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EDITORIAL

Our dear readers,

We are proud to publish the last issue of our journal for 2021 with 36 articles. In this issue, there are 35 research articles, and 1 case reports. Although COVID-19 pandemic is still goes on, we are studying hard day by day. Our principal aim is to contribute international literature at an increasing scientific level. We would like to thank all authors for submitting articles contributing to both domestic and international literature with their comprehensive scientific content for publication in our journal. We hope that this issue will be useful to our readers.

Sincerely yours

Prof. Murat KEKİLLİ, MD
Editor

CONTENTS

Original Article

CONTENTS

Original Article

CONTENTS

Original Article

Case Report

Original Article

HEALTH SCIENCES
MEDICINE

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Comparison of the effect of the intracameral lidocaine anesthesia and subconjunctival lidocaine anesthesia on the development of intraoperative floppy-iris syndrome in cataract surgery

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ABSTRACT

Aim: To evaluate the effect of intracameral lidocaine anesthesia (ILA) and subconjunctival lidocaine anesthesia (SLA) administered during cataract surgery on the development and prevalence of intraoperative floppy iris syndrome (IFIS).

Material and Method: The study involved the medical records of 86 cataract patients having no risk factors other than small pupil size for IFIS whom ILA and/or SLA were applied during phacoemulsification surgery. While 45 patients were administered intracameral lidocaine anesthesia ILA (1%), 41 patients in the other group were administered subconjunctival lidocaine anesthesia (SLA) (2%). Floppy iris syndrome findings such as iris billowing, iris incarceration in the wound site, or progressive myosis, if any, were recorded. The groups were compared in terms of the prevalence of IFIS development.

Results: While IFIS ratio was 33.7% in the ILA group, it was 17.1% in the SLA group (p=0.084). Patients with smaller pupil diameter observed a higher IFIS rate (when pupil diameter threshold value was 6.5 mm p=0.011 and 6 mm p=0.009).

Conclusion: During cataract surgery, surgeons should care for the development of IFIS in patients with small pupil diameters. However, the effect of intracameral lidocaine use on the development of IFIS has not been determined.

Keywords: Cataract, phacoemulsification, iris diseases, local anesthesia

INTRODUCTION

Cataract is the most common cause of blindness worldwide, and cataract surgery is one of the most frequently performed surgeries. Although many causes are counted in the etiology, the mechanisms of cataract formation are not fully elucidated. Today, the only treatment option is surgery. The preferred method in cataract surgery is phacoemulsification combined lens implantation due to reduced complication rates and high success rates (1). Patients could be administered general anesthesia or local anesthesia during cataract surgery. Local anesthesia could be in the form of topical, retrobulbar, peribulbar or sub-Tenon's blocks. Topical anesthesia could be combined with subconjunctival anesthesia or intracameral anesthesia to increase patient comfort. Unpreserved lidocaine (1%) 0.3-0.5 mL could be used for this purpose in intracameral anesthesia (2,3). Applicability of intracameral anesthesia is easy. This very commonly used anesthesia method yield good results with the right patient selection (4). Subconjunctival anesthesia could be applied in pterygium excision, conjunctiva surgeries, glaucoma and cataract surgeries (5).

Intraoperative floppy-iris syndrome (IFIS) is a condition associated with iris anomalies that might emerge during cataract surgery. Many factors are considered responsible for its etiology. IFIS could develop depending on the use of various systemic medications. The most important group among these is α-receptor antagonists (6). IFIS could emerge in the forms of floppy iris stroma billowing with the liquid flow, the tendency of iris tissue to be prolapsed from the wound site, and progressive intraoperative pupillary constriction. IFIS was first defined in patients who used tamsulosin (7). Other studies conducted later confirmed that tamsulosin could cause IFIS (8,9). It was also reported that apart from tamsulosin, IFIS

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could develop depending on α-1 blockers, 5-α reductase inhibitors, some herbal medicine (saw palmetto), and some antipsychotic medicine (10-14).

The complication ratio is reported to increase during surgery in the eyes with IFIS. Cases that developed IFIS could experience iris trauma due to unexpected iris prolapses and myosis, iris capture during phacoemulsification or irrigation, iridodialysis, hyphema, posterior capsule damage, and vitreous loss (15). Some studies report that complication ratios might not increase if the condition is predicted and patients receive operation from experienced surgeons (16). When IFIS develops during surgery, the use of intracameral epinephrine and iris hooks in tandem could enable to enlarge the pupil adequately and complete the surgery successfully (17). In addition, soft-shell viscoelastic technique, low liquid flow velocity, visco-adaptive viscoelastic use, pupil expansion rings, and staining the capsule with trypan blue are recommended when IFIS develops (18). This study aims to identify whether intracameral lidocaine has effects on the floppy iris syndrome that might develop during surgery.

MATERIAL AND METHOD

The study was carried out with the permission of Hitit University Medical Faculty Non-interventional Clinical Researchs Ethics Committee (Date: 01.03.2019, Decision No: 2019-10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The case-control study conducted retrospectively (via file scan) included 86 cataract patients whom ILA and/or SLA were applied during phacoemulsification surgery in the eye clinic of our hospital and having no risk factors (patients who did not have a history of use of drugs such as tamsulosin, α-1 blockers, 5-α reductase inhibitors, saw palmetto and some antipsychotic medicine) other than small pupil size for IFIS. The patients systemic diseases (HT, DM, etc.) were noted. Patients' best-corrected visual acuity, biomicroscopic findings, intraocular pressure (IOP) measured with applanation tonometry, and fundus oculus examination findings were recorded. The patients' cataracts were classified according to the Lens Opacities Classification System III (19). In our study, the potential of the two anesthesia types to perform IFIS was compared with each other.

The patients who had a history of prostate disease and therefore used α - adrenergic antagonist, who had over the 3.00 D refractive error, who had white cataract, who had grade ≥ 3 nuclear cataract and traumatic cataract (as the total surgery durations of these patients are long, they are at a higher risk of developing myosis during surgery), who had pseudoexfoliation, who had a uveitis findings history, and who had posterior synechia in iris were excluded from the study.

Preoperative dilatation of the patients was enhanced using 1% cyclopentolate, 2.5% phenylephrine and 1% tropicamide dripped 3 times in 10-minute intervals (triple dilatation is routinely performed in our clinic to ensure rapid and long-term dilatation). Preoperative pupil diameters were measured with a caliper with an operating microscope. Topical anesthesia of both groups was done with 0.5 % proparacaine. The operations were performed in the same center, by two experienced surgeons (TŞ,SIK). The surgeons randomly chosed the anesthesia type.

The patients involved in the study were divided into two groups. After the site-port of 45 patients was performed, they were administered 0.3-0.5 ml 1% unpreserved lidocaine in the anterior chamber. After the 41 patients in the second group were administered proparacaine, they were given 2% lidocaine 0.5 ml with a 27 gauge injector from the upper nasal to under conjunctiva. Subconjunctival anesthesia is performed as 0.5 ml lidocaine injection with a 27 gauge needle from 5 mm posterior of the limbus to superior conjunctiva . The type of anesthesia performed during surgery was chosen randomly. The wound site was pressed with cigarette sponge for 1 minute and waited to prevent leakage. Before the capsulorhexis, 3% sodium hyaluronate viscoelastic was used. The phacoemulsification procedure was done using the 'stop and chop' technique (Infinity Alcon System, Alcon). Peristaltic pump was used during the phacoemulsification procedure (to keep the pressure control system more stable). The following stages of surgery utilized 1.4% sodium hyaluronate viscoelastic. If any IFIS findings reported in the study conducted by Chang et al. (iris billowing, incarceration in the wound site, or progressive myosis) were seen, these patients were accepted to have developed IFIS (7). The stage when IFIS developed was also recorded. The cases that developed myosis during surgery were given 1 mL (1:1000, 1mg/mL) epinephrine. Despite this, the cases that continued to have myosis were administered iris retractor if necessary. No mydriatic method was applied until the IFIS was detected. If posterior capsule rupture or vitreous loss were experienced during surgery, these were also recorded.

Statistical method: Statistical analyses of the data obtained in the study was performed using SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA) package program. In line with the data distribution, descriptive statistics were reported as median (minimum-maximum) and mean±standard deviation for the continuous variables. Descriptive statistics of the categorical data were presented as numbers and percentages (%). The normality distribution for the statistical test choice was assessed using the Shapiro-Wilk test. A comparison of

the independent two groups measurements was done using the non-parametric Mann Whitney U test as the data did not distribute normally. Relationships and ratio comparisons between the categorical variables were done using Chi-square test. Statistical significance was taken as $p < 0.05$.

Power analysis: Power analysis results indicated 80 % power (1-β=0.80) and α=0.05 margin of error (95% confidence interval) according to 0.335 medium effect size calculated using the Cohen Criteria; 86 patients were involved in the study to identify the statistically significant differences in terms of the ratio of IFIS development in the intracameral lidocaine and subconjunctival lidocaine groups.

RESULTS

Of all the patients involved in the study, 49 were males (57 %) and 37 were females (43%). The age range was between 37 and 85. The average age of the patients was 65.93±9.21. No significant differences were found between the groups in terms of the average age (intracameral 63.80±9.01, subconjunctival 63.88±9.09; $p > 0.05$). The number of patients with hypertension was equal in both groups (as it is known that hypertension could cause IFIS) (6). Pre-operative dilate pupil diameters were between 4 mm and 8.3 mm. While the beginning pupil diameter was 6.21±0.92 mm in the intracameral group, it was 6.43±0.84 mm in the subconjunctival group. In the beginning, there were no significant differences between the groups in terms of pupil diameters (p=0.160).

No statistically significant relationships were found in the IFIS development ratios according to the anesthesia type (p=0.084). IFIS development ratios between the groups were compared in **Table 1**.

Pupil diameters of those who developed IFIS were lower in both anesthesia groups compared to those who did not; however, this difference was not statistically significant (p=0.093, p=0.116). (**Table 2**, Comparison of the IFIS prevalence according to the pupil diameters).

When the pupil diameter of 6.5 mm was accepted as the threshold value, a significant increase was found in the IFIS prevalence in patients who had pupil smaller than 6.5 mm (p=0.011) (**Table 3**). IFIS prevalence increased even more in those who had a pupil diameter of less than 6 mm (p=0.009) (**Table 4**).

None of the patients experienced capsule perforation. The cases that demonstrated pupil incarceration or myosis in the wound site during surgery were given epinephrine and 1.4% viscoelastic. These patients were administered adequate dilation again. None of the patients needed to receive iris retractor.

Table 1. Intraoperative floppy-iris syndrome (IFIS) development ratios according to the anesthesia type groups

Groups	Intracameral (n=45)	Subconjunctival (n=41)	P value
IFIS			0.084
Yes	15 (33.7%)	7 (17.1%)	
No	30 (66.7%)	34 (82.9%)	

Table 2. Comparison of the intraoperative floppy-iris syndrome (IFIS) prevalence according to the pupil diameters

Groups	N	Mean±SD (mm)	Median (min-max) (mm)	P value[a]
Intracameral				0.093
Yes	15	5.95 ± 0.59	6 (5-7)	
No	30	6.34 ± 1.03	6.25 (4-8.3)	
Subconjunctival				0.116
Yes	7	6.07 ± 0.61	6 (5-7)	
No	34	6.50 ± 0.87	6.55 (4-7.5)	

[a]Mann Whitney U test

Table 3. The relationship between the pupil diameter and intraoperative floppy-iris syndrome (IFIS) development when the 6,5 mm pupil diameter was accepted as the threshold value.

		IFIS		Total	P value
		No	Yes		
Pupil					0.011*
≤ 6.5 mm	n	36	19	55	
	%	65.5	34.5	100	
> 6.5 mm	n	28	3	31	
	%	90.3	9.7	100	

* Chi-square test statistically significant ($p < 0.05$)

Table 4. The relationship between the pupil diameter and intraoperative floppy-iris syndrome (IFIS) development when the 6 mm pupil diameter was accepted as the threshold value.

		IFIS		Total	P value
		No	Yes		
Pupil					0.009*
≤ 6 mm	n	26	16	42	
	%	61.9	38.1	100	
> 6 mm	n	38	6	31	
	%	86.4	13.6	100	

* Chi-square test statistically significant ($p < 0.05$)

DISCUSSION

To the best of our knowledge, this study is the first study in the literature that investigated the effect of intracameral lidocaine administered during surgery on the floppy iris syndrome. In comparison to the subconjunctival lidocaine group, IFIS was found to be more common at a proportion of 16.6% in the group that was given intracameral lidocaine.

Lidocaine is known to prevent the development of the action potential through the inhibition of fast sodium channels in the cell membrane and cause mydriasis independently of the sympathetic and parasympathetic effect (20). Mydriatic effect of lidocaine varies according

to the way it is administered and the amount of injection (21). To prevent the IFIS development during surgery in patients who used tamsulosin, a study conducted before administered intracameral lidocaine- epinephrine to a group of patients and administered nothing in the anterior chamber to another group of patients. IFIS development ratio was 38.5% in the group that was administered lidocaine-epinephrine and 25.5% in the other group. Contrary to what was expected, IFIS was found to be higher in the group that was administered lidocaine – epinephrine. This finding was considered to result from the smaller beginning pupil diameter in the group that was administered lidocaine- epinephrine (22). Another study conducted with patients who used tamsulosin and received cataract surgery administered sub-Tenon's lidocaine (2%) to one group and intracameral lidocaine (1%) to the other group. The group that was administered sub-Tenon's lidocaine was found to develop IFIS at a proportion of 8.8%, while this ratio was 48.6% in the group administered intracameral lidocaine. It was concluded that this finding resulted from the longer effect of sub-Tenon's lidocaine due to its accumulation around the eyeball and longer maintenance of mydriasis (23). Savino et al. (20) gave sub-Tenon's ropivacaine to a group of patients who received strabismus surgery and sub-Tenon's saline accompanied by general anesthesia to the other group. The group that was given sub-Tenon's ropivacaine was found to develop mydriasis approximately 5 minutes later. This effect was found to occur as local anesthesia (ropivacaine) caused a blockage in ciliary ganglion and ciliary nerves. Lidocaine amount decreases in a short time in intracameral anesthesia due to high liquid flow in the anterior chamber and viscoelastic substance administration. Mydriatic effect of intracameral lidocaine is known to end within 10-15 minutes (24). We observed that IFIS findings were more common in patients who underwent intracameral anesthesia in our cataract surgeries. In order to understand whether there is a difference between anesthesia types, we excluded the factors (tamsulosin use, …) that may affect IFIS in our study. We think that the reason for intracameral lidocaine to cause IFIS could be explained with this possible mechanism: lidocaine given to the anterior chamber inhibits both circular and meridional fibers of iris, which decreases the parasympathetic system activities dominant in eyes and develops mydriasis. However, tonus of the iris decreases depending on the inhibition of two muscle groups, and iris becomes flask. In this case, the iris becomes more sensitive against fluid flow and manipulations in the anterior chamber, and floppy iris syndrome findings might occur. We thought that this might also be meaningful, but we could not obtain a meaningful result in our study.

In our study, we observed that the IFIS ratio increased as the pupil diameter decreased. However, the difference was not statistically significant. IFIS development was more common in pupil diameters of less than 6.5 mm, and the difference between them was significant. As to the pupil diameters of less than 6 mm, the prevalence of IFIS development was even more. Casuccio et al. (25) reported that a small pupil diameter was a good indicator of IFIS development. In their study that was conducted later, Chen et al. (22) detected that preoperative small pupil diameter (less than 6.5 mm was accepted as small) increased the probability of IFIS development during surgery. It was reported that IFIS might have developed because the iris surface area was more sensitive against the liquid flow in the anterior chamber as the pupil diameter gets smaller. Our study also confirmed that small pupil diameter is a risk factor for IFIS.

This study has some limitations. First, our study was retrospective. Secondly, before the study, a power analysis was performed for the number of patients to be involved in the study. However, we think that prospective studies to be conducted with a higher number of patients would yield more meaningful results.

CONCLUSION

While performing cataract surgery, patients with small pupil diameters should be careful about the development of IFIS. The effect of intramaral lidocaine use on IFIS is controversial. Future prospective studies to be conducted with a higher number of cases could be a guide on this issue.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Medical Faculty Non-interventional Clinical Researchs Ethics Committee (Date: 01.03.2019, Decision No: 2019-10).

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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Complications with moderate-to-severe COVID-19 during hospital admissions in patients with pneumonia

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is an infectious disease that has caused significant mortality and morbidity worldwide. COVID-19 is known to cause complications, such as myocardial damage, acute coronary syndrome, deep vein thrombosis, pulmonary embolism, arrhythmia, heart failure, acute ischemic stroke, liver damage, cytokine storms, ischemia-reperfusion damage and side effects of drug treatments. In our study, we aimed to evaluate the complications that developed during hospitalizations in patients with moderate-to-severe COVID-19 related pneumonia who were hospitalized in our COVID-19 service.

Material and Method: This study included patients with moderate-to-severe COVID-19 pneumonia with a positive reverse transcriptase polymerase chain reaction (RT-PCR) test who were treated in our COVID-19 service between November 2020 and January 2021. Their demographic characteristics, treatment regimens, baseline laboratory values and complications during their hospitalization were retrospectively recorded.

Results: The study group had a mean age of 62.92± 14.60 years and 40.6% (n= 55) were female. Approximately 35.3% (n= 48) of the patients developed complications due to COVID-19 during their follow-up period. Of the patients with complications, 63.8% (n: 30) were male, most common complications were elevated liver enzymes (47.9%) and pulmonary thromboembolism (20.8%). Of those patients with complications, the most common comorbidities were hypertension (40.4% [n= 19]), diabetes mellitus (25.5% [n=12]) and cardiovascular disease (23.4% [n=11]). There were no significant relationships between the presence of complications and age, sex or comorbid diseases (p>0.05 for each).

Conclusion: There are more underlying conditions, such as hypertension, diabetes mellitus, cardiovascular disease, chronic lung disease in hospitalized patients with moderate-to-severe pneumonia compared to outpatients with COVID-19 pneumonia. Complications develop, especially in the management of COVID-19 pneumonia, which affect the treatment process and patient mobilization.

Keywords: COVID-19, pneumonia, complication

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), began in China at the end of 2019. COVID-19 is an infectious disease that has caused significant mortality and morbidity worldwide. Although it progresses with mild symptoms in most patients, serious complications, such as a cytokine storm, multiple organ failure, septic shock and acute respiratory distress syndrome (ARDS) have been observed in some cases (1-3).

COVID-19 is known to cause complications, such as myocardial damage, myocarditis, acute coronary syndrome, deep vein thrombosis, pulmonary embolism, arrhythmia, heart failure, acute ischemic stroke and cardiogenic shock (4,5). In addition, it has been shown to cause liver damage by systemic inflammatory responses, cytokine storms, ischemia-reperfusion damage and side effects of drug treatments. The liver damage may also stem from an underlying liver disease or by direct actions

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on liver cells via angiotensin converting enzyme (ACE) receptors (6). Furthermore, the risk of a spontaneous pneumothorax is increased in patients with COVID-19, with an average incidence of approximately 1% (7,8).

In our study, we aimed to evaluate the complications that developed during hospitalizations in patients with moderate-to-severe COVID-19related pneumonia who were hospitalized and treated in our COVID-19 service.

MATERIAL AND METHOD

The study was conducted in compliance with the criteria of the Helsinki Declaration. It was approved by the Ethics Committee of the University of Health Sciences, Kecioren Training and Research Hospital (Date: 2021, Decision No: 2366). Written informed consent was obtained from all participants who participated in this study. Included in this study were patients with moderate-to-severe COVID-19 pneumonia with a positive reverse transcriptase polymerase chain reaction (RT-PCR) test who were treated in our COVID-19 service between November 2020 and January 2021. We followed the Republic of Turkey Ministry of Health COVID-19 Diagnosis and Treatment Guidelines to classify our patients as having moderate-to-severe pneumonia. This included patients with tachypnoea (respiratory rate> 30/min), poor prognostic criteria in blood tests and oxygen saturation levels < 90% in room air or at admission. Other criteria included a blood lymphocyte count < 800/µl; C-reactive protein (CRP)>40 mg/L; ferritin>500 ng/ml; or D-dimer >1.000 ng/ml. Additionally, patients with bilateral pneumonia, as determined with a chest X-ray or a thoracic computed tomography, were recorded as cases with moderate-to-severe pneumonia. Demographic characteristics, treatment regimens, baseline laboratory values and complications during their hospitalization of all patients were recorded retrospectively. Among the complications were elevations in the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that exceeded the upper limits of the normal values.

Statistical Analysis

Data were evaluated using the IBM-SPSS (version 20.0) statistical package. For descriptive statistics, the following values were used: number, percentage, mean, standard deviation (SD), median and interquartile range (IQR: 25–75). Chi-square tests were used to compare categorical data, while Mann-Whitney U tests were used to compare continuous data. A logistic regression model was created with variables found to be significant in the bivariate analysis ($p < 0.05$). Binary logistic regression analyses (were used in the model analysis. For statistical significance, $p < 0.05$ was accepted.

RESULTS

The study group had a mean age of 62.92±14.60 years and 40.6% (n=55) were female.. Approximately 35.3% (n=48) of the patients developed complications due to COVID-19 during their follow-up period. Of the patients with complications, the two most common complications were elevated liver enzymes (47.9%) and pulmonary thromboembolism (20.8%) in 63.8% (n=30) of the patients (**Table 1**). Of those patients with complications, the most common comorbidities were hypertension (40.4% [n=19]), diabetes mellitus (25.5% [n=12]) and cardiovascular disease (23.4% [n=11]). While 14.9% (n=7) of those with complications were diagnosed with chronic obstructive pulmonary disease (COPD), 10.6% (n=5) were diagnosed with asthma. There were no significant relationships between the presence of complications and age, sex or comorbid diseases ($p>0.05$ for each). **Table 2** shows the sociodemographic characteristics of the study group based on the presence of complications.

Table 1. Distribution of complications seen in the study group

Complication	n	%
Acute artery thombosis	1	2.1
Acute renal failure	2	4.2
Bradicardy	1	2.1
Deep vein thrombosis	1	2.1
Elevations of liver enzymes	23	47.9
Hydrocephalia	1	2.1
Pneumotorax	4	8.4
Pulmonary thromboembolism	10	20.8
Otitis media	3	6.3
Cerebrovascular event	1	2.1
New diabetes mellitus	1	2.1
Total	48	100.0

Table 2. Sociodemographic characteristics of the study group according to the presence of complications

		Complication				p
		No		Yes		
		n	%	n	%	
Gender	Male	49	57.0	30	63.8	0.442
	Female	37	43.0	18	36.2	
Age	<65 Age	42	48.8	24	51.1	0.806
	≥65 Age	44	51.2	23	48.9	
COPD	No	72	83.7	40	85.1	0.834
	Yes	14	16.3	7	14.9	
Asthma	No	78	90.7	42	89.4	0.804
	Yes	8	9.3	5	10.6	
Hypertension	No	42	48.8	28	59.6	0.236
	Yes	44	51.2	19	40.4	
Diabetes mellitus	No	53	61.6	35	74.5	0.135
	Yes	33	38.4	12	25.5	
Malignancy	No	74	86.0	43	91.5	0.356
	Yes	12	14.0	4	8.5	
Hypotyroid	No	80	93.0	45	95.7	0.528
	Yes	6	7.0	2	4.3	
Romatological disease	No	82	95.3	44	93.6	0.669
	Yes	4	4.7	3	6.4	
Cardiovascular disease	No	63	73.3	36	76.6	0.673
	Yes	23	26.7	11	23.4	

Although 38.3% (n=18) of the patients who experienced complications had previously been admitted to intensive care, 34% (n=16) were on pulse steroids. Pulse steroid use and the presence of desaturation were more frequent in those with complications (p=0.012, p=0.009, respectively).

In our study, we observed that 129 of 134 patients were given low molecular weight heparin (LMWH) treatment as venous thromboembolism prophylaxis. No relationship was found between the use of LMWH, acetylsalicylic acid (ASA), colchicine, ground glass in radiological imaging, the presence and extent of consolidation and the development of complications (p > 0.05 for each). **Table 3** shows the need for intensive care, drug use and radiological characteristics of the study group based on the presence of complications.

