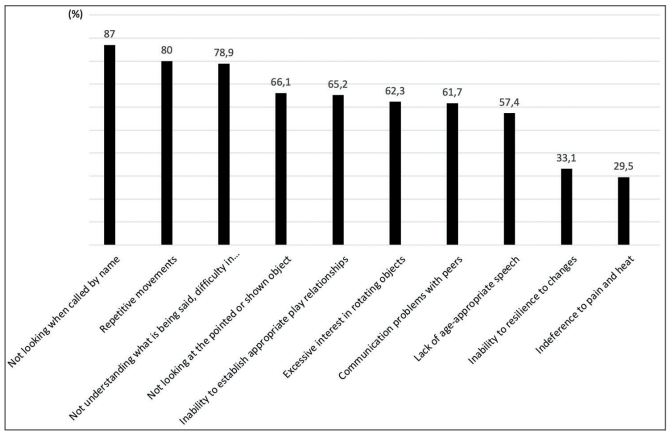

Figure 2. Symptoms highly stimulating for age 2-6

It is shown in **Tables 2**, **3** whether the level of experience (10- years /10+ years), gender, having a child, type of specialization (specialist / subspecialist) and the sense of competence have a significant effect on the knowledge levels and clinical approaches of physicians.

DISCUSSION

This study revealed that paediatricians do not have sufficient knowledge about the causes, clinical symptoms and treatment of ASD, as well as serious deficiencies in routine screening in outpatient clinics, identifying risky children and referring them to the Child Psychiatry Clinic.

Autism Related Experience and Past-Training

Approximately two-thirds of the participants (67.2%) self-reported that they did not have enough experience with ASD. This result is consistent with the results of Austriaco et al.[16] and Citil et al.[10] which shown that paediatricians experience discomfort in the field. Although these data suggest that paediatricians have more experience in the field, the sense of competence is limited as it is evaluated subjectively and can be reconsidered in studies where competence is evaluated objectively.

41.7% of the participants received a one-month rotation training during the residency. It was determined that receiving rotation training didn't increase the sense of subjective competence and didn't cause significant changes in general knowledge and clinical approaches. Although this information is consistent with the results of Citil et al.[10] Song et al.[8] found that rotation training significantly increased the general knowledge about ASD. It has been shown that an interactive video and practice-based training program for paediatric resident increases ASD-related competence.[17] The results of our study may be related to insufficient rotation training, as well as the loss of long-term gains. The low rate of receiving post-specialization education (8.8%) and the relative inadequacy of personal efforts, such as following up-to-date literature, seem to support this information. In a study conducted in our country, it has been reported that healthcare professionals who participated in training related to ASD shown an improvement in their knowledge levels.[18] While almost all participants (94.8%) agreed on the necessity of Child Psychiatry rotation, 90.1% found the current one-month duration insufficient. Based on this information, it is recommended to review the duration and content of paediatricians rotation training during the residency, and develop strategies for continuing educational efforts in the post-specialty period.

Clinical Approach

Although ASD symptoms appear in the first two years,[19] the mean age at diagnosis still seems high in the world and in our country. In a retrospective examination of diagnosed children conducted by Erden et al.[15] it was revealed that only 4% of the patients seen by paediatricians were referred. In studies conducted around the world, it is mentioned that there are serious problems with the referral of patients, for various reasons, even when standardized tools like M-CHAT are used to identify at-risk individuals.[13,14]

The fact that 87% of the participants considered ASD screening as part of their duties indicates a high level of awareness in the field. However, the rate of routine screening of developmental stages in the 0-2 age group remained at 54.3%. It was found that 9.7% of the participants had never referred a patient, 30.1% referred 1-2 patients per month, 37.2% referred 1-2 patients per year. The frequency of referral was not related to factors such as year of residency, title, gender, having children or a sense of competence. Given that participants evaluate

an average of 30-50 paediatric patients per day, it can be concluded that referrals are relatively infrequent. One limiting factor for referrals may be a lack of time, as 87.2% of the participants stated that they did not have enough time in the outpatient clinic for ASD screening. Dosreis et al.[20] and Gabrielsen et al.[21] reported that not having sufficient time was the biggest obstacle to screening patients for ASD. As a matter of fact, in our study, both the general knowledge of the physicians about ASD and their familiarity of clinical signs that can aid in diagnosis were insufficient. However, there are studies showing that even qualified paediatricians who can administer tools like M-CHAT often fail to refer at-risk patients to specialized centers.[13] In these studies, besides the organizational problems with the referral system, factors such as the physician's insecurity, fear of labeling the patient, and lack of communication skills to talk to the family about the issue came to the fore.[22] In the group we studied, these factors might also have presented challenges in terms of patient referrals.

53.5% of the paediatric specialists indicated that they preferred to refer suspected ASD cases between the ages of 2-3, while 36% stated that they preferred to refer them over the age of three. Men and those without children are significantly more likely to refer over the age of three years This pattern might be explained by the idea proposed by Mao et al.[9] which suggests that women and those with parenting experience tend to possess greater knowledge about developmental milestones. Contrary to the ideal timing, delayed referral may be associated with various factors mentioned earlier (lack of time, lack of knowledge and experience, etc.). Alternatively, it could be linked to the wait-and-see strategy, which is still frequently preferred by physicians, although it is not recommended in the field.[23]

A similar delay in referral appears to be applicable to patients presenting with speech delay as well. In our study, 41.4% of the paediatricians stated that they referred patients with speech delays for psychiatric evaluation after the age of three. Considering that individuals with ASD are often brought to the outpatient clinic due to a lack of age-appropriate speech,[24] the high rate of referrals at age three and older can be regarded as a serious issue for early diagnosis. Although the participants stated that ASD symptoms started before the age of three with a high rate (89.7%), the delay in referrals may be due to paediatricians not perceiving speech-related impairments as significant enough. Among physicians, 64.3% for ages 0-2 and 57.4% for ages 2-6 considered speech delay as a strong indicator for ASD. This approach to speech delay aligns with previous studies in our country. According to Erden et al.[15] the families of some of the children with speech delay are given early assurance that there is no problem leading to wasted time, especially for boys, by assuming they will talk over time.

Further studies are needed to investigate the frequency of referral for formal assessment of ASD, the age range of referral, and the factors that hinder referral.

Knowledge and Beliefs About ASD

Relatives of patients experience serious confusion about the causes and treatment of ASD.[25,26] Misleading explanations regarding the causes of autism can lead to increased feelings of guilt among families, while unverified treatment attempts can result in families misallocating their resources. Considering the close contact of paediatricians with families, it is critical for physicians to have accurate knowledge of etiology and treatments, and to effectively communicate this information to families to ensure proper treatment adherence.

In our study, it was determined that were considered the false beliefs in the society about the etiology of ASD were also accepted among paediatricians to a certain extent. Specifically, screen exposure (69.6%), important life events (36.8%), insufficient family involvement (61.4%), inappropriate parental attitudes (55.3%), heavy metals (69.3%), autoimmunity (21.1%), and vaccines (9.6%) were mentioned as potential causes of ASD. General paediatricians cited the insufficient care of the family and inappropriate parental attitudes as significantly more causes than the subspecialties ($p<0.05$). For other parameters, duration of expertise, title, gender, sense of competence or having children were not associated with a significant difference. Insufficient knowledge of paediatricians about etiology reported by Citil et al.[13] is in line with the results of our study.

The most critical of the misconceptions regarding etiology were those related to vaccines. Although the percentage of paediatricians who believe vaccines are the cause of ASD appears low, it is still worrisome. It has been shown that the trust of healthcare professionals in vaccines directly affects their willingness to recommend vaccines to their patients.[27] Associating vaccines with ASD can lead parents to reject vaccines, emphasizing the importance of dispelling such misconceptions among healthcare providers.

Considering the opinions of paediatricians regarding the treatment of ASD, approximately two-thirds of the participants view ASD as treatable. Although the study did not outline the specific curability framework, it is encouraging to see that the majority of physicians consider ASD treatable. This perspective is likely influenced by the progress made in treating ASD in the country. It's noteworthy that 64% of the participants identified special education as the primary treatment, indicating that special education practices are widely accepted

among pediatricians. Furthermore, 29.6% of participants stated that family involvement is the primary treatment, possibly related to their perception of insufficient parental attention as a cause of the condition and attributing it to incorrect parental attitudes. A total of 56.6% of the paediatricians believed that dietary interventions were effective in the treatment, while 43.9% believed in the effectiveness of vitamin-mineral supplementation. It is possible that parents frequently opt for dietary practices to address gastrointestinal system problems, which in turn may have influenced the physicians' perceptions regarding the effectiveness of such interventions in managing ASD symptoms. It was observed that physicians with more than 10 years of expertise considered family support, dietary interventions and vitamin-mineral supplements to be significantly more effective in the treatment than those with less than 10 years of experience. This suggests that physicians with less than 10 years of experience may have more accurate knowledge about treatment options. It's a positive sign that awareness about ASD treatment has increased in recent years.83.8% of the participants self-reported that hyperbaric oxygen therapy was not effective. The high level of awareness on this issue is gratifying.

Warning Signs

In the 0-2 age group, the three symptoms that were seen as highly stimulating at the highest rate were as follows: not responding to the name (85.2%), difficulty in following instructions (80.9%) and not speaking in accordance with their age (64.3%). The fact that ASD diagnostic criteria in the field of social communication and interaction are the most widely recognized symptoms is consistent with the literature.[10] However, inflexibility to changes was reported by 48.2%, insensitivity to pain by 30.7%, and sniffing objects by 14.3% as high stimuli. The lower recognition of symptoms in the restricted, repetitive behaviours domain may indicate a knowledge gap in this area. These findings are consistent with a study indicating low awareness related to sensory dysregulation.[16] This could be because general education tends to focus more on social communication and interaction, possibly neglecting sufficient emphasis on symptoms related to limited and repetitive behaviour.

The highest rate of non-stimulant (non-stimulant) symptoms in the 0-2 age group were breast refusal (64.9%), gastrointestinal system problems (60.2%), and absence of separation anxiety from the mother (53.1%). Although these symptoms are not diagnostic criteria, they often accompany with ASD. Weaning is more frequent in children with ASD than in normally developing children.[28] Again, approximately 70% of these children have eating behaviour problems and 36% of these are serious problems.[29] Abnormal eating habits and problems with GIS may be a manifestation of limited interest and repetitive behaviours of ASD. In addition, despite the knowledge that motor developmental defects can be observed in patients with ASD from an early period,[19,24] 43% of paediatricians did not consider them significant. Although these symptoms may not be directly related to ASD, their dismissal as potential clinical indicators by paediatricians can be viewed as a deficiency.

In the 2-6 age group, the three symptoms that considered highly stimulating at the highest rate were not responding to the name (87.0%), repetitive movements (80.0%) and difficulty in following instructions (78.9%), while the three symptoms that not considered as stimulant (non-stimulant) were hyperlexia (63.2%), sniffing objects (38.6%) and excessive playing with water (35.1%). Not responding to the name and difficulty following instructions are the two symptoms with the highest sensitivity in the 2-6 age group, just like in the 0-2 age group.

On the other hand, among the other ASD diagnostic criteria related to social communication and interaction, the lack of joint attention was highly stimulating for 66.1%, unable to play appropriate games with peers was highly stimulating for 65.2%, having problems in peer communication was highly stimulating for 61.7% and age-appropriate speech deficiency was highly stimulating for 57.4%. These symptoms were not as stimulating as the two mentioned earlier.

Among the diagnostic criteria of ASD, resistance to changes was marked as high-level stimulus for 33.1%, insensitivity to pain for 29.5%, and sniffing objects for 14.9%. These symptoms seem to be overlooked in this age group as well as in the 0-2 age group. It has been shown that 84% of hyperlexia cases have ASD.[30] However, only 4.4% of paediatricians see hyperlexia as highly stimulating, while 63.2% see it as a non-stimulating symptom, which can be considered a deficiency.

In conclusion, it can be said that paediatricians attribute a high level of importance to symptoms like unresponsiveness to name and difficulty in following directions in both age groups. However, other diagnostic criteria related to social communication and interaction, criteria defined in the field of restrictive and repetitive patterns of behaviour except for repetitive movements, and other clinical conditions accompanying the clinical presentation don't seem to be adequately recognized as significant indicators.

The present study has several limitations that should be considered when interpreting the results obtained. Cross-sectional design and small sample size may be a limitation. Since our results represent samples from two city centres, the results may not fully represent the perspective of all paediatricians in Türkiye. Additionally, the working conditions of the participants were not

standardized and subspecialists from different fields were grouped together, which could impact the interpretation of the results. Furthermore, the questionnaire we used in this study was created by the authors specifically for this research and its psychometric properties were not tested. Additionally, the questionnaire was not piloted. However, this study still provides valuable insights into paediatricians' knowledge and clinical approaches to the field. The questionnaire is planned to be used in future research after its psychometric validity has been studied. There is a need for nationwide, multicentre prospective studies with larger samples, in which standardized tools will be used.

CONCLUSION

Our study shown that the level of knowledge of paediatricians in the field of ASD is insufficient; although they are aware of the importance of the subject, they cannot find enough time in the outpatient clinic, they don't know enough about the symptoms that are expected to be warning signs in the clinic, and the number of patients referred to the child psychiatry clinic with the suspicion of ASD remains very low. It's thought that increasing the competency of paediatricians in the field by improving the trainings received during the residency and after specialization, in a qualitative and quantitative manner, as well as arranging the working conditions in a way that facilitates patient screening and referral to child psychiatry for formal assessment, will provide important advances in the early diagnosis and treatment of ASD.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the University of Health Sciences Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date: 12.09.2019, Decision No: 20/16).

Informed Consent: Written informed consent form was obtained from participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Improving the performance of EM and K-means algorithms for breast lesion segmentation

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ABSTRACT

Aims: Breast cancer is the most common type of cancer in women and accounts for a large portion of cancer-related deaths. As in the other types of cancer, the prevention and early diagnosis of breast cancer gain importance day after day. For this purpose, the artificial intelligence-based decision support systems become popular in recent years. In this study, an automatic breast lesion segmentation process is proposed to detect breast lesions in the images taken with magnetic resonance imaging (MRI) protocol.

Methods: Two most popular segmentation methods: expectation maximization (EM) and K-means algorithms are used to determine the region of breast lesions. Furthermore, superpixel based fuzzy C-means (SPFCM) algorithm is applied after EM and K-means methods to improve the lesion segmentation performance.

Results: The proposed methods are evaluated on the private database constructed by the authors with ethical permission. The performances of the utilized methods are analyzed by comparing the lesion areas determined by a radiologist (ground-truth) and areas that are achieved by automatic segmentation algorithms.

Conclusion: Dice coefficient, Jaccard index (JI), and area under curve (AUC) metrics are calculated for performance comparison. According to the simulation results, EM, K-means, EM+SPFCM, and K-means+SPFCM methods provides good segmentation performance on breast MRI database. The best segmentation results are obtained by using EM+SPFCM hybrid method. The results of the EM+SPFCM method are 0,8711, 0,8979, and 0,9981 for JI, Dice, and AUC, respectively.

Keywords: Breast cancer, automatic segmentation, magnetic resonance imaging, image processing

INTRODUCTION

Cancer can be defined simply as abnormal cell formation and uncontrolled proliferation of these cells, beginning in almost any organ or tissue of the body, and can spread to other organs. Since cancer is the second leading cause of death worldwide, cancer research is increasing daily and gaining importance. Among women, the most prevalent cancer types include breast, colorectal, lung, cervical, and thyroid cancers, whereas men commonly encounter lung, prostate, colorectal, stomach, and liver cancers. It's noteworthy that the global incidence and mortality rates of breast cancer are on the rise.[1] Nevertheless, early detection plays a crucial role in reducing the fatality associated with breast cancer, as it does with many other cancer types. Various methods are employed for diagnosing and assessing the progression of breast cancer. Magnetic Resonance Imaging (MRI) is a widely utilized screening technique for breast cancer that offers the advantage of being radiation-free and painless during the imaging procedure.[2]

In recent studies, the researchers aim to detect breast lesions, segment lesions, and classify them as benign and malignant, automatically. To reach this aim, several image processing, machine learning and deep learning techniques are utilized.[3-6] In this study, our goal is to detect breast lesions and determine lesion region as correct as possible.