In the group with complications, the length of stay was significantly longer than in the group without complications (p=0.001). White blood cells (WBC), lymphocytes, platelets (PLT), albumin, lactate dehydrogenase (LDH), CRP, troponin, ferritin, D-dimer, pro-brain natriuretic peptide (pro-BNP) and haemoglobin A1c (HgA1c) values were evaluated according to the presence of complications in the study community. There was no correlation between the presence of complications and these parameters (p>0.05 in each case). **Table 4** shows the age, duration of hospital stay and laboratory values of the sample group based on the occurrence of complications.

The length of hospital stay (OR: 1.066, 95% CI: 1.004–1.132) was a risk factor for the existence of complications when the significant variables were analysed according to the presence of complications in the logistic regression analysis (**Table 5**).

Table 3. Intensive care need, drug use and radiological characteristics of the study group according to the presence of complications

		Complication				p
		No		Yes		
		n	n	n	n	
ICU	No	56	65.1	29	61.7	0.695
	Yes	30	34.9	18	38.3	
Use of pulse steroid	No	73	84.9	31	66.0	0.012
	Yes	13	15.1	16	34.0	
Desaturation	No	24	27.9	4	8.5	0.009
	Yes	62	72.1	43	91.5	
LMWH	No	3	3.5	1	2.1	0.661
	Yes	83	96.5	46	97.9	
Acetylsalicilic acid	No	59	68.6	31	66.0	0.755
	Yes	27	31.4	16	34.0	
Colchicine	No	72	83.7	33	70.2	0.068
	Yes	14	16.3	14	29.8	
Ground glass opacity	No	7	8.1	3	6.4	0.713
	Yes	79	91.9	44	93.6	
Consolidation	No	32	37.2	11	23.4	0.104
	Yes	54	62.8	36	76.6	
Radiological infiltration	<%50	54	62.8	22	46.8	0.075
	>50	32	37.2	25	53.2	
Final	Alive	77	89.5	42	89.4	0.975
	Exitus	9	10.5	5	10.6	

LMWH: Low molecular weight heparin. ICU: Intensive care unit

Table 4. Age, length of hospital stay and laboratory values of the study group according to the presence of complications

	Complication						p
	No			Yes			
	median	IQR 25	IQR 75	median	IQR 25	IQR 75	
Age	65.0	55.0	75.0	64.0	53.0	73.0	0.675
Length of hospital stay	9.0	6.0	14.0	14.0	8.0	19.0	0.001
Length of ICU stay	6.0	2.0	9.0	9.0	5.0	14.0	0.083
WBC	7595.0	5680.0	10620.0	8465.0	6120.0	11980.0	0.234
Lymphocyte	1015.0	690.0	1780.0	955.0	700.0	1420.0	0.599
Lymphocyte %	16.1	8.4	24.6	12.2	7.8	18.7	0.099
Platelet	239.0	186.0	301.0	245.5	190.0	330.0	0.474
Albumin	33.6	29.0	37.5	31.6	29.8	34.7	0.333
LDH	295.5	217.0	423.5	342.0	280.0	487.0	0.106
CRP	86.5	28.0	145.0	99.9	47.0	186.8	0.166
Troponin	5.9	3.2	17.3	7.7	4.1	20.6	0.413
Ferritin	226.5	91.8	555.0	368.1	220.2	589.4	0.085
D-dimer	.8	.4	1.5	1.0	.6	2.7	0.080
Pro-BNP	65.9	35.9	133.0	58.6	30.9	198.8	0.904
HgA1C	7.0	5.8	9.7	6.9	6.4	7.9	0.850
Number of lung lobes affected	4.0	3.0	5.0	5.0	3.0	5.0	0.079

LDH: Lactate dehydrogenase. WBC: White blood cells. pro-BNP:pro-brain natriuretic peptide. HgA1c: Haemoglobin A1c. CRP: C-reactive protein

Table 5. Risk factors determined according to the presence of complications in logistic regression analysis

	β	S.E.	p	OR	%95 CI
The length of hospital stay	0.064	0.031	0.036	1.066	1.004-1.132
Use of pulse steroid	0.298	0.509	0.558	1.347	0.497-3.656
Desaturation	0.985	0.642	0.125	2.678	0.761-9.421

DISCUSSION

In our study, 134 patients who were RT-PCR positive, had moderate-to-severe COVID-19 pneumonia and were hospitalized between November 2020 and January 2021 were analysed retrospectively. The patients were divided into two groups: those with complications and those without complications. Males comprised 59.3% of our hospitalized COVID-19 patients and 63.8% (n=30) of the patients who developed complications. Previous studies have shown that men experience a more severe disease and have a higher mortality rate compared to women (9). Higher rates of comorbidities, such as hypertension (HT) and diabetes mellitus (DM), are also observed in patients hospitalized for severe disease (1). The most common comorbidities in our study were HT and DM. However, no significant effects of comorbidities on the development of complications were found. Lymphopenia is seen with COVID-19 as in other viral infections. The severity of lymphopenia is related to the severity of the disease and is effective in the decision of hospitalization (7,10). In our study, lymphopenia was present in most of the patients, but no significant relationship was found between this condition and the development of complications.

Moderate liver enzyme elevations (especially AST and ALT) are common in patients with COVID-19. These may be associated with severe disease and increased inflammation, but they generally do not result in liver dysfunction; thus, no liver-directed therapy is required (11). Liver enzyme levels vary between 4%–33% in patients with COVID-19; however, this rate has reached 39% in some studies (12-14). In our study, liver enzyme elevations were observed in 47 patients (47.9% of patients with complications, 35.3% of all patients). The most frequently reported adverse events related to Favipiravir use in clinical studies are increased serum uric acid levels, diarrhoea, decreased neutrophil counts and liver enzyme elevations. In light of these findings, it was difficult to determine if the increases in liver enzymes in our patients were due to the medication or to the moderate-to-severe COVID-19 pneumonia. In patients with COVID-19, pulmonary thromboembolisms occur at a rate of 10%–28%, with the average age of onset being 57–61 years (15,16). In a study by Bompard et al. (17), the cumulative rate for thromboembolism was 24% and 50% in COVID-19 patients followed in intensive care.

D-dimer levels are significantly higher in a group with thromboembolisms (17). In another study, D-dimer values were associated with acute thrombosis, and generally>1 IU was found to be significant (18,19). In two separate studies evaluating 314 and 124 patients with COVID-19 who were hospitalized outside the intensive care unit, the venous thromboembolism (VTE) rate was 6.4% and 3%, respectively (20,21). In our study, the pulmonary thromboembolism (PTE) rate was 7.5% for all patients and 20.8% in the group with complications. D-dimer values were also higher in the group with complications, but not significantly higher, and a threshold D-dimer value could not be defined for determining the risk of PTE. This is due to the fact that all of our patients were hospitalized with a diagnosis of moderate-to-severe pneumonia. This suggests that high D-dimer levels (> 0.5 IU) may indicate an increased risk of PTE, as well as be associated with the severity of pneumonia. We think our PTE rate was lower than that in the literature since all our patients used prophylactic LMWH.

Studies have shown that COVID-19 has a 1% rate of causing a spontaneous pneumothorax. There are also reports that it is seen less frequently (7,8). The mechanism of a pneumothorax in COVID-19 infections remains unclear. Risk factors for a spontaneous pneumothorax include male sex, slim and tall body build, smoking, trauma and infection. In our study, the four patients who developed a pneumothorax were male. Parenchymal lesions, such as cysts and bullae, were not found in the first thoracic CT images. In the literature, a case with a pneumothorax due to a giant bulla (22) and a case with cystic lung lesions causing a pneumothorax, both resulting from of a COVID-19 infection, were published by Liu K et al. (23). In a study with 3,000 patients, Massa Zantah et al. (8) reported the presence of lymphopenia and increased inflammatory markers, such as CRP, LDH, ferritin, D-dimer and interleukin 6 (IL-6) in almost all patients who developed a spontaneous pneumothorax. When a pneumothorax developed in our patients, inflammatory parameters were high and consistent with the literature, but no significant relationships were found with the development of complications.

In COVID-19, as in other viral infections, there is a risk of infectious particles spreading from the nasopharynx to the middle ear. Viral infections may lead to otitis media either as a single middle ear pathogen or by causing a eustachian tube dysfunction. There have been several cases with otitis media detected during the course of COVID-19 (24,25). In our study, otitis media was detected in three patients (2.2% of all patients and 6.3% of those with complications).

Mao et al. (26) reported that cerebrovascular disease develops in 5.7% of patients with severe infections. In another study by Li Y et al. (27), the incidence of cerebrovascular events in patients with COVID-19 was approximately 5% with a mean age of 71.6 years. In our study, this rate was 2.1% in patients with complications, and 0.7% in the entire study group.

The duration of hospital stay was higher in the group with complications than in the group without complications. This suggests that, with more immobilization, the higher the risk of complications.

The limitations of our study include the limited number of patients in our study, the evaluation of only inpatients, inadequate follow up after discharge and the retrospective nature of the study.

CONCLUSION

The main points in our study are: The most common complications in our inpatients with covid 19 pneumonia; liver enzyme elevation and pulmonary thromboembolism were consistent with the literature. It was observed that the most common underlying diseases in the development of complications in COVID-19 patients were hypertension, diabetes mellitus and cardiovascular diseases, again in line with the literature. The duration of hospital stay was higher in the group with complications compared to the group without complications. It showed that we should be careful in terms of complication development as the immobilization time gets longer.

There are more underlying conditions, such as hypertension, diabetes mellitus, cardiovascular disease, chronic kidney disease or chronic lung disease in hospitalized patients with moderate-to-severe pneumonia compared to outpatients with COVID-19 pneumonia. Hypertension is the most common underlying condition in patients with moderate-to-severe COVID-19 pneumonia. Complications develop, especially in the management of COVID-19 pneumonia, which affect the treatment process and patient mobilization. Therefore, it is important to perform laboratory follow up in the clinical follow up and to be especially careful in terms of the development of coagulopathy, pneumothorax and neurological events.

ETHICAL DECLARATIONS

Ethics Committee Approval: It was approved by the Ethics Committee of the University of Health Sciences, Kecioren Training and Research Hospital (Date: 2021, Decision No: 2366).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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

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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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The spectrum of underlying diseases in children with autoimmune hemolytic anemia

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ABSTRACT

Aim: Autoimmune hemolytic anemia (AIHA) is characterized by the production of antibodies directed against red blood cells. We aimed to describe the clinical presentation, hematologic and biochemical profiles, treatment modalities, underlying diseases and outcomes in children suffering from AIHA.

Material and Method: In a retrospectively manner, we consecutively recrutied 62 children (aged 1 month-18 years) with AIHA who had been followed in Erciyes University Child Hospital between January, 2000 and November, 2017.

Results: The mean age at time of diagnosis was 61.9±51.9 months (range:1-192) in 62 children including 28 girls and 34 boys. The most common complaints included fatigue and fever while the most common findings were jaundice and hepatosplenomegaly. In 22 children (35.4%), there was a comorbid, active, non-specific infection (upper respiratory tract infection, lower respiratory tract infection, diarrhea, urinary tract infection). At time of diagnosis, mean hemoglobin (Hb) level was 6.9±2.5 g/dL (range: 5-9). The glucocorticoid therapy was given in majority of the patients while no medical treatment was given to 4 patients. Of the patients with primary AIHA, 6 patients were unresponsive to the treatment while one patient responded partially. It was found that there were underlying risk factors in 36 patients with secondary AIHA, as immunodeficiency and autoimmune disorders being the most common risk factors.

Conclusion: The immunodeficiencies were highly prevalent in children included. By advance of whole exome sequencing technology, we believe that primary immunodeficiencies was the most common underlying disease in our study detected quite high in presented children.

Keywords: Autoimmune hemolytic anemia, children, immunodeficiency

INTRODUCTION

Autoimmune hemolytic anemia (AIHA) is characterized by the production of antibodies directed against red blood cells, in which the red cell lysis occurs either by the mononuclear phagocytic system or by the complement system. The AIHA prevalence is still unknown at childhood, but it likely increases by advancing age as for most autoimmune disorders. In some studies, the AIHA prevalence was estimated as 0.2-0.8per in 100,000 person-years (1,2).

In infantile period, it mostly develops due to viral and bacterial infections or vaccination. However, in teenagers and young adults, there is an increased association with an underlying systemic illnesses, most commonly with immunodeficiency, malignancy and autoimmune disorders (systemic lupus erythematosus (SLE), collagen vascular diseases) (3).

The AIHA diagnosis generally depends on clinical features, positive direct anti-globulin test (DAT) / Coombs test, laboratory analysis for hemolysis,anemia, hyperbilirubinemia, reticulocytosis. (4).

In hemodynamically stable patients with Hb level <7 g/dL, the erythrocyte transfusion is based on the American Association of Blood Banks (AABB) guidelines. If an AIHA patient has severe anemia and cardiopulmonary symptoms due to anemia,erythrocyte transfusion is required (5).

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In general, glucocorticoids are first-line treatment options due to their immunosuppressive effect. The additional treatment options may include intravenous immunoglobulin (IVIG) therapy, splenectomy, danazol, or immunosuppressive agents (6).

In this study, we aimed to describe the clinical presentation,hematologic and biochemical profiles, treatment options, underlying diseases and outcomes in children suffering from AIHA in Kayseri, Central Anatolia, Turkey

MATERIAL AND METHOD

We retrospectively reviewed children (aged 1 months-18 years) who were diagnosed as AIHA at Pediatric Hematology and Oncology Department of Erciyes University, Medicine School between January 2000 and November 2017. From medical records and outpatient clinic database, we initially searched data regarding history and physical examination, first-line tests including hemogram, White blood cell (WBC), peripheral smear for red cell morphology for hemolysis, reticulocyte count and reticulocyte smear, biochemical hemolysis parameters (DAT, haptoglobin, lactate dehydrogenase (LDH), indirect bilirubin), blood typing, hepatic and renal function tests, and urinalysis. In addition, we also searched data regarding second-line tests including viral markers (hepatitis B, C and HIV, EBV, CMV, Parvovirus B19, HSV serology), immune-hematological parameters (C3, C4, CH50, autoimmune markers including thyroid auto-antibodies, immunoglobulin class, lymphocyte subpopulation, double-negative T cells, immune disease panel) in patients diagnosed AIHA.

The patients with clinical findings and laboratory results of AIHA such as anemia (including elevated reticulocyte count and blood smear with hemolysis), positive DAT, low haptoglobin levels, increased indirect bilirubin and LDH levels were reviewed. For all records reviewed, the study was carried out with the permission of Erciyes University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 16.06.2017, Decision No: 2017/348). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The AIHA classification was made based on different serological antibodies (IgG, IgM, IgA, C3, and/or combinations) for different serological types, disease severity and treatment outcome. Patients diagnosed with AIHA were classified as primary and secondary AIHA according to associated and potentially causative disorders including infections, autoimmune disorders, and malignancies. Whole exome sequencing (WES) was performed in the patients diagnosed with AIHA, in whom etiology could not be determined.

The children aged 1 month ≤ or ≥ 18 years were excluded from the study because of unique features. The patients with transfusion-related AIHA, during bone marrow transplantation or those congenital hemolytic disorders were also excluded.

For treatment response, the complete response was defined as achieving Hb concentration ≥ lower limit of normal for age with no signs of hemolysis, i.e. normal reticulocyte count and bilirubin concentration while the partial response was defined as an increase in Hb level by ≥2 g/dLbut not reaching a normal value for age. No response was defined as an increase in Hb level by<2 g/dL and/or dependence on transfusions (7).

Statistical Analysis

To summarize data, descriptive statistics were given in tables as mean ± standard deviation or median and interquartile range for continuous variables according to the distribution. The categorical variables were summarized as counts and percent. In comparisons between categorical variables, if the values observed in 2×2 tables are 5 and above, Pearson Chi-Square was used while Fisher Exact test was used cases where it is below 5. The RxC tables were used in Fisher's Freeman Halton Test. The statistical analyses were performed using Jamovi project software [(2018, Version 0.9.2.8), (Computer Software), (Retrieved from https://www.jamovi.org)]. The significance level was set as 0.05 (p value).

RESULTS

The study included 62 patients who were diagnosed as AIHA between 2000 and 2017. Of the patients included, 34 (54.8%) were boys and 28 (45.2%) were girls. The mean age at time of diagnosis was 61.9±51.9 months (range: 1-192).

There was comorbid, active, non-specific infection (upper respiratory tract infection, lower respiratory tract infection, diarrhea, urinary tract infection) in 22 patients (35.4%). **Table 1** summarizes clinical features of the patients. The patients were classified into two groups as primary and secondary AIHA according to the etiologic causes. When groups were compared regarding age, gender, blood groups, complaints on admission, physical examination findings, relapsefrequency and frequency of accompanying diseases, no significant difference was found.

When the complete blood counts and biochemical parameters were compared between the patients with primary and secondary hemolytic anemia, no significant difference was found inWBC, Hb level, platelet count, reticulocyte percentage, lymphocyte count, eosinophil count, neutrophil/lymphocyte rate, haptoglobin level,

Table 1. Mean values of some demographic characteristics, complaints, physical examination findings and some blood values for all patients

Age, month (Range: 1-192 months)	61.9±51.9
Gender	
Male	34 (54.8%)
Female	28 (45.2%)
AIHA	
Primary	26(41.9%)
Secondary	36(49.1%)
Complaints	
Fatigue	12 (19.4)
Fever	5 (8.1)
Concentrated urine	4 (6.5)
Uneasiness	3 (4.8)
Loss of appetite	2 (3.2)
Diarrhea	1 (1.6)
Vomiting	1 (1.6)
Pain	1 (1.6)
Convulsion	1 (1.6)
Physical examination findings	
Icterus	34 (54.8)
Hepatosplenomegaly	33 (53.2)
Paleness	31 (50)
Murmur	17 (27.4)
Tachycardia	7 (11.3)
Splenomegaly	6 (9.7)
Hepatomegaly	3 (4.8)
Microcephaly	1 (1.6)
Oral moniliasis	1 (1.6)
Laboratory results of AIHA patients	
WBC (10^3/μL)	11855.8±7379.5
Hgb (g/dL)	6.9±2.5
PLT (10^3/μL)	335400±186080.7
% RTC	10.8±7.5
NEU (10^3/μL)	5972.1±5534.1
LYMP (10^3/μL)	3802.9±2634
EOS (10^3/μL)	176.1±186.4
NEU/LYMPH (%)	2±1.8
Monocyte (10^3/L)	598.2±601.1
Total bilirubin (mg/dL)	4.4±4.4
Free bilirubin(mg/dL)	0.8±1.3
Haptoglobulin (g/L)	16.9±36.9
LDH (u/L)	698.3±872.1

and LDH level. When the neutrophil counts were compared between groups, it was found that neutrophil count was significantly lower in patients with secondary AIHA than in patients with primary AIHA ($p<0.05$). It was found that total and free bilirubin levels were higher in patients with primary AIHA compared to those with secondary AIHA ($p<0.05$).

DAT was performed in all the patients for the presence of antibody. A significant difference was found between DAT positivity and Hb level in patients with both primary and secondary AIHA. When the distribution of antibody types was evaluated in 32 cases (51.6%), the DAT was positive for IgG and negative for C3d,while in 19 cases (30.7%) both were positive. Distributions of AIHA groups, DAT positivity scores, mean treatment durations and response conditions to treatment according to Hb levels were given in **Table 2**.

As the first-line treatment, 12 patients (19.4%) received IVIG therapy while 31 patients (50%) received high-dose steroid treatment (10-30 mg/kg/day), and 15 patients (24.2%) received low-dose steroid treatment (2 mg/kg/day). No medical treatment was given to 4 patients (6.4%). The patients unresponsive to IVIG were given high-dose steroid treatment (10-30 mg/kg/day) or low-dose steroid treatment (2 mg/kg/day). Only one patient achieved remission with IVIG treatment. Regarding treatment response, there was no significant difference between the two groups received low-dose steroid treatment (2 mg/kg/day) and high-dose steroid treatment (10-30 mg/kg/day). Eleven patients (17.7%) did not respond to the treatment. One of the patients died. The relapse was seen in 8 patients (29.6%) in the group received IVIG and/or low-dose steroid (2 mg/kg/day) whereas in 5 patients (16.1%) in the group received high dose steroid (10-30 mg/kg/day). There was no significant difference in relapse between groups. The combined treatments were often used in treatment. Apart

Table 2. Distributions of the groups with AIHA, DAT positivity scores, mean treatment durations and response to treatment conditions according to hemoglobin levels

	Hemoglobin level (gr/dL)				p
	0-4	4-8	8-12	Total	
AIHA					0.166*
Primary	1(2.9)	24 (70.6)	9 (26.5)	34	
Secondary	4(14.3)	14 (50)	10 (35.7)	28	
DAT					0.028*
+	0(0)	1(50)	1(50)	2	
++	2(12.5)	5(31.3)	9(56.3)	16	
+++	3(13)	16(69.6)	4(17.4)	23	
++++	0(0)	16(76.2)	5(23.8)	21	
Duration of treatment (day)	180 (30-180)	142.5 (90-280)	40 (10-75)	-	<0.001**
Response to treatment					0.104*
CR	4(8.3)	29(60.4)	15(31.3)	48	
NR	1(9.1)	9(81.8)	1(9.1)	11	
PR	0(0)	0(0)	1(100)	1	
No treatment	0(0)	0(0)	2(100)	2	

* Fisher's Exact Test was used. Descriptive statistics were given as numbers (%). ** Kruskal-Wallis H Test was used. Descriptive statistics were given as median (IQR). CR:Complet Remission, NR:Non response, PR: Poor Response

from corticosteroid and IVIG treatments, 7 patients who were refractory or not responding to the treatment or with relapse received cyclosporine treatment while 3 patients received mycophenolate mofetil (MMF) treatment, 2 patients received rituximab (RTX), 3 patients underwent plasma exchange and 4 patients underwent splenectomy. No relapse was observedin patients who received RTX and plasmapheresis. Relapses were seen all 3 patients who underwent splenectomy.The duration of treatment in patients underwent RTX and plasmapheresis was longer than those received steroid or IVIG treatment as first-line treatment.

However, in group with DAT ++ results, the most patients had Hb levels between 8-12 mg/dL (high), indicating a significant difference (p=0.028). When duration of treatment was evaluated, it was significantly shorter in the group with high level of Hb compared to the other groups (p<0.001), which was an expected condition as high severity of anemia would cause low levels of Hb and require longer duration of treatment. There was no significant difference in treatment response according to Hb levels.

The mean number of erythrocyte transfusions was 3.4±6.2 in the group with primary AIHA while it was 2±1.8 in the group with secondary AIHA, indication to significant difference. Of the patients with primary AIHA, 6 (54.5%) were unresponsive to the treatment, and one patient responded partially. Of the patients with secondary AIHA, 5 (45.5%) were unresponsive to the treatment. There was no significant difference in treatment response between the groups with primary and secondary AIHA .

The rate of drug-related side effects was 44.4% in low-dose steroid group and 32.3% in high-dose steroid group, indicating no significant difference between groups. The most common side effects were gastrointestinal complaints, increased appetite, stria formation, hypertrichosis, Cushingoid appearance, and thrombosis. In our study, one patient had cortical venous sinus thrombosis induced by steroid treatment which was previously reported (8).

When the underlying diseases were assessed in patients who were treated for secondary AIHA, it was found that there was immunodeficiency in 14 (38.9%), Evans syndrome in 6 (16.6%), Hodgkin's lymphoma in 3 (8.3%), SLE in 4 (11.1%), diabetes mellitus (DM) in one, autoimmune hepatitis in one and infection secondary AIHA in 4 patients. **Table 3** presents immunodeficiencies detected in patients who were followed up due to secondary AIHA.

Relapse was seen in 13 patients (21.0%) while 29 patients (46.8%) had a disease that had already been followed up.