Recent segmentation algorithms that are used to determine breast lesions' regions are investigated to show the main contributions of the presented study. According to the literature, the majority of studies conducted in recent years use deep learning techniques. However, deep learning-based techniques require very large databases and high-performance processors because of their computational complexities. So, some of the researchers prefer conventional image processing, machine learning, and pattern recognition methods to reduce computational complexity and also computation time.[7,8]

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Although there are various studies, the most relevant and effective ones can be summarized as follows: In[9], the authors propose a CNN-SVM network. In the testing phase of the study, the CNN's labeled output and the grayscale test images are passed through the SVM classifier for segmentation. The database of the study is collected from the Second Affiliated Hospital of Fujian Medical University. DSC, precision, and sensitivity metrics are used to evaluate the performance of the proposed segmentation approach. According to the simulation results, the achieved DSC, precision, and sensitivity values are 0.93, 0.95, and 0.92, respectively. In reference[10], a Res-UNet CNN based automatic segmentation method is used to measure the size and volume of breast masses over MRI database that is constructed by the authors. The DSC metric is calculated to evaluate the segmentation phase of the study. According to the results, improved Res-UNet got the best DSC of 0.89 among different networks. In reference[11], a breast tumor segmentation-generative adversary network (BTS-GAN) is proposed to segment breast tumors in MRI scans automatically. The generator of the proposed GAN architecture learns to detect tumors and generates a binary mask; on the other side, the discriminator tries to learn the discriminating ground truth and synthetic mask. The authors use the public RIDER breast cancer MRI dataset and achieve 85% DSC and 93% sensitivity. The highest values of analyzed single and hybrid segmentation methods are 0.90 (DSC), 0.95 (precision), and 0.95 (sensitivity), respectively.

In this study, an automatic breast lesion segmentation process based on two most popular segmentation methods: EM and K-means algorithms are used to determine the region of breast lesions. Then, SPFCM algorithm is applied after EM and K-means methods to augment the lesion segmentation performance.

The main contributions of the study can be summarized as follows:

i. Breast MR database used in the study is a private database that is built to design a decision support system for breast lesion segmentation. The database includes various types of benign and malignant lesions.
ii. The segmentation performances of the two popular methods are investigated.
iii. Performance improvement is achieved by utilizing SPFCM algorithm as a post processor.
iv. Three common metrics are calculated to evaluate the success of the proposed segmentation methods.

The study is organized as follows: The utilized methods are introduced in Section 2. The experimental results of the study are shown in Section 3. Finally, the main contributions of the study are discussed in Section 4.

METHODS

The study was carried out with the permission of the Sakarya University Non-interventional Ethics Committee (Date: 21.12.2016, Decision No: 201-7). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. In addition, the proposed single and hybrid segmentation algorithms are explained to better understand the background of the study.

Database

RI serves to enhance our understanding of the suspicious regions identified through mammography, ultrasonography, or palpation. In this particular investigation, Dynamic Contrast-Enhanced MRI (DCE-MRI) is conducted by using a 1.5 Tesla GE Healthcare-Signa Voyager machine, equipped with a specialized breast surface coil. To begin the examination, T2-weighted spin-echo sequences in the axial plane is acquired. Subsequently, a T1-weighted sequence is captured following the administration of a Gadolinium-based contrast agent, dosed at 0.1 mmol/kg with a flow rate of 1.5 ml/s. For each patient, ensuring a temporal resolution of at least 30 seconds, resulting in the acquisition of approximately 5000 images, five scans are utilized. The dynamic T1 series' parameters included a repetition time (TR) of 7.2 ms, a scan-specific echo time (TE) of 2.0 ms, a flip angle (FA) of 11 degrees, a slice thickness of 1.4 mm, and a field of view (FOV) frequency of 35.0 along with a FOV phase of 1.0. The imaging process produced a maximum of 1618 slices, each with a pixel size of 0.9 × 1.2. We used a single excitation (NEX 1.0) and a matrix size of 192 × 300. It's important to note that these parameter values may be adjusted for individual patients based on their structural characteristics. The radiologist carefully selected the most appropriate slices that would aid in accurately delineating the lesion's location to ensure the most informative results.

Lesion Segmentation

Before performing lesion segmentation process, the left and right breast regions were separated according to the location of hearth. In the lesion segmentation step, two single algorithms: EM and K-means are employed to determine the lesion area as correct as possible. These methods are selected according to their segmentation performances on the breast MRI database. Then, a hybrid model was constructed by performing SPFCM method after K-means and EM algorithms, respectively. **Figure 1** shows the lesion segmentation process.

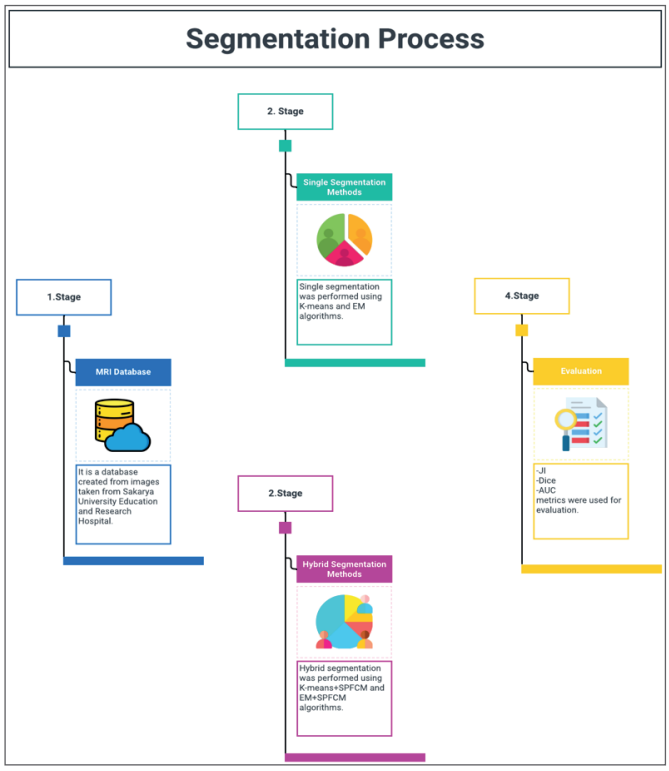

Figure 1. Breast lesion segmentation process of the study.

The main steps of the utilized algorithms are given in the following subsections.

Expectation maximization: Maximum likelihood estimation (MLE) is a statistical method that is used to predict the parameters of an assumed probability distribution under adequate number of observations. The EM algorithm is also a statistical technique which is based on MLE and used to segment images. EM method constructs a likelihood function and tries to maximize this function according to the given problem. However, maximizing the likelihood function according to the parameters in many real-world problems is a challenging task.

The EM algorithm solves this problem by degrading the problem to two simple small steps referred as expectation (E) and maximization (M). The E is the expectation of the unknown underlying variables. On the other side, M step provides a new estimate of the parameters. Steps E and M are performed consecutively until convergence is achieved.[12] EM algorithm can be summarized as follows:

Assume that, n training data points having d attributes $\{x_1, x_2, \dots, x_n\} \in R^d$ are observed. In this case, for the sake of simplicity, it is supposed that the observations are generated by a Gaussian Mixture Model (GMM) of which parameters are needed for algorithm initialization. The probability density function of GMM is expressed as follows:

$$f(\boldsymbol{x}|\theta) = \sum_{i=1}^{K} \alpha_i f_i(\boldsymbol{x}|\theta_i) \quad (1)$$

In Equation (1), K shows the number of Gaussian distributions in the model and α_i is the weight of the mixture. Each Gaussian in the mixture is characterized by its mean μ_i and variance σ_i^2. The probability density function of Gaussian distribution can be expressed as follows

$$P(\boldsymbol{x}|\theta_i) = \frac{1}{\sqrt{2\pi}\sigma_i} exp\left\{-\frac{(x-\mu_i)^2}{2{\sigma_i}^2}\right\} \quad i = 1,2,\dots,K. \quad (2)$$

where $\theta_i = (\mu_i, \sigma_i)$ is the Gaussian mixture distribution parameter. In this stage, the target is to find $\boldsymbol{\varphi} = \alpha_1, \alpha_2, \dots, \alpha_k, \mu_1, \mu_2, \dots, \mu_K, \dots, \sigma_1^2, \sigma_2^2, \dots, \sigma_K^2$ parameters. So, MLE approach must be carried out. But, because of the high computational complexity of MLE, EM algorithm is preferred.

After parameter initialization, the posterior probabilities given in Equation (3) are calculated.

$$\boldsymbol{\varphi}(i|x_j,\theta) = \frac{p_i f_i(x_j|\alpha_i)}{\sum_{k=1}^{K} p_k f_k(x_j|\alpha_k)} \quad (3)$$

In Equation (3), i and j are the data point and Gaussian component indices, respectively. The posterior probability of jth Gaussian component of each data point is estimated by performing E-step. The likelihood of observations is maximized by re-estimating all the parameters to achieve a better parameter estimation. So, M-step updates the prior p_i and parameters with Equations (4-6).

$$p_i^{new} = \frac{1}{N}\sum_{j=1}^{N} \varphi\left(i|x_j,\theta^{old}\right) \quad (4)$$

$$\mu_i^{new} = \frac{\sum_{j=1}^{N} x_j \varphi(i|x_j,\theta^{old})}{\sum_{j=1}^{N} \varphi(i|x_j,\theta^{old})} \quad (5)$$

$$\sigma_i^{2,new} = \frac{\sum_{j=1}^{N} \varphi\left(i|x_j,\theta^{old}\right)(x_j-\mu_i^{new})(x_j-\mu_i^{new})^T}{\sum_{j=1}^{N} \varphi(i|x_j,\theta^{old})} \quad (6)$$

Steps E and M are performed consecutively until the difference between the new and old parameter values is smaller than the predetermined error quantity.

$$||\theta^{new} - \theta^{old}|| \leq \varepsilon \quad (7)$$

The details of the EM algorithm can be investigated with reference.[12]

K-Means: K-means is a known unsupervised clustering method that aims to reach locally optimal solutions that provides minimum clustering error. As aimed in the most clustering based segmentation methods, K-means tries to group homogenous data points in a given image or data set. Each group constitutes a region where the object's density is locally different from other regions. Suppose that the vector $X = \{x_1, x_2, \dots x_N\}$, $x_n \in R^d$ is the data set. The aim of the K-means clustering algorithm is to obtain k partitions (clusters) which optimizes the clustering objective function. K-means algorithm's steps are explained as follows:

i. Divide n data into k clusters and randomly determine the centers of clusters $C_1, C_2, \ldots, C_k$.

ii. Measure the Euclidean distance between each data point and cluster center.

iii. Assign each data point to the nearest cluster.

iv. Calculate the value of objective function J with Equation (8). Note that, to reach the best clustering performance J must be minimized.

$$J = \sum_{i=1}^{k} \sum_{X_i \in C_i} ||X_j - C_i||^2 \quad (8)$$

v. Assign the mean of each cluster as a new centroid by calculating means with the Equation (9).

$$C_i = \frac{1}{n_i} \sum_{j=1}^{n_i} X_j \quad (i = 1,2,\ldots,k) \quad (9)$$

vi. Repeat steps (ii-v) to reach the determined convergence criterion.

If the value of objective function converges to zero, it can be said that the best clustering performance is achieved. In other words, the ideal J value is zero. In addition, the convergence criterion mentioned in Step (vi) can be either the number of iterations or the value of J.[13]

Super pixel fuzzy C-means: Superpixels arise from perceptual grouping of image pixels and carry more information than pixels. The use of superpixels becomes popular in image processing applications, as it can significantly reduce the complexity of post-processing tasks.

A superpixel must have main properties to be implemented in the FCM algorithm, which assigns each pixel in an image to a cluster. A superpixel extraction algorithm must be fast and simple to overcome time consuming problem. Once superpixels are constructed, information such as the number of pixels of each superpixel, and the adjacent relationship between the superpixels is stored for the next clustering process. The steps of the SPFCM algorithm is given below:

Superpixel objective function J is generated by the information obtained from the original image.

$$J = \sum_{i=1}^{C} \sum_{j=1}^{Q} \gamma_j u_{ij}{}^m ||\zeta_j - v_i||^2 + \frac{a}{N_R} \sum_{i=1}^{C} \sum_{j=1}^{Q} u_{ij}{}^m \left(\sum_{s_r \in N_j} \gamma_r ||\zeta_r - v_i||^2\right) \quad (10)$$

In Equation (10), Q denotes the number of image superpixels, γ_j and ζ_j are the number of pixels in superpixel and the average color value of superpixel s_j, respectively. u_{ij} is the membership of superpixel s_j to the ith cluster. N_j shows the set of neighboring superpixels adjacent to s_j. N_R stands for the cardinality of N_j. v_i, is the ith cluster center. $||\cdot||$ presents the Euclidean distance between pixels and cluster centers. The parameter inspects the effect of the neighbor element.

The cluster centroids $(v_i, i = 1,2,\ldots,C.)$ are obtained.

The membership of superpixel is updated by using Equation (11)

$$u_{ij} = \left(\sum_{k=1}^{C} \left(\frac{\gamma_j ||\zeta_j - v_i||^2 + \frac{a}{N_R} \sum_{s_r \in N_j} \gamma_r ||\zeta_r - v_i||^2}{\gamma_j ||\zeta_j - v_k||^2 + \frac{a}{N_R} \sum_{s_r \in N_j} \gamma_r ||\zeta_r - v_k||^2} \right)^{1/(m-1)} \right)^{-1}$$

Equation (12) calculates the new cluster centroids as follows:

$$v_i = \left(\sum_{j=1}^{Q} u_{ij}{}^m \left(\gamma_j \zeta_j \frac{a}{N_R} \sum_{s_r \in N_j} \gamma_r \zeta_r \right) \right) \left(\sum_{j=1}^{Q} u_{ij}{}^m \left(\gamma_j + \frac{a}{N_R} \sum_{s_r \in N_j} \gamma_r \right) \right)^{-1} \quad (12)$$

Finally, steps ii-v are repeated until the difference between the new and old parameter values is smaller than the given error value ε.

$$||V^{new} - V^{old}|| \leq \varepsilon$$

According to the database, modifications such as inserting a bias parameter can be required to generate the objective function.[14]

In the presented study, K-means and EM single segmentation algorithms are applied to breast MR images to perform given lesion segmentation task. Then, to improve the performance of these algorithms SPFCM method is used as a post processing step. In the following section, the performances of the EM, K-means, EM+SPFCM, and K-Means+SPFCM segmentation algorithms is evaluated by calculating three performance metrics.

RESULTS

In this section, our goal is to show the success of the proposed methods on the constructed private dataset. For this purpose, several MR images are analyzed that include benign and/or malignant breast lesions. Dice coefficient, JI, and AUC metrics are used for performance evaluation.

At first, pixel-level confusion matrix must be obtained to calculate performance metrics. The lesion area determined by the radiologist (manual segmentation) is compared with the lesion area determined by the applied segmentation method (automatic segmentation) when constructing the pixel-level confusion matrix. The manual segmentation region selected by the expert is assumed as the ground truth lesion region. For example, with a segmentation algorithm, pixels that are claimed to be in the lesion area and are actually in the lesion area, according to the expert, are evaluated as true positive (TP). True negative (TN), false positive (FP), and false negative (FN) assessments are done similarly. After obtaining the pixel-level confusion matrix, the metrics can be calculated to evaluate segmentation algorithm.

The JI is a statistic used to measure similarity and diversity. The JI can be expressed in terms of TP, FP, and FN as:

$$JI = \frac{TP}{TP+FP+FN} \quad (13)$$

The Jaccard similarity coefficient of two sets A and B (also known as intersection over union or IoU). In our study, A is the lesion region manually selected by the radiologist and B shows the lesion region determined by the proposed segmentation methods. The Dice coefficient is a statistic used to measure similarity and can be calculated as follows:

$$Dice = \frac{2*JI}{1+JI} \quad (14)$$

The AUC represents the degree or measure of separability. In **Table 1**, the proposed breast lesion segmentation methods are compared by means of three criteria. Note that, the highest values of JI, Dice, and AUC metrics are 1.

The rows of the **Table 1** give JI, Dice coefficient, and AUC values, respectively. According to the table, EM+SPFCM hybrid method provides the best segmentation results. The values of JI, Dice, and AUC for EM+SPFCM are 0,8177, 0.8979, and 0,9981, respectively. On the other hand, K-Means+SPFCM hybrid method gives 0,7805, 0,8756, and 0,9986 for JI, Dice, and AUC, respectively. As can be seen from the table, hybrid methods perform the segmentation task more successful than single methods. So, applying SPFCM method after K-Means and EM algorithms improves the segmentation ability of methods.

Table 1. Performance comparison of proposed single and hybrid methods

	EM	EM+SPFCM	K-Means	K-Means+SPFCM
JI	0.7149	0.8177	0.6821	0.7805
Dice	0.8264	0.8979	0.8062	0.8756
AUC	0.9937	0.9981	0.9933	0.9986

Furthermore, to illustrate the lesion area determined by the utilized segmentation algorithms **Figure 2** is drawn. In **Figure 2**, original MR image, the images obtained by applying single and hybrid segmentation methods, binary images, and ground truth image are illustrated from left to right. **Figure 2** is given to show the performances of the utilized segmentation methods on an MR image exist in the database. According to the figure it is clear that the hybrid methods determine the lesion are more compact and correct than single methods.

Finally, **Table 2** is built to compare utilized segmentation approaches with the recent studies that are performed to segment breast MR images. **Table 2** shows the lesion segmentation results of references[9,10] and our higher-performance methods. In **Table 2**, the comparison is made with only Dice metric which is the common metric used in our study and compared studies. According to the table the proposed method achieves the breast lesion task better that the compared studies with respect to Dice coefficient.

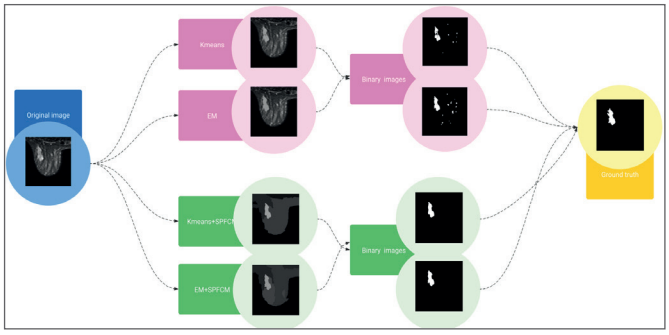

Figure 2. Illustration of detected lesion areas achieved by utilized methods

Table 2. Performance comparison

Method	Dice
[9]	0.93
[10]	0.89
Proposed	0.90

DISCUSSION

In this study, at first, two popular segmentation methods; EM and K-means are applied to MR images to determine the breast lesions' regions. Then SPFCM algorithm is utilized to improve the performance of these segmentation methods.

In the presented study, **Table 1** and **Figure 2** are given to show the segmentation results of the methods. **Table 1** compares the performances of single and hybrid methods with respect to JI, Dice coefficient, and AUC criteria. The higher Dice coefficient and JI values imply that the breast lesion region determined by the automatic segmentation algorithms are very close to that of manual segmentation performed by an expert radiologist. As can be seen from **Table 1**, the proposed single and hybrid segmentation methods provide good performance on the constructed database. Furthermore, the performances of EM and K-means methods increase by carrying out SPFCM algorithm after these methods. K-means+SPFCM and EM+SPFCM provide the most successful segmentation results. So, K-means+SPFCM and EM+SPFCM hybrid methods can reliably be used to determine the breast lesion region in MR examinations.

Figure 2 illustrate the determined lesion region for ductal carcinoma in situ malignant lesion type. **Figure 2** give an idea about the segmentation ability of all performed methods. According to the figure, hybrid methods give a more distinct and compact lesion region than single methods. Thus, the lesion area determined by hybrid methods is closer to the manually determined area.

Finally, **Table 2** compares the proposed study with recent studies that are performed to segment breast MR images. **Table 2** shows the lesion segmentation results of references[9,10] and our higher-performance methods. According to the table the proposed method performs the breast lesion task better that the compared studies with respect to Dice coefficient.

CONCLUSION

In addition, the proposed segmentation process needs a shorter computation time than deep learning based segmentation methods and does not requires high-performance processors. In our future studies, our goal is to use deep learning based segmentation methods by modifying them to decrease the computational load. For this purpose, also the constructed database must be extended. The main limitation of the study is the size of constructed database. The number of MR images will be increased and various benign and malignant lesion types will be included to the database to obtain more reliable results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Sakarya University Non-interventional Ethics Committee (Date: 21.12.2016, Decision No: 201-7).

Informed Consent: Written informed consent form was obtained from participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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Autonomic symptoms in early-stage Parkinson's patients and their relationship with cognition and disease parameters

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ABSTRACT

Aims: Autonomic dysfunction is a prevalent feature throughout various stages of the disease and can significantly exacerbate the overall impact of the condition. Moreover, it is linked to accelerated disease advances and diminished vitality rates in individuals with Parkinson's disease (PD). The main goal of this study is to evaluate the prevalence of autonomic symptoms and cognitive findings and investigate their associations with disease-related factors in early-stage PD patients.

Methods: A total of 49 individuals diagnosed with PD were enrolled in this study. Disease severity was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS), and the disease stage was determined through the modified Hoehn & Yahr Rating Scale (mHYRS). By the mH&Y scale, only individuals in the early stages (≤2.5) of the disease were included in this investigation. The evaluation of autonomic symptoms in PD was conducted using the Scales for Outcomes in Parkinson's Disease for Autonomic Symptoms (SCOPA-AUT) scale. The cognitive functions of the patients were assessed utilizing the Turkish version of the Montreal Cognitive Assessment Scale (MOCA-TR).

Results: The study included 44% (n=22) females and 56% (n=27) males as participants. The average age was 61.5±10.1 years. The mean SCOPA-AUT score was 18.9±9.36, with the most prevalent autonomic symptoms related to the gastrointestinal system. A positive correlation was shown with autonomic symptoms and disease stages (p=0.024, r=0.322). However, no significant relationship was found between autonomic symptoms, other disease parameters, and cognition. We observed a notable inverse correlation between the disease stage and cognitive status (p=0.003, r=-0.417).

Conclusion: Our study concluded that autonomic dysfunctions manifest from the early stages of Parkinson's disease and can intensify as the disease progresses. Identifying and addressing these dysfunctions at an early stage would play a pivotal role in lessening the overall impact of the disease.

Keywords: Autonomic dysfunction, Parkinson's disease, SCOPA-AUT, MOCA

INTRODUCTION

Non-motor symptoms in Parkinson's disease (PD) are just as prevalent as motor symptoms, with recent research indicating their presence in nearly all PD patients. Interestingly, non-motor symptoms contribute significantly to morbidity, even in the early stages of PD, surpassing the impact of motor symptoms.[1] In PD, there is evidence of involvement of sympathetic and parasympathetic branches of the autonomous nervous system, with pathology affecting both its peripheral and central components.[2] Dysautonomia, one of the non-motor manifestations, has been found to occur in approximately 50-70% of individuals with PD.[3,4] Autonomic dysfunction can be observed in almost all stages and may increase the burden of the disease. The spectrum of autonomic dysfunction in PD manifests a wide range, including cardiovascular, gastrointestinal, urological, sexual, and thermoregulatory impairments.[5] In a study involving early-stage PD patients, at least one autonomic dysfunction symptom was observed in 71% of them.[6] As previously demonstrated, autonomic dysfunction is linked to an accelerated progression of disease milestones and reduced survival among individuals with PD.[7] There is a limited body of literature evaluating the impact of autonomic dysfunction on cognition in PD, and the results are conflicting.[8,9]

In this study, we aimed to evaluate the prevalence of autonomic symptoms, cognitive findings, and their correlation with disease parameters in early-stage PD patients following up by our movement disorders clinic.

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METHODS

The study was carried out with the permission of the İstanbul Fatih Sultan Mehmet Training and Research Hospital Clinical Researches Ethics Committee (Date: 23.08.2023, Decision No: 2023/121). All procedures were conducted in compliance with ethical guidelines and adherence to the principles of the Declaration of Helsinki.

The study is a cross-sectional study involving 49 individuals admitted to the neurology outpatient clinic of Fatih Sultan Mehmet Training and Research Hospital in August and September 2023. These individuals had been predicted with PD based on the criteria outlined in the clinical diagnostic criteria of the movement disorder association.[10] A brief anamnesis form was created, encompassing the disease stage, clinical motor score, body mass index (BMI), initial motor syptoms (tremor/bradykinesia), medications used, equivalent dose of levodopa, and disease duration for all patients. In addition to levodopa, the levodopa equivalent daily doses (LEDD) of patients using drugs such as rasagiline and pramipexole were calculated according to Tomlison et al.[11] Disease severity was evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS), and the disease stage was determined using the modified Hoehn & Yahr Rating Scale (mHYRS). By the mH&Y scale, only individuals in the early stages of the disease (stage ≤ 2.5) were included in this study.[12]

Individuals diagnosed with atypical/secondary parkinsonism, those with mH&Y stage > 2.5, a history of cerebrovascular disease or brain surgery, those had diabetes mellitus, or those unable to comply with tests, as well as individuals diagnosed with psychiatric disorders or those with cardiac arrhythmias or pacemakers, were not included in the study.

The assessment of autonomic symptoms in Parkinson's patients was performed using the SCOPA-AUT (Scales for Outcomes in Parkinson's Disease for Autonomic Symptoms) scale.[13] The SCOPA-AUT scale consists of 25 items covering various domains of dysfunction, including the gastrointestinal system (7 items), urinary (6 items), cardiovascular (3 items), thermoregulatory (4 items), pupillomotor (1 item), and sexual (2 items for males, 2 items for females). Respondents select from options "never, sometimes, often, and frequently" to answer the questions, and a scoring system ranging from 0 (never) to 3 (frequently) is applied based on these choices. The highest possible total score is 69, with a higher score indicating a more pronounced degree of autonomic dysfunction.

To evaluate patients' cognitive functions, we employed the Turkish version of the Montreal Cognitive Assessment Scale (MOCA-TR). The MOCA-TR assesses various cognitive domains, including visuospatial and executive functions, abstraction, naming, delayed recall, attention, language, and orientation.[14]

Statistical analysis was carried out using IBM SPSS Statistics 23 software, which was provided by IBM SPSS Turkey. To summarize the data, we utilized descriptive methods such as mean, standard deviation, median, frequency, ratio, minimum, and maximum. The normality of data distribution was evaluated using the Shapiro-Wilk Test. In the case of two-group comparisons involving quantitative data with a normal distribution, we employed Student's t-test. We used the Mann-Whitney U Test to compare qualitative data between the two groups. Categorical data were compared using Pearson's Chi-Square test. To examine the relationship between variables, Pearson's correlation was applied. Statistical significance was determined at a significance level of $p<0.05$.

RESULTS

The research encompassed 49 patients, comprising 44% (n=22) females and 56% (n=27) males. The participants' ages ranged from 32 to 84 years, with an average age of 61.5±10.1 years. Comprehensive demographic and clinical findings, including disease duration, LEDD, UPDRS, and mHYRS scores, initial motor syptoms, BMI, MOCA-TR, and SCOPA-AUT scores are detailed in **Table 1**.

Table 1. Clinical characteristics of the patients

	Mean±SD	Median (min-max)
Age	61.5±10.1	62 (32-84)
Sex (F/M)	22 (44%)/27 (56%)	
Duration of disease (years)	5.43±5.04	4 (1-20)
Initial motor syptoms (B/T)	B: 22 (44%)/T: 27 (56%)	
LEDD	550±245	525 (200-1200)
UPDRS	28.4±14.4	23 (8-69)
m H&Y	1.65±0.5	1.50 (1-2.5)
BMI	26.3±3.89	26 (20.8-37.3)
MOCA-TR	21.2±4.9	22 ((9-28)
SCOPA-AUT	18.9±9.36	17 (5-44)

SD: standard deviation, F: female, M: male, LEDD: Levodopa equivalent daily dose,, BMI: Body mass index, B: Bradykinesia, T: tremor, UPDRS: Unified Parkinson's disease rating scale, m H&Y: modified Hoehn and Yahr scale, MOCA-TR: Turkısh version of Montreal Cognitive Assessment scale, SCOPA-AUT: Scales for Outcomes in Parkinson's Disease for Autonomic Symptoms

The Mean SCOPA-AUT scores were 18.9±9.36, and the most common autonomic symptoms were about the gastrointestinal system. This was followed by the urinary and thermoregulatory systems, respectively. The occurrence of autonomic symptoms is displayed in **Table 2**.

Table 2. Frequency of autonomic symptoms in patients

	Patients (N:49) Mean±SD	Frequency (%)
SCOPA-AUT-Total	18.9±9.36	100%
Gastrointestinal system	5.49±3.37	29%
Urinary	5.18±3.92	27%
Cardiovascular	1.27±1.37	6%
Thermoregulatory	4.69±3.44	24%
Pupillomotor	1.06±1.25	5%
Sexual	1.20±1.66	6%

N: number, SD: standard deviation, SCOPA-AUT: Scales for Outcomes in Parkinson's Diseas for Autonomic Symptoms

The study did not find a statistically significant rate of difference in autonomic symptoms between male and female patients. See in **Table 3**.

Table 3. Comparison of autonomic symptoms in female and male patients

	Female (N:22)	Male (N:27)	p value
SCOPA-AUT-Total	19.73±9.84	18.26±9.09	0.590
Gastrointestinal system	6.05±3.17	5.04±3.51	0.302
Urinary	5.14±4.07	5.22±3.88	0.940
Cardiovascular	1.59±1.30	1.0±1.39	0.133
Thermoregulatory	4.86±3.52	4.56±3.42	0.758
Pupillomotor	1.0±1.23	1.11±1.28	0.760
Sexual	1.0±1.38	1.37±1.86	0.443

*T- test, N: Number, SCOPA-AUT: Scales for Outcomes in Parkinson's Disease for Autonomic Symptoms

Autonomic symptoms were positively correlated with disease stages (p=.024 r=.322). However, we did not find a relationship between autonomic symptoms and other disease parameters and cognition. See in **Table 4**. We also did not find any relationship between autonomic symptoms and the following areas: visuospatial and executive functions, naming, attention, language, abstraction, delayed recall, and orientation (p>0.05).

While a weak positive correlation was observed between cognition and LEDD, a significant moderate negative relationship was detected between disease stage and cognitive status (p<0.05). This is shown in **Table 5**.

DISCUSSION

We examined autonomic symptoms among early-stage PD patients and explored their connections with disease-related factors and cognitive function. The gastrointestinal system exhibited the highest prevalence of autonomic symptoms. Additionally, a positive correlation was found between the severity of autonomic symptoms and disease stages. No significant relationship was found between autonomic symptoms, other disease parameters, and cognition. However, we did observe a noteworthy inverse correlation between disease stage and cognitive status.

Autonomic dysfunctions, first described by James Parkinson, present with symptoms of constipation, excessive salivation, and urinary incontinence.[15] Histopathological examinations have indicated the presence of Lewy bodies (LB), which are pathological protein aggregates, in various areas of the disease's pathology, including the brainstem, hypothalamus, sympathetic system (thoracic inter mediolateral column and sympathetic ganglia), and parasympathetic system (dorsal, vagal, and sacral parasympathetic nuclei). Additionally, LB has been observed in neural plexuses innervating the adrenal medulla, intestines, heart, and pelvic region.[16] Furthermore, if synuclein pathology impacts the cardiac sympathetic fibers, it may lead to conditions like orthostatic hypotension and postural hypotension. Conversely, exposure to parasympathetic fibers can induce variations in heart rate.[17]

PD patients experience a range of dysautonomia: gastrointestinal issues, cardiovascular irregularities, urinary problems, sexual dysfunction, thermoregulation abnormalities, and pupillomotor abnormalities.[5] Gastrointestinal symptoms are remarkably prevalent in PD, even in the early stages of the condition. Studies indicate that a significant 88.9% of individuals with PD experience gastrointestinal symptoms before the emergence of classic Parkinsonian motor symptoms.[18]

Table 4. Correlation of autonomic symptoms with disease parameters and cognition

	Correlation of SCOPA-AUT						
SCOPA-AUT Total	**Age**	**Duration of disease**	**BMI**	**LEDD**	**UPDRS**	**m H&Y**	**MOCA-TR**
Pearson's r	0.138	0.244	0.205	0.020	0.264	0.322*	0.037
p-value	0.343	0.091	0.158	0.890	0.066	0.024	0.801
N	49	49	49	49	49	49	49

* p < 0.05, LEDD: Levodopa equivalent daily dose, BMI: Body mass index, UPDRS: Unified Parkinson's disease rating scale, m H&Y: modified Hoehn and Yahr scale, MOCA-TR: Turkish version of Montreal Cognitive Assessment scale, SCOPA-AUT: Scales for Outcomes in Parkinson's Disease for Autonomic Symptoms, N: Number

Table 5. Correlation of cognition with disease parameters

Correlation of MOCA-TR						
MOCA-TR	**Age**	**Duration of disease**	**BMI**	**LEDD**	**UPDRS**	**m H&Y**
Pearson's r	-0.092	- 0.157	0.186	0.283*	-0.265	- 0.417*
p-value	0.527	0.281	0.201	0.049	0.066	0.003
N	49	49	49	49	49	49

* p < 0.05, LEDD: Levodopa equivalent daily dose, BMI: Body mass index, UPDRS: Unified Parkinson's disease rating scale, m H&Y: modified Hoehn and Yahr scale, MOCA-TR: Turkish version of Montreal Cognitive Assessment scale, N: Number

The frequency of gastrointestinal symptoms in our study was lower, and this difference may be related to the use of different measurement scales. Two independent research studies, which utilized the same measurement tool as our study (SCOPA-AUT), consistently reported high gastrointestinal and urinary symptoms. Our research results corroborate and support the findings from these studies.[19,20]

A recent study has uncovered a distinct progression of dysautonomia symptoms that appears to be independent of motor disability. In addition, this study included only stage one patients and observed them for three years, yet they associated dysautonomia findings with non-motor symptoms and age.[6] However, we did not find a relationship between autonomic symptoms and parameters such as age, disease severity, disease duration, medication doses, and cognition. We speculate that this discrepancy could be attributed to the cross-sectional design of our research. In a retrospective study of 100 PD patients with autopsy-confirmed diagnoses, researchers found that autonomic dysfunction was linked to certain factors.[7] These factors included older age at the time of PD diagnosis, being male, having a poor initial response to levodopa treatment, and belonging to the motor PD subtype characterized by postural instability and gait difficulty. Additionally, the study revealed that the onset of autonomic dysfunction at an earlier stage of PD was associated with a faster disease progression and reduced survival.[7] Nevertheless, we observed that autonomic symptoms were exclusively linked to the disease stage.