Table 3. Diseases found in patients diagnosed with secondary AIHA

	n (%)
PrimaryAIHA	26 (41.9)
Secondary AIHA	36 (49.1)
Autoimmune Diseases	
Evans'syndrome	6 (9.7)
SLE	4 (6.5)
Autoimmune hepatitis	1 (1.6)
Type-1 Diabetes mellitus	1 (1.6)
Hashimoto's thyroiditis	1 (1.6)
Autoimmune neuropathy	1 (1.6)
Immunodeficiencies	
SCID (undefined)	3 (4.8)
SCID (defined)	7 (11.2)
PGM3 mutation	2 (3.2)
LRBA deficiency	2 (3.2)
RAG 1 deficiency	1 (1.6)
ZAP 70 deficiency	1 (1.6)
XLF-2 deficiency	1 (1.6)
CVID	2 (3.2)
ALPS	2 (3.2)
Malignancies	
Hodgkin's lymphoma	3 (4.8)
Infection	
HBV	1 (1.6)
HEV	1 (1.6)
Varicella	1 (1.6)
Tuberculosis	1 (1.6)

CVID: Common variable immunodeficiency,SCID:Severe combined immunodeficiency

DISCUSSION

The AIHA causes erythrocyte destruction as a result of autoantibodies binding to erythrocyte surface membrane. Its incidence is higher than that of aplastic anemia. The primary AIHA is seen in young children. Although mean age varies according to the regions in recent studies, it was reported as 5.3 years in a study performed in Turkey, 11 years in a study in India, 3.8 years in a study in France and 10 years in a study by Mayo Clinic (USA) (9-12).

A mild fever may be seen in children. Paleness and scleral icterus are commonly seen in patients. Anemia-induced systolic murmur, tachycardia and hepatosplenomegaly are commonly seen physical examination findings. In agreement with pediatric series in literature, the most common complaints on admission were fatigue, fever and concentrated urine while paleness, jaundice and hepatosplenomegaly were the most common physical examination findings in our patients (11). Apart from expected clinical presentations, we also had patients who presented with life-threatening heart failure caused by severe anemia. Life-threatening hemolytic complications may be seen during erythrocyte transfusions to the patients with AIHA (13,14). We generally had cross-matching problems in our patients requiring erythrocyte transfusion and no serious hemolytic reaction transfusion was observed.

In children, the warm AIHA is generally provoked by viral infections and hemolysis may occur. In the literature, warm AIHA cases caused by basic viral infections such as mycoplasma, parvovirus, HBV and HCV, cytomegalovirus, varicella, toxoplasma and Ebstein-Barr virus were reported (3,9,15-18). While non-specific infections were detected in most of our patients, 1 had varicella, 1 had HBV, 1 had HEV and 1 had tuberculosis infection. Patients who were admitted with AIHA presentation must be questioned for previous infections and serological and microbiological studies must be performed on patients with an active infection in order to reveal a potential agent.

Neutrophil count was significantly lower in the patients diagnosed secondary AIHA than those in patients with primary AIHA. Total bilirubin level was higher in patients with primary AIHA than those in patients with secondary AIHA. When the patients with primary and secondary AIHA were compared regarding hemogram and biochemical parameters, no difference was found. High DAT positivity (+++/++++) was higher in patients with primary AIHA than in patients with secondary AIHA. We considered that low neutrophil count in patients with secondary AIHA may be due to the underlying immunodeficiency and auto-inflammatory disease. DAT positivity and clinical findings suggest the presence of AIHA, however, it should be kept in mind that DAT may be positive in chronic liver diseases, malignancies, patients who frequently undergo transfusion due to drug use and in patients who have underwent bone morrow or organ transplantation. Moreover, DAT may also be positive in auto-inflammatory diseases (SLE, rheumatoid arthritis, etc.) and immunodeficiencies (autoimmune lymphoproliferative disease (ALPS), SCID, etc.) (19-26). DAT may be found as negative in approximately 3-11% of the patients with AIHA.20,25 Patients with negative DAT result, patients who underwent transfusion and patients who underwent a transplantation were excluded.

AIHA caused by warm antibodies is the most common type of AIHA seen in children and occurs with IgG-typeantibodies activated at room temperature or 37oC. In cold AIHA, there are IgM-type antibodies typically binding to erythrocytes at temperatures between 0oC and 37oC. Similar to the series in literature and as expected, the positivity rate of IgG-type antibodies was high. In patients with high positive results of DAT, low levels of Hb were found while need for transfusion was higher; the duration of the treatment was longer; response to the treatment lasted longer; and prognoses were worse. Moreover, DAT positivity was higher in patients with primary AIHA than in patients with secondary AIHA (3,9,11,12).

AIHA may result in death. The therapeutic decisions should be made according to the growth rate and severity of anemia. The recommended treatments rely on patient-based individual experiences. Observation may be sufficient in a patient with moderate level of anemia due to a viral infection. The treatment should be initiated in patients with more severe anemia and in patients with rapid decrease in Hb level (1-4). If the patient has a serious intravascular hemolysis, renal blood flow and the amount urine should be carefully monitored. In children with warm antibody-related AIHA, the treatment should start with monitoring, erythrocyte transfusion and corticosteroid. Intravenous immunoglobulin, plasma exchange in chosen patients, and RTX as targeted treatment can be added to the treatment (4,27). We tried to prefer corticosteroid treatments as first-line treatment in our patients diagnosed with AIHA. In cases (patients with active reactions, etc.) in which corticosteroid therapy could not be used, we preferred IVIG treatment as second option. In patients who were refractory to the treatment or who relapsed, we used other immunosuppressive treatments (MMF, cyclosporine, and RTX), splenectomy and recently plasma exchange. Cushing's syndrome, stria, hypertrichosis, and gastrointestinal side effects were observed secondary to corticosteroid treatment. Venous sinus thrombosis secondary to high-dose steroid treatment was found in one patient and IVIG treatment was carried on. In cyclosporine treatment, hirsutism, and high blood pressure were found. MMF had fewer side effects and its use was more readily than cyclosporine. Side effects occurring due to the drugs and insufficient treatments affected our treatment alterations. No complication was observed in patients who received RTX. In our study, one patient received 4 doses (375 mg/m2) while another patient received 6 doses (375 mg/m2). Among 4 patients, one underwent 4 plasma exchange sessions whereas 3 underwent 3 plasma exchange sessions. When the literature is reviewed, high-dose steroid, IVIG, cyclosporine, MMF and splenectomy were performed in previous studies while RTX and plasmapheresis have become treatments preferred (28,29). In patients who develop complications due to corticosteroid, RTX , cyclosporine and MMF, immunosuppressive drugs such as azathioprine and campath-1H and chemotherapeutics such as 6-merkaptopurin and cyclophosphamide can be used. Autologous stem cell transplantation may be performed in refractory AIHA (30).

Secondary AIHA is a condition that occurs secondary to inflammatory or autoimmune diseases such as Sjogren's syndrome, scleroderma, dermatomyositis, ulcerative colitis, Crohn'sdisease, and autoimmune thyroid; however it is most commonly seen in SLE (31). In the study from Mayo Clinic and in the largest pediatric study by Aladjiji et al., autoimmune/inflammatory diseases were the

most commonly detected secondary diseases in patients diagnosed with AIHA (11,12). Similarly, the most common causes of AIHA were autoimmune diseases in the study by Vaglio et al. (32) SLE, autoimmune hepatitis, anti-phospholipid syndrome, primary sclerosing cholangitis, Evans' syndrome and rheumatoid arthritis were main autoimmune diseases detected. In the study by Aladjiji et al. (11), secondary AIHA incidence was 63% whereas it was 11% for autoimmune disorders. In our study, secondary AIHA was found in 58% of the patients. The most commonly detected causes of secondary AIHA were immunodeficienciesand autoimmune diseases. Evans syndrome and SLE were the most common autoimmune diseases. While some of our patients were followed up with autoimmune/auto-inflammatory diseases, immunodeficiency was diagnosed in their follow-ups.

In malignancies such as chronic myeloid, leukemia, lymphoma, multiple myeloma and myelodysplasia, AIHA may develop before or during the disease. In the study from Mayo Clinic, one patient had chronic myeloid leukemia but not lymphoma. Three patients had non-Hodgkin lymphoma in the study by La Sapienza and 8 patients had a malignancy (breast cancer, thyroid cancer, lymphoma, colon cancer and acute myeloid leukemia) in the study by Aladjiji et al. (11). In our study, Hodgkin's lymphoma developed in 3 patients. Two of the patients in whom Hodgkin's lymphoma was detected were observed to have been followed up with AIHA secondary to ALPS and 1 patient with Evans syndrome. It may also be seen with congenital immunodeficiencies accompanied by immune cytopenia. It is particularly seen in immunodeficiency syndrome that often changes and in Wiskott-Aldrich syndrome. AIHA may also be seen in acquired immunodeficiencies occurring after human immunodeficiency virusor organ transplantation (33). In our study, immunodeficiency was found in 19% of the patients diagnosed with AIHA and in about 43% of the patients diagnosed with secondary AIHA. PMG3 mutation, LRBA deficiency, RAG1 deficiency, ZAP70 deficiency and XLF-2 deficiency were primary immunodeficiencies that can be typed with the use of examinations of all genes-analysis. As a result of WES, LRBA deficiency was found in 2 patients followed up with AIHA secondary to ALPS and ZAP70 deficiency was found in one patient followed up with AIHA secondary to SLE. We had patients followed with immunodeficiency that could not be typed in the era where WES technology was unavailable. In the study by Aladjiji et al (11), humoral immunodeficiency was detected in 17 patients, non-identified primary immunodeficiency in 5, combined immunodeficiency in 2, adenosine deaminase deficiency in 2 and HLA class 2 deficiency in 1 patient.

Evans syndrome and ALPS that should be considered in the distinctive diagnosis of organomegaly, lymphadenopathy, and immunodeficiencies (34,35). Evans syndrome was found in 99 patients in a French study, in 20 patients in the study of Junjie Fan et al. (36) and in 6 patients in the study of Naithani et al. (11, 37). In our study, Evans syndrome was found in 6 patients.

Although immune hemolytic anemia caused by drugs is not common in children. methyldopa, penicillin, cephalosporin, tetracycline, erythromycin, ribavirin, acetaminophen and ibuprofen may also be the cause of hemolytic anemia (38). Paroxysmal nocturnal hemoglobinuria (PNH) which is rarely seen in children should be considered (39,40). In our study, PNH was rarely seen in childhood, and no AIHA caused by drugs was observed.

In conclusion, AIHA may be either primary or secondary to underlying diseases such as autoimmune diseases, malignancies, infections and immunodeficiencies. In our study, the rate of patients with immunodeficiency was quite high. By advent of WES technology, we believe that primary immunodeficiencies may be the most common underlying diseases in patients especially with autoimmune-induced AIHA. Patients diagnosed with AIHA should be examined for immunodeficiencies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 16.06.2017, Decision No: 2017/348).

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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Aortic arch calcification is strongly associated with obstructive sleep apnea

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ABSTRACT

Aim: Obstructive sleep apnea (OSA) is a common clinical condition that causes an increase in cardiovascular morbidity and mortality. OSA is likely to show increased arterial stiffness and progressive systemic atherosclerosis. Chest radiography reveals atherosclerotic changes in the aorta.The aim of this study was to investigate the relationship between aortic arch calcification (AAC) on chest radiography and OSA.

Material and Method: 204 patients (age: 55±14 years; 78 men) who were diagnosed with OSA by performing night polysomnography were evaluated. On the other hand 200 (age: 48±15 years; 94 men) patients were selected to the group non OSA. AAC was evaluated with chest radiography and inter-observer agreement was analyzed by using kappa statistics. Univariate and multivariate logistic regression analysis was conducted to assess the association of AAC and OSA. P-value <0.05 was considered statistically significant.

Result: The prevalence of AAC was 207 (51,2%). OSA group had significantly higher prevalence of AAC (79% vs. 32.5%, p <0.0001) as compared to the normal group. Presence of AAC was a strong and independent predictor of OSA (OR 3.923, 95%CI 2.396 to 6.328) in multivariate analysis.

Conclusion: Presence of AAC on plain chest radiography is strongly and independently associated with the presence of OSA.

Keywords: Aorta, thoracic, calcinosis, sleep apnea, obstructive.

INTRODUCTION

Obstructive sleep apnea (OSA) is a disease that causes complete or partial airway collapse during sleep and thus repetitive ventilation interruptions (1). OSA has been demonstrated to be involved with increased prevalence of cardiovascular disease (CVD) due to intermittent sleep interruptions, intermittent chronic hypoxia, and changes in intra-chest pressure (2,3). Oxidative stress and sympathetic activation occur in OSA due to fast reoxygenation following periods of hypoxia. These also lead to inflammation resulting in endothelial dysfunction and thus subclinical and clinical atherosclerosis (4).

In some studies, the relationship between OSA and coronary artery calcium level has been revealed and, as it is known, coronary artery calcium level is one of the indicators of subclinical atherosclerosis (5). There are previous studies evaluating the relationship between aortic calcification, coronary artery calcium level, and CVD (6). Calcium accumulation in the aortic arch can be evaluated by chest radiography. Calcification of the aortic arch gives preliminary clues about atherosclerosis. Based on all these, further investigation of the relationship between OSA and subclinical atherosclerosis markers is valuable in that it leads to evaluations for reducing possible future adverse events related to CVD in patients with OSA.

In this study, it was aimed to research its relationship with OSA by evaluating aortic arch calcification (AAC) on chest radiography.

MATERIAL AND METHOD

The study was carried out with the permission of Tokat Gaziosmanpaşa University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 19.08.2020, Decision No: 20-KAEK-122). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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Study Populations

The aortic arch was evaluated with posterior-anterior (PA) chest radiography of all participants included in the study. Patients older than 18 years of age and had night polysomnography from July 2018 to May 2020 were included in the study. The conditions for not being included in the study were as follows: inadequate chest x-ray, known aortic, coronary artery, cerebrovascular and peripheral vascular disease, open chest surgery, heart failure, moderate to severe cardiac valve disease, pregnancy, malignancy, and receiving dialysis. All participants were evaluated by transthoracic echocardiography. Participants were evaluated with medical history, physical examination and laboratory data.

Demographic and Laboratory Data

Biochemical parameters were automatically evaluated with the aid of the Beckman Coulter LH-750 Hematology Analyzer (Beckman Coulter, Inc, Fullerton, California). Routine biochemical parameters were evaluated by standard methods. Hypercholesterolemia was described as an initial cholesterol level of> 200 mg/dL and/or a low-density lipoprotein cholesterol level of> 130 mg/dL or pre-diagnosed and treated hypercholesterolaemia. Diabetes mellitus and hypertension were defined as drug users for the treatment of these diseases. In addition to these, presence of diabetes mellitus was determined based on when glucose level was ≥126 mg/dL in several measurements for fasting or glucose level >200 mg/dL at any measurement and hypertension was diagnosed based on when repeated systolic/diastolic blood pressure measurements was ≥140/90 mmHg. Current smoking was defined as smoking in the previous 6 months. Body mass index was expressed as kg/m².

Evaluation of Obstructive Sleep Apnea

All subjects were evaluated using polysomnography overnight using an equipped digital polygraph (SomnoStar Alpha Sleep System, SensorMedics Corp., Yorba Linda, CA, USA). Patients who were diagnosed with OSA according to the accepted definition and scoring methods were included in the study (7). Hypopneas were defined as a 30-80% reduction in nasal pressure flow for at least 10 seconds with oxygen desaturation ≥4%. A flow reduction of 80% in the nasal pressure signal for at least 10 seconds was defined as apneas. Oxygen desaturation scored if there is a ≥4% decrease. The apnea-hypopnea index (AHI) was defined as the number of apnea and hypopneas per hour of the analyzed recording time and it was used to determine the risk of OSA.

The Aortic Arch Assessment

To all participants included in the study; standard PA chest radiography was taken while the participants were standing (AXIOM Aristos MX, SIEMENS, Germany). The AAC was evaluated in four categories: grade 0, no visible calcification; grade 1, the calcification appears as a small dot or thin line; grade 2, calcification at one or more points or in the form of a thick line; grade 3, circular prominent calcification of the aortic arch (8). AAC for each patient was assessed by two independent, experienced cardiologists in a blinded fashion.

Echocardiographic Examination

All echocardiographic evaluations (General Electric Vivid S5, Milwaukee, WI, USA) were performed on all participants in the left decubitus position using a 2.5-3.5 MHz transducer. The examination was performed using two-dimensional and pulsed Doppler measurements according to the American Echocardiography Association and the European Society of Cardiovascular Imaging criteria. Simpson's method was used to evaluate left ventricular ejection fraction (9).

Statistical Analysis

Statistical analyses were carried out using the SPSS 18.0 Statistical Package Program for Windows (SPSS Inc., Chicago Illinois, USA). Qulitative data are presented as medians with interquartile ranges. Quantitative variables were expressed as a mean value standard deviation and categorical variables as percentages. The distribution of continuous variables was determined with the Kolmogorov-Smirnov test. Student t-test was used to evaluate normally distributed data and Mann-Whitney U test was used to evaluate non-normally distributed data. Fisher's exact or chi-square tests were used to evaluate categorical variables . Multivariate logistic regression analyses were applied to determine independent factors. Data from univariate and multivariate logistic regression analyses were reported as odds ratios with 95% CI. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 404 participitians were included in this study. The mean patient age was 55±14 years, and 172 (42.5%) of the patients were men. Two hundred four patients (50.4%) with OSA were evaluated as an OSA (+) group. Those without OSA diagnosis, symptoms or signs were determined as the OSA (-) group. As compared to the OSA (+) group, the OSA (-) group was older (p<0.001). Basic characteristic features of the groups, demographic and laboratory data were similar except age. Patient characteristics are summarized in **Table 1**.

In the evaluations made on chest radiography, the frequency of AAC in the group with OSA (+) was statistically higher than the group with OSA (-) (p<0.001) (**Table 2**).

In univariate analyses (**Table 3**), AAC was positively and significantly correlated with age(r=0.172, p< 0.001, BMI (r=0.201, p<0.001), AHI (r=0.212, p<0.001), arousal index (r =0.282, p<0.01), and 3% ODI (r=0.224, p<0.01), as well as several clinical variables.

Table 1. Baseline characteristics of the study groups

	OSA (+) (n=204)	OSA (-) (n=200)	P value
Age (years)	55±14	48±15	<0.001
Male, n (%)	78 (38.2)	94 (47)	0.064
BMI, kg/m2	29±5	28±4	0.062
Smoker, n (%)	65 (31.8)	52 (26)	0.110
Hypertension, n (%)	104 (50.9)	83 (41.5)	0.051
Glucose (mg/dL)	106±30	102±29	0.086
Diabetes mellitus, n (%)	46 (22.5)	34 (17)	0.286
Total cholesterol (mg/dL)	189±40.6	194±40.4	0.198
Triglyceride (mg/dL)	191±39	174±81	0.019
Low-density lipoprotein (mg/dL)	116±38	118±37	0.902
High-density lipoprotein (mg/dL)	46±12	45±13	0.498
Creatinine (mg/dL)	0.82±0.18	0.82±0.27	0.880
Antihypertensive drug, n	47	35	0.049
Antidiabetic drug, n	2	1	0.988
AHI, /h	50.5±14.4	2.1±1.7	<0.001
Aurosal index, /h	48±17.2	19.8±9.6	<0.001
ODI (3%), /h	40.1±20.2	0.8±0.7	<0.001
Lowest SpO2, %	75±9.7	90.5±3.5	<0.001

AHI, apnea-hypopnea index; BMI, body mass index; ODI, oxygen desaturation index; SpO2, peripheral oxygen saturation

Table 2. Aortic arch calcification grades in the study groups

Aortic arch calcification (n, %)	OSA (+) (n=204)	OSA (-) (n=200)	P value
Grade 0	62 (30.3)	135 (67.5)	<0.0001
Grade 1	86 (42.1)	50 (25)	<0.001
Grade 2	45 (22)	14 (7)	<0.001
Grade 3	10 (4.9)	1 (0.5)	<0.001

OSA, obstructive sleep apnea

Table 3. Univariate analysis for obstructive sleep apnea

Variables	β	P value
Age	0.172	<0.001
Male	0.006	0.056
BMI	0.201	<0.001
Smoker	-0.186	0.103
Hypertension (%)	0.526	0.048
Diabetes mellitus (%)	0.438	0.301
Total cholesterol (mg/dL)	-0.012	0.235
Triglyceride (mg/dL)	-0.002	0.089
Antihypertensive drug	0.490	0.044
Antidiabetic drug	0.042	0.382
AHI	0.212	<0.001
Aruosal index	0.282	<0.001
ODI (%3)	0.224	<0.001
Lowest SpO2	-0.134	0.001

AHI, apnea-hypopnea index; BMI, body mass index; ODI, oxygen desaturation index; SpO2, peripheral oxyen saturation

ACC was associated with OSA in univariate logistic regression analysis (**Table 4**). According to the results of multivariate regression analysis, AAC was also identified as an independent predictor for OSA (r =1.362, OR 3.923, 95%CI 2.396 to 6.328) (**Table 5**).

Table 4. Correlations between OSA and AAC

Aortic arch calcification (%)	β	P value
Grade 0	Reference category	
Grade 1	1.384	<0.0001
Grade 2	1.846	<0.0001
Grade 3	2.698	0.006

ACC, aortic arch calcification; OSA, obstructive sleep apnea

Table 5. Multivariate analysis for obstructive sleep apnea

Variables	β	OR	Lower	Upper
Age	0.014	1.014	0.978	1.034
Body mass index	0.035	1.036	0.987	1.089
Hypertension	0.060	1.062	0.658	1.690
Triglyceride	-0.002	0.985	0.989	0.996
Presence of aortic arch calcification	1.362	3.923	2.396	6.328
AHI	0.15	1.018	0.985	1.049
Male	0.15	1.008	0.964	1.027

AHI, apnea-hypopnea index

DISCUSSION

As far as we know, this study clearly reveals the relationship between AAC and OSA, which was detected for the first time via chest radiography. Therefore, the detection of ACC, which is one of the indicators of subclinical atherosclerosis, more frequently in OSA patients provides early treatment and approaches. Accordingly, this situation; it is important in terms of improving the morbidity and mortality of CVD.

The pathophysiology of OSA is not fully explained. Factors that reduce the width of the upper respiratory tract can cause OSA (10). The most important complications seen in patients with OSA are associated to the cardiovascular system (11). Cardiovascular diseases seen with OSA in order of frequency: hypertension (30-60%), CAD (20-30%), pulmonary hypertension (20-30%) (12,13).

Many risk factors for atherosclerosis are common with OSA; age, male gender, smoking, obesity, metabolic syndrome are parameters specific to both situations (14). Oxidative stress in OSA; there are data that it causes both endothelial dysfunction and LDL oxidation (15). CRP, fibrinogen, IL-6 are risk factors for atherosclerosis and these have also been shown to increase in OSA (16). In patients with CAD proven by coronary angiography, OSA has been found frequently (17).

Local endothelial damage, inflammation, oxidative stress, and vascular calcification are involved in the pathogenesis of atherosclerosis (18). Calcification of the

arterial wall is part of atherosclerosis and is not found in normal vessels (19).

Oxidative stress and inflammatory reaction in OSA lead to increased arterial stiffness , carotid intima-media thickness and CAD progression (20-22). OSA is frequently seen in patients with aortic aneurysms and dissection and it has been suggested that negative intrathoracic pressure may play a role in the development or worsening of these pathologies. Additionally, negative intrathoracic pressure is thought to cause basal atherosclerotic changes in the aorta (23,24).

AAC has been presented as a substitution marker for atherosclerosis to better reflect the total burden of atherosclerosis (25). Atherosclerosis may be suspected if ACC is present in the chest radiography evaluation in OSA patients, and so patients can be stratified by their risk of atherosclerosis (26). In addition, Patients with higher risk for atherosclerosis can be distinguished by investigating the presence of ACC in patients with OSA. Most of the parameters that are indicators of subclinical atherosclerosis do not provide additional information other than the prediction of subclinical atherosclerosis (27-29). On the other hand, chest radiography is generally used in OSA, health checks unrelated to OSA, and in various clinical settings.

In this study, we have demonstrated that the presence of AAC is strongly associated with OSA. New studies are also needed to confirm our findings and, in addition, to assess the possible relationship of AAC and OSA by classifying by severity.

Study Limitations

The main limitation of the study is the small number of participants included in the study. OSA is categorized as no/mild, moderate, and severe. However, we did not examine OSA by classifying it by severity.

CONCLUSION

OUA is strongly and independently associated with ACC presence on plain chest radiography. This simple assessment allows us to distinguish patients with OSA based on their risk of developing subclinical atherosclerosis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tokat Gaziosmanpaşa University Faculty of Medicine Clinical Research Ethics Committee (Date: 19.08.2020, Decision No: 20-KAEK-122).