The available literature on the influence of autonomic dysfunction on cognitive function in Parkinson's disease is limited, and the findings are inconclusive.[8,9] In one study, participants were evaluated for postprandial hypotension (PPH), heart rate responses during deep breathing (HR (DB)) and orthostatic hypotension (OH) in addition to the SCOPA-AUT scale.[8] The presence of orthostatic OH or PHP did not show a crucial association with cognition. However, although PD patients with dementia experience more cardiovascular symptoms, no significant association between autonomic manifestations and cognitive decline could be found.[8] In a similar research performed in our country, no relationship was found between autonomic symptoms and cognitive tests.[9] In both studies, Mini-Mental State Exams (MMSE) were utilized for cognitive assessment. However, a comparative investigation suggests that the Montreal Cognitive Assessment Scale (MoCA) is a more effective tool than the MMSE within the PD population.[21] Turkish-validated MOCA scale was used in our study, and although we compared all cognitive domains and autonomic symptoms, we did not find any relation between them. This indicates that the progression of cognitive and autonomic symtoms in PD occurs independently of each other and varies between individual PD. Furthermore, in our study, the degree of cognitive impairment was found to be associated with the disease stage, and our results supported the existing literature.[8,9]

Among the limitations of our study are the absence of a control group, the presentation of cross-sectional data, and the assessment of autonomic symptoms solely based on subjective patient responses.

Its strengths include the investigation of early-stage PD and the use of cognitive assessments with the MOCA-TR test. Particularly, future research would benefit from longitudinal designs that include patients in premotor stages or those who are treatment-naive, incorporate objective data like orthostatic hypotension and PPH, and involve a larger number of participants.

CONCLUSION

Based on our current knowledge, no study has been conducted in our country, particularly in the early stages and newly diagnosed PD patients, that incorporates the frequency of autonomic dysfunction. Detecting and managing these dysfunctions in the early stages would be instrumental in reducing the overall impact of the disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the İstanbul Fatih Sultan Mehmet Training and Research Hospital Clinical Researches Ethics Committee (Date: 23.08.2023, Decision No: 2023/121).

Informed Consent: Written informed consent form was obtained from participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

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Conditioned media of tonsil derived mesenchymal stem cells shows different rates of cytotoxicity on solid cancer cells

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ABSTRACT

Aims: Mesenchymal stem cells (MSCs) are the apple of the eye of cancer studies. It was indicated that the secreted factors, especially released by MSCs, have tumoral or anti-tumoral effects on tumor progression. MSCs obtained from different sources show different anti-tumoral effects, while MSCs originating from the same source also show different tumoral effects in different cancer cells. Here, we investigated the anti-tumor effects of soluble factors secreted from palatine tonsil MSCs (TMSC) as a new source of MSC on human lung carcinoma (A549) and pancreatic cancer (PANC-1) cell lines.

Methods: Conditioned medium (CM) was obtained from TMSCs isolated from palatine tonsil tissue, and the cytotoxic effect of CM on the growth of A549 and PANC-1 in a dose-dependent manner was demonstrated by MTT analysis. In addition, the function of CM treatment on the cell cycle status of cancer cells and the apoptosis process were investigated through cell cycle analysis with propidium iodide (PI) and Annexin-V/PI detection method by flow cytometry analysis, respectively.

Results: We demonstrated that TMSC-CM treatment significantly decreased the viability of A549 and PANC-1 cell lines in a dose-dependent manner post-48 hours. In addition, CM treatment differentially induced the apoptosis on A549 and PANC-1 cells and also, caused G2/M arrest in the cells.

Conclusion: In light of these findings, our study is the first to show that TMSC-CM has an anti-tumoral effect by stimulating apoptosis on A549 and PANC-1 cells. These findings reveal that the usage of CM has a cell-free cellular therapeutic potential.

Keywords: Cancer, cellular therapy, cytotoxicity, mesenchymal stem cell, palatine tonsil

INTRODUCTION

Cancer remains a serious health problem with considerable morbidity and mortality rates worldwide despite the rapid progress in diagnostic and therapeutic research.[1] Mesenchymal stem cells (MSCs) have important curative potential because of their self-renewal and multilineage differentiation properties and are broadly investigated in tissue engineering and regenerative medicine.[2] The use of stem cells in regenerative medicine has many advantages; however, the usage also brings with it a series of problems, such as a low survival rate after transplantation, immunological responses after administration, and a decrease in regenerative potential.[3] Recent research has shown that the therapeutic potential of MSCs is due to the different paracrine effects that they secrete. Based on the principle of paracrine signaling mechanism, MSC conditioned medium (MSC-CM) has intensive paracrine factors containing a variety of cytokines, chemokines, growth factors and nano-scale cellular vesicles exosomes.[4] The conditioned medium (CM) as a cell-free cellular therapy has an important advantage due to no requirement of donor-recipient compatibility. Besides, MSC-CMs are more accessible and applicable because of the requirement of less controlled conditions for application procedures than stem cells, which require special production and application conditions. In addition, they can be easily packaged and commercialized by freeze-dried or lyophilized, and they can be accessed and transported more easily as they eliminate the cryopreservation requirement compared to cell-based therapy.[4] Based on all these properties, CMs are promising candidates for biological pharmaceuticals, but many unknowns remain unclear.[5]

Stem cells from different sources have been shown to have the potential to secrete different paracrine factors to treat a variety of degenerative diseases. The paracrine factors released from cells vary according to the age of the cells, culture status, and tissue. Therefore, tissue selection plays a main role in therapeutic applications using stem cells.[3,4]

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Tonsil tissue provided as waste tissue after tonsillectomy is recommended as an important source of MSC because of no need for unnecessary surgical procedures compared to bone marrow and its relatively high proliferation rate and low immunogenicity properties.[6] TMSCs not only show the expression of MSC-specific cell surface antigens, including CD29, CD44, CD73, CD90, and CD105, but also have their distinctive markers, such as CD106, CD108, and CD166, associated with adhesion, migration, and immunomodulation.[7] Besides, the palatine tonsil is important because it is the secondary lymphoid tissue that provides the formation of an effective immune response against antigens. In addition, TMSCs have faster proliferation and shorter doubling times than other stem cell types, and also, TMSCs from multiple donors can be used together. Its stem cell properties are well preserved and are not damaged after freezing and thawing. These properties make TMSCs important candidates for stem cell banking and are advantageous for their use in regenerative medicine.[8]

Different studies showing different results have been carried out to determination of the effects of MSC-CMs on cancer cell growth.[9-11] CMs obtained from human lung-derived MSCs suppress tumor cell growth,[12] human Wharton's jelly-derived MSC (WJMSC)-CM did not show any effect on proliferation and apoptotic potential of A549 lung cancer cells,[13] bone marrow-derived MSC (BMMSC)-CM has been shown to suppress the proliferation of lung cancer cells and cause apoptosis on the tumor cells in vitro.[14] Another study, evaluating the anti-cancer effect of the paracrine factors of umbilical cord-derived MSCs on MCF-7 tumor cells, showed a dose-dependent cytotoxic effect of MSC secretome on the breast cancer cell line.[15] The limited studies are showing the growth inhibitory effect on TMSC cancer cells. The study on head and neck squamous cell carcinoma (HNSCC) cell lines has demonstrated that TMSCs can cause notably growth inhibitory effects on HNSCC cell lines through number-dependent increased cell cycle arrest and apoptosis.[16] In the studies of the authors investigating the effect of TMSC-CM on hematological cancer cell lines, it was shown that CM induces cell death in hematological cancers after heat induction of TMSCs.[17] It causes changes in cancer behavior depending on the variability of the tumor niche in hematological and solid cancers.[18] Studies have supported that different therapeutic responses to the same therapeutic agent occur in hematological and solid cancers.[19] In this direction, in our study, we aimed to determine the concentration-dependent effects of the secretome released by TMSCs on the solid cancer cell line proliferation and further elucidate their anti-cancer properties and mechanism of function.

METHODS

The study was carried out with the permission of Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 08.07.2021 Decision No: 2021/347). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Human tonsil derived mesenchymal stem cell isolation

Tonsil tissues were obtained from donors who underwent tonsillectomy with the approval of the donor and the clinical research ethics committee. TMSCs were enzymatically isolated and characterized as described in our previous study,[17] and cultured with DMEM supplemented with 10% Fetal Bovine Serum (FBS, Gibco), and 1% antibiotic-antimycotic (Sigma Aldrich). Characterization was done in the second or third passages. Stem cells from passages four or five were used in the experiments.[17]

Immunophenotyping of Tonsil Derived Mesenchymal Stem Cells

Characterization of TMSCs was done based on specific cell surface antigens. For that, the cells were incubated with anti-human CD90, CD73, CD166, CD34, and CD45 monoclonal antibodies (Biolegend) according to the Manufacturer's directions. Next, the cells were analyzed by flow cytometry (Cytoflex S, Beckman Coulter).[6]

Evaluation of Multilineage Differentiation Potential of Tonsil Derived Mesenchymal Stem Cells

The adipogenic and osteogenic differentiation protocol was performed as in our previous study.[17] Briefly, the 10×10^4 cells were cultured in the six well plate with the specific differentiation mediums for 3 weeks. The differentiation mediums were changed every two days. Then, the differentiated cells were washed with PBS and fixed in 4% paraformaldehyde (PFA; Sigma Aldrich). After fixation, the cells were stained with 2% Oil Red O (Sigma Aldrich) solution and also, were stained with 2% Alizarin Red S (Sigma Aldrich) at room temperature (RT). Intracellular lipid droplets for adipogenic differentiation, and extracellular matrix calcification for osteogenic differentiation were observed under inverted microscopy.

Collection of Tonsil Derived Mesenchymal Stem Cells Conditioned Medium

To collect CM from TMSCs in passage four, the cells were cultured until 80-90% confluence. Then the cells were washed with PBS and with serum-free culture medium. After washing, they were incubated for 48 h in a serum-free culture medium at 37°C, and CMs were collected. The cell debris removed with centrifuged at 1500 rpm for 10 min. Afterward, they were passed through a 0.22 µm filter, aliquoted, and stored at -80°C to be used in the next experiments.[20]

Cell Lines and Cell Cultures

Human lung carcinoma cell line A549 and pancreatic carcinoma cell line PANC-1 cells were used in this study. The cells were cultured with DMEM supplemented with 10% FBS (FBS; Gibco), and 1% antibiotic-antimycotic (Sigma Aldrich) at 37°C in 5% CO2.

MTT Proliferation Assay on Cancer Cells

The Methylthiazolediphenyl-Tetrazolium Bromide (MTT-Sigma-Aldrich) assay was performed to determine the effect of TMSC-CM treatment on cancer cell proliferation. For that, the cancer cells were seeded on 96-well plates at a density of 10×10^3 cells/well. The medium was exchanged for each well with 100 µl of TMSC-CM (at a ratio of 1:1, 1:2) and incubated for 48 h. Then, 10 µL of MTT (5 µg/ml stock solution) was added to each well. After 4 h of incubation, the formazan crystals were dissolved by adding 100 µl of solvent solution (0.01 N HCl with 10% SDS; Sigma-Aldrich) and incubated at 37°C for 16 h. The absorbance values were measured at a wavelength of 570 nm in a plate reader (BioTek Epoch). The absorbance data were analyzed using Prism 7.00 (GraphPad Software, Inc) program compared to untreated control cells.[17] All experiments were performed in triplicate.

Annexin-V/PI Apoptosis Detection Assay

Apoptotic cell death was performed with FITC-Annexin-V/ PI detection kits. 5×10^4 cells were seeded in 24-well plates and after a day treated with CM at a ratio of 1:1. Normal culture medium was added to the control group. After 48 h incubation, the cells were trypsinized and stained with FITC-Annexin-V and PI for apoptosis analysis (Invitrogen) according to the manufacturer's instructions. The stained cells were analyzed by flow cytometry.[17]

Cell Cycle Assay

Cell cycle analysis was performed with flow cytometry following PI staining. After 48 h post-treatment of 5×10^4 A549 and PANC-1 cells with TMSC-CM, the cells were suspended in cold 75% ethanol at -20°C 1 h. After fixation, cells were washed with PBS and stained with 50 µg/ml propidium iodide (PI) and 50 µg/ml RNase A dissolved in 0.5 µmL PBS. The stained cells were analyzed by flow cytometry after incubation at 37°C for 30 min. in the dark.[17]

Statistical Analysis

The results are expressed as mean ± SEM. "2-tailed Student's t-test" was used to determine the level of significance. If the values had $p<0.05$, the results were considered statistically significant.

RESULTS

Tonsil Derived Mesenchymal Stem Cells Have Stem Cell Characteristics

MSCs successfully isolated from palatine tonsil tissues exhibited typical standard fibroblastic cell morphology (**Figure 1A**). One of the characteristic features of MSCs is their multi-lineage differentiation capacity. The adipogenic and osteogenic differentiation potentials of the isolated TMSCs were evaluated by Oil red O and alizarin red S staining, demonstrating the ability to differentiate into adipocytes and osteocytes (**Figure 1A**). In addition, specific cell surface antigens of sub-cultured TMSCs up to passage three were determined by flow cytometry. More than 90% of the cells showed positive expression levels for the typical MSC antigens CD73, CD90, and CD166, while markedly low expression levels were detected for the hematopoietic stem cell markers CD34 and CD45 (**Figure 1B**). These results confirmed the mesenchymal stem cell characteristic of cells isolated from palatine tonsil tissue.[17]

TMSC-CM Showed the Anti-proliferative Effect on A549 and PANC-1 Cancer Cells

To determine the cytotoxic effect of TMSC-CMs on cancer cell lines, we treated them with CM at two different concentrations (1:1 and 1:2). Accordingly, the cytotoxic effects of CMs applied at two different concentrations after 48 h were evaluated by MTT analysis. The cell viability was significantly reduced in cancer cells post the treatment of a 1:1 ratio compared to a 1:2 ratio. For A549 cells, the cell viability was decreased at 2.6-fold post-treatment at a ratio of 1:1 compared to the control and 1:2 ratio CM treatment ($p<0.001$). Besides, the cell viability declined approximately 4-fold in PANC-1 cells post-treatment at a ratio of 1:1 compared to control ($p<0.001$). We did not find any significant decrease in PANC-1 cell viability post-treatment of a 1:2 ratio (**Figure 2**). However, It was observed that the application at a ratio of 1:2 was statistically more effective in A549 cells. These findings demonstrate the dose-dependent growth inhibitory effect of TMSC-CM on cancer cells.

TMSC-CM Highly Induced Apoptosis in A549 and PANC-1 Cancer Cells

After indicating the cytotoxic effect of 1:1 CM concentration on cancer cells by MTT analysis, we performed the apoptosis assay to understand the mechanism of the anti-proliferative effect of TMSC-CM on A549 and PANC-1 cancer cells at this concentration. After the treatment with CM, no significant differences were found for the post-apoptotic and necrotic cell populations in A549 cells, while pre-apoptotic cells increased approximately 2-fold post-TMSC-CM treatment ($P<0.05$) (**Figure 3A, B**).

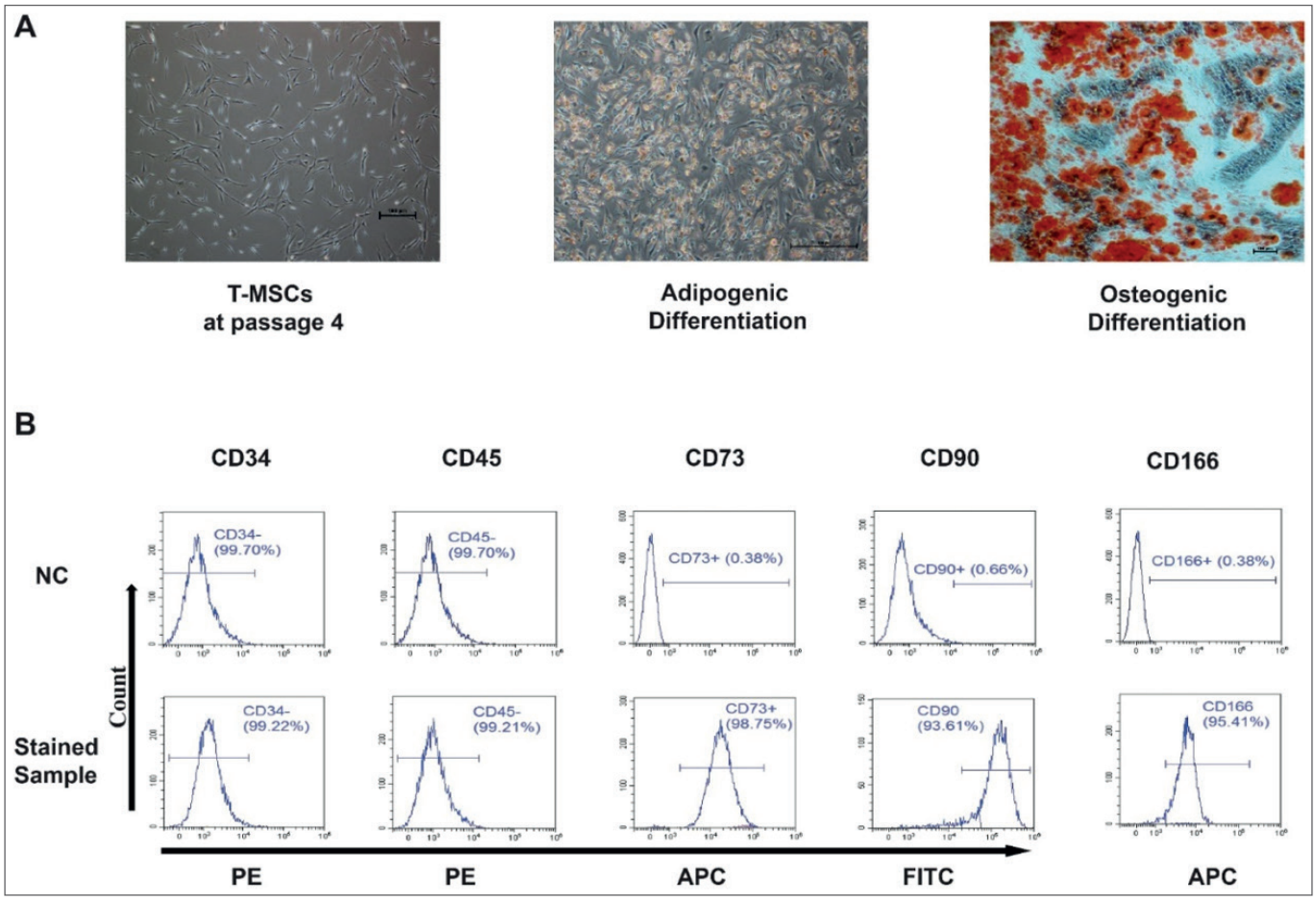

Figure1. Characterization of T-MSCs by determination of the multi-lineage potential and cell surface CD markers (A) the microscopic views of multi-lineage differentiation post specific staining to differentiation types and (scale bar 100 μm) (B) the percentage of T-MSCs carrying CD markers on the cell surface. PE: Phycoerythrin; APC: Allophycocyanin; FITC: Fluorescein isothiocyanate.