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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The relationship of sensorimotor and function with activities of daily living and disease specific parametres in patients with rheumatoid arthritis

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ABSTRACT

Objective: To evaluate the level of effect on the sensorimotor functions of the hand in patients with rheumatoid arthritis (RA) and investigate the relationship between this level and patients' daily life activities and disease-specific parameters.

Material and Method: This case-control study involved the evaluation of 80 patients with RA and 50 healthy volunteers aged 20-65 years. The coarse and fine grip strength of the patients was evaluated with hand dynamometer and pinch meter. The sensory evaluation of the hand was undertaken using the Semmes-Weinstein monofilament test (SWMT). The hand skill level was evaluated with Duru Oz Hand Index (DHI). The daily living activities of the patients were investigated using the Health Assessment Questionnaire (HAQ). The Disease Activity Score 28 (DAS28) was used to evaluate disease activity.

Results: The mean DAS28 score of the RA group was at a mild level (DAS 28 <3.2). No statistically significant difference was found between the RA group and the control group in terms of daily life activities (HAQ scores). The grip strength and the hand skill level (DHI) were statistically significantly lower in the RA group compared to the control group. In the sensory examination of the RA group using SWMT, while 83% of the patients were evaluated as normal, 15% were determined to have diminished light touch sensation. However, there was no statistical difference between the RA and control groups in terms of sensory evaluation.

Conclusion: As a result of the quantitative evaluations used in our study, the RA group was determined to have impaired fine skills and grip strength. Consistent with the literature, these findings had a negative effect on the daily life activities and function of these patients. In our knowledge there is no study with SMWT in RA patients. In our study, there wasn't statistical difference between the RA and control groups in terms of sensory evaluation with SMWT. Because of our patients have low disease activity, further studies are needed in patients with higher disease activity for evaluation sensorial functions.

Keywords: Rheumatoid arthritis, health assessment questionnaire, disease activity score, Semmes-Weinstein monofilament test

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic disease characterized by symmetrical chronic arthritis that can affect all synovial joints, especially the wrist and small joints of the hand (1,2). Impairment of anatomical integrity, limitations in the range of motion, loss of muscle strength, possible sensory problems, pain, and swelling are the main problems that impair the ability to use hands in RA (1,3).

Since the focus is mostly on pain and inflammation control in patients with RA, a sensorimotor evaluation can be overlooked. In this study, we aimed to evaluate the level of effect on the sensorimotor functions of the hand in patients with RA and to investigate the relationship between this level and patients' daily life activities and disease-specific parameters (4,5). Accordingly, it is very important to perform detailed motor and sensory examinations in this patient group. Since impairment in the hand functions of these patients can negatively affect their daily lives and make them dependent on others, they should be closely monitored and early rehabilitative treatment methods should be applied before the loss of function (6,7).

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MATERIAL AND METHOD

The study was carried out with the permission of Ankara Yıldırım Beyazıt University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 17/12/2014, Decision No: 243). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This case-control study involved the evaluation of 80 patients diagnosed with RA and 50 healthy volunteers that presented to the physical medicine and rehabilitation outpatient clinic of Training and Research Hospital between January 2015 and June 2015. All individuals were informed about the study and signed the informed consent form. Patients who were diagnosed with RA according to the EULAR/ACR criteria and had cognitive function sufficient to comprehend evaluations to be made during the study were included in the sample while those with orthopedic, traumatic, neurological and endocrinological disorders that previously caused sensory or motor impairment in the hand and upper extremities were excluded.

Hand Function Assessment

The Duru öz Hand Index (DHI) was used to evaluate the fine skill functions of the hands.

DHI is a self-reported questionnaire developed to evaluate the hand function limitations of patients with RA. It consists of 18 items inquiring about hand skills in kitchen, dressing, personal hygiene and work activities and other general movements. The function of both hands is scored as follows= 0-40 for kitchen work, 0-10 for dressing, hygiene and office work, and 0-20 for the 'other' category. The score ranges between 0 (no difficulty) and 5 (impossible) in performing daily life activities. (8,9)

The Semmes-Weinstein monofilament test (SWMT) was used for the sensory evaluation of the hands. SWMT is a diagnostic test used to reveal sensory problems and objectively measure the touch threshold. SWMT has two kits of 20 and five pieces. In the current study, the patients were evaluated with a monofilament set consisting of five monofilaments at four different power levels. In this set, the green monofilament (log1.65-2.83) is evaluated as normal, blue monofilament (log3.22-3.61) as diminished light touch, purple monofilament (log3.84-4.31) as diminished protective sensation, red monofilament (log4.56-6.65) as loss of protective sensation, and white monofilament (>log6.65) as un detectable sensation.

Grip strength was evaluated with a digital hand dynamometer and pinch meter. A Baseline® hydraulic hand dynamometer was used to evaluate the coarse grip strength of the hands. Finger grip strength was assessed with a Baseline® pinch meter. Measurements were made bilaterally from three different positions (lateral, fingertip, and palmar). Each bilateral measurement was performed three times and averaged. (10)

Disease Activity Score (DAS28)

In this study, disease activity was evaluated with the DAS28 scoring system. The basic criteria of this system are the sensitivity of the joints, number of swollen joints, sedimentation, and general health assessment using the Visual Analog Scale (VAS). (11)

Health Assessment Questionnaire (HAQ)

HAQ was used to evaluate the daily life activities of the patients. HAQ is a health assessment questionnaire developed to evaluate rheumatic diseases, especially RA. It consists of 20 questions under eight activity domains inquiring about daily tasks, namely dressing/general care, sitting/standing up, eating, walking, hygiene, lying down, and grip. Each response is graded from 0 to 3. The HAQ score reflects functional status and its score has been shown to correlate with disease activity indicators. (12)

Statistical Analysis

Statistical analyses were carried out using the Statistical Package for the Social Sciences (IBM SPSS Statistics 14). Frequency tables and descriptive statistics were used in the interpretation of findings. Non-parametric methods were used for measurement values that were not suitable for normal distribution. The Mann-Whitney U test (Z-table value) was employed to compare the measurement values of two independent groups in accordance with the parametric methods. The Spearman correlation coefficient was used to examine the relationships between two non-normally distributed quantitative variables. P value <0.05 was accepted as statistically significant.

RESULTS

The demographic characteristics of the 80 patients with RA and 50 healthy volunteers constituting the control group are given in **Table 1**.

Table 1. Sociodemographic data table

	RA Group		Control Group	
	n	%	N	%
Age, years				
20-35	6	7.5	5	10
35-50	22	27.5	22	44
50-65	52	65	23	46
Gender				
Female	66	82.5	39	78
Male	14	17.5	11	22

RA, rheumatoid arthritis

While 65% of 80 patients with RA were seropositive, 35% were seronegative. There was no patient with systemic involvement. The mean disease duration in the RA group was 7.09±5.64 months. The dominant hand was right in 78 patients (96%) and left in two (4%) while all controls were right-handed. The mean DAS28 value of the patients with RA was 3.17±0.82, and according to this result, their disease activity was detected to be at a mild (<3.2) level. As an inclusion criterion, patients without hand deformities were included in the sample.

The mean HAQ score was 0.37±1.45for the RA group and 0.61±0.42 for the control group. No statistically significant difference was found between the two groups in terms of daily life activities (p> 0.05). The mean DHI was 4.67±7.58 and 0.02±0.14 in the RA and control groups, respectively, indicating a statistically significant deterioration in the fine skills of the hands in the former compared to the latter (p <0.001).

DAS28, HAQ and DHI Scores and Their Relationship with Disease Duration

The relationship of the DAS28, HAQ and DHI scores with disease duration among the 80 patients in the RA group is presented in **Table 2**. A positive significant correlation was found between disease activity and DHI in the RA group (r=0.483, p=0.001). There was also a significant positive correlation between disease activity and quality of life scores (r=0.504, p=0.001). However, no significant correlation was observed between disease duration and disease activity level (p> 0.05). P value <0.05 was accepted as statistically significant.

Relationship between the SWMT Results, DAS28, DHI and HAQ Scores and Disease Duration in the RA Group

In the sensory examination of the RA group using SWMT, 83% (n=67) of the patients were evaluated as normal with the green monofilament (log 1.65-2.83), 15% (n=12) as diminished light touch sensation with the blue monofilament (log 3.22-3.61), and only 1.3% (n=1) as diminished protective sensation with the purple filament. For all the healthy controls, the SWMT sensory evaluation result was normal with the green monofilament. However, no statistical difference was found in the sensory evaluation results of the RA and control groups (p> 0.05). P value <0.05 was accepted as statistically significant.

In the RA group, we also divided the SWMT sensory examination results into group 1 (green monofilament/ normal) and group 2 (blue monofilament/diminished light touch sensation) for further analyses. We found no statistical difference between groups 1 and 2 in terms of the DAS28, HAQ and DHI scores and disease duration (p> 0.05).

Relationship between Grip and Pinch Values and DAS28, DHI, HAQ Scores of the RA Group

A negative correlation was found between the grip and pinch values and DAS28, DHI and HAQ scores, and this was a statistically significant level (p <0.001). Increased disease activity and decreased hand dexterity significantly affected the grip function of the hands in the patients with RA. P value <0.05 was accepted as statistically significant.

Table 2. DAS28, HAQ and DHI scores and their relationship with each other and disease duration in the RA group

	DAS28	HAQ	DHI
DHI	P=0.001 r=0.483	p=0.001 r=0.865	
DAS28		p=0.001 r=0.504	p=0.001 r=0.483
HAQ	p=0.001 r=0.504		p=0.001 r=0.865
Disease duration	p=0.087 r=0.193	p=0.031 r=0.241	p=0.036 r=0.234

RA, rheumatoid arthritis; DAS, Disease Activity Score; HAQ, Health Assessment Questionnaire; DHI, Duru Oz Hand Index

Table 3. Relationship between the SWMT results, DAS28, DHI and HAQ scores and disease duration in the RA group

		DAS28	DHI	HAQ	Disease duration
SWMT	Group 1 (green)	3.15±077	4.71±7.9	0.4±1.5	6.46±4.7
SWMT	Group 2 (blue)	3.26±1.08	4.58±5.48	4.58±5.48	10.5±8.8
	P value	0.6	0.54	0.54	0.19

RA, rheumatoid arthritis; DAS, Disease Activity Score; HAQ, Health Assessment Questionnaire; DHI, Duru Oz Hand Index; SWMT, Semmes-Weinstein monofilament test

Table 4. Relationship between grip and pinch values and DAS28, DHI, HAQ scores of the RA group

	Right hand grip	Left hand grip	Right lateral pinch	Left lateral pinch	Right tip pinch	Left tip pinch	Right palmar pinch	Left palmar pinch
DAS 28	p=0.001 r=-0.497	p=0.001 r=-0.526	p=0.001 r=-0.459	p=0.001 r=-0.439	p=0.001 r=-0.371	p=0.001 r=-0.348	p=0.001 r=-0.491	p=0.001 r=-0.450
DHI	p=0.001 r=-0.545	p=0.001 r=-0.583	p=0.001 r=-0.601	p=0.001 r=-0.572	p=0.001 r=-0.487	p=0.001 r=-0.402	p=0.001 r=-0.575	p=0.001 r=-0.531
HAQ	p=0.001 r=-0.556	p=0.001 r=-0.581	p=0.001 r=-0.566	p=0.001 r=-0.513	p=0.001 r=-0.482	p=0.001 r=-0.452	p=0.001 r=-0.598	p= 0.001 r=-0.575

RA, rheumatoid arthritis; DAS, Disease Activity Score; HAQ, Health Assessment Questionnaire; DHI, Duru Oz Hand Index

Relationship between Wrist Dorsiflexion Limitation and HAQ, DHI and DAS28 Scores

The relationship between wrist dorsiflexion limitation and HAQ, DHI and DAS28 scores was evaluated with the Spearman correlation test. There were no volunteers with wrist dorsiflexion limitation in the control group. In the RA group, we observed that as wrist dorsiflexion limitation increased, the HAQ, DHI and DAS28 scores increased, but this increase was not statistically significant ($p>0.05$). P value <0.05 was accepted as statistically significant.

Table 5. Relationship between wrist dorsiflexion limitation and HAQ, DHI and DAS28 scores in the RA group

	Right wrist dorsiflexion limitation	Left wrist dorsiflexion limitation
HAQ	p=0.596 r=0.06	p=0.679 r=0.04
DHI	p=0.201 r=0.144	p=0.385 r=0.098
DAS28	p=0.157 r=0.16	p=0.172 r=0.154

RA, rheumatoid arthritis; HAQ, Health Assessment Questionnaire; DHI, Duru Oz Hand Index; DAS, Disease Activity Score

DISCUSSION

In this study, we evaluated hand sensorimotor functions in patients with RA and investigated their relationship with daily life activities and disease specific parameters. In the literature, the sensory evaluation of the hands in patients with RA has not been addressed as much as motor evaluation. However, it has been reported that proinflammatory cytokines and proteolytic enzymes, which are shown to be produced by tenosynovium in patients with RA, may cause sensory disturbances by affecting mechanoreceptors found in the tendons and myotendinous junctions (13). Therefore, the second stage of our study was designed to include a sensory evaluation.

In the literature, many tests and scales have been used in the evaluation of sensorimotor functions. DHI, which was developed by Duru Oz et al. (8,14,15) in 1996, has entered clinical practice as a self-report scale for the assessment and monitoring of functional disability in rheumatoid hands. In a study conducted with 102 patients with RA, Dedeoğlu et al. (16) find a strong relationship between daily life activity (HAQ) and hand function (DHI) . In our study, there was no statistical difference between the RA and control groups in terms of the HAQ scores. However, a significant correlation was detected between daily life activities (HAQ) and hand skill functions (DHI). This finding is an indication that hand functions occupy an important place in daily life activities. In our study, the mean DHI of the RA group was higher than that of the control group. Although there was no difference in the HAQ scores between the two groups, the statistical difference observed in the DHI averages proves once again that tests evaluating manual skills better reflect hand functions in rheumatoid hands than a general health assessment.

In a study in which 151 female and 45 male patients with RA were included, it was shown that increased disease activity significantly affected the daily life in RA patients (17). In another study 752 patients with RA were followed up for three years and the patients with low DAS28 scores were found to have higher quality of life(18). In our study, consistent with the literature, a significant correlation was observed between the daily life activities and disease activity scores of the patients. Similar to our findings, Birtane et al. (19) who evaluated 48 patients with RA, reported strong correlations between disease activity, daily life activities and hand functions. In another study, Özeri et al. (20) determined that functional impairment of the hand, disability, and joint damage had a strong relationship with disease duration, wrist range of motion, and grip strength in patients with RA.As a result, the authors argued that wrist range of motion and grip strength could be used to predict disability and joint damage in clinical practice.

Silva et al., comparing patients with RA and controls, found that the grip strength decreased in the RA group with high DAS28 activity (21). Similarly, in our study, a significant correlation was found between both fine skill and grip functions of the hands and disease activity scores. The increase in disease activity negatively was observed to negatively affect the quality of life and hand functions the patients.

In a study in which 153 patients with RA were retrospectively evaluated, there was a significant correlation between the HAQ and DAS28 scores, and a strong correlation between increased disease duration and disease activity (22). In our study, daily life activities and hand functions were significantly affected in the RA group among the patients with a longer disease duration, while a weaker correlation was observed between disease duration and activity. We consider this to be due to the low disease activity scores of the RA cases included in our study.

There was a negative correlation between the grip and pinch values and disease activity, daily life activities, and fine skill level in our patients with RA, and this was found to be at a statistically significant level. Consistent with the literature (23), We consider that diminished grip strength significantly affects daily life activities and hand dexterity functions and is an important cause of disability. Supporting our findings, Dedeoğlu et al. (16) also found a significant negative relationship between hand grip strength and finger grip strength, as well as hand functions and general disability level.

It was determined that the increase in wrist dorsiflexion limitation affect the daily life activities and hand functions of the patients and resulted in an increase their disease activity scores. In parallel with our study, in a study conducted by Taştekin et al. (24), limitations in the range of motion and impaired hand functions were determined to cause difficulties in daily life activities.

SWMT is a quantitative method for sensory evaluation. In the literature, it has mostly been employed in diseases such as tendon damage, diabetic neuropathy, and carpal tunnel syndrome (25-27). In a study by Silva et al. (28), patients with systemic sclerosis accompanied by distal phalanx deformities were compared with another patient group without deformity, and the former was found to have diminished light touch. In our knowledge there is no study with SMWT in RA patients. In our study, there was no statistical difference between the RA and control groups in terms of sensory evaluation with SMWT.

Potential limitations of our study are that the number of patients was relatively small and have low disease activity and no hand deformity. Another limitation of our study is that subgroups with chronic diseases such as DM, cervical radicular pain etc. were not differentiated.

CONCLUSION

As a result of the quantitative evaluations used in our study, the RA group was determined to have impaired fine skills and grip strength. Consistent with the literature, these findings had a negative effect on the daily life activities and function of these patients. SMWT sensory examination results were not statistically different between RA patients and control group. Because of our patients have low disease activity, further studies are needed in patients with higher disease activity for evaluation sensorial functions.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Yıldırım Beyazıt University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 17/12/2014, Decision No: 243).

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

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Knowledge and attitude of physicians for the diagnosis and management of obstructive sleep apnea

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ABSTRACT

Introduction: Obstructive sleep apnea (OSA) is a disease characterized by recurrent complete (apnea) or partial (hypopnea) upper respiratory tract obstructions episodes during sleep and often a decrease in blood oxygen saturation. The knowledge and attitude of physicians about OSA is very important as it influences the level of clinical suspicion of OSA which can lead to diagnose. Therefore, aim of this study was to evaluate the knowledge of and attitude to OSA of physicians.

Material and Method: This descriptive, cross-sectional survey study included 105 physicians in a university hospital. Participants completed the Obstructive Sleep Apnea Knowledge and Attitudes (OSAKA) questionnaire through the face-to-face interview method.

Results:The study respondents comprised 55 (52.8%) males and 50 (47.6%) females with a mean age of 34.4±7.3 years (range, 23-59 years). The years of experience was mean 10.45±7.37 years (range, 1–35 years). The knowledge level of physicians was mean score of 11.56±2.70 (64.1%). Attitude section, the mean score was 16.34±2.47 from a possible maximum of 25. A positive correlation was determined between the knowledge and the attitude scores of the physicians (r: 0.467 p<0.001).

Conclusion: These findings suggest that importance of the level of knowledge of physicians about OSA for early diagnosis and provision of treatment.

Keywords: Obstructive sleep apnea, physician, knowledge, attitude, survey, questionnaire, assessment

INTRODUCTION

Obstructive sleep apnea (OSA) is a disease characterized by recurrent complete (apnea) or partial (hypopnea) upper respiratory tract obstructions episodes during sleep and often a decrease in blood oxygen saturation (1). OSA is most common type of sleep-related breathing disorder (2). As a consequence of increasing obesity rates in the general population, the frequency of OSA is also rising (3). Untreated OSA causes cardiovascular diseases, decreases cognitive functioning, glucose intolerance and leads to metabolic diseases (4-7). OSA is an independent risk factor for developing hypertension (8). Daytime tiredness resulting from OSA may also cause traffic accidents resulting in morbidity and mortality (9).

Obstructive sleep apnea is quantified by the apnea–hypopnea index (AHI), which reflects the number of apneas and hypopneas per hour. Apnea–hypopnea index is derived after scoring data obtained using multi-channel polysomnography (PSG). PSG is considered the gold standard method for diagnosing OSA (10). Many individuals worldwide suffer from undiagnosed OSA (11). At ≥5 events/h apnea-hypopnea index (AHI), the overall population prevalence ranged from 9% to 38% and was higher in men (12). OSA is affecting nearly 9% of women and 24% of men in the general middle-aged population (13). Studies have suggested that educational interventions for physicians are needed to improve the identification and treatment of patients with OSA (14). The knowledge and attitude of physicians about OSA is very important as it influences the level of clinical suspicion of OSA which will lead to diagnose. The knowledge level and attitude of physicians about OSA and it has been emphasized that these differences affect the management of OSA (15-17). This study aims to evaluate the knowledge and attitude of physicians about obstructive sleep apnea.

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MATERIAL AND METHOD

This study was designed as a prospective, descriptive, cross-sectional questionnaire study, which was conducted in Afyonkarahisar Health Sciences University Medical Faculty hospital between February 2020 and April 2020.

Ethical Approval

Approval for this study was granted by the Clinical Researchs Ethics Committee of Afyonkarahisar Health Sciences University (Date: 07.02.20, Decision No: 2020/2 KAEK-2). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Sampling and Data Collection

The study included 105 physicians who worked in Afyonkarahisar Health Sciences University Medical Faculty Hospital and agreed to participate. In face-to-face interviews, the study participants completed the Obstructive Sleep Apnea Knowledge and Attitudes (OSAKA) questionnaire. The OSAKA questionnaire was developed by Schotland and Jeffe to evaluate the knowledge and attitudes of physicians about OSA syndrome (OSAS) (18). In a study of medical faculty students by Çelik, the Turkish version of the OSAKA questionnaire was determined to be a valid measurement tool with Turkish translation and cultural adaptation according to the criteria of Guillemin et al (19,20).

The OSAKA questionnaire consists of 18 items measuring the level of knowledge of OSAS, and 5 items to evaluate attitudes to OSAS (**Table 1**). The items which measure the level of knowledge include questions related to epidemiology, pathophysiology, symptoms, diagnosis and treatment, which are answered with 3 options of "true", "false", and "I don't know". The response of "I don't know" is accepted as false. In the calculation of the total knowledge points, a correct response is scored with 1 point and false or I don't know with 0 points. The total points are divided by 18 to provide a percentage. Of the 5 items evaluating attitudes to OSAS, the first 2 items are related to the clinical importance of OSAS. The reponse to these items are scored on a 5-point Likert-type scale from 1 (not important) to 5 (extremely important) (not important=1, somewhat important=2, Important=3, very important=4, extremely important=5).The other 3 items are related to confidence about diagnosis and treatment, with response scored from 1 (I strongly do not agree) to 5 (I strongly agree) (strongly disagree=1, disagree=2, neither agree nor disagree=3, agree=4, strongly agree=5). The demographic characteristics of the study participants were recorded.

Table 1. The obstructive sleep apnea knowledge questionnaire

1. Women with obstructive sleep apnea may present with fatigue only.
2. Uvulopalatopharyngoplasty is curative for a majority of people with obstructive sleep apnea.
3. The estimated prevalence of obstructive sleep apnea among adults is between 2% and 10%.
4. The majority of patients with obstructive sleep apnea snore.
5. Obstructive sleep apnea is associated with hypertension.
6. An overnight sleep study is the gold standard for diagnosing obstructive sleep apnea.
7. CPAP (continuous positive airway pressure) therapy may cause nasal obstruction.
8. Laser-assisted uvuloplasty is an appropriate treatment for severe obstructive sleep apnea.
9. The loss of upper airway muscle tone during sleep contributes to obstructive sleep apnea.
10. The most common cause of obstructive sleep apnea in children is the presence of large tonsils and adenoids.
11. A craniofacial and oropharyngeal examination is useful in the assessment of patients with suspected obstructive sleep apnea.
12. Alcohol at bedtime improves obstructive sleep apnea.
13. Untreated obstructive sleep apnea is associated with a higher incidence of automobile crashes.
14. In men, collar size 17 inches or greater is associated with obstructive sleep apnea.
15. Obstructive sleep apnea is more common in women than in men.
16. CPAP is the first-line therapy for severe obstructive sleep apnea.
17. Fewer than 5 apneas or hypopneas per hour is normal in adults.
18. Cardiac arrhythmias may be associated with untreated obstructive sleep

Statistical Analysis

Data obtained in the study were analyzed statistically using IBM SPSS 25 software (IBM Corpn., Armonk, NY, USA). Descriptive statistics of the data were stated as mean±standard deviation values. Conformity of the data to normal distribution was assessed with the Kolmogorov-Smirnov test. In the analysis of independent quantitative data, the Mann Whitney U-test was used, and for the analysis of qualitative independent data, the Chi-square test was applied. A value of $p<0.05$ was accepted as statistically significant. It was determined that a sample size of 84 was required to represent the population, with a sampling error of 5% at a 80% power.

RESULTS

The 105 physicians included in the study comprised 55 (52.8%) males and 50 (47.6%) females with a mean age of 34.4±7.3 years (range, 23-59 years). The mean years of experience were recorded 10.45±7.37 years (range, 1–35 years). The demographic data of the study participants are shown in **Table 2**.

Table 2. Sociodemographic characteristics of the participants

Socio-demographic characteristics	Frequency. n (%) n=105	Mean (SD)
Age (year)		34.4±7.3
Years of Experience		10.45±7.37
Men	55 (52.8%)	
Women	50 (47.6%)	

OSAS Knowledge Level

The mean score of the OSAKA responses for the knowledge section was 11.56±2.70 (64,1%). The knowledge section points are shown in **Table 3**. No statistically significant difference was determined between the mean knowledge points of males (11.67±2.86, 64,7%,) and females (11.44±2.54, 63,4%) (p=0.056). No statistically significant difference was determined between the years of professional experience and the knowledge points (p>0,05). The percentages of true and false responses in the knowledge category of the OSAKA questionnaire are shown in **Figure 1**. The highest rate of correct responses (92%) was given to the item, "untreated OSAS is associated with an increased incidence of traffic accidents". The lowest rate of correct responses (20%) was given to the item, "uvulopalatopharyngoplasty is curative in most OSAS patients".

Table 3. Knowledge scores of participants regarding OSA

Knowledge Score, N	(%)	Mean (SD)
Gender		
Men (n:55)	64.76	11.67±2.86
Women (n:50)	63.49	11.44±2.54

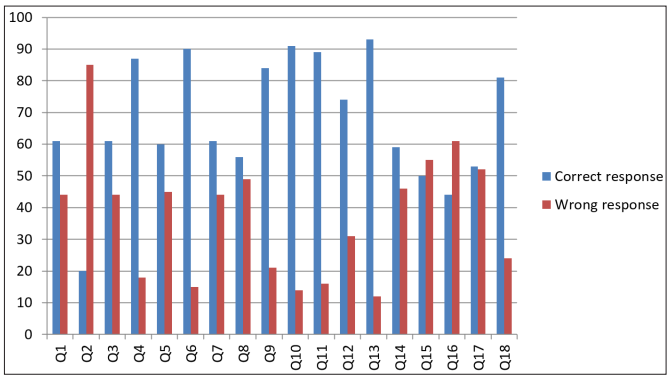

Figure 1. The frequency and percent of correct responses to questions on knowledge among the participants regarding OSA

OSAS Attitude Level

In the attitude section of the OSAKA questionnaire, the mean score was 16.34±2.47 from a possible maximum of 25. The highest points were determined in response to the item, "OSAS is important as a clinical disorder", to which 72% of the respondents stated that it was very important and extremely important. The lowest points (23%) were in reponse the item "I am confident in my management of patients receiving CPAP".

Correlation between the Knowledge and Attitude Levels

A moderate level positive correlation was determined between the knowledge and the attitude scores of the respondents (r: 0.467 p<0.001).

DISCUSSION

The level of knowledge and attitudes of physicians to OSAS were evaluated in this study. In this study, the total correct mean knowledge score was 64%. Physicians do not routinely engage in formal postgraduate training programs. The level of physicans knowledge at the time of graduation from medical school affect the quality of their practice after graduation. In a study by Schotland and Jeffe (18), who developed the OSAKA questionnaire, the level of knowledge of physicians in a university hospital in the USA was determined to be 76% . Wang et al. (21) measured the level of knowledge of anesthesia specialists in China and reported a score of 62%, while in another study of anesthesia specialists in Italy, the knowledge level score was found to be 66% (22). In a study by Deveraj (23) in Kuala Lumpur, the knowledge level score was determined to be 64.38% for primary level physicians in healthcare clinics treating inpatients and outpatients. Medical Faculty students in Nigeria were evaluated in another study and the knowledge level score was found to be 42% (24). In a previous study in Turkey, the knowledge level score of medical faculty students was reported to be 61% (19).

The score for the level of knowledge obtained in the current study was seen to be lower than that of the study by Schotland and Jeffe. In this study level of knowledge was similar to the scores reported by Wang and Deveraj and the study of medical faculty students in Turkey. Our results was higher than that of the medical faculty students in Nigeria. In the study by Schotland and Jeffe (18), a negative correlation was observed between age and the knowledge level score. No such correlation was determined in the current study, and there was no correlation between the years of professional experience and knowledge level. Furthermore, consistent with the findings of previous studies, there was no statistically significant difference in the knowledge level scores according to gender.

Within the items related to attitude, 72% of the respondents in the current study stated that OSAS was very important or extremely important as a clinical disorder. This response rate was determined to be higher compared to the study by Deveraj (23) (59%) and similar to the rates reported by Southwell (17) (68%) and Ojeda et al. (25) (72%). In the previous study of Turkish medical students, this rate was found to be 79% (18). Of the current study

participants, 21% stated that they were comfortable with their management of OSAS patients, which was similar to the result of the study by Deveraj (23%). In that same study, 7% of the primary level physicians were confident in their management of CPAP treatment, while this rate was found to be 23% in the current study. The responses of primary level physicians to the same question were determined to be 22% by Ojeda et al. (25) and 26% by Al-Khafaji et al. (16). This rate was determined as 21,3% in the study of Turkish medical students (19). The confident in their management of CPAP obtained in the current study was seen to be similar to the previusly studies.

Previosly studies have demonstrated that the positive correlation between knowledge and attitude scores. Al-Khafaji (16) found that primer care physicians have weak level correlation (r:0.142). Medical Faculty students in Nigeria have weak level correlation (r:0.142) (24). Solanski (14) and Corso (22) evaluated anesthesia specialists they found moderate correlation (respectively r:0.37; r:0.40). In a previous study in Turkey, correlation level of medical faculty students was reported weak (r:0.156) (19). Our results (r:0.467) was higher than that medical faculty students, similar that anesthesia specialists. The positive correlation between knowledge and attitude scores suggests that physicians with a better understanding of OSA-related problems are more confident in selecting the safest method for its management.

Limitations

This study had some limitations. The findings of this study must be interpreted in light of its limitations. First this was a cross-sectional survey of physicians in a university hospital.

Study group was formed only of physicians in a university hospital, and so there was a relatively low number of participants. Thus, we cannot infer causation from any of the associations we observed, and we also cannot generalize our results to physicians. It is a cross-sectional study and cannot establish the causal relationship between low knowledge and low education programs. Other potential factors, not assessed in this study, that may have influenced responses include the specialization and access to sleep specialists and sleep laboratories. However, a strong aspect of the study was that to avoid systematic prejudice, the questionnaires were completed in face-to-face interviews.

CONCLUSION

The knowledge and attitudes about OSAS of physicians in a university hospital were evaluated in this study. The results demonstrated a positive correlation between the knowledge level scores and the attitude scores. Although the knowledge and attitude levels were seen to be similar to those reported in previous studies, they were not at the expected level. This likely contributes to the rate of OSA underdiagnosis. Management of patients with OSA is linked to a variety of factors, including the personal knowledge, clinical experience. The results of this study emphasize the importance of the level of knowledge of physicians about treatment options for OSA for early diagnosis and provision of treatment to be able to bring the disease under control. Physicians need to enhance awareness of OSA. Education of OSA for physicians need to improve knowledge and management of this important and prevalent disorder.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for this study was granted by the Clinical Research Ethics Committee of Afyonkarahisar Health Sciences University (Date: 07.02.20, Decision No: 2020/2 KAEK-2).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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Original Article

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First-line palliative chemotherapy clinical benefit is a key determinant of survival in metastatic uterine leiomyosarcoma

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ABSTRACT

Aim: To evaluate whether obtaining a clinical benefit with first-line therapy in patients receiving palliative systemic therapy with a diagnosis of metastatic uterine leiomyosarcoma (ULMS) provides a survival benefit and the factors that may predict first-line therapy response.

Material and Method: This study was a retrospective observational single-center analysis conducted with patients diagnosed with metastatic ULMS. Patients who received palliative chemotherapy with an ECOG PS of 0 or 1 at the time of diagnosis of metastatic disease were included in the study. Main patient characteristics, first-line palliative treatment responses, progression-free survival, and overall survival (OS) were reviewed retrospectively. Multivariate analyses were performed to determine the independent predictive factors of first-line palliative treatment response and overall survival.

Results: Of the 36 patients whose medical records were evaluated retrospectively, 24 patients who were eligible for the study were included in the study. Gemcitabine plus docetaxel combination chemotherapy was the most commonly used treatment protocol (n=12, 50%) for first-line palliative treatment. While a complete response as a first-line treatment response could not be achieved, clinical benefit (partial remission and stable disease) and progressive disease were observed in 15 (62%) and 9 (37.5%) patients, respectively. Binary logistic regression analysis failed to detect any independent predictive factors for the clinical benefit of first-line palliative therapy. Median (OS) was 19.7 (95% CI, 4.1-35.3) months for all patients (N=24). Median OS was 25.6 (95% CI, 21.0-30.2) months and 6.9 (95% CI, 1.7-12.2) months for patients with and without the clinical benefit of first-line palliative chemotherapy (p=0.004). Cox-regression analysis revealed that increasing age at diagnosis of metastatic disease (HR=0.929, 95% CI 0.870-0.992, p=0.027), pulmonary metastasectomy (HR=0.162, 95%CI 0.031-0.863, p=0.033), and presence of first-line palliative chemotherapy clinical benefit (HR=0.195, 95% CI 0.063-0.606, p=0.005) were independent predictive factors for a better OS.

Conclusion: In metastatic ULMS, for which the survival benefit is not clear with palliative chemotherapy, prolonged survival can be obtained in patients with clinical benefit with first-line palliative chemotherapy. There is a need for new studies to determine the factors that will predict the clinical benefit of first-line palliative chemotherapy.

Keywords: Metastatic uterine leiomyosarcoma, palliative chemotherapy, clinical benefit, overall survival

INTRODUCTION

Uterine sarcomas are highly aggressive mesenchymal tumors and make up less than five percent of all malignant uterine tumors (1). Although there are numerous histologic subtypes, leiomyosarcomas are the most common (nearly 70%) subgroup of uterine sarcomas (2,3). The main goal in treating early-stage disease is complete surgical resection of the tumor bulk (4). The clinical course of uterine leiomyosarcomas (ULMSs) is quite aggressive; even with successful surgical treatment, there is a chance of recurrence of up to 70% (5). Approximately twenty percent of patients are stage IV at diagnosis (6). In the presence of isolated metastases, a metastasectomy accompanying systemic palliative treatments with and without radiotherapy may be an appropriate treatment approach (7). Also, systemic therapy should be considered in patients who are not suitable candidates for surgery because of poor performance status or high tumor burden. Although these are among the treatment options, it is suggested that leiomyosarcomas are not sensitive to radiotherapy and chemotherapy (8). In a study in which 5-year disease-specific survival was reported as 28.7% for stage IV ULMS, chemotherapy and radiotherapy could

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not have been shown to provide a survival benefit (6). Although there is no agreed-upon chemotherapy regimen for the treatment of advanced ULMS, the addition of docetaxel to gemcitabine monotherapy resulted in both better objective treatment response and improved survival for soft tissue sarcomas (9).

In our study, we aimed to evaluate whether obtaining a clinical benefit with first-line therapy in patients receiving palliative systemic therapy with the diagnosis of metastatic ULMS provides a survival benefit and the factors that may predict first-line therapy response.

MATERIAL AND METHOD

This study was a retrospective observational single-center analysis conducted with patients with a diagnosis of metastatic ULMS.

Ethical approval was obtained University of Health Sciences Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Clinical Researchs Ethics Committee (Date: 13.01.2021, Decision No: 2021-01/942). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients who received palliative chemotherapy in the medical oncology clinic of the University of Health Sciences Dr. Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital between December 2010 and February 2021 were included. Medical records in the hospital data processing system and patient files were reviewed retrospectively. Patients older than 18 years of age and only those with an ECOG PS of 0 or 1 at the time of diagnosis of metastatic disease were included. Patients with an ECOG PS of 2 or more were excluded from the study. Patients' demographic characteristics, stages at the time of diagnosis, adjuvant treatment modalities, recurrence time, metastatic sites, whether metastasis surgery was performed, systemic palliative treatments, systemic treatment response rates were recorded. Time to metastasis was estimated as the time from surgery to the development of metastasis for patients who underwent curative surgery and zeroed for metastatic patients at diagnosis. Patients who achieved clinical benefit (complete response, partial remission, stable disease) or progressive disease response with first-line therapy were identified. Factors that may affect the clinical benefit of first-line therapy were evaluated. For first-line palliative systemic therapy, progression-free survival was defined as the time from initiation of therapy to progression. Overall survival (OS) was defined as the time from diagnosis of metastatic disease to death or last follow-up. Overall survival analysis was performed of patients with and without clinical benefit from first-line palliative therapy. Prognostic factors that could predict OS were analyzed. Study results were compared with the literature data.

Statistical Analysis

Descriptive statistics were used to show the distribution of the main characteristics of the population. A binary logistic regression model was constructed that includes factors that could predict the clinical benefit of first-line palliative systemic therapy. Survival rates were estimated using the Kaplan-Meier method, and groups were compared using the log-rank test for difference in survival. A Cox regression model was carried out that includes crucial factors that could predict death. Statistical analysis was performed using SPSS software (SPSS for Windows, version 24.0, SPSS Inc., Chicago, USA). All statistical tests were two-sided, and a $P < 0.05$ value was considered statistically significant.

RESULTS

Medical records of 36 patients with metastatic ULMS were reviewed retrospectively. Eight patients with an ECOG PS of 2 or higher and four patients with insufficient medical records were excluded. Twenty-four patients with a median age of 54.9 (35.8-70.7) were included in our study. The median follow-up time was 13.3 (range, 2.3-58.8) months. Four (16.7%) patients were in the metastatic stage, and 20 (83.3%) patients underwent curative primary surgical treatment with an early-stage disease at the initial diagnosis. Median time to the metastatic stage was 17.1 (range, 0.0-123.6) months. The most common site of metastasis (n=20, 83.3%) was lung, and 5 (20.8%) patients underwent pulmonary metastasectomy. Main patient and disease characteristics are shown in **Table 1**.

Table 1. Main patient and disease characteristics

Parameter	Number (N=24)	Percent (%)
Age, median (range)	54.9	35.8-70.7
Menopausal status		
Premenopausal	10	41.7
Postmenopausal	14	58.3
Stage at first diagnosis		
Stage I	14	58.3
Stage II	1	4.2
Stage III	4	16.7
Stage IV	5	20.8
Presence of metastases at diagnosis	4	16.7
Primary surgery	20	83.3
Adjuvant chemotherapy	10	41.7
Adjuvant radiotherapy	2	8.3
Metastasis sites		
Lung	20	83.3
Peritoneum	7	29.2
Bone	4	16.7
Liver	4	16.7
Lymph node	2	8.3
Only Lung	10	41.7
Lung with other sites	10	41.7
Only extrapulmonary	4	16.7
Metastasectomy	5	20.8

Twenty-four patients included in the study were given various palliative chemotherapy regimens. In addition, patients had received one to four lines of palliative systemic chemotherapy. Gemcitabine plus docetaxel combined chemotherapy was given most frequently (n=20, 50.0%) as first-line treatment. While clinical benefit was obtained with first-line chemotherapy in 15 (62.5%) patients, the first-line treatment response of 9 (37.5%) patients was progressive disease. Median progression-free survival with first-line chemotherapy was 6.1 (95% CI, 1.5-10.7) months. First-line chemotherapy regimens, the responses obtained with first-line treatment, and the number of patients receiving further treatments are shown in **Table 2**.

Median OS was 19.7 (95% CI, 4.1-35.3) months for all patients (N=24) (**Figure 1A**). The OS rate at 36 months was 22.3% for all patients. Median OS was 25.6 (95% CI, 21.0-30.2) months and 6.9 (95% CI, 1.7-12.2) months for patients with and without the clinical benefit of first-line palliative chemotherapy, respectively (p=0.004) (**Figure 1B**). The OS rate at 36 months was 28.3% and 11.1% for patients with and without the clinical benefit of first-line palliative chemotherapy, respectively. In the binary logistic regression analysis, in which factors (age, menopausal status, stage at diagnosis, primary surgical treatment, adjuvant chemotherapy, adjuvant radiotherapy, site of metastasis, time to metastasis) that may affect the benefit of first-line palliative chemotherapy were included, no independent predictive factor was found to predict the benefit of first-line palliative chemotherapy.

In Cox-regression analysis including factors that may predict OS, increasing age at diagnosis of metastatic disease (HR=0.929, 95% CI 0.870-0.992, p=0.027), pulmonary metastasectomy (HR=0.162, 95%CI 0.031-0.863, p=0.033), and presence of first-line palliative chemotherapy clinical benefit (HR=0.195, 95% CI 0.063-0.606, p=0.005) were independent predictive factors for a better OS (**Table 3**).

Table 2. Features associated with palliative chemotherapies

Parameter	Number (N=24)	Percent (%)
First- line chemotherapy		
Gemcitabine plus docetaxel	12	50.0
IMA	8	33.3
Others	4	16.7
Best response with first-line chemotherapy		
Partial remission	4	16.7
Stable disease	11	45.8
Progressive disease	9	37.5
Clinic benefit rate (CR + PR + SD)	15	62.5
Patients receiving second-line therapy	13	54.2
Patients receiving third-line therapy	9	37.5
Patients receiving fourth-line therapy	3	12.5

IMA, chemotherapy scheme consisting of ifosfamide, mesna and adriamycin; CR, complete response; PR, partial remission; SD, stable disease

Table 3. Cox-regression analysis results including factors that may predict overall survival

Parameter	HR	95% CI		p-value
		Lower	Upper	
Age	0.929	0.870	0.992	0.027
Presence of metastases at diagnosis (Yes vs No)	2.706	0.525	13.952	0.234
Time to metastasis	1.005	0.980	1.030	0.726
Only extrapulmonary metastasis (Yes vs No)	3.205	0.660	15.549	0.148
Metastasectomy (Yes vs No)	0.162	0.031	0.863	0.033
First-line CTx clinical benefit (Yes vs No)	0.195	0.063	0.606	0.005

CTx, chemotherapy

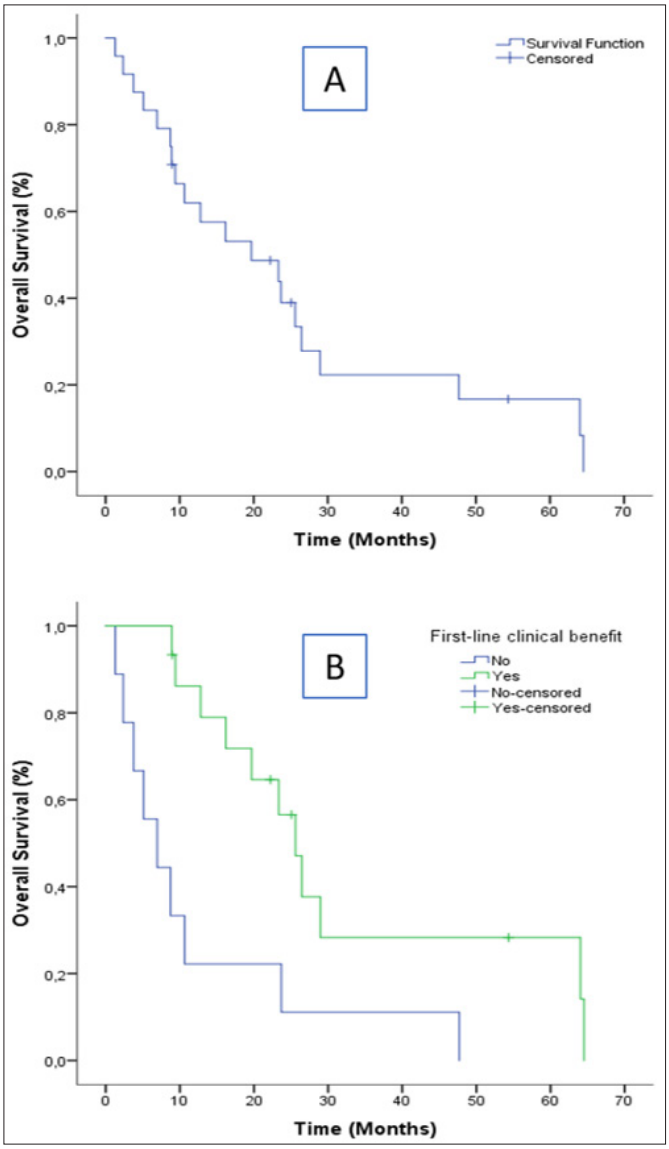

Figure 1. Kaplan-Meier curves of overall survival, A: Plot of all patients receiving palliative chemotherapy, B: Comparative plot of patients with and without the clinical benefit of first-line palliative chemotherapy.

DISCUSSION

In our study, we observed that OS of patients who achieved clinical benefit with first-line palliative chemotherapy was better than those whose chemotherapy response was in the form of progressive disease. However, we were unable to demonstrate any independent predictive factors that could predict first-line therapy response. In multivariate analysis, we found that increasing age at diagnosis of metastatic disease, metastasectomy, and clinical benefit from first-line palliative chemotherapy were independent predictive factors for a better OS.

Leiomyosarcomas constitute the most common histological subtype of all soft tissue sarcomas (10). However, studies mainly cover all soft tissue sarcomas and include gastrointestinal stromal tumors that differ in their clinical course and treatment. The majority of studies evaluating the efficacy of chemotherapy in patients with advanced or metastatic uterine sarcoma concluded with negative results and no survival benefit (11). Moreover, since ULMS is a very rare disease, it is challenging to conduct a study in this patient group, and the literature data on chemotherapy efficacy for ULMS is limited. In a phase II study in which Hensley et al. (12) evaluated the efficacy of gemcitabine plus docetaxel treatment in 29 patients with metastatic ULMS, they achieved an objective response (complete and partial remission) in 53% of the patients. Also, PFS and OS were reported at approximately six months and 18 months, respectively, in the same study (12). Although the treatment response rate in our study was considerably lower than that of Hensley et al., the PFS and OS in our study were almost the same as in this study. In addition, in another phase II study by Hensley et al. (13), an objective response rate of 35.8%, approximately 4.5 months, and 16 months PFS and OS was obtained with fixed-dose rate gemcitabine plus docetaxel as first-line therapy for metastatic ULMS. The OS range in this study was quite wide, ranging from 4 to 41.3 months, and some patients lived very long with gemcitabine plus docetaxel therapy (13). Similarly, there was a fairly wide OS range in our study. In addition, there is a 17% difference between patients who are alive at the end of the third year compared to patients who have clinical benefit from first-line chemotherapy and those who do not. The literature data as mentioned above and our results suggest that chemotherapy may be beneficial for OS in some patients with metastatic ULMS. To the best of our knowledge, no prospective randomized study in which a significant OS benefit with chemotherapy for metastatic ULMS can be clearly demonstrated. However, in an observational cohort study of 7455 patients based on the 1998-2013 National Cancer Database by Seagle et al. (14), chemotherapy was associated with 8.5 (19.4 vs. 10.9, $p<0.001$) months increased survival of women with metastatic leiomyosarcoma.

In a phase III study in which patients with a metastatic soft tissue sarcoma diagnosis showed a significant improvement of 12% in the overall response rate and nearly three months in PFS with the addition of ifosfamide to doxorubicin monotherapy, but no significant difference in OS (15). In this study, ULMS was not examined as a separate group, and factors that could predict death were not evaluated statistically. However, in the phase III GeDDiS study involving 257 patients with treatment-naive metastatic soft tissue sarcoma, no difference was observed between patients treated with gemcitabine plus docetaxel and doxorubicin in terms of treatment response rates, PFS, and OS (16). Although a subgroup analysis was performed comparing leiomyosarcomas with other sarcoma subtypes and ULMS with other sarcoma subtypes, no subgroup analysis was performed in terms of factors that would predict treatment response (16). In order to minimize the effect of other factors on survival, we included patients with an ECOG PS of 0 or 1 at the start of treatment. Although we demonstrated in our study that providing disease control with first-line palliative chemotherapy was associated with more prolonged survival, we could not detect any factor that could predict treatment response. This result can be explained by the small number of patients and the retrospective nature of our study. However, there is a lack of literature data regarding the factors that can predict the palliative systemic treatment response in ULMS.