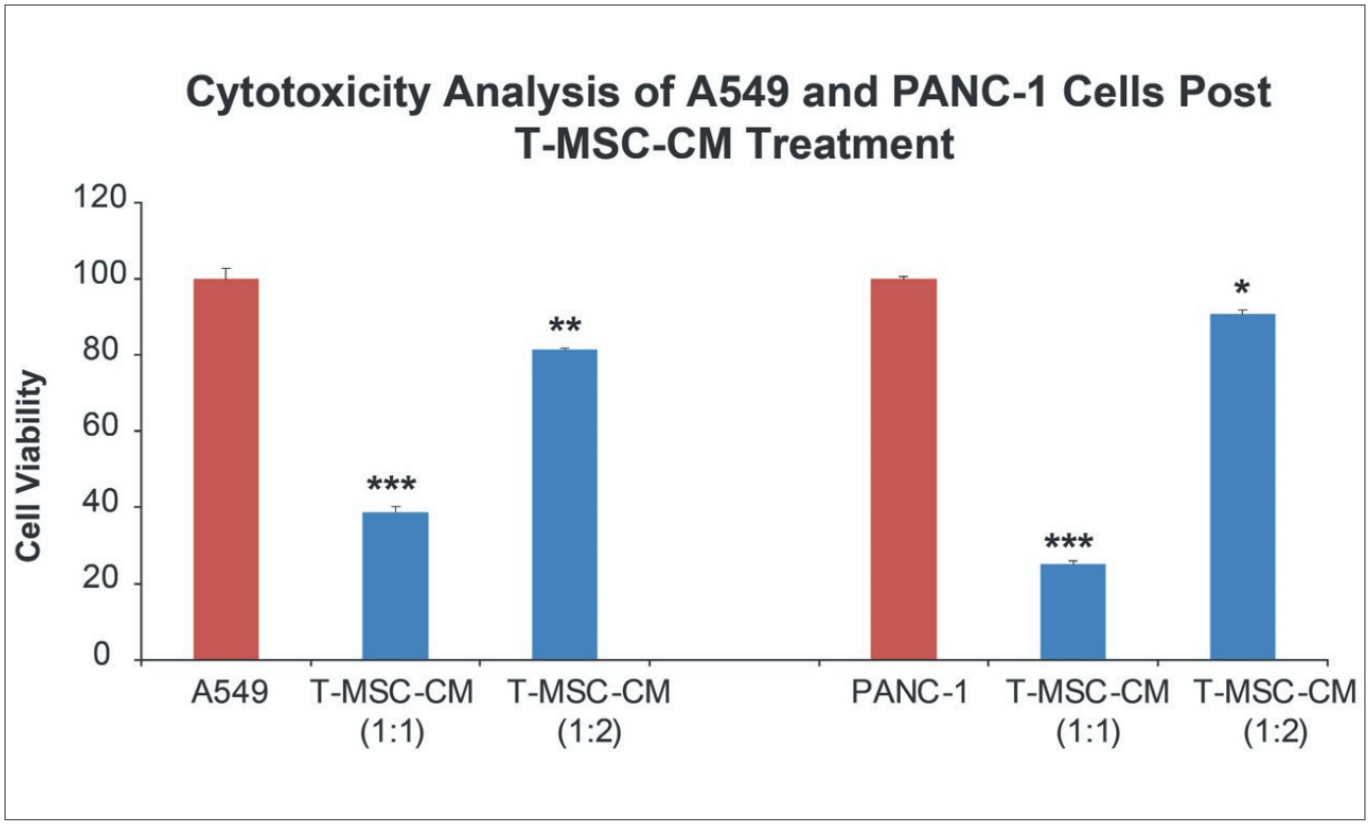

Figure 2. Cytotoxic effect of T-MSC-CM treatment on cancer cell lines. 48 h administration of 1:1 ratio T-MSC-CM significantly reduced cell viability in A549 and PANC-1 cell lines. n=3. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

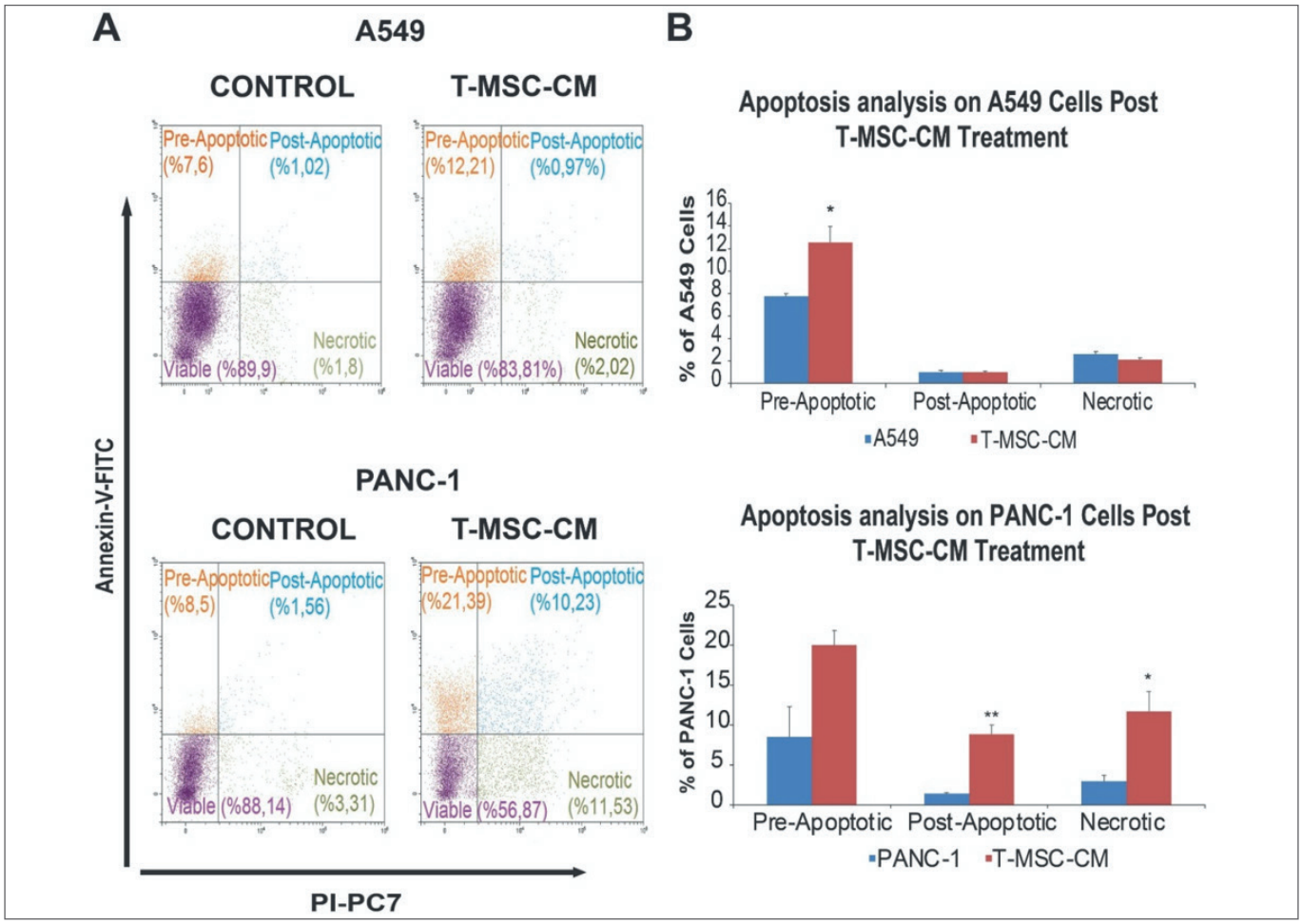

Figure 3. The apoptotic effect of T-MSC-CM administration on A549 and PANC-1 cells was determined by flow cytometry after Annexin-V-PI staining. The flow plots (A) and the quantification results as a graph (B) were shown. T-MSC-CM applied at a ratio of 1:1 induced apoptosis on cancer cells at different rates. n=3, * p<0.05 and ** p<0.01.

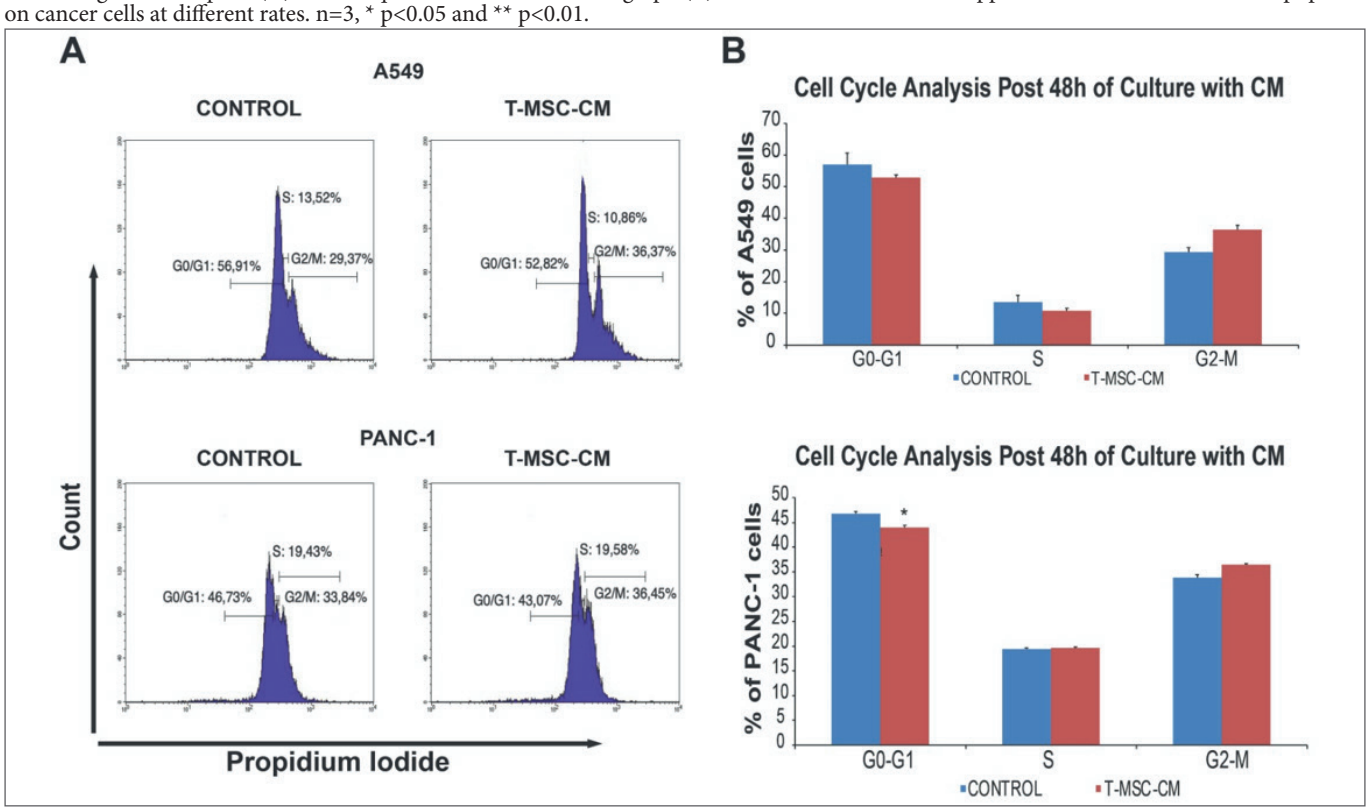

Figure 4. Cell cycle analysis with PI staining on A549 and PANC-1 cells post T-MSC-CM treatment. The cell cycle arrest of TMSC-CM on A549 and PANC-1 cancer cells was determined by flow cytometry. The flow plots (A) and the quantification results as a graph (B) were shown. T-MSC-CM applied at a ratio of 1:1 induced G2/M arrest of the cell cycle on cancer cells. n=3, * p<0.05.

When the apoptotic effect of TMSC-CM treatment on PANC-1 cells was examined, a significant increase (approximately 6.5-fold) in the post-apoptotic cell ratio ($P<0.01$) was observed compared to the control group. In line with this finding, a 28% reduction in PANC-1 viable cell rate ($P<0.01$) was detected after TMSC-CM treatment (**Figure 3A, B**). We also found that the treatment of TMSC-CM increased the pre-apoptotic and necrotic PANC-1 cell ratio at 2.5-fold and 3.8-fold, respectively (**Figure 3A, B**). These findings reveal that TMSC-CM induces apoptosis at different rates in A549 and PANC-1 cancer cells and is more efficient in PANC-1 cells.

A549 and PANC-1 Cells were Arrested in the G2/M Phase of the Cell Cycle Post-TMSC-CM Treatment

Representative images of cell cycle analysis results for TMSC-CM are summarized in Figure 4. After treating A549 cells with TMSC-CM, it was determined that the cells in the G0/G1 and S phases decreased, and cell cycle arrest occurred in the G2/M phase. While 29% of the cells in the control group were in the G2/M phase, this increased to 36% of the rate cells after treatment. A similar cell cycle profile was observed in PANC-1 cells as with A549. After the treatment, a statistically significant decrease was observed in the number of cells in the G0/G1 phase. Similarly, there was G2/M cell cycle arrest, but it was not statistically significant.