Previous studies have demonstrated that big tumor size, high mitotic index, high grade, advanced stage, advanced age, and inadequate surgical treatment are negative prognostic factors for ULMS (5,6,17,18). However, none of these studies evaluated patients receiving palliative chemotherapy for the advanced disease alone. Our study differs from these studies in that the patient profile is a more specific group, and for this reason, we think that our study is valuable. In our study, increasing age at diagnosis of metastatic disease, metastasectomy, and clinical benefit from first-line palliative chemotherapy were positive prognostic factors. Bernstein-Molho et al. (19) observed a survival difference of approximately 14 months with metastasectomy in a study involving 33 patients with metastatic ULMS. In an analysis of 128 patients with recurrent ULMS, secondary cytoreductive surgery prolonged time to recurrence (20). Moreover, in the same analysis, neither chemotherapy nor radiation showed improved outcomes in patients with recurrent ULMS (20). Also, considering the high hazard ratios in our analysis, we can say that the presence of only extrapulmonary metastases and metastatic disease at the time of diagnosis may be negative prognostic factors. The inadequacy of the number of patients in our study may be the reason for not showing statistical significance for these factors.

CONCLUSION

In metastatic ULMS, for which the survival benefit is not clear with palliative chemotherapy, prolonged survival can be obtained in patients with clinical benefit with first-line palliative chemotherapy. There is a need for new studies to determine the factors that will predict the clinical benefit of first-line palliative chemotherapy.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for the study was given by the University of Health Sciences Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Clinical Researchs Ethics Committee (Date: 13.01.2021, Decision No: 2021-01/942)

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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Dapsone can be a new treatment option for reducing the detrimental effect of priapism

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ABSTRACT

Aim: This study aims to analyze the effect of dapsone against ischaemia-reperfusion injury on corporal tissue in a model of induced-priapism in rats.

Material and Method: A total of 24 rats were randomized into three groups. Group 1 was defined as the control group. Ischaemia-reperfusion injury was evaluated following the priapism model in Group 2. Group 3 had similar procedures to the rats in Group 2. Group 3 additionally had 12.5 mg/kg dapsone administered intraperitoneally 30 minutes after priapism.

Results: Biochemical analysis of blood indicated a significant increase in Group 3 in terms of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activity and total antioxidant status (TAS) values compared with Group 2 (p:0.002, p:0.029 and p:0.009, respectively). The highest values of malondialdehyde (MDA), protein carbonyl (PC) and total oxidant status (TOS) were recorded in Group 2 ($p<0.001$). Interleukin 1beta (IL-1beta), Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF alpha) levels were found to be significantly decreased in Group 3 compared with Group 2 (p:0.022, p:0.049 and $p<0.001$, respectively). Direct microscopic evaluation determined an improvement in inflammation, edema, desquamation and vasocongestion scores in Group 3 compared to Group 2 ($p<0.05$).

Conclusion: Dapsone has a protective effect on ischaemia-reperfusion injury in corporal tissue.

Keywords: Dapsone, priapism, ischaemia reperfusion injury, treatment

INTRODUCTION

Priapism is an urgent urologic pathology defined by full or partial tumescence lasting longer than 4 hours without sexual stimulation (1). Priapism is characterised by three different categories as ischaemic (low flow, veno-occlusive), non-ischaemic (high flow, arterial) and stuttering (intermittent, recurrent ischaemic) priapism. Ischaemic priapism is the most common form of priapism that accounts for 95% of the cases (2). It is predicted that the incidence of ischaemic priapism is between 0.34-1.5 per 100,000 person in a year (3). Basic pathophysiology of ischaemic priapism includes venous obstruction, and stasis in penile corpus cavernosa. This clinical presentation, which is known as, penile compartment syndrome, poses a threat for the vitality of penile tissues by developing anoxic, hypercarbic, acidic and glucopenic environment (2). Reoxygenation of penile corpus cavernosa is essential for restoration of normal sexual functioning. On the other hand, after priapism is corrected with the effective treatment approaches, ischaemia-reperfusion injury occurs which leads to severe cellular damage paradoxically following the revascularization. Several different pathways are involved in the pathophysiology of ischaemia-reperfusion injury. The basic mechanism can be explained by reactive oxygen species (ROS) which are produced rapidly when dense molecular oxygen enters into the cell. ROS, which are abundant in the environment during the ischaemia-reperfusion period, render natural antioxidant mechanisms ineffective. Critically elevated levels of ROS lead to a severe tissue destruction by interacting with several vital units such as membrane lipids, macro proteins and nucleic acids (4). In this regard, keeping the ischaemia-reperfusion injury

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due to termination of priapism at the lowest levels holds critical importance for the cases to maintain a healthy sexual life afterwards. Different experimental priapism models of previous years has focused that many different molecules were used to minimise ischaemia-reperfusion injury in penile corpus cavernosa (4-9).

Dapsone (4,4'-diaminodiphenylsulfone) is a derivative of aniline that belongs to the group of synthetic sulfones exhibiting antibacterial and antiparasitic properties. It inhibits the growth of microorganisms by suppressing folic acid synthesis in a competitive manner with paraaminobenzoate, which is a critical substrate of dihydropteroate synthase. Since 1940's, it has been included in the multidrug therapy protocol for leprosy treatment by World Health Organization (10). Additionally, different infectious diseases are also preferred in Malaria disease and Pneumocystis carinii pneumonia which is manifested in immune-suppressive patients (11). Apart from its antimicrobial properties, Dapsone shows an anti-inflammatory activity by blocking the leukocyte enzyme myeloperoxidase (10). It also activates the scavenging enzyme system and inhibits apoptosis (12,13). Recent studies have revealed that dapsone can be used effectively and safely in infective pathologies, as well as in minimizing tissue damage by exhibiting anti-inflammatory, anti-apoptotic and anti-oxidant properties against oxidative stress in many different systems such as skin, kidney, testis, heart, brain and spinal cord (11-18).

The aim of the present study is to determine the possible protective effect of dapsone against ischaemia-reperfusion injury in penile corpus cavernosa. According to our knowledge, this is the first experimental study in the English literature administering dapsone to rats with an induced-priapism model.

MATERIAL AND METHOD

After obtaining approval from the Tokat Gaziosmanpaşa University Animal Studies Ethics Committee (Date: 08.04.2021, Decision No: 2021-HADYEK-06), a total of twenty four male Wistar albino rats, 7–7.5 months old and weighing 310–350 g, were used in this study. The rats were handled in the laboratory according to institutional guidelines as well as the Guide for Care and Use of Laboratory Animals of the National Research Council. The animals were housed under standard vivarium conditions in a climate-controlled room (18–22°C, 40–60% humidity, and 12-h light/dark cycle), with free access to water and standard rodent chow. Experimental animals were divided into three groups. Group 1 was defined as the control group. Group 2 was designed as the ischaemia-reperfusion group. Group 3 included the treatment group.

All surgical procedures were performed in sterile conditions and under the appropriate depth of anesthesia. To this aim, ketamine 1 mg/kg xylazine and 50 mg / kg ketamine were used intraperitoneally.

Group 1 rats were sham group and only penectomy was performed in this group. Penile tissues were sent to pathology laboratory for histopathological examination. Additionally, blood samples were taken from inferior vena cava for biochemical analysis.

A model of priapism was performed for rats in Group 2. This model was implemented using the method previously defined by Ciftci et al (8). Accordingly, constriction bands were prepared by dividing 16 Fr silicone foley catheter into straight pieces with an approximate length of 2 mm. A 50 cc syringe was used as vacuum erection device on the penises of rats. A full penile erection was achieved by applying negative pressure with the syringe. Afterwards, a priapism model was constructed by placing constriction bands at the root of the penis (**Figure 1**) (6,8). Constriction bands were removed after maintaining priapism for an hour in total. Rats rested for an hour for the evaluation of ischaemia-reperfusion injury (8). Finally, penectomy was performed for histopathological examination and blood samples were obtained from their inferior vena cava for biochemical analysis.

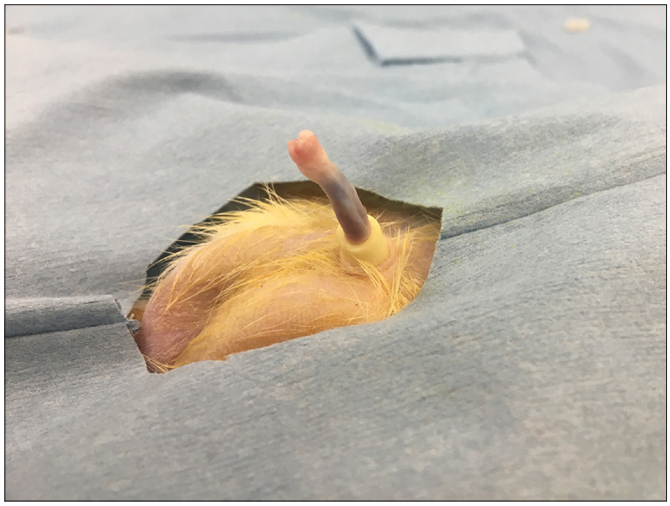

Figure 1. Experimental priapism model in rat

Group 3 had similar procedures to the rats in Group 2. Group 3 additionally had a single dose of dapsone (12.5 mg /kg) administered intraperitoneally 30 minutes after priapism (11,13). Dapsone was suspended in 4.5% v/v poly (ethylene glycol)/water. Ninety minutes after the administration of dapsone, a blood sample was taken from the inferior vena cava by applying penectomy. Dapsone were provided from Sigma-Aldrich (CAS Number: 80-08-0).

At the end of the experiment, cervical dislocation was applied to all rats and their vital functions were terminated.

Biochemical Evaluation

Measurement of Malondialdehyde (MDA) Level

MDA is the latest product of lipid peroxidation and is a frequently used marker as an indicator of oxidative stress. MDA reacts in an acidic environment under high temperatures with thiobarbituric acid (TBA), forming a pink compound. The optical density of this compound was measured at a wavelength of 532 nm and the MDA level was calculated. The results were measured using a standard graph prepared with standard 1,1,3,3-tetraethoxypropane serial dilutions. MDA level was expressed as μmol/L (19).

Measurement of Protein Carbonyl (PC) Level

Evidence of oxidative stress manifests an increase in reactive carbonyl groups in protein oxidation. PC groups were analyzed and assessed by spectrophotometrical means at 370 nm based on the principle that hydrazine occurs as a result of the reaction of carbonyl groups of proteins with 2.4 Dinitrophenylhydrazine (DNPH) (20). PC level was expressed as nmol/ml.

Measurement of Superoxide Dismutase (SOD) Activity

The principle of this experiment is based on the formation of violet-colored formazan compound via the superoxide radical, which is created through xanthin-xanthin oxidase system, by reducing the nitro-blue tetrazolium (NBT) compound in the environment. This compound gives maximum absorption at a wavelength of 560 nm on a spectrophotometer. In the serum added to the reaction environment, the enzyme SOD removes the formed superoxide radicals from the environment, preventing the reduction of NBT in direct proportion to its activity. Enzyme activity was calculated by comparing the absorption value found by adding SOD to the environment with the absorbent of the blind experiment in which the enzyme was not added (21). The SOD activity unit was expressed as U/L.

Measurement of Glutathione Peroxidase (GSH-Px) Activity

The glutathione reductase enzyme downgrades the upgraded GSH while at the same time increasing nicotinamide adenine dinucleotide phosphate (NADPH) and transforming it into nicotinamide adenine dinucleotide (NADP). It provides NADPH absorption at a wavelength of 340 nm on the spectrophotometer. The reduction in absorption due to conversion to NADP allows measurement of GSH-Px activity (22). GSH-Px activity unit was expressed as U/L.

Measurement of Tumour Necrosis Factor-Alpha (TNF-alpha), Interleukin-1 Beta (IL-1 Beta), Interleukin-6 (IL-6) Levels

The kits were supplied from Atlas Biotechnology (Ankara, Turkey). TNF- alpha (Cat.No E0764Ra), IL-1 beta (Cat.No E0119 Ra), and IL6 (Cat.No E0135Ra) levels measured in serums using ELISA kits according to the instructions of manufacturers. Measurement was carried out with Multiskan™ FC Microplate Photometer device. TNF- alpha and IL-1 beta were expressed as pg/ml while IL-6 ng/ml.

Measurement of Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) Level

TAS and TOS levels were measured using a novel automated colorimetric method developed by Erel (23,24). TAS were expressed as mmol Trolox equivalent/L while TOS μmol H_2O_2 equivalent/L.

Histopathological Evaluation

The penile tissues of rats were kept in 10% buffered formalin solution for 2 days. Samples were cut by 4 μm by microtome and stained with haematoxylin and eosin solutions following the grossing and tissue preparation. Tissue slides were examined via upright light microscope (Nikon Eclipse E600). Tissue slides were assessed and scored in the categories of vasocongestion, inflammation, desquamation and edema. A semiquantitative scoring system was employed for scoring histopathological alterations in parallel to the one used by Senturk et al. These parameters were scored between 0 and 3 as follows: 0: normal, 1: mild, 2: moderate and 3: severe (25).

Statistical Method

Statistical analysis in this study were conducted using the SPSS (SPSS Inc., Chicago, IL, USA) package program. Descriptive statistics were presented as mean ± standard deviation and median (min-max) depending on the data's normal distribution. Normality distribution of the data was evaluated using the Shapiro-Wilk test. Levene's test was used to test the homogeneity of variances. ANOVA was used for normally distributed data and Kruskal-Wallis test was used for non-normally distributed data in continuous variable comparisons between three independent groups. In order to determine the different groups after ANOVA analysis, Tukey post-hoc test was used when the assumption of homogeneity of variances was met and Games-Howell post-hoc test was used when the assumption of homogeneity of variances was not met. After the Kruskal-Wallis test, the Mann-Whitney U test with Bonferroni correction post-hoc test was used to determine the different groups. P values < 0.05 were considered statistically significant.

RESULTS

Our histopathological results are documented in **Table 1**. When penile tissue samples in Group 2 were examined in detail, it was determined that inflammation, edema, desquamation and vasocongestion scores were extremely negatively affected compared to Group 1 (p<0.001). The mean inflammation score in Group 3 was calculated as 0.88±0.354 and inflammation scores decreased dramatically compared to Group 2 (p: 0.031) (**Figure 2**). No penile tissue samples in Group 3 had severe edema and vasocongestion. These scores were significantly lower in Group 3 than in Group 2 (p:0.018 and p:0.037, respectively) (**Figure 3** and **Figure 4**). In Group 2, the desquamation score was observed as 1.88±0.354. This value was significantly higher than Group 3 (p:0.038) (**Figure 5**).

Our serum biochemical analysis results are detailed in **Table 2**. In Group 2, MDA and PC levels, the latest products of lipid peroxidation and protein oxidation, were calculated as 1.53±0.33 and 606.4±59.06. These values were recorded as considerably higher than Group 1 (p:0.001 and p<0.001 respectively). In Group 3, it was noted that there was a dramatic decrease in MDA and PC levels compared to Group 2 (p:0.003 and p:0.015, respectively). The levels of the pro-inflammatory cytokines including IL-1 beta, IL-6, and TNF-alpha were observed to be highest in Group 2 (p<0.001). On the other hand, serum samples of rats in Group 3 showed a significant decrease in these inflammatory markers (p:0.022, p:0.049 and p<0.001, respectively). TOS levels were recorded as 11.2±1.78 in Group 2. This level was quite high compared to Group 1 (p<0.001). In Group 3, the TOS level was 8.37±1.58 and these values were recorded as significantly lower than Group 2 (p:0.002). When the antioxidant enzyme level is analyzed, SOD and GSH-px Px activity were significantly higher in Group 3 compared to Group 2 (p:0.002 and p:0.029, respectively). Similarly, the TAS level was noted as 1.39±0.30 in Group 3, which was recorded as significantly increased compared to Group 2 (p:0.009).

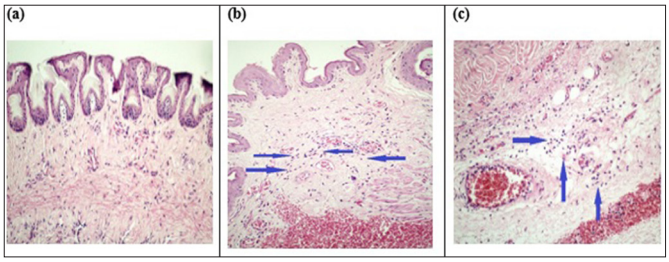

Figure 2. Inflammation between groups **a.** No inflammation in Group 1. **b.** Moderate inflammation foci accompanying congested vascular structures and hemorrhage areas in Group 2. **c.** Local and mild inflammation in Group 3. Magnification is x10 for all images, blue arrows indicate inflammatory cells and neutrophills.

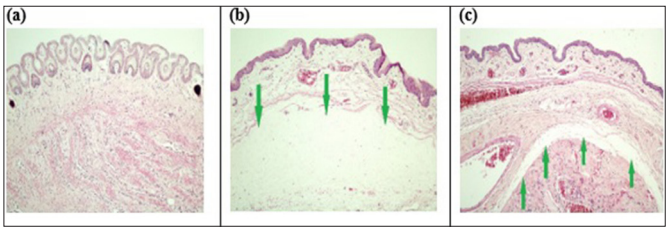

Figure 3. Evaluation of edema between groups. **a.** No edema in Group 1. **b.** Extensive edema in Group 2. **c.** Mild and focal edema in Group 3. Green arrows indicate edematous areas, magnification is x10 for all images.

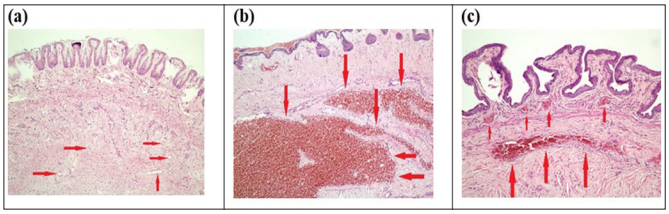

Figure 4. Comparison of vasocongestion between groups. **a.** Only normal caliber vascular structures (red arrows), scored as no vasocongestion in Group 1. **b.** Extensive vasocongestion and focal haemorrhage in Group 2 (red arrows). **c.** Vasocongestion of medium and small sized vascular structures in Group 3 (red arrows). Microscopic magnification is x10 for all photos.

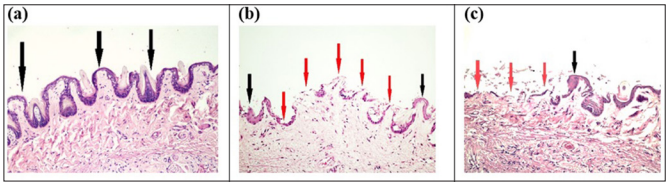

Figure 5. Desquamation alterations are shown. **a.** No desquamation in Group 1. **b.** Medium desquamation occurring in Group 2. **c.** Focal and mild desquamation detected in Group 3 Black arrows indicate normal thickness epithelium, whereas red arrows show desquamated areas. Magnification is x20 for both images.

Table 1. Comparison of Inflammation, Desquamation, Edema, and Vasocongestion scores between rat groups

	Groups	N	Mean±SD	Median (min-max)	*P* values	Post-hoc *P* values
Inflammation	1	8	0.25±0.463	0 (0-1)	<0.001[a]*	1-2[b]: <0.001*
	2	8	1.88±0.354	2 (1-2)		1-3[b]: 0.327
	3	8	0.88±0.354	1 (0-1)		2-3[b]: 0.031*
Desquamation	1	8	0.13±0.354	0 (0-1)	<0.001[a]*	1-2[b]: <0.001*
	2	8	1.88±0.354	2 (1-2)		1-3[b]: 0.422
	3	8	0.75±0.707	1 (0-2)		2-3[b]: 0.038*
Edema	1	8	0.38±0.518	0 (0-1)	<0.001[a]*	1-2[b]: <0.001*
	2	8	2.88±0.354	3 (2-3)		1-3[b]: 0.312
	3	8	1.13±0.354	1 (1-2)		**2-3[b]: 0.018***
Vasocongestion	1	8	0.5±0.535	0.5 (0-1)	<0.001[a]*	**1-2[b]: <0.001***
	2	8	2.75±0.463	3 (2-3)		1-3[b]: 0.249
	3	8	1.38±0.518	1 (1-2)		**2-3[b]: 0.037***

SD: standard deviation, *Statistically significant (p<0.05), [a] Kruskal Wallis test, [b] Post-hoc test: Mann Whitney U test with Bonferroni correction

Table 2. Comparison of GSH-px, SOD, TAS, PC, MDA, IL-1beta, IL-6, TNF- alpha and TOS values from serum between rat groups

	Groups	N	Mean±SD	Median (min-max)	*P* values	Post-hoc *P* values
GSH-px (U/L)	1	8	1030±141	1002.5 (832 - 1295)	<0.001[a*]	1-2[c]:<0.001*
	2	8	792.05±64.28	807.6 (706 - 887)		1-3[c]:0.087
	3	8	923.6±54.27	920.1 (845 - 1000)		2-3[c]:0.029*
SOD (U/L)	1	8	12.78±1.72	12.89 (9.22 - 15.2)	<0.001[a*]	1-2[c]:<0.001*
	2	8	7.59±0.96	7.34 (6.34 - 8.96)		1-3[c]: 0.001*
	3	8	10.03±0.89	10.07 (9.11 - 11.87)		2-3[c]:0.002*
TAS (mmol Trolox equivalent/L)	1	8	1.68±0.23	1.75 (1.3 - 2)	<0.001[a*]	1-2[c]:<0.001*
	2	8	0.98±0.17	0.95 (0.8 - 1.35)		1-3[c]:0.063
	3	8	1.39±0.30	1.37 (1 - 1.8)		2-3[c]:0.009*
PC (nmol/ml)	1	8	477.47±22.72	479.4 (445.6 - 519.1)	<0.001[a*]	1-2[c]:<0.001*
	2	8	606.4±59.06	615.1 (500.12 - 682.5)		1-3[c]:0.134
	3	8	528.5±60.82	513.45 (422.1 - 607.3)		2-3[c]:0.015*
MDA (µmol/L)	1	8	0.79±0.07	0.81 (0.67 - 0.86)	<0.001[a*]	1-2[d]:0.001*
	2	8	1.53±0.33	1.47 (1 - 1.92)		1-3[d]:0.001*
	3	8	0.95±0.06	0.94 (0.87 - 1.07)		2-3[d]:0.003*
IL-1 beta (pg/ml)	1	8	260.65±37.94	265.4 (205.3 - 312.1)	<0.001[b*]	1-2[e]:0.001*
	2	8	367.47±33.94	384.65 (296.7 - 399.1)		1-3[e]:1.000
	3	8	282.48±31.71	287.7 (214.4 - 321.2)		2-3[e]:0.022*
IL-6 (ng/ml)	1	8	5.81±0.84	5.92 (4.22 - 7.2)	<0.001[b*]	1-2[e]:<0.001*
	2	8	11.26±2.19	11.04 (8.2 - 15)		1-3[e]:0.206
	3	8	7.49±0.96	7.94 (5.56 - 8.21)		2-3[e]:0.049*
TNF- alpha (pg/ml)	1	8	313.03±43.15	310.65 (234.5 - 376.5)	<0.001[a*]	1-2[c]:<0.001*
	2	8	445.57±48.98	439.45 (376.2 - 522.1)		1-3[c]:0.503
	3	8	337.96±38.79	328.15 (288.9 - 411)		2-3[c]:<0.001*
TOS (µmol H_2O_2 equivalent/L)	1	8	7.31±0.70	7.5 (5.9 - 8.1)	<0.001[a*]	1-2[c]:<0.001*
	2	8	11.2±1.78	11 (9.2 - 15.1)		1-3[c]:0.321
	3	8	8.37±1.58	8.1 (6.1 - 10.9)		2-3[c]:0.002*

GSH-px: Glutathione Peroxidase, SOD: superoxide dismutase, TAS: total antioxidant status, PC: protein carbonyl, MDA: malondialdehyde, IL-1 beta: Interleukin 1 beta, IL-6: Interleukin 6, TNF-alpha: tumor necrosis factor alpha, TOS: total oxidant status, SD: standard deviation
*Statistically significant (p<0.05), [a] ANOVA test, [b] Kruskal Wallis test, [c] Tukey's post hoc multiple comparison test, [d] Games-Howell post hoc multiple comparison test, [e] Mann Whitney U with Bonferroni correction post hoc test

DISCUSSION

Priapism represents a true erection physiology disorder. Although there are three different types, the most important form is ischemic priapism, which is high prevalence and causes quite detrimental effects (26). Ischemic priapism is an urological emergency characterized by persistent and painful erection in which cavernosal blood flow is little or no (3,26). A clear etiological factor cannot be detected in 60% of cases with ischemic priapism. Hematologic diseases, oncological pathologies, neurological disorders and the use of antipsychotics, antidepressants, systemic or intracorporeal vasoactive agents are commonly known causes of priapism (2). The degree of destruction in ischemic priapism is directly associated with its duration. In patients with long-term untreatable priapism, permanent damage occurs in the smooth muscle fibers, which are deemed absolutely necessary for penile dynamics (26,27). The durability of the penile tissues for the ischaemia occurring in penile corpus cavernosa is quite limited. In ischaemic priapism, ultrastructural alterations occur after 12 hours in smooth muscles, focal necrosis takes place after 24 hours and finally, necrosis and transformation of fibroblast-like cells occur after 48 hours (5). In present study, it was determined that dapsone, an antimicrobial agent, has a protective effect on ischaemia – reperfusion injury occurring after priapism.