DISCUSSION

MSCs have been shown to have anti-tumor effects in vitro and in vivo cancer models.[21,22] The therapeutic potential of MSCs is mediated by direct interaction with paracrine factors released from MSCs.[23] The latest research has indicated that the anti-tumor properties of MSCs on different cancer cells are related to the various factors released by MSCs.[24] CMs from MSC cultures have been shown to have an inhibitory effect on the proliferation of hepatoma cells. According to the study results, further treatment of liver cancer cell lines with an MSC-conditioned medium resulted in decreased expression of β-catenin, Bcl-2, c-Myc, PCNA, and survivin genes.[21] In the study reported by Eiró et al., the effects of MSCs derived from the human uterine cervix (hUCMSCs) on different cell types in tumor tissue, including cancer cells, fibroblasts, and macrophages, were investigated. The researchers demonstrated that hUCMSCs stimulated apoptosis and reduced cell proliferation in human breast cancer cells. In addition, the lyophilized hUCMSCs-CMs were applied to the MDA-MB-231 cell line, which exhibits aggressive cancer properties, and as a result, a dose-dependent inhibition of cell proliferation in these cells has been demonstrated. Besides, it is known that cancer-associated fibroblasts are very effective in cancer development and aggressiveness. Therefore, the administration of hUCMSCs-CM to cancer-associated fibroblasts has resulted in induced apoptosis and reduction of cell proliferation and migration abilities.[25]

It was indicated that the anti-tumoral function of MSCs from different sources was variable as their pro- or anti-tumor potential both in vitro and in vivo due to the MSC sources. These different tumoral effects have been associated with the heterogeneity of MSCs depending on the used source. Accordingly, it is important to find alternative MSC sources, which have anti-tumor effects, for cancer therapeutics.[25] The anti-tumoral effects of MSCs and their CMs from different sources have been studied in divergent solid and hematological cancers. In the study investigating the effect of human WJ-MSC secretome on the proliferation of lung cancer cells, it was determined that WJ-MSC secretome did not have any important results on tumor cell proliferation and apoptosis.[13]

We applied TMSC-CM on A549 and PANC-1 cells to determine the tumoral effects of paracrine factors of MSCs derived from the tonsil, which is a new source of MSCs and has a high proliferation potential, in our study. In contrast to the research carried out by Hendijani et al.[13] we found that TMSC-CMs reduced cancer cell viability through the induction of apoptosis in a concentration-dependent manner. In our study, TMSC-CMs were treated at a ratio of 1:1 and 1:2 with cancer cells, and a significant reduction in cancer cell viability was found at 1:1 concentration treatments by MTT analysis. These results are similar to the results of Pan et al.[26] In addition, we found that TMSC-CM treatments induced apoptosis in A549 cells. The TMSC-CM treatment also significantly induced post-apoptotic cell ratio in treated PANC-1 cells compared to control groups. Interestingly, this induction found for PANC-1 cells is higher than the apoptotic induction found for A549 cells. Consistently with our findings, the study reported by Bagheri et al. in 2021 showed that CMs obtained from bone marrow and amniotic membrane MSCs (AMMSCs) reduced the cell viability and the proliferation of squamous carcinoma cells (SCC) at different rates depending on the source. Also, CMs induced apoptosis in SCC cell lines depending on the treatment time.[23] AMMSCs downregulated the expression of cell cycles progression-related genes such as cyclin and cyclin-dependent kinase in some solid and hematological cancer cell lines, and thus, they have shown an anti-proliferative effect through cell cycle arrest in the G0/G1 phase.[27] In our study, cancer cells were arrested at the G2/M phase, although there was no statistically significant difference after treatment with CM. In a study using the amnion as the source of MSC (AMSC), the anti-tumoral potential of AMSC-CMs was tested

on prostate cancer cell lines, and it was reported that a time-dependent decrease in cancer cell proliferation was achieved by preventing its progression through the cell cycle.[28] Limited studies are showing the growth inhibitory effect on TMSC cancer cells. The study on HNSCC cell lines has shown that TMSCs can stimulate the growth inhibitory effects on HNSCC cell lines through number-dependent increased cell cycle arrest and apoptosis.[16] On the other hand, no other study was found reporting the effect of TMSC-CMs on cancer proliferation. In our study, we showed that CMs obtained from tonsillar tissue, as a new source of MSC, induced apoptosis at different rates in A549 and PANC-1 cell lines depending on the concentration. Our findings are consistent with the previously carried out studies on TMSC and cancer cells. Besides, our study shows consistent results with the study performed by Li et al. on 2 different lung cancer cell lines. Researchers reported that BMMSC-CMs decreased the proliferation in cancer cells due to some secretable factors and induced apoptosis in vitro. In addition, in vivo, studies on BALB/c nude mice showed a lower incidence of tumors when tumor cells were injected into mice after administration with MSC-CMs compared to control groups.[14]

We suggest that tonsillar tissue is more advantageous as a source of MSC because bone marrow supply requires a more invasive and painful procedure. The donor age is an important parameter in MSC isolation, and the numbers of MSCs collected from bone marrow are significantly decreased with donor age.[29] However, the potential importance of TMSCs for obtaining more cells before clinical applications were emphasized due to higher proliferation capacity and the presence of immune modulatory properties.[30]

CONCLUSION

Lung and pancreatic cancers are known as the major cause of cancer death worldwide. The 5-year survival rate is still low considering the standard therapies applied in the clinic. Besides, the standard treatments have very important side effects. Therefore, new therapeutical strategies, which have fewer side effects and are better tolerated, are needed. TMSC-CMs, which are easily accessible as waste tissue and show a high proliferation potential, showed anti-proliferative properties by inducing apoptosis in some solid cancers in our study. The various paracrine factors released from TMSC-CMs, such as several chemokines, cytokines, growth factors, and exosomes as nanometer-sized vesicle, play a key role in the emergence of this effect. Therefore, the relation between this therapeutical function and paracrine factors needs to be investigated in detail.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 08.07.2021, Decision No: 2021/347).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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Original Article

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The relationship between digital game addiction, cyberloafing, and psychological well-being in primary school students

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ABSTRACT

Aims: Today, with the advances in technology, the use of devices such as computers, digital game consoles, phones, and tablets has also increased. However, with the widespread use of internet access, digital platforms have started to be used frequently in interpersonal interaction and relations. The use of digital games and the internet, where the user age is in a wide range, has decreased to very young ages, and the time spent by individuals in games and on the internet has gradually increased. In this context, the duration of stay in the digital environment can be seen as an important criterion in terms of addiction. Spending excessive time in the digital environment can cause individuals to be adversely affected by psychosocial aspects. The purpose of our study is to reveal the reasons for the complex and multifaceted relationship between cyberloafing, digital game addiction, and well-being, and to discuss potential solutions.

Methods: This study was designed as a cross-sectional study. The study group was formed of 1330 students in the second stage of primary education in grades, 5, 6, 7, and 8, in state schools. The sample Group consisted of 614 females and 716 males in almost the same age group. The game addiction scale, perceived cyberloafing scale, and psychological well-being scale were applied to students. The necessary permission to conduct the study was obtained from, then the data were collected in face-to-face interviews on a voluntary basis. The data obtained in this study were analyzed statistically using SPSS vn. 23 and AMOS 23 software.

Results: The Gaming Addiction Scale score was determined to be mean 42.09 for the whole group, 36.96 for females, and 46.96 for males. The Cyberloafing Scale score was determined to be a mean of 24.01 for the whole group, 21.94 for females, and 25.79 for males. The Psychological Well-Being Scale score was found to be a mean of 30.60 for the whole group, 32.02 for females and, 29.39 for males. A positive correlation was observed between gaming addiction and, cyberloafing, (p=0.00) and both of these conditions were determined to be negatively correlated with psychological well-being. (p=0.00)

Conclusion: Activities to support students' psychological well-being can help prevent vicious cycles between cyberloafing, game addiction, and psychological well-being by contributing to controlling cyberloafing and game addiction tendencies.

Keywords: Cyberloafing, digital game addiction, psychological well-being

INTRODUCTION

The longer time spent by people on the internet and using information technology for both entertainment and work purposes has also led to a series of negative effects. Intense and frequent use contributes to the development or progression of various negative behaviors and psychological problems. Cyberloafing and digital game addiction are the primary of these negative effects.[1] Especially during the COVID-19 pandemic when people had to stay at home, there was an increase in cyberloafing because of the social isolation and uncertainties experienced in that period, and online games became a form of entertainment and a means of passing the time.[2,3] Both during the pandemic and afterward, it was noticed that cyberloafing was the leading negative effect of prolonged screen time and more time spent on internet-based applications. Cyberloafing can be defined in the most general form as unproductive, unplanned internet browsing for personal aims during inappropriate periods such as at work or during lessons, and it was understood to have shown an increase during the COVID-19 pandemic. These activities that can be evaluated as cyberloafing include more frequent visits to news and discussion sites or social media platforms, frequent checking of e-mail, downloading more files, playing online games, and doing online shopping, and

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these are undertaken at times when an individual has responsibilities such as in working or lesson hours.[4] The repetitive nature of these digital activities leading to neglect of responsibilities naturally gives rise to different problems. Studies related to cyberloafing have shown that it is associated with low academic performance, loss of concentration, wasting time, low learning motivation, low levels of well-being, short attention span, and low memory retention.[5,6]

Digital gaming addiction is a type of behavior addiction that is characterized by overuse and compulsive use of games and manifests with negative effects.[7] These effects, which occur together with risks such as low academic success, communication problems within the family, and problems in peer relationships, are negatively reflected in the lifestyles of children and adolescents in particular.[8,9] When recent studies are examined, digital gaming addiction can be seen to create a risk for cardiovascular health problems including behaviors such as a sedentary lifestyle, poor nutrition, lack of exercise, smoking, and increasing stress levels, and at the same time has a negative effect on mental health, academic performance and success, physical socialization, sleep regularity and quality, communication skills, physical health, and self-respect.[10,11]

When the factors affecting the formation of gaming addiction are examined, it can be seen that just as for other types of addiction, reasons such as loneliness, isolation, exclusion, and trying to escape problems can lead people to behaviors that can form addiction. It has also been reported that gaming addiction could be a coping mechanism for individuals experiencing stress and anxiety.[12]

The majority of research that has dealt with gaming addiction and cyberloafing has been observed to have emphasized the negative effects of these two variables and to have focused on diseases in particular when evaluating mental health. However, as indicated by the World Health Organization (WHO), being healthy does not only mean the absence of any sign of disease or disability, but includes reaching a complete state of well-being in physical, mental, and social aspects. In this sense, a holistic health status, including mental health, should be aimed for.

The WHO emphasis on mental health well-being has become increasingly important with the efforts of positive psychology studies to focus on positive elements. In this context, "psychological well-being" is seen to be the leading concept reflecting complete well-being in mental health. This is because psychological well-being highlights more positive and strong aspects rather than negative characteristics. In other words, the person has life aims to overcome mental problems, can establish healthy communication with others, is conscious of the responsibilities related to their life, and obtains satisfaction by fulfilling these.[13] According to this perspective, psychological well-being is not limited to the state of happiness of the individual but is a concept that can also be associated with characteristics such as acquiring a specific place in society, being productive, and contributing to the meaning of life. When there is any type of addiction, the control mechanism of the individual is impaired, and as a result of this negative states develop such as withdrawal, decreased self-respect, pessimism, high levels of anxiety, low self-care, coping through avoidance, and psychological stress, leading to a decrease in the level of psychological well-being.14 Therefore, it has been emphasized that cyberloafing and gaming addiction are not just variables associated with academic success, but it is important that they are evaluated as a part of the psychological well-being of students.[15]

Although previous research has investigated the negative concepts of cyberloafing and digital gaming addiction, there is a limited number of studies related to psychological well-being, which have dealt with mental health as a whole. Most of the studies related to psychological well-being have been conducted on university students and workplace employees. Nevertheless, the extent of cyberloafing and digital gaming addiction in children and adolescents has been reported to be at extremely worrying rates.[16] From this starting point, this study aimed to expose the complex and multidirectional relationships between cyberloafing, digital gaming addiction, and psychological well-being, and to discuss recommendations for a solution. Our study examines the effects of cyberloafing and gaming addiction on the psychological well-being of individuals, rather than investigating whether the individuals participating in the study have any mental illnesses. The research hypotheses formulated within this framework are as follows:

Hypothesis 1: Cyberloafing behaviors directly predict the level of psychological well-being at a statistically significant level.
Hypothesis 2: Cyberloafing behaviors directly predict the level of game addiction at a statistically significant level.
Hypothesis 3: The level of game addiction directly predicts psychological well-being levels at a statistically significant level.

METHODS

The study was carried out with the permission of the Sakarya University Educational Researches and Publication Ethics Committee (Date: 20.09.2023, Decision No: 05). All procedures were carried out following the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Participants

This study was conducted in a survey model. To reach a sample that reflects the research variables more within the literature, a purposive sampling method was used rather than randomly selecting participants. Because the rate of technology use in city and district centers is higher than in rural areas.[17] In this case, it was aimed to reach the largest four districts in terms of population in Sakarya province and chose one public and one private school from each district's center to collect data.

The study was conducted between 2018 and 2020. The study group was formed of 1587 students in the second stage of primary education in grades, 5, 6, 7, and 8, in state and private schools of Sakarya. 257 students did not participate in the study because permission could not be obtained from their families, and as a result, 1330 students constituted the sample of the study. 340 students were in 5th grade, 334 in 6th grade, 324 in 7th grade and 332 in 8th grade students, and the age range of the students was between 10-14 years old. The sample consisted of 750 students attending public schools and 580 students attending private schools.

Data Collection and Data Collection Tools

The data were collected face-to-face by the researchers going to the schools and filling out the scale forms using paper and pencil.

The necessary permission to conduct the study was obtained, then the data were collected in face-to-face interviews on a voluntary basis. Three measurement tools were used in the study; the Perceived Cyberloafing Scale, the Psychological Well-Being Scale, and the Gaming Addiction Scale. The details of the scales are given below.

The Perceived Cyberloafing Scale

This scale was developed by Blanchard and Henle (2008)[18], and reliability and validity studies of the scale in Turkish were made by Kalayci and Altun (2010).[19] Scale consists of 13 items with 5-point Likert-type responses. The scale examines internet use in 3 sub-categories of "personal tasks", "following the news", and "socializing". This scale allows individuals to determine how often and in what way they use the internet outside the area they are responsible for during work or education.

The Psychological Well-being Scales

This scale was developed by Diener et al. (2010)[20] and the reliability and validity studies of the Turkish version were conducted by Telef (2013).[21] The 8-item scale describes important elements of human functioning, from positive relationships to feelings of competence, and having a meaningful and purposeful life.

The Gaming Addiction Scale

The Gaming Addiction Scale for Children was developed by Horzum et al. (2008).[22] The scale consists of 21 items and has been determined to be representative and differentiating as a whole. Gaming addiction is examined in the 4 sub-groups of Self-control, Reward/reinforcement, Problems, and Involvement with statements such as "I cannot resist playing videogames even if it negatively affects my life", "In videogames, defeating my enemies/leaping up a level gives me pleasure", "Playing videogames prevents me from fulfilling my responsibilities", and "I always talk about videogames with my friends", respectively

Data analysis

The data obtained on a voluntary basis in this study were analyzed statistically using SPSS vn. 23 and AMOS 23 software. In the evaluation of differences between groups, the Student's t-test was applied to continuous variables and the Pearson chi-square analysis to discrete variables. Continuous variables were stated as mean±standard deviation values and categorical variables as numbers and percentages. Relationships between variables were examined with Pearson correlation analysis. The mediating effect of gaming addiction on the relationship between cyberloafing and psychological well-being with the direct effect of cyberloafing behaviors on gaming addiction and psychological well-being was examined using a structural regression technique. A value of $p<0.05$ was accepted as statistically significant. "Structural regression technique" refers to a statistical method or approach used to analyze the relationships between variables in a structural equation modeling framework. It's a technique used to investigate and understand the causal relationships between variables in a complex system. It allows researchers to assess the direct and indirect effects of variables on each other, helping to model and understand complex systems. In our study, we aimed to contribute to a better understanding of the relationship between data by using this technique.

RESULTS

The study sample group consisted of 614 females and 716 males in almost the same age group. The average age of the sample group was found to be 12.29±1.31, with women having an average age of 12.28±1.29, and men having an average age of 12.30±1.34. There was no statistically significant difference between men and women in terms of age. The sociodemographic and clinical data of the study group are shown in **Table 1**. The Gaming Addiction Scale score was determined to be a mean of 42.09 for the whole group, 36.96 for females, and 46.96 for males. The Cyberloafing Scale score was determined to be a

mean of 24.01 for the whole group, 21.94 for females, and 25.79 for males. The levels of gaming addiction and cyberloafing were found to be significantly higher in the male participants than in the females.

Table 1. Clinical and sociodemographic characteristics of the sample group

Gender (n=1330)		Game addiction (mean±SD)	Cyberloafing (mean±SD)	Psychological well-being (mean±SD)
Female n (%)	Male n (%)			
614 (46.2)	716 (53.8)	42.09±14.65	24.01±9.42	30.60±5.76

SD: standard deviation

The Psychological Well-Being Scale score was found to be a mean of 30.60 for the whole group, 32.02 for females, and 29.39 for males. This score was determined to be significantly higher for females than males (**Table 2**). A positive correlation was observed between gaming addiction and cyberloafing, and both of these conditions were determined to be negatively correlated with psychological well-being (**Table 3**).

Table 2. Comparison of game addiction, cyberloafing, and psychological well-being by gender

	Female (n=614)	Male (n=716)	Statistics	
			t	p
Game addiction (mean±SD)	36.96±12.96	46.49±14.59	-12.49	0.00*
Cyberloafing (mean±SD)	21.94±8.69	25.79±9.66	-7.5	0.00*
Psychological well-being (mean±SD)	32.02±5.43	29.39±5.77	8.51	0.00*

t: Student t-test, *: p < 0.05, SD: standard deviation

Table 3. Evaluation of the relationship between game addiction, cyberloafing, and psychological well-being

	Game Addiction	Cyberloafing	Psychological Well-Being
Game Addiction	-	r=0.38 p=0.00*	r=-0.27 p=0.00*
Cyberloafing	r =0.38 p=0.00*	-	r=-0.17 p=0.00*
Psychological Well-Being	r=-0.27 p=0.00*	r=-0.17 p=0.00*	-

r: Pearson correlation value, *: p < 0.05

The independent variable of cyberloafing was determined to have a direct effect on the dependent variable of gaming addiction (β=0,444, p<0,001), and a direct effect on the dependent variable of psychological well-being (β=-0.114, p<0.05) and an indirect effect together with the intermediate variable of gaming addiction (β=-0.117, p<0.05, VAF=50.43%). In the test model, the independent variable of gaming addiction was seen to have a direct significant effect on the dependent variable of psychological well-being (β=-0.264, p<0.001). The Variance Accounting Formula (VAF) values calculated for cyberloafing were found to be 50.43% (20% ≤ VAF ≤ 80%). This result showed that gaming addiction has a partial mediating role in the relationship between cyberloafing and psychological well-being (**Table 4**).