Different treatment steps have been determined in ischaemic priapism. The primary objective of the treatment is to eliminate the compartment syndrome that occurs in the penile corpus cavernosa. The first-line treatment approach in ischaemic priapism is the aspiration of corporal blood. This intervention can be combined with saline irrigation. The success rate is reported to be approximately 30%. The second-line treatment approach is the local intracavernosal sympathomimetic administration. It is thought that the success rate is up to 80% on average. The third treatment step is surgical shunts (2). Even if detumescence is successfully achieved, cases may present with several sexual dysfunctions, especially erectile dysfunction, in the later stages. El-Bahsanawy et al. (27) reported that only 43% of these cases were able to maintain their erectile function during long-term follow-up in a series of 35 cases with an average duration of 48 hours, which was directly linked to penile tumescence duration. Pal et

al. (28) concluded in their study involving 19 patients that 66.7% of the cases of priapism which were successfully treated with intracorporeal phenylephrine injection and corporal aspiration had normal erectile functions in the follow-up. Kulmala et al. (29) reported in their large-scale study of 124 cases that erectile dysfunction was observed in 39% of cases after priapism treatment. In the same study, the rate of erectile dysfunction was 8% in cases with a duration of less than 24 hours, while this rate was 78% in patients with a duration of more than 1 week. An important reason for encountering problems in the sexual life of the majority of the cases in long-term follow-ups after priapism can be explained by ischaemia-reperfusion injury due to revascularization of penile tissues.

Ischaemia-reperfusion injury is observed at a very remarkable level in this clinical implication due to the fact that penile corpus cavernosa is reoxygenated quite quickly after the treatment of ischemic priapism (30,31). Critical metabolic and structural changes occur in penile tissues due to lack of oxygenation during ischemic priapism. The function of cellular oxidative phosphorylation is reduced to low levels. This energy deficiency results in the inhibition of Na+, K+-ATPase pump and intracellular sodium and calcium levels increase. This altered ion balance brings tissue edema and cytotoxic effect. This makes corporal tissues highly vulnerable to ischaemia reperfusion injury (4). During the ischemia period, a significant increase in the level of purine metabolites such as adenosine, hypoxanthine, xanthine and inosine is observed in the by-products of adenosine triphosphate (ATP) catabolism. These molecules accumulated in the tissue under ischemic conditions by using oxygen oxidants, which are abundant in the environment, become unstable and form intensely ROS by revascularization of the tissues (32,33). It is extremely important to keep the duration of the ischaemia short, reducing the amount of reaction that low-energy molecules enter with oxygen and keeping ROS formation at controllable limits (6). On the other hand, ischaemia-reperfusion injury leads to both a local and a systemic acute inflammatory response characterized by neutrophil activation (9,31). With this response, inflammatory cells become a second source for ROS. On the other hand, cystoxic molecules such as protease and myeloperoxidase released from activated neutrophils contribute to tissue damage. For all these reasons, anti-inflammatory molecules are thought to play an extremely important role in protecting cells against tissue damage in ischaemia-reperfusion damage (11). ROS levels are normally kept under control by antioxidant defense systems such as SOD, catalase and GSH-Px. These antioxidant systems are extremely ineffective in cases where oxygen flow is very rapid, such as the termination of priapism. In this context, it is evident that the use of molecules that show antioxidant efficacy has recently played a significant role in minimizing the harmful effects of ischaemia reperfusion injury (8). In this context, it has been biochemically and histopathologically demonstrated that Dapsone, which is a very strong anti-inflammatory and antioxidant property used in the study, effectively regresses the ischemia-reperfusion damage in penile corpus cavernosa.

In previous experimental studies, the harmful effects of ischemia-reperfusion injury on penile corpus cavernosa tissue have been clearly demonstrated. Although numerous pharmacological agents such as curcumin, dipyridamole, melatonin, pentoxifylline, lycopene, quercetin have been shown to protect against tissue injury in different animal models, treatment routine protocols for ischaemia reperfusion injury in penile corpus cavernosa have not yet been developed across the world (4-9). In the priapism model where Yilmaz et al. evaluated ischaemia reperfusion injury, found that TAS decreased in experimental animals due to ischaemia reperfusion injury, while there was a significant increase in edema, necrosis and hemorrhage scores in penile tissue (4). Similarly, our study indicated a decrease in the level of antioxidant enzymes such as SOD and GSH-Px and in TAS due to ischemia reperfusion injury. Karagüzel et al. documented histopathologically that after ischaemia-reperfusion injury, the endothelial cells covering the penile corpus cavernosa were severely damaged and quite a lot of tears were observed in tunica albuginea (5). In our study, it was observed that desquamation, edema and vasocongestion were detected high levels in penile tissues after ischaemia-reperfusion injury. The experimental study of the Ciftci et al. (8) reported that antioxidant defense systems such as SOD, catalase and GSH-Px were decreased in corporal ischaemia reperfusion injury, while TBARS (thiobarbituric acid reactive substances), an important marker of lipid peroxidation, was increased. Similarly, Uluocak et al. (6) reported that MDA level increased significantly in penile ischemia reperfusion injury. In our study, an increase in MDA and PC levels, the most important markers of lipid peroxidation and protein oxidation, was observed after ischaemia reperfusion injury. In another experimental study, Munarriz et al. (33) found that polymorphonuclear leukocyte infiltration was observed in penile corpus cavernosa with re-oxygenation of post-ischaemia, however, myeloperoxidase activity and lipid peroxidation increased. Similarly, in our study, it was observed that the inflammation scores in penile tissues increased significantly after ischemia reperfusion, and in our serum analysis, there was a dramatic increase in proinflammatory cytokines such as IL-1beta, IL-6 and TNF alpha.

Dapsone was synthesized from p-nitro-thiophenol in the early 1900s and was heavily used in the chemical industry in the production of azo dyes during its initial use (10,14). In the following periods, it has been discovered that this pharmacological agent plays a important role in maintaining the vital functions of the cell against tissue damage apart from antimicrobial property (16). This activity is performed via many different mechanisms. Inhibition of inflammation has a significant place among these pathways. Previous studies have revealed in great detail that dapsone has anti-inflammatory properties equivalent to nonsteroidal anti-inflammatory drugs (18). Dapsone demonstrates this effect in many different aspects. Dapson inhibits the activity of myeloperoxidase, which converts hydrogen peroxide into water and hypochlorous acid. In this way, it causes the hypochlorous acid level to be reduced, which leads to severe tissue damage (10,18). On the other hand, this pharmacological agent stabilizes neutrophil lysosome, suppresses the expression of proinflammatory cytokines, neutrophil chemotaxis and neutrophil adherence function (34,35).

Liang et al. (15) analyzed the efficacy of dapsone therapy in 79 cases with chronic spontaneous urticaria and experienced improvement in 78% of cases. Abe et al. also reported in their in vivo study examining the pharmacological effect of dapsone on cutaneous lupus erythematosus that this pharmacological agent produced an anti-inflammatory response by suppressing TNF-alpha release from activated mononuclear cells (17). Dapsone, on the other hand, leads to a significant decrease in ROS levels by activating scavenging enzymes such as glutathione reductase, GSH-Px, SOD and catalase (12). In addition to its anti-inflammatory and antioxidant efficacy has been shown to have a strong anti-apoptotic properties in previous studies. Dapson demonstrates this effect by inhibiting both intrinsic apoptosis, which is triggered by the activation of the caspase in direct connection with the exposure of mitochondria to a very high amount of calcium, as well as extrinsic apoptosis, in which inflammatory mechanisms play a leading role (13).

The effects of dapsone on oxidative tissue damage on different tissues have been analyzed in detail in the previous years. Rios et al. (36) reported neuroprotective efficacy by reducing the infarction volume of dapsone therapy by more than 90% in the rat model in which they formed middle cerebral artery occlusion. In their experiment that resulted in intracranial kainic acid toxicity, Diaz-Ruiz et al. (16) demonstrated that dapsone prevents neuronal death of pyramidal cells by showing antioxidative and anticonvulsive properties. In our study, it was observed that dapson caused a significant increase in the level of antioxidant enzymes such as SOD and GSH-Px, which are a very important defense system against ischemia reperfusion damage after priapzm treatment. A similar experimental study also found that dapsone demonstrated neuroprotective activity in brain ischaemia reperfusion injury by lowering levels of nuclear factor erythroid 2-related factor 2 and reactive oxygen species, as evidenced with magnetic resonance images (37). In our study, a significant increase in TAS and a decrease in oxidative damage were observed after the dapsone treatment. Nezamoleslami et al. (18) observed that dapsone exhibited reno-protective properties on the kidney ischemia reperfusion injury and indicated that there was considerable evidence that this activity was carried out by modulating the inflammatory cascades. Similarly, in the experimental testicular torsion/detorsion model performed by Debjan et al. (11), a significant decrease in TNF-alpha levels of the rats applied dapsone was observed while a significant increase in SOD activity was indicated. In our study determined that TOS decreased to very low levels after dapsone treatment, while proinflammatory cytokine levels such as IL-1beta, 6 and TNF alpha were also reduced dramatically. In the study analyzing the efficacy of dapsone in cardiotoxicity caused by doxorubicin in rats, Sheibani et al. (12) found that dapsone significantly reduced oxidative stress and inflammation. The same study reported that it reversed the elevated cardiac enzyme levels due to cardiotoxicity and papillary muscle contractility. Although penile dynamics could not be evaluated in our study, it was found that edema, desquamation, hemorrhage and inflammation scores that increased due to ischemia-reperfusion damage in the penile corpus cavernosa after dapsone treatment significantly regressed.

The main limitation of our study is to evaluate only the early effects of dapsone treatment after priapism. In our study, dapsone was administered only in a single dose, and its long-term effects could not be demonstrated. Although previous studies have reported that dapsone does not have a dangerous side effect profile, our study failed to document the changes that this pharmacological agent may potentially cause in penile corpus cavernosa in healthy experimental animals. Finally, penile dynamics could not be revealed and immunohistochemical analyses could not be presented due to the technical incapacity of our laboratory conditions.

CONCLUSION

In the light of the data obtained in our study, we are in the opinion that dapsone therapy can be considered as an alternative approach to minimise ischaemia-reperfusion injury in penile corpus cavernosa during priapism. Although our study adds a new function in the treatment approach of priapism with this use of dapsone, it is

essential to elaborate the molecular mechanism of this activity and determine the dose curves. Furthermore, our study was based solely on the experimental priapism model and our results should be validated by prospective, randomized and controlled clinical trials in the future.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tokat Gaziosmanpaşa University Animal Studies Ethics Committee (Date: 08.04.2021, Decision No: 2021-HADYEK-06).

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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Evaluation nutritional status and anthropometric parameters in patients with chronic hepatitis B

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ABSTRACT

Introduction: Hepatitis B continues to be a major health problem around the world. 257 million people are estimated to be chronically infected with hepatitis B worldwide. Chronic hepatitis B (CHB) patients are likely to develop various comorbidities, including diabetes, insulin resistance, hyperlipidemia, nonalcoholic fatty liver disease, and obesity. Proper nutrition is essential for the management of both hepatitis B and its associated comorbidities.

Material and Method: The study was completed a total of 105 patients. The universe of the study comprised of CHB patients who were admitted to the nutrition and dietetics outpatient clinic of a public hospital in Turkey between 1 October 2019 and 31 December 2019. Biochemical and ultrasound results, anthropometric measures, demographic characteristics, dietary habits, and 1-day food records were retrospectively recorded from patient files.

Results: Female patients were more likely to consume 2 main meals per day (57.8%) whereas most male subjects (75.6%) consumed 3 meals. Both female and male patients had above-normal Body Mass Index (BMI) (31.2 kg/m² and 29.2 kg/m², respectively), and they also had high dietary fat (%) and cholesterol consumption than recommendation. In addition, males had borderline The fasting blood glucose (FBG), total cholesterol, and triglyceride levels, and both sexes were at risk for abdominal obesity. Dietary carbohydrate, fiber, B1, B6, calcium, magnesium, and phosphorus intake were higher in males than in females.

Conclusion: This study was found on above-normal BMI values, and high dietary fat (%), and cholesterol consumption in both males and females. Moreover, males had borderline FBG, total cholesterol, and triglyceride levels, and both sexes were at risk for abdominal obesity. In the setting of CHB, it is crucial to maintain an adequate and balanced diet to control body weight, prevent nutritional disorders, protect the liver, and improve overall well-being. More comprehensive studies are needed to better understand the link between nutrition and hepatitis B.

Keywords: Chronic hepatitis B virus infection, nutritional status, body composition, body weights and measures

INTRODUCTION

Hepatitis B virus (HBV), a major infectious disease, continues to be a leading global public health concern. Despite vaccination efforts, millions of people become infected with the HBV every year (1). According to the World Health Organization (WHO), 257 million people are chronically infected with HBV worldwide as of 2015. As of 2016, 27 million people – only 10.5% of all people estimated to be chronically infected with hepatitis B (HB) – are aware of their infection, while only 4.5 million (16.7%) of the diagnosed individuals are receiving treatment (2). The prevalence of CHB varies among geographical regions. The prevalence of CHB is <2% in Northern America and Western Europe, 2-7% in Mediterranean countries, the Middle East, Japan, Central Asia, and parts of South America, and is up to ≥8% in South Sudan and West Africa (3). In Turkey, 3.3 million individuals (4.57% of the general population) are estimated to be chronically infected with HB (4).

Hepatitis B infection is often divided into three types: acute, chronic, and occult. The most important indicator for the definitive diagnosis of chronic HBV infection is the presence of HBsAg in the blood for longer than six months (5). All patients with chronic HBV infection are at risk of developing hepatocellular carcinoma (HCC) and cirrhosis depending on host and viral factors (1). Obesity is known to be associated with HCC and also contributes to the development of , non-alcoholic fatty liver disease (NAFLD), hepatic steatosis, and non-

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alcoholic steatohepatitis due to its negative effects on the liver (6). The main purpose of HBV treatment is to improve survival and quality of life by preventing the progression of the disease, and therefore the development of HCC (1). CHB infection can be treated with medications, which can slow the progression of cirrhosis, reduce the risk of developing HCC, and improve long-term survival. WHO recommends oral tenofovir or entecavir treatment as the most potent approach to suppress HBV (2).

Patients with CHB may or may not require medication, but adequate and balanced nutrition is essential for liver health and overall well-being. The diet should be rich in protein, energy, and vitamins, and also include fresh fruits, whole grains, fish, lean protein, and plenty of vegetables (7).

In the setting of acute or chronic liver diseases, the risk of developing eating and malabsorption disorders, nausea, anorexia, and malnutrition are increased. Moreover, CHB patients are likely to develop various comorbidities, including diabetes, insulin resistance, hyperlipidemia, nonalcoholic fatty liver disease, and obesity. Proper nutrition is prominent in the management of both HB and the associated comorbidities. There are very few studies on the nutritional status of patients with hepatitis B both in Turkey and worldwide. In this study, we aimed to investigate the general eating habits, nutritional status, and anthropometric parameters of patients with CHB, and to compare the sexes.

MATERIAL AND METHOD

This study is a retrospective descriptive cross-sectional study. The universe of the study comprised of CHB patients who were admitted to the nutrition and dietetics outpatient clinic of a public hospital in Turkey between 1 October 2019 and 31 December 2019. Patient files were retrospectively reviewed and biochemical test results, anthropometric measures (body analysis results, height, hip and waist circumference), dietary habits, and food consumption records were retrospectively recorded from patient files. Because the study was designed retrospectively, no written informed consent form was obtained from patients.

This study was granted ethical approval by the İstanbul Okan University Non-Interventional Research Ethics Committee (Date 29.04.2020, Decision No: 56665618-204.01.07). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

For every patient that presents to the dietetics outpatient clinic, demographic characteristics, overall nutritional habits, and physical activity, sleep duration, drug use, and a 1-day food record are electronically recorded prior to the prescription of a diet. Moreover, height and waist and hip circumference and body composition are measured. The BMI was computed by dividing body weight (kg) by height (m^2). According to the WHO, the BMI classification was used. The BMI was defined as underweight if it was less than 18.5 kg/m^2, normal if it was between 18.5-24.9 kg/m^2, overweight if it was between 25.0-29.9 kg/m^2, and obese if it was more than 30.0 kg/m^2 (8). The midpoint between the lower border of the last palpable rib and the top of the iliac crest was used to calculate waist circumference. Hip circumference was measured the circumference going through the highest point on the hip from the right side of the patients. The waist/hip ratio was determined by dividing the waist circumference (cm) by the hip circumference (cm). The waist/height ratio was assessed according to the classification according to WHO recommendations. Waist circumference measurements were classified as ≥94 cm risky in men, ≥102 cm high risk, ≥80 cm risky in women, and ≥88 cm high risk. A waist/hip ratio >1.0 in men and >0.8 in women was considered android obesity (9).

Body composition parameters were assessed using Tanita BF-350 Total Body Composition Analyzer, and heights were measured with a Seca stadiometer. An inflexible measuring tape was used for waist and hip measurements.

The International Physical Activity Questionnaire (IPAQ-Short Form) was used to determine the patients' physical activity status. The IPAQ technique was used to score physical activity. Patients with a total score of 600 Metabolic Equivalent Minutes (MET) on the physical activity scale were classified sedantary, patients with 600-3000 MET were classified moderate active, and patients with >3000 MET were classified very active (10).

Daily energy and nutrient intake were analyzed using "Computer Assisted Nutrition Program, Nutritional Information Systems Package Program (BEBIS)" developed for the Turkish population. TUBER was used to evaluate the data collected.

The ultrasonography findings of the patients were used to make the diagnosis of steatosis. The classification was based on ultrasonography data.

The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0. All statistical analyses were performed with parametric tests. In addition to descriptive statistical methods (mean, standard deviation), the t test was used to determine the difference between two means, and the chi-square test and Pearson's correlation analysis were used to investigate correlations. $p<0.05$ was accepted as statistically significant.

RESULTS

The study included a total of 105 patients (64 females [61%] and 41 males [39%]). 78.1% of female participants and none of male patients were unemployed. Most female (54.7%) and male (58.5%) patients had completed only primary education. 28.1% of females and 36.6% of males were on medication for hepatitis B. Female patients were more likely to consume 2 main meals per day (57.8%) whereas most male subjects (75.6%) consumed 3 meals (**Table 1**). Grade 1 and 2 steatoses were more common among females. About half (48.6%) of all patients had a chronic disease other than hepatitis. 23% of the patients had been diagnosed with diabetes, the majority of which (74.4%) were female. 15.7% of the patients had concomitant heart disease, which was more common in women (62.5%) than in men.

The anthropometric measurement and body analysis results were evaluated. For females, the mean height was 157.2 cm, mean body weight was 77.0 kg, mean BMI was 31.2 kg/m², mean lean mass was 46.9 kg, and mean fat mass was 30 kg. For males, the mean height was 171.5 cm, mean body weight was 85.9 kg, mean BMI was 29.2 kg/m², mean lean mass was 63.4 kg, and mean fat mass was 22.4 kg. Females had significantly higher BMI, hip circumference, and fat mass and percentage whereas males had significantly higher lean body mass and percentage ($p<0.05$) (**Table 2**).

More than half of females and 41.5% of males were obese. When the waist-to-hip ratio was evaluated, 70.3% of female patients were evaluated to be at risk, and 73.2% of male patients were not at risk. Patients of both sexes most commonly reported moderate physical activity. Sex was significantly associated with BMI, waist-to-hip ratio, and MET levels ($p<0.05$) (**Table 3**).

Table 1. Distribution of some characteristics of the patients according to the gender

Characteristic	Women (n=64)		Men (n=41)	
	n	%	n	%
Job				
Unemployed	50	78.1	-	-
Employed	14	21.9	41	100.0
Education				
Literate	11	17.2	1	2.4
Primary school	35	54.7	24	58.5
Secondary school	6	9.4	4	9.8
High school	6	9.4	10	24.4
Associate degree	2	3.1	0	0.0
University	4	6.3	2	4.9
Marital status				
Married	49	76.6	41	100.0
Single	6	9.4	0	0.0
Widowed/divorced	9	14.1	0	0.0
Hepatitis medication use				
Yes	18	28.1	15	36.6
No	46	71.9	26	63.4
Number of main meals				
2	37	57.8	10	24.4
3	27	42.2	31	75.6
Number of snacks				
None	1	1.6	8	19.5
1	24	37.5	15	36.6
2	33	51.6	13	31.7
3	6	9.4	5	12.2
Meal skipping				
Yes	53	82.8	36	87.8
No	5	7.8	4	9.8
Sometimes	6	9.4	1	2.4
Liver fatty degree (n=63)				
Normal	15	37.5	5	21.7
Grade 1	15	37.5	13	56.5
Grade 2	7	17.5	3	13.0
Grade 3	3	7.5	2	8.7
Sleep duration				
Short sleep (<7 hour)	27	42.2	22	53.7
Moderate sleep (7-8 hour)	24	37.5	16	39.0
Long sleep (>8 hour)	13	20.3	3	7.3

Table 2. Comparison of anthropometric measurement and body analysis values of the patients according to the gender

	Women (n=64)	Men (n=41)	t	p
Body weight (kg)	77.05±14.22	85.9±10.93	-3.599	0.001*
Height (cm)	157.25±6.53	171.51±6.96	-10.645	<0.001*
BMI (kg/m²)	31.26±5.89	29.24±3.58	2.173	0.032*
Waist circumference (cm)	97.46±14.09	100.2±8.15	-1.269	0.207
Hip circumference (cm)	108.56±11.25	104.34±6.09	2.487	0.015*
Waist-hip ratio	0.89±0.09	0.96±0.044	-4.729	<0.001*
Lean mass (kg)	46.99±5.19	63.43±6.42	-14.419	<0.001*
Lean mass (%)	61.99±7.08	74.27±5.56	-9.397	<0.001*
Fat mass (kg)	30.07±10.40	22.49±6.97	4.108	<0.001*
Fat mass (%)	37.97±7.13	25.73±5.60	9.306	<0.001*
Water ratio (%)	45.25±5.01	54.61±4.42	-9.772	<0.001*

Independent Sample T Test, *p<0,05

Table 3. Comparison of BMI, waist/hip risk, MET level classifications of the patients according to gender

	Women		Men		X2	p *
	n	%	n	%		
BMI					6.092	0.048*
Normal	13	20,3	5	12,2		
Overweight	15	23,4	19	46,3		
Obese	36	56,3	17	41,5		
Total	64	100,0	41	100,0		
Waist-hip ratio risk					18.985	<0.001*
Yes	45	70,3	11	26,8		
No	19	29,7	30	73,2		
Total	64	100,0	41	100,0		
MET level					13.520	0.001*
Sedantary	6	9,4	3	7,3		
Moderate	58	90,6	30	73,2		
Vigorous	0	0,0	8	19,5		
Total	64	100,0	41	100,0		

Chi Square test, *p<0,05

Dietary carbohydrate, fiber, B1, B6, magnesium, and phosphorus intake, and glycemic index were higher in males than in females (p<0.05). Women consumed significantly less calcium than males (648.47±291.39 mg vs. 860.93±423.33 mg). Vitamin D intake was higher in males, but this finding was not statistically significant (p>0.05) (**Table 4**).