DISCUSSION

This study examined the effects of cyberloafing behaviors and gaming addiction on psychological well-being in pre-adolescent students in the second stage of primary education.

The first direct effect that can be dealt with in the models examined is the effect of cyberloafing behaviors on psychological well-being. The study results showed that cyberloafing behavior plays an explanatory role in psychological well-being, thereby demonstrating that with an increase in the level of cyberloafing, the psychological well-being levels of the students decreased. In this context, the results obtained in this study were seen to be consistent with the findings and explanations in the literature.[23] When the current study findings and the explanations in publications in this field are evaluated as a whole, it can be said that students with low motivation in school, who are bored and seeking entertainment exhibit cyberloafing behaviors in the form of preferring to play digital games.

Inappropriate use of technology can cause higher levels of depression, increased stress, and a decrease in psychological well-being.[24] It has been suggested in some studies that the lack of motivation, low performance, and low academic success observed in these individuals could be associated with the misuse of technology.[13] This is reflected in the reciprocal vicious circle between cyberloafing and academic success. Moreover, these negative effects are not limited to learning environments and academic success but also decrease the levels of psychological well-being of students. Based on the results

Table 4. Effects of variables on each other

Relationship	Effect	Path Coefficient (β)	Critical Ratio (t)	VAF	p
Cyberloafing-Game Addiction	Total effect/direct effect	0.444	14.288	-	***
Cyberloafing-Psychological Well-Being	Total effect	-0.232	-3.059	50.43%	*
Game Addiction-Psychological Well-Being	Total effect/direct effect	-0.264	-6.910	-	***
Cyberloafing-Game Addiction-Psychological Well-Being	Indirect (with the intermediator)	-0.117	2.638	50.43% (partial mediator)	*
Cyberloafing -> Psychological Well-Being	Direct effect	-0.114	2.638	-	*

***: p<0.001, **: p<0.01, *: p<0.05; Variance Account For (VAF): indirect effect/ total effect 100

of a recent study of students in 2023 that examined the relationships between the positive and negative effects of cyberloafing, it was emphasized that cyberloafing is not a variable only related to academic success, but it should be evaluated as a part of the psychological well-being of students.[25]

One of the results of the current study was that cyberloafing behavior effects the level of gaming addiction. The results showed that cyberloafing behavior has an explanatory role on gaming addiction, as it was seen that with an increase in the level of cyberloafing of the students there was an increase in the level of gaming addiction. In a recent study that examined the mutually predictive relationships between cyberloafing and online gaming addiction, it was determined that cyberloafing was a predictor of gaming addiction and gaming addiction was a predictor of cyberloafing.[26] Just as this reciprocal relationship was indicated by the correlation values in the current study, it was also seen that both conditions triggered each other. Similarly, in another study, it was determined that an increase in smartphone cyberloafing in a classroom environment caused an increase in smartphone addiction.[27]

Another effect examined in the model formed with structural regression analysis was the direct effect of the relationship between gaming addiction and psychological well-being. The study results showed that gaming addiction had an explanatory role in psychological well-being. These results and the negative relationships between the variables demonstrated that an increase in the level of gaming addiction of the students was negatively reflected in psychological well-being. When previous studies are examined, it is noticeable that they have generally focused on negative effects and negative mental health, and very few studies have been conducted related to gaming addiction and psychological well-being.[28,29] School success is the main negative effect examined and it has been reported that playing video games has a negative effect on school success and can lead to hostile behaviors and problems in the family and social life.[30]

Studies conducted on the overuse of the internet, including online gaming, have shown that uncontrolled or compulsive use is negatively correlated with psychological problems and psychopaths such as depression, anxiety, and emotional eating.[30] Another study examined the direct relationship between the duration of playing games and psychological well-being and reported that an increase in the use of digital games created a decreasing effect on the level of psychological well-being, and digital users were more vulnerable to negative, social, psychological, and physical outcomes.[28]

The research hypothesis was formed from the starting point of the examination of inhibiting factors and sources of strength on the path to psychological well-being, and one of the findings related to this was the mediating effect of gaming addiction on the relationship between cyberloafing and psychological well-being. These findings showed that gaming addiction increased the negative effects of cyberloafing in the process of reaching psychological well-being. Therefore, gaming addiction can be evaluated as having a function of supporting poor well-being and strengthening the negative effect of cyberloafing on psychological well-being.

The results of this study also revealed that male students showed statistically significantly higher game addiction and cyberloafing than female students and that female students' psychological well-being scale scores were higher than those of male students. However, our study did not investigate familial, social, economic, and other related factors that could have shed light on the reasons for this situation. It does not seem possible for game addiction or cyberloafing alone to produce these results. This should be considered an important limitation of our study. To completely understand the reasons behind these findings, further studies are needed to assess genetic, social, and cultural factors, as well as the economic framework.

Another limitation of our study is that the answers given by the participants regarding cyberloafing behaviors and game addiction were evaluated based on the student's perceptions. In addition, since our study was designed to uncover a cross-sectional situation, mental status was assessed solely using the psychological well-being scale. Future studies can provide more accurate data by including observations of the participants' families, teachers, and mental health professionals, as well as different evaluation tools in the study.

CONCLUSION

As a result, our study presented cross-sectional data on psychological well-being, cyberloafing, and game addiction in a large sample group. Considering the limited number of studies conducted on children and young people in particular that have focused on psychological well-being fully reflecting positive mental health, it can be said that a sufficient explanation has not yet been reached of the reciprocal effects between cyberloafing, gaming addiction, and psychological well-being. The increasing rates of cyberloafing and gaming addiction together with the negative effects these bring for children and pre-adolescents, who are at an especially sensitive stage of development, show that this situation has reached a worrying level for parents and teachers.

Therefore, it can be thought that the results of this study will be helpful in the process of designing interventions to increase the awareness of families and educators. By contributing to the control of tendencies for cyberloafing and gaming addiction, activities to support the psychological well-being of students may help prevent the vicious circle between cyberloafing, gaming addiction, and psychological well-being.

Low motivation, social isolation, and psychological problems such as wishing to escape from problems, the use of avoidance coping strategies, thrill-seeking, and loneliness in children direct them towards cyberloafing behaviors and lay the ground for gaming addiction. Thus, pre-existing problems increase cyberloafing and gaming addiction and by further decreasing psychological well-being, contribute to maintaining the vicious cycle.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Sakarya University Educational Researches and Publication Ethics Committee (Date: 20.09.2023, Decision No: 05).

Informed Consent: Written informed consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Effects of chronic obstructive pulmonary disease stage on muscle oxygenation during exercise

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ABSTRACT

Aims: The aim of this study was to investigate peripheral muscle oxygenation in patients with chronic obstructive pulmonary disease (COPD) at rest, during submaximal exercise, and during recovery, and to determine the effects of disease stage on peripheral muscle oxygenation.

Methods: Of the 35 stable COPD patients (62.49±8.45 years), 18 patients in GOLD 1 and 2 were assigned to Group 1 and 17 patients in GOLD 3 and 4 were assigned to Group 2. Dyspnea perception of the patients was evaluated with the Modified Medical Research Council (mMRC) Dyspnea Scale, severity of the disease affecting daily life was evaluated with the COPD Assessment Test (CAT-COPD Assessment Test), respiratory function was evaluated with the Pulmonary Function Test, and quadriceps muscle strength was evaluated with a manual muscle testing device. Muscle oxygenation of the patients was measured with Near-infrared spectroscopy (NIRS) for 5 minutes at rest, 6 minutes during the 6-Minute Walk Test (6-MWT), and 5 minutes during recovery after the end of the test. The results of the two groups were compared.

Results: Intragroup comparisons of muscle oxygenation at rest, during 6-MWT and during recovery; in Group 1, there was a statistically significant decrease between resting SmO_2 mean and test SmO_2 mean ($p=0.001$), a increase between test SmO_2 mean and recovery SmO_2 mean ($p<0.001$), and a significant increase between resting SmO_2 mean and recovery SmO_2 mean ($p=0.022$). In Group 2, there was a statistically significant decrease between resting SmO_2 mean and SmO_2 mean during the test ($p=0.002$), increase between resting SmO_2 mean and recovery SmO_2 mean ($p<0.001$*), and resting SmO_2 mean and recovery SmO_2 mean ($p=0.024$). There was no significant difference between the groups in Δ Rest-Test SmO_2mean, Δ Recovery-Test SmO_2mean, and Δ Recovery-Rest SmO_2mean ($p>0.05$). In the SmO_2 comparison of Group 1 and Group 2 at rest, during 6-MWT, and during recovery, it was observed that the test SmO_2mean value was statistically higher in Group 2 ($p=0.023$).

Conclusion: When the disease stage increases in individuals with COPD, muscle oxygen utilization metabolism during submaximal exercise worsens, demanding more oxygen to the muscle to produce the same movement as in individuals with a lower disease stage. This may be explained by the fact that energy metabolism and endurance are affected due to the decrease in the oxygen level of the muscle and its capacity to utilize the available oxygen with increasing disease severity.

Keywords: COPD, near infrared spectrometry, severity, submaximal test

INTRODUCTION

According to the "Global Initiative for Chronic Obstructive Lung Disease (GOLD)-2023", Chronic Obstructive Pulmonary Disease (COPD) is defined as "a heterogeneous condition characterized by chronic respiratory symptoms (dyspnea, cough, and sputum), persistent and often progressive airway obstruction caused by airway (bronchitis/bronchiolitis) or alveolar (emphysema) abnormalities".[1] Historically, COPD has mainly been considered a lung disease and treatment has focused on the lung alone. Large cohort studies have shown that COPD is a complex, heterogeneous, and multicomponent disease with both pulmonary and extrapulmonary manifestations contributing to the burden of the disease.[2] Changes in body composition, such as muscle, fat, and bone wasting, are a cluster of extrapulmonary manifestations in COPD.[2,3] Muscle wasting is common in COPD patients and its frequency varies according to the patient's condition.[4] The prevalence of muscle wasting increases with airflow severity. One study showed that the prevalence of muscle weakness increased from 25% to 38% in patients with GOLD 1 to GOLD 4 COPD, respectively.[4]

Limited aerobic capacity is a characteristic feature of patients with chronic obstructive pulmonary disease (COPD). The magnitude of the limitation in

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aerobic capacity is determined by the interaction of three main factors: impaired pulmonary function, abnormal cardiopulmonary interactions, and skeletal muscle dysfunction.[5] Skeletal muscle dysfunction is associated with impaired peripheral muscle circulation, loss of muscle oxidative capacity, and mitochondrial dysfunction.[6,7] Despite these well-known systemic consequences of the disease, the association of skeletal muscle dysfunction with disease severity and severity of pulmonary impairment is unclear. This is due to the fact that measurements of skeletal muscle structure and function require complex methodological approaches.[8]

Patients with COPD breathe faster and more superficially at higher volumes than healthy individuals. This situation causes dyspnea, impaired pulmonary gas exchange, increased breathing, respiratory muscle fatigue, decreased exercise capacity and daily living activities, and loss of strength in peripheral muscles due to increased mechanical work and oxygen consumption on the respiratory muscles.[9,10] Decreased activity causes social isolation, causing this patient to become depressed. All of these create a vicious circle, decreasing exercise capacity and quality of life.[11,12] Dyspnea, exercise capacity, and peripheral muscle strength, which are of great importance in individuals with COPD, are frequently used parameters in studies where measurements are made, and the 6 MWT and mMRC Dyspnea scale used to evaluate these have minimal clinical significance values for COPD.[13] Although these evaluations provide important data regarding the patient's condition and the course of the disease, they are insufficient to elucidate the metabolic effects of the skeletal muscle and the oxygen metabolism of the muscle, and it is emphasized that further research is needed.[14,15]

Near-infrared spectroscopy (NIRS) has emerged as a non-invasive method to assess oxidative capacity and blood flow and metabolism in skeletal muscles in patients with COPD.[16] Studies have been conducted on the potential applicability of NIRS during exercise programs in COPD patients.[7,17] However, studies have resulted in limited information to analyze the role of muscle metabolism and muscle oxygenation given the multifactorial basis of muscle dysfunction in COPD.[7]

Assessment of skeletal muscle performance and metabolism by studying muscle oxygenation is an emerging field. Muscle oximetry based on NIRS of peripheral oxygen consumption in COPD patients can non-invasively provide information about these changes in oxygenation and hemodynamics in muscle tissues based on the oxygen-dependent properties of near-infrared light.[18] In the literature, studies with NIRS technology have been used to determine the blood flow and oxygenation level of muscle at rest and during exercise, and it has been one of the current methods used to assess blood flow and muscle oxygen availability in COPD patients, especially in recent years.[16] However, to date, in studies conducted with NIRS in COPD patients, muscle oxygenation has been evaluated by comparing individuals with healthy controls or by comparing different pulmonary rehabilitation programs, and the effect of disease severity on muscle oxygenation has remained an issue that has not yet been elucidated.

The aim of this study was to investigate peripheral muscle oxygenation in patients with COPD at rest, during submaximal exercise, and during recovery, and to determine the effects of disease stage on peripheral muscle oxygenation.

METHODS

The study was carried out with the permission of the Bandırma Onyedi Eylül University Health Sciences Non-Interventional Researches Ethics Committee (Date: 13.04.2023, Decision No: 2023-72), and registered at ClinicalTrials.gov (Identifier: NCT06041126). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Participants

This prospective cross-sectional study was conducted between April 2023 and July 2023 in individuals who were followed up with a diagnosis of COPD in the Chest Diseases outpatient clinic of Balıkesir Bandırma Training and Research Hospital and whose conditions were stable. The study was conducted in the application unit of Bandırma Onyedi Eylül University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation.

Inclusion criteria were being diagnosed with COPD (according to GOLD Staging: GOLD 1, GOLD 2, GOLD 3, or GOLD 4), being between 40-80 years of age, having a Body Mass Index (BMI)<35, and having no history of an acute exacerbation in the last 3 months. Patients who did not want to participate in the study; patients with various conditions causing weakness in the lower extremities, such as arthritis, neurologic disease, deep vein thrombosis, peripheral arterial disease, muscle weakness, fracture; patients with extensive parenchymal damage such as malignancy, pulmonary embolism, vasculitis, collagen tissue diseases, interstitial fibrosis, and severe pneumonia; patients with diffuse parenchymal damage such as malignancy, pulmonary embolism, vasculitis, collagen tissue diseases, interstitial fibrosis, and severe pneumonia; patients on continuous oxygen therapy; patients with dyspnea and hemodynamic instability severe enough not to allow them to perform the 6 Minute Walk Test were excluded from the study. Patients with GOLD 1 and GOLD 2 were classified into Group 1, and those with GOLD 3 and GOLD 4 were classified into Group 2.

The patients who were diagnosed with COPD at Bandırma Training and Research Hospital Chest Diseases outpatient clinic and whose disease severity was determined by their physicians according to GOLD staging and who were referred to Bandırma Onyedi Eylül University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation (GYG, CD) were first questioned about demographic information and disease history. Dyspnea perception of the patients who met the inclusion criteria and volunteered for the study was evaluated with the Modified Medical Research Council (mMRC) Dyspnea Scale, severity of the disease affecting daily life was evaluated with the COPD Assessment Test (CAT-COPD Assessment Test), respiratory function was evaluated with the Pulmonary Function Tests (PFTs), and quadriceps muscle strength was evaluated with a manual muscle testing device. Muscle oxygenation of the patients was measured with NIRS for 5 minutes at rest, 6 minutes during the 6-Minute Walk Test (6-MWT), and 5 minutes during recovery after the end of the test.

Measurements

Modified medical research council (mMRC) dyspnea scale: The Modified Medical Research Council (mMRC) Dyspnea Scale was used to assess the dyspnea levels of the patients. mMRC is a 0-4-point category scale in which patients select the statement that best describes their dyspnea levels from among 5 statements about shortness of breath.[19] mMRC increases, especially values of 2 and above, are considered as increased mortality risk.[20]

COPD assessment test (CAT): The CAT, which measures the effects of COPD and deterioration in health status and is easy to apply in clinical practice, consists of eight items questioning "cough, sputum, chest symptoms, fatigue, and confidence in leaving home". The validity and reliability study of the CAT scale was conducted by Yorgancıoğlu et al.[21] in 2012 in Turkey. In the study, Cronbach's alpha internal consistency coefficient was 0.91, item-total score correlation coefficients were between 0.62 and 0.79, and test-retest correlation coefficient was 0.96. In the same study, the construct validity of the scale was examined by exploratory factor analysis and it was reported that the scale showed a single-factor structure with factor loadings ranging between 0.71 and 0.85 and an eigenvalue of 4.95, explaining 61.9% of the variance. In addition, it has been reported that CAT total score has significant discriminative power according to different disease stages, severity, and levels and correlated significantly with pulmonary function tests.[21] A minimum score of 0 and a maximum score of 40 can be obtained from the scale. In the study, the Cronbach alpha coefficient of the CAT scale was found to be 0.86.

Pulmonary function tests (PFTs): PFTs were performed with a COSMED brand Pony FX model portable spirometer (COSMED, Italy). In PFTs performed according to the American Thoracic Society (ATS) and the European Respiratory Society (ERS) criteria, Force Vital Capacity (FVC), Forced expiratory volume in the first second (FEV1), FEV1/FVC, peak expiratory flow rate (PEFR), and forced expiratory volume 25%-75% (FEV25%-75%) flow rate values were recorded.[22] Individuals were informed about the test and values such as age, gender, height, and weight were entered into the device to compare them with standard values. The test was performed 3 times in a sitting position. It is a valid and reliable test for the assessment of respiratory function.[22]

Muscle oxygenation measurement with muscle oxygen monitor: The muscle oxygen monitor is a lightweight (42 g) and small (dimensions: 61 × 44 × 21 mm) device that measures regional blood flow and oxygenation using NIRS by non-invasively placing it on the skin. It has data acquisition and telemetric capabilities, enabling O_2 measurement in non-laboratory settings and field-based research. The muscle oxygen monitor has been shown to be a valid and reliable device to assess muscle oxygenation. Its validity in measuring muscle oxygenation (SROC: r=0.842-0.993, ICC: r=0.773-0.992, $p < .01$) was found to be strong or very good, and its reliability was moderate to high in low-intensity exercise.[23] Patients were first placed in a long sitting position on the stretcher and the NIRS device was connected to the vastus lateralis part of the quadriceps to measure muscle oxygenation. Measurements were taken for 5 minutes at rest. Then the 6-MWT was performed with the device connected to the leg. At the end of the test, the patient rested for 5 minutes and the NIRS device was removed. The 6-Minute Walk Test was performed in accordance with the guidelines. Muscle oxygen saturation (SmO_2) of the patients is shown with minimum, maximum, and mean values.

Quadriceps muscle strength measurement: Quadriceps muscle strength of the patients was evaluated using a manual muscle strength measuring device (Lafayette Instrument, USA) and recorded in kg/force. Maximal voluntary isometric contraction (a make test), which was reported to be more reliable and frequently used in the literature was used for muscle strength measurements.[24-26] In the muscle strength measurements, principles stated by Otman and Köse were taken into consideration, and the rest was performed three times 30 seconds apart in both lower extremities and the highest measurement was recorded.[27] For quadriceps muscle strength measurement, patients were seated on the treatment table with legs hanging down and a rolled towel was placed under the knee joint of the side to be evaluated and the thigh was fixed. The patient was asked to perform extension in this position until the knee was locked, and resistance was given slightly above the ankle joint.

6-minute walk test (6-MWT): It is a submaximal exercise capacity measurement test. The patient is asked to walk as briskly as possible on a 30-meter flat surface for 6 minutes.

During the test, the patient is reminded of the time every minute with standardized commands. Before and at the end of the test, oxygen saturation, heart rate, Borg fatigue scoring, and distance walked are recorded. It is a frequently used exercise test in the clinic because it requires little equipment and is easy to perform.[28] In our study, the test was performed in a 30-meter corridor in accordance with the guidelines. It was explained to the patients that they should walk as briskly as they could and that they could stop in case of shortness of breath or excessive fatigue, but that the test period would continue during this time. Resting saturation (SpO_2), heart rate, and modified Borg fatigue score were recorded before the test. Saturation and heart rate were continuously monitored with finger oximetry during the test. Measurements and scoring were taken again at the end of the test and 3 minutes after recovery. The distance traveled was recorded.

Statistical Power and Analysis

For the sample size in our study; similar to our study, the study of Barberan-Garcia et al.[5] which evaluated quadriceps muscle oxygenation with the NIRS device, was taken as an sample. According to this study, it was determined in G*Power 3.1.9.7 that a total of 32 people should be included, 16 people in each group, with a 95% confidence interval and 80% power. As a result of the power analysis performed at the end of our study, it was found that the power of our study was 85%.

Statistical analyses were performed using the IBM SPSS Statistics 26 (Version 26.0. Armonk, NY: IBM Corp). The Shapiro Wilk test was used to check whether the data fit the normal distribution. For descriptive statistics, numerical variables are given as mean and standard deviation if they met the parametric assumption, median and minimum-maximum values if they did not, and as frequency and percentage values for qualitative variables. Before the statistical evaluation, all continuous measurements were evaluated for conformity to normal distribution in the groups, and parametric tests were used when the normality assumption was met, and nonparametric tests were used when the normality assumption was not met. Independent groups t test was used for those that met the normality assumption and the Mann Whitney-U test for those that did not. The significance level was accepted as $p<0.05$ for all tests.

RESULTS

Of the 35 stable COPD patients (62.49±8.45 years), 2 were in GOLD 1 (5.7%), 16 in GOLD 2 (45.7%), 14 in GOLD 3 (40.0%), and 3 in GOLD 4 (8.6%). Eighteen patients in GOLD 1 and 2 were assigned to Group 1 and 17 patients in GOLD 3 and 4 were assigned to Group 2. Demographic and clinical characteristics of all patients and groups are given in **Table 1**. Age, body mass index (BMI), smoking status, CAT score, 6-MWT, right and left quadriceps muscle strength, and FEV1/FVC values of the groups were similar ($p>0.05$). The mMRC score was significantly higher in group 2 compared to group 1 ($p<0.001$); resting SpO_2 ($p=0.028$), SpO_2 at the end of the test ($p=0.010$), FEV1 ($p<0.001$), and FVC ($p<0.001$) values were significantly lower in Group 2.

Intragroup comparisons of muscle oxygenation at rest, during 6-MWT and during recovery are given in **Table 2**. In Group 1, there was a statistically significant decrease between resting SmO_2 mean and test SmO_2 mean ($p=0.001$), a statistically significant increase between test SmO_2 mean and recovery SmO_2 mean ($p<0.001$), and a statistically significant increase between resting SmO_2 mean and recovery SmO_2 mean ($p=0.022$). In Group 2, there was a statistically significant decrease between resting SmO_2 mean and SmO_2 mean during the test ($p=0.002$), a statistically significant increase between resting SmO_2 mean and recovery SmO_2 mean ($p<0.001$*), and a statistically significant increase between resting SmO_2 mean and recovery SmO_2 mean ($p=0.024$).

Table 1. Demographic and clinical characteristics of all patients and groups

	Total (n=35)	Group 1 (GOLD 1 and 2) (n=18)	Group 1 (GOLD 3 and 4) (n=17)	p
Age (year)	62.49 ±8.45	60.11±9.18	65.00±7.01	0.85
BMI (kg/m²)	26.99±4.78	26.29±5.41	27.73±4.02	0.377
Smoking (pack/year)	45.91±24.63	44.94±21.07	47.06±29.06	0.815
mMRC	1.68± 0.99	1.05±0.72	2.35±0.78	p<0.001
CAT	12.69±7.08	10.35±5.99	15.41±7.51	0.075
6-MWT (m), median (min-max)	472 (250-600)	478.50 (360-600)	450.00 (250.00-570.00)	0.305
SpO_2 rest	96.17±3.46	98 (82-100)	96 (91-98)	0.028
SpO_2 post-test	93.51±5.96	97 (71-99)	92(84-98)	0.010
Right Quadriceps Muscle Strength	19.00 (8.40-32.80)	19.00 (13.30-26.40)	18.30 (8.40-32.80)	0.815
Left Quadriceps muscle strength	19.97± 5.01	20.03±5.17	19.91±5.02	0.944
FEV1,%	51.39±16.02	63.00±10.70	37.11±7.95	p<0.001
FVC, %	69.84± 20.49	83.05±16.99	54.00±10.75	p<0.001
FEV1/FVC	62.46±12.48	64.72±12.80	59.75±11.93	0.258

BMI, body mass index; FEV1, volume expired in the 1st second of forced expiration; FVC, forced vital capacity; mMR,: Modified Medical Research Council Dyspnea scale; CAT, COPD Assessment Test; 6-MWT, six minute walking test; SpO_2, peripheral oxygen saturation; n, number of participants; X, mean value; SD, standard deviation.

Table 2. Intragroup comparison of muscle oxygenation at rest, during 6-MWT, and during recovery

Values	X ±SD	p
Group 1 (GOLD 1 and 2) (n=18)		
Rest SmO_2 Mean Test SmO_2 Mean	44.92±8.11 34.76 ±13.82	0.001*
Test SmO_2 Mean Recovery SmO_2 Mean	34.76±13.82 48.71±9.67	<0.001*
Rest SmO_2 Mean Recovery SmO_2 Mean	44.92±8.11 48.71±9.67	0.022*
Group 2 (GOLD 3 and 4) (n=17)		
Rest SmO_2 Mean Test SmO_2 Mean	50.51±11.049 44.63±10.23	0.002*
Test SmO_2 Mean Recovery SmO_2 Mean	44.63±10.23 55.51±12.72	<0.001*
Rest SmO_2 Mean Recovery SmO_2 Mean	50.51±11.049 55.51±12.72	0.024*

6-MWT, six minute walk test; n, number of participants; X, mean value; SD, standard Deviation; SmO_2, muscle oxygen saturation; p, level of significance; *, p<0.05

There was no significant difference between the groups in Δ Rest-Test SmO_2 mean, Δ Recovery-Test SmO_2 mean, and Δ Recovery-Rest SmO_2 mean (p>0.05) (**Table 3**). In the SmO_2 comparison of Group 1 and Group 2 at rest, during 6-MWT, and during recovery, it was observed that the test SmO_2 mean value was statistically higher in Group 2 (p=0.023), while there was no difference in resting and recovery SmO_2 mean values (p>0.05) (**Table 3**).

Table 3. Comparison of muscle oxygenation Δ values and muscle oxygenation at rest, during 6-MWT, and during recovery

	Group 1 (GOLD 1 and 2) (n=18)	Group 2 (GOLD 3 and 4) (n=17)	p
Δ Rest-Test SmO_2 mean	13.95±7.60	10.87±7.65	0.242
Δ Recovery-Test SmO_2 mean	10.16±11.09	5.87±6.49	0.175
Δ Recovery-Rest SmO_2 mean	3.78±6.37	5.01±8.31	0.628
Rest SmO_2 mean	44.92±8.11	50.50±11.04	0.101
Test SmO_2 mean	34.76±13.82	44.63±10.23	0.023*
Recovery SmO_2 mean	48.71±9.67	55.51±12.72	0.087

6-MWT, six minute walk test; n, number of participants; X, mean value; SD, standard Deviation; SmO_2, muscle oxygen saturation; p, level of significance; *, p<0.05; Δ, difference.

DISCUSSION

In the present study, 35 COPD patients were reached and the participants were divided into 2 groups according to GOLD Staging. The evaluation results showed that both groups were similar in terms of age, BMI, smoking, functional capacity, right-left quadriceps muscle strength, and CAT scores. The dyspnea level was higher and peripheral oxygen saturation was lower in the group with higher disease stage (GOLD 3 and 4). In both groups, muscle oxygenation decreased significantly during the 6-MWT compared to rest and increased during recovery. In addition, muscle oxygenation increased in recovery than at rest. When the differences (Δ) of the groups during these changes were compared, it was seen that there was no difference between the groups. However, when the muscle oxygenation mean values of the groups obtained during the test were compared, it was observed that the average muscle oxygenation of the group with high disease stage was significantly higher than the other group. In other words, the muscle oxygen utilization metabolism of people with high COPD stage was poorer during submaximal exercise; considering that the functional capacities of the groups were similar, it was thought that the muscle demanded more oxygen to produce the same movement.

Measurement of muscle oxygenation with near-infrared technology is a relatively new method for noninvasively obtaining physiological information on peripheral muscle metabolism and muscle oxygen utilization. Muscle oxygenation is expressed as SmO_2 and can take different values between 0 and 100 depending on the oxygen supply to the muscle via peripheral blood flow and the oxygen demand of the muscle, which varies depending on whether the muscle is at rest or during/after exercise. SmO_2 mean value shows the mean value during the measured time period. Muscle oxygenation decreases with exercise in healthy individuals compared to its value at rest, reaching the resting level in recovery after exercise.[29] In our study, normal physiological responses occurred and muscle oxygen saturation decreased with exercise in both groups and increased above the resting level during recovery.

In our study, there was no difference between the SmO_2 mean values of the groups at rest and during recovery, but there was a difference during exercise. Although there was no difference in the exercise capacity of the participants in both groups, the fact that the mean muscle oxygenation during exercise was lower in patients in GOLD stages 1 and 2 compared to those in stages 3 and 4 shows that they can provide the same level of exercise performance using less oxygen. This is an important clue that the peripheral muscles of patients in the early stages of COPD are able to balance the oxygen supply and demand status using oxygen more efficiently and effectively during exercise, which is how Szucs et al. explained the improvement in muscle oxygenation after pulmonary rehabilitation in patients with COPD.[30] These results obtained in muscle oxygen saturation are explained by the physical fitness status of healthy individuals, which varies according to whether they are sedentary or active, and by the fact that energy production-consumption systems have the potential to perform more work at the same oxygen levels.[31] In our study, although muscle oxygen saturation during exercise was higher in COPD patients with increasing disease severity, they performed functionally the same task as the group with less disease severity, which may be explained by the fact that energy metabolism and

endurance status were affected due to decreased oxygen level and oxygen utilization capacity of the muscle with increasing disease severity.

Oxygen saturation, which is an indicator of whole body oxygenation, is a parameter that provides important information about whether oxygen is sufficient in body tissues, and decreases of 4 percent or more indicate general deoxygenation in the body.[32] When the participants were analyzed in terms of SpO_2 levels in our study, deoxygenation was observed in the SpO_2 values of the group with high disease severity after the test in accordance with the clinical characteristics. According to the literature, this is an indication that metabolic adaptation to exercise decreases with increasing disease severity in COPD and the general oxygenation status of the body deteriorates.[33,34] The deoxygenated state of the patients during exercise may also have affected the oxygen utilization metabolism of the muscles during exercise. Since we worked with a select group of patients in terms of our exclusion criteria (such as patients receiving continuous oxygen therapy, patients with diffuse parenchymal damage, patients with dyspnea and hemodynamic instability so severe that they could not perform the 6-MWT, the SpO_2 and SmO_2 values of these patients were similar to those of the group with lower disease severity at rest and during recovery, even if the disease severity was high.

Tateishi et al.[35] compared muscle oxygenation at rest and during exercise in COPD patients with healthy controls and found that muscle oxygenation was impaired during exercise in COPD patients. They stated that this may be due to muscle fiber changes caused by the systemic effects of the disease and decreased oxidative capacity of the muscle, as well as increased oxygen consumption in respiratory muscles during exercise in COPD, and reported that future studies should focus on the causes of impaired muscle oxygenation. Evaluation of peripheral muscle oxygenation during exercise by spatially resolved spectroscopy in patients with chronic obstructive pulmonary disease.[36] Patients with COPD often develop skeletal muscle and vascular abnormalities as a complication of the disease, similar to patients with heart disease.[37,38] Studies have shown different parameters such as chronic immobilization, abnormal metabolic regulation, and decreased muscle oxidative capacity as the cause of impaired oxidative metabolism.[39] In our study, when we look at the CAT scores of our patients, which also reflect daily life, we see that they were moderately affected, and the 6-MWT result, which reflects the activities of daily living, was not too low. Therefore, the change in muscle oxygenation in both groups was similar. This result supports the above study and shows that when there is no condition such as chronic immobilization, that is, when the activities of daily living of COPD patients are not affected, there are no serious differences in muscle oxygenation changes between disease stages.

Our study has some limitations. First of all, the distribution of patients according to GOLD staging was not equal. In other words, a homogeneous number of patients with GOLD 1, 2, 3, and 4 would have allowed us to reach clearer results and the results of each stage could have been compared separately. Our study was conducted with a stable and select patient group. Separate evaluation of patients receiving continuous oxygen therapy could have revealed different perspectives. In addition, muscle oxygenation measurement was performed only on the lateral part of the quadriceps femoris due to insufficient number of devices. The results could have been strengthened by studying more than one muscle point. To the best of our knowledge, our study is the first study to evaluate muscle oxygenation in COPD patients according to staging. Although we have limitations, we think that our study is a pioneering study and the literature needs studies that offer different perspectives on this subject.

CONCLUSION

When the disease stage increases in individuals with COPD, muscle oxygen utilization metabolism during submaximal exercise worsens, demanding more oxygen to the muscle to produce the same movement as in individuals with a lower disease stage. This may be explained by the fact that energy metabolism and endurance are affected due to the decrease in the oxygen level of the muscle and its capacity to utilize the available oxygen with increasing disease severity. Our study is a pioneering study. Considering that COPD is a systemic disease and primarily affects the musculoskeletal system, studies with larger numbers of COPD patients with different clinical characteristics are needed to explain muscle metabolism.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Bandırma Onyedi Eylül University Health Sciences Non-Interventional Researches Ethics Committee (Date: 13.04.2023, Decision No: 2023-72).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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

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