The blood biochemistry results of the patients were analyzed, and it was observed that male patients had elevated FBG levels (106.61±50.33 mg/dL), but the FBG results of two sexes were not significantly different. Males had significantly higher ALT levels, and females had significantly higher HDL levels compared to males (54.16 mg/dL vs. 44.46 mg/dL, p<0.05) (**Table 5**).

The correlation between steatosis and BMI classes and waist-to-hip ratio was not significantly different for the two sexes (p>0.05). There was a significant positive correlation between BMI and serum cholesterol (r=0.253, p=0.44), LDL cholesterol (0.286, p=0.22), and triglyceride (r=0.341, p=0.006) levels in female patients. BMI was not significantly associated with biochemistry parameters in males.

Table 4. Comparison of the mean intakes of energy, macro and micronutrients of the patients according to the gender

Nutrients	Women (n=64)	Men (n=41)	t	p*
	Mean±SD	Mean±SD		
Energy (kcal)	1876.50±1735.46	2387.46±1016.56	-1.705	0.091
Protein (g)	70.46±85.15	89.48±43.05	-1.324	0.188
Protein (%)	14.98±3.99	15.34±4.42	-0.428	0.669
Fat (g)	85.88±136.76	87.30±36.71	-0.065	0.949
Fat (%)	38.54±12.96	34.22±10.82	1.776	0.079
Carbohydrate (g)	201.49±116.29	304.74±166.89	-3.460	0.001*
Carbohydrate (%)	46.54±13.52	50.39±11.43	-1.507	0.135
Glycemic Index	194.93±158.60	304.55±239.82	-2.587	0.012*
Fiber (g)	20.69±12.42	28.08±16.64	-2.600	0.011*
Alcohol	0.01±0.04	0.01±0.08	-0.425	0.672
Vitamin E (mg)	12.26±9.61	12.76±9.57	-0.261	0.795
Vitamin D (µg)	1.93±2.96	3.16±7.79	-1.140	0.257
Vitamin K (mg)	95.05±110.09	83.10±83.43	0.594	0.554
Vitamin B12 (µg)	9.26±24.27	3.26±2.17	1.967	0.054
Vitamin A (µg)	2382.83±8301.76	1000.70±616.45	1.326	0.189
Vitamin C (mg)	108.95±125.9	113.82±125.65	-0.193	0.847
Cholesterol (mg)	352.93±365.11	342.56±190.97	0.167	0.867
Polyunsaturated fatty acid (g)	13.84±9.74	15.88±9.91	-1.039	0.301
Monounsaturated fatty acid (g)	32.58±66.59	30.23±13.64	0.223	0.824
Vitamin B1 (Thiamine) (mg)	0.79±0.35	1.09±0.49	-3.467	0.001*
Vitamin B2 (Riboflavin) (mg)	1.42±1.11	1.46±0.58	-0.252	0.801
Vitamin B6 (mg)	1.05±0.55	1.35±0.71	-2.435	0.017
Folate (µg)	283.27±171.08	340.45±138.47	-1.795	0.076
Sodium (mg)	4148.37±7377.51	4617.45±2515.63	-0.392	0.696
Potassium (mg)	2237.05±1312.10	2762.72±1469.29	-1.911	0.059
Calcium (mg)	648.46±291.38	860.92±423.33	-3.047	0.003*
Magnesium (mg)	264.18±150.15	349.17±154.05	-2.801	0.006*
Phosphorus (mg)	1004.76±576.54	1295.25±542.25	-2.577	0.011*
Iron (mg)	10.45±9.97	12.60±5.67	-1.255	0.212
Zinc (mg)	10.37±13.08	12.15±5.45	-0.826	0.410

Independent Sample T Test, *p<0,05

Table 5: Comparison of blood test results of patients according to gender

Blood test results	Women (n=64)	Men (n=41)	Reference range	t	p*
	Mean±SD	Mean±SD			
The fasting blood glucose (mg/dL)	101.81±23.12	106.61±50.33	70-105	-0.662	0.509
Alanine aminotransferase (ALT) (IU/L)	22.72±11.17	34.68±22.22	0-50	-3.198	0.002*
Aspartate aminotransferase (AST) (IU/L)	25.69±18.65	26.90±9.80	0-39	-0.384	0.702
Total cholesterol (mg/dL)	199.41±48.59	201.85±39.68	0-200	-0.270	0.788
LDL cholesterol (mg/dL)	124.88±40.43	136.68±52.87	0-160	-1.293	0.199
HDL cholesterol (mg/dL)	54.16±16.67	44.46±10.38	<40 (risk for male) <50 (risk for female)	3.672	<0.001*
Triglyceride (mg/dL)	111.48±55.63	156.24±193.53	35-150	-1.443	0.156
Vitamin D (ng/mL)	18.96±10.69	19.15±7.71	0-20 Deficiency 20-30 Insufficiency 30-100 Sufficiency >150 Toxic	-0.099	0.921

Independent Sample T Test, *p<0,05

DISCUSSION

In this study, we investigated the anthropometric measures, nutritional status, and eating habits of CHB patients. There was a total of 105 patients, and the mean age was 45.6 years. 64% of the patients were female, and more than half of all participants had completed only primary education. The mean BMI of the patients was 30.47 kg/m² and there was a statistically significant difference between the mean BMIs of females and males. One study from Pakistan evaluated the educational status of 100 HB patients and found that 71% were illiterate and only 1% had completed post-secondary education. They concluded that educational status was significantly associated with the risk of developing hepatitis B (11). These data suggest that HB may be more common among individuals who are poorly educated.

Waist circumference is an important indicator of visceral obesity and has been cited as a risk factor for numerous metabolic diseases. In one study, it was reported that CHB patients who had steatosis had higher BMI and waist circumference values compared to those who did not (12). Another study found that 67.7% of patients with HCV-associated NAFLD were obese, and 80.2% had a large waist circumference (13). In this study, we found that males were at risk and females at high risk of abdominal obesity. This also suggests that these patients are at risk for developing cardiovascular diseases. Obesity is also associated with an increased risk of cirrhosis-related hospitalization or death, as well as a higher risk of developing HCC (6). There are very few studies on the prevalence of steatosis in the setting of hepatitis B. One such study demonstrated that in CHB patients, steatosis was associated with the diagnostic criteria for metabolic syndrome, and BMI (14). In this study, we were able to access liver ultrasound results of 63 patients and we did not find a significant relationship between steatosis and BMI classes or waist-to-hip ratio. Among these 63 individuals, 68.2% had steatosis of various severity (grade 1 44.4%, grade 2 15.9%, 2 and grade 3 7.9%). A study on the prevalence of fatty liver in individuals with and without chronic hepatitis found that the prevalence of NAFLD was 13.5% in HB patients and 28.3% in the control group (15). The prevalence of fatty liver was quite high in our study, but this result may be due to not having accessed the ultrasound results of all patients. On the other hand, we found a positive correlation between BMI values and total cholesterol, LDL cholesterol and triglyceride values in female patients. Moreover, male patients had risky triglyceride and total cholesterol levels. Regarding the impact of HBV infection on the lipid profile, one study compared HBV patients with healthy individuals and observed elevated total cholesterol and LDL levels in patients with HBV infection (16). In our study, 15.7% of HB patients had been diagnosed with heart disease. In the 2010 report of the Turkish Nutrition and Health Survey, it is emphasized that a healthy, adequate and balanced diet should include no more than 300 mg of cholesterol intake every day (17). In our study, the cholesterol intake of both female and male patients significantly exceeded this recommended level. Excessive dietary cholesterol raises blood cholesterol levels and increases the risk of cardiovascular disease (17). This underlines the significance of adequate nutrition for patients chronically infected with HB in terms of cardiovascular diseases.

Another chronic disease associated with HB is diabetes. It has been shown that the prevalence of HB is higher among individuals with diabetes compared to non-diabetics (18). In addition, patients with chronic HBV infection have a higher risk of developing diabetes (19). In this study, 23% of patients had been diagnosed with diabetes. Another major factor in the development of diabetes is obesity, and therefore, adequate and balanced nutrition. Bodyweight management, as well as antiviral therapy, is essential for the management of HB (6). According to Turkey-Specific Food and Nutrition Guide

(TUBER), 55-60% of daily calorie intake should come from carbohydrates, 10-15% from protein, and 25-30% from fats (20). In this study, the percentage of energy from carbohydrates was below, and the percentage of energy from fat was above recommended levels for both sexes. This finding was ascribed to fad diets that promote food rich in proteins and fats and poor in carbohydrates.

Physical activity is a significant component of HBV treatment. Adequate physical activity can prevent the development of metabolic syndrome and weight gain and subsequently produce therapeutic effects. One study found that individuals with NAFLD were less physically active compared to healthy individuals (21). In our study, both male and female patients most commonly reported moderate physical activity. Hepatic disease is associated with an increased risk of early mortality. One study demonstrated that every 10 minutes of moderate to vigorous physical activity decreased mortality risk by 89% (22).

The limitations of our study are due to its retrospective nature, including the small sample size, incomplete ultrasound data, and lack of a healthy control group. Further larger controlled studies are needed.

CONCLUSION

To conclude, we found above-normal BMI values, and high dietary fat (%) and cholesterol consumption in both male and female CHB patients. In addition, males had borderline FBG, total cholesterol, and triglyceride levels, and both sexes were at risk for abdominal obesity. In reference to our findings, we conclude that it is essential to maintain an adequate and balanced diet in order to control body weight and prevent nutrition-related diseases in the setting of CHB.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was granted ethical approval by the İstanbul Okan University Non-Interventional Research Ethics Committee (Date 29.04.2020, Decision No: 56665618-204.01.07).

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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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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Type D personality and self-esteem in conversion disorder: a case-control study

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ABSTRACT

Aim: In the present study, we aimed to investigate the differences in type D personality and self-esteem between patients with conversion disorder (CD) and the control group.

Material and Method: We carried out the study with 100 patients diagnosed with CD and 100 matching healthy individuals. We used a sociodemographic information form, the Type D Personality Scale (DS-14), and the Rosenberg Self-Esteem Scale (RSES) as the data collection tools. Considering that the scales should have high discriminative powers, we calculated the cut-off points on each scale performing the ROC analysis.

Results: The results showed that the patients with CD had significantly higher negative affectivity (NA), social inhibition (SI), and DS-14 total scores than the control group ($p<0.001$). Also, the patient group obtained significantly higher scores on the RSES than the control group, indicating lower self-esteem among the patients ($p<0.001$). Finally, we concluded significant positive correlations between the RSES scores and NA ($r=0.549$ $p<0.001$), SI ($r=0.410$ $p<0.001$) and DS-14 total scores ($r=0.521$ $p<0.001$).

Conclusion: Overall, we found that CD patients had type D personality traits and low self-esteem. As the patients had decreased self-esteem, their DS-14 scores increased. Uncovering SI and NA to be high in these patients may drive physicians to take measures to mitigate these situations and adopt a different perspective on CD. In addition, the results may contribute to better addressing patients' conversion symptoms and personality traits compatible with CD.

Keywords: Conversion disorder, type D personality, self-esteem

INTRODUCTION

Conversion disorder (CD) is a psychiatric disorder that causes functionality loss, usually following a stressful life event, with evidence of divergent symptoms of one or more voluntary motor/sensory function alterations with neurological or general medical conditions (1). The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) covers CD as a diagnosis under somatoform disorders (2). In the fifth edition of the manual (DSM-V), it is defined as "Functional Neurological Symptom Disorder" under "Somatic Symptom Disorders and Related Disorders" (1). The lifetime prevalence of somatoform disorders is 0.1-0.5% (3). Besides, the CD is the most prevalent somatoform disorder; its incidence in Turkey was previously reported to be 4.5-32% (4). Previous large-scale population-based studies reported the incidence rates to range from 0.04 to 12% (5). The conversion disorder is often accompanied by another mental disorder; major depressive disorder is shown to be the most common accompanying disorder at a rate of 17-29% (6).

Personality is conceived of temperament and character traits (7). Some personality traits are considered significant predisposing factors for psychiatric disorders. In this respect, it was reported that patients with somatic symptoms in somatization disorder have more primitive, dependent, and egocentric traits (7). In the relationship between CD and personality, relevant research showed that difficult temperament causes internalization of behavioral problems in children, whereas easy temperament works in preventing the

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emergence of psychological disorders (8). Another study on the relationship between character traits and CD reported that children with CD had a more dominant negative mood (frustrated, angry, discontented/irritated) compared to controls (9). The same study also mentioned that temperament is a likely factor for CD development in children (9). Yet, there are quite limited studies investigating personality traits in CD in adult groups (10, 11). Some of such studies reported low neurotic tendencies and personal adjustment among the patients (11).

Individuals with type D personality are those who can easily have negative emotions, such as anger and tension, and experience such emotions more than positive ones (12). At the same time, these individuals are likely to be introverted and tend to be disturbed in settings with strangers. Type D personality consists of social inhibition (SI) in conjunction with negative affectivity (NA). NA is defined as a tendency to experience depressive affect, restlessness, irritability, and hostility. SI, on the other hand, is the tendency to prevent the expression of emotions in social spheres. Individuals with high NA experience dysphoria, anxiety, and irritability more frequently. Those with high SI often feel anxious and insecure when they are with others (12). Substantial evidence shows that these individuals have higher levels of stress and experience more physical complaints when compared to the general population (13). A recent study reported that 91% of patients with type D personality had at least one somatic disorder, and 10% had at least three somatic disorders (14). Type D personality prevalence is often reported to be 13%-24% in the general population (15). In addition, the literature points out that type D personality traits are associated with suicidal thoughts in depressive disorder (16, 17). In type D personality, negative thoughts may also impair self-esteem.

Self-esteem is the ability to value oneself and make accurate assessments of oneself; it is a measure of seeking approval, acceptance, and self-confidence (17). It is known that psychological disorders adversely affect self-esteem is affected (18). When it comes to CD, patients embody their feelings and thoughts, which they perceive to be unacceptable, through symptoms. Using these signals, one may attract the attention of those around them and make their own existence accepted (1). Individuals with type D personality traits are often dissatisfied with their lives and have low self-esteem (12).

Although studies exploring CD frequently address temperament and character traits, they seem to miss type D personality patterns in CD (10, 11, 19). Therefore, this study aimed to compare type D personality traits and self-esteem in individuals diagnosed with CD upon DSM-5 criteria with the control group. We hypothesized that CD patients might show type D personality traits, consisting of a combination of NA and SI, more than healthy controls and that the same group might have lower self-esteem than the control group.

MATERIAL AND METHOD

This study was approved by Non-interventional Researchs Ethics Committee of Fırat University (Date: 14.01.2021, Decision No: 2021/01-07). After being informed about the purpose of the study, all the participants provided their written consent to participate in the study voluntarily. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We recruited a total of 100 patients who applied to the Fırat University psychiatry clinic and were treated as either inpatient or outpatient and diagnosed with CD by a psychiatrist according to DSM-5 diagnostic criteria. We carried out the study with only those 18-65 years, without any important physical pathology or physical disorder that may affect the distribution of psychiatric symptoms, without alcohol and substance abuse, and without any other accompanying psychiatric disorder.

For the healthy control group, we selected a total of 100 individuals among healthcare staff and patient relatives who could match the patient group regarding sociodemographic data, such as age, sex, educational attainment, and did not have any psychiatric disorder.

Initially, we reached out to 153 CD patients for the study but could not include five people because they refused to participate in the study voluntarily. Of the remaining 148 patients, we had to exclude 42 due to another comorbid psychiatric disorder. Finally, we did not evaluate the data from 6 patients due to missing data on their questionnaires. A psychiatry specialist collected the data using a sociodemographic information form, the DS-14, and the RSES. It took approximately 35 minutes each to administer the questionnaires to the participants.

Data Collection Tools

1) Sociodemographic Information Form: We prepared the form in line with the overall purpose of the study. It is a form covering demographic information, such as age, marital status, educational attainment, place of residence, and employment and economic status, as well as clinical evaluation inquiries, such as duration of the psychiatric disorder, presence of any psychosocial stress factors, inpatient treatment history, and smoking or alcohol use.

2) Type D Personality Scale (DS-14): The scale was developed particularly to assess NA, SI, and Type D personality. The items of DS-14 were derived from DS-16 (20), but the scale also contains new items aimed at

enhancing the assessment of NA and SI (12). The DS-14 includes 14 items in total equally distributed to two subscales measuring NA and SI. It is a four-point (0-4) Likert-type scale. The total score ranges from 0 to 28 for both subscales, and the cut-off point is ≥ 10 for each subscale (15). Öncü et al. (21) carried out the validity and reliability study of the scale in the Turkish context. Accordingly, the researchers found Cronbach's alpha values to be α=0.82 for the NA subscale and α=0.81 for the SI subscale. In this study, we calculated these values to be 0.80 and 0.79, respectively.

3) Rosenberg Self-Esteem Scale (RSES): The scale developed by Rosenberg (22) attempts to determine the self-esteem levels of patients based on their own perceptions. The 63-item scale has 12 subscales; we used only the 10-item "Self-Esteem" subscale in this study. The items on this subscale are rated on a scale ranging from 0 to 6 points. Accordingly, those scoring 0-1, 2-4, and 5-6 on the subscale are considered to have high, moderate, and low self-esteem, respectively. Çuhadaroğlu (23) performed the Turkish validity and reliability study of the scale. In the Turkish version, the researcher found Cronbach's α value to be 0.76. In this study, we calculated the internal consistency of the "Self-Esteem" subscale to be 0.75.

Statistical Analysis

We performed all statistical analyses on SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL). In the study, we presented categorical variables in number and percentage, while continuous variables were shown in mean±standard deviation, median, interquartile range (25-75 percentiles). We run a Chi-square analysis to compare categorical data. Besides, we performed a Kolmogorov-Smirnov test to check whether the continuous variables showed a normal distribution. Then, for non-normally distributed data, we used a Mann-Whitney U test to compare non-normally distributed variables between two groups, while the data were compared using a Kruskal-Wallis test between more than two groups. We calculated Spearman's correlation coefficients to reveal relationships between continuous variables. We drew Receiver-operating characteristic (ROC) curves to measure the diagnostic values of DS and RSES. Finally, we considered p<0.05 statistically significant in all statistical analyses.

RESULTS

We carried out the study with a total of 200 individuals, 100 CD patients and 100 healthy controls. While the mean age of the patient group was 29.3±7.8 (min=18, max=50), the control group had a mean age of 28.6±7.9 (min=18, max=47). We detected 67% of the patients had a psychosocial stress factor.

The results revealed no significant differences between the patient and control groups by sex, marital status, educational attainment, place of residence, economic status, occupation, and organic disorder (p>0.05) (**Table 1**).

We found that the patient group had significantly more psychiatric treatment history, family history of psychiatric disorder, and smoking than the control group (p<0.001) (**Table 1**). In addition, we discovered the participants with secondary school and below education (35.5%) had significantly more multiple symptoms than those with high school and above education (16.2%) (p=0.039).

Table 1. Sociodemographic and disorder-related characteristics of the groups

	Patients		Controls		p
	n	%	n	%	
Age, Median (IQR)	28 (23-35)		28 (22-34)		0.461*
Sex					0.339**
Female	70	70.0	76	76,0	
Male	30	30.0	24	24,0	
Marital status					0.777**
Single	51	51.0	53	53.0	
Married	49	49.0	47	47.0	
Educational Attainment					0.884**
Secondary school and below	62	62.0	61	61.0	
High school and above	38	38.0	39	39.0	
Place of Residence					0.885**
District	40	40.0	41	41.0	
City	60	60.0	59	59.0	
Economic status					1,000**
Low	46	46.0	46	46.0	
Middle	54	54.0	54	54.0	
Profession					0.874**
Housewife	28	28.0	29	29.0	
Student	5	5.0	5	5.0	
Civil servant	16	16.0	16	16.0	
Worker	28	28.0	22	22.0	
Unemployed	23	23.0	28	28.0	
Physical disorder					0.065**
Yes	7	7.0	1	1.0	
No	93	93.0	99	99.0	
Psychiatric treatment history					<0.001**
Yes	32	32.0	4	4.0	
No	68	68.0	96	96.0	
History of family psychiatric disorder					<0.001**
Yes	37	37.0	6	6,0	
No	63	63.0	94	94.0	
Smoking					<0.001**
Yes	31	31.0	6	6,0	
No	69	69.0	94	94.0	
Childhood trauma					<0.001**
Yes	45	45.0	22	22.0	
No	55	55.0	78	78.0	

Mann Whitney U, ** Chi-square analysis; IQR: Interquartile range

We discovered that the NA, SI, DS-14 total, and RSES scores of the patient group were significantly higher than those of the control group (p<0.001) (**Figure 1**). Besides, those with motor symptoms had significantly higher RSES scores than those not showing a motor symptom (p=0.02) (**Table 2**).

The NA (p=0.01), SI (p=0.031), and DS-14 total scores (p=0.044) of those who had attempted suicide were significantly higher than those who had no suicidal ideation (**Table 2**). Moreover, we found these scores to be significantly higher in the participants with childhood trauma than those without (p<0.001) (**Table 2**).

The results of the correlation analysis showed that there was a positive and significant relationship between the age of onset of treatment and age in the patient group (r=0.574 p<0.001). Similarly, in the patient group, the RSES scores were positively correlated with the NA (r=0.549 p<0.001), SI (r=0.410 p<0.001) and DS-14 total scores (r=0.521 p<0.001) (**Figure 1**).

The results of the ROC analysis on the DS-14 total scores by conversion disorder revealed the cut-off point to be 18. At this cut-off point, we calculated the sensitivity to be 85%, the specificity to be 63%, the positive predictive value to be 69.67%, and the negative predictive value to be 80.77%. Finally, we concluded the area under the curve to be 0.784. On the other hand, we found the cut-off point to be 1.08 for the RSES scores. At this cut-off point, we calculated the sensitivity to be 57%, the specificity to be 74%, the positive predictive value to be 68.67%, and the negative predictive value to be 63.25%. We found the area under the curve to be 0.659 (**Table 3**, **Figure 2**).

Table 2. Participants' scores by various parameters

		NA		SI		DS-14 total		RSES	
		Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p
Group	Patient	17 (12-20)	<0.001	11 (9-16)	<0.001	30 (21-36)	<0.001	1.1 (.8-1.7)	<0.001
	Control	8 (6-12.5)		8 (5-11)		16 (12-22)		1.0 (.8-1.1)	
Psychosocial stress factor	Yes	18 (13-23)	0.111	11 (9-16)	0.685	31 (22-36)	0.123	1.2 (1.0-1.8)	0.239
	No	16 (8-20)		12 (7-17.5)		29 (17-33.5)		1.1 (.8-1.4)	
Secondary benefit	Yes	17.5 (11-23.5)	0.601	11 (9-16.5)	0.830	30 (20.5-36)	0.806	1.2 (.9-1.7)	0.457
	No	17 (13-20)		12 (9-16)		30 (21-35)		1.1 (.8-1.7)	
Motor symptom	Yes	17 (15-20)	0.457	11 (10-15)	0.344	30 (26-35)	0.458	1.3 (1.1-1.8)	0.02
	No	17.5 (10-21)		11.5 (8-17)		31 (19-36)		1.1 (.8-1.6)	
Sensory symptom	Yes	18 (13-20)	0.655	12 (9-15)	0.926	30 (20-36)	0.917	1.1 (.8-1.5)	0.330
	No	17 (12-20)		11 (9-16)		30 (21-36)		1.2 (.8-1.8)	
Pseudo seizure	Yes	18 (11-23)	0.769	11 (8-16)	0.331	29.5 (20-36)	0.694	1.2 (.8-1.8)	0.259
	No	17 (13-20)		13 (10-18)		30 (22-35)		1.1 (.8-1.5)	
Treatment	None	16.5 (11-20)	0.194	11 (8-17)	0.671	29.5 (19-35)	0.393	1.2 (.8-1.7)	0.474
	Irregular treatment	18 (12-24)		14 (9-16)		31 (20-36)		1.1 (1.0-1.5)	
	Regular treatment	18.5 (16-20)		11.5 (10-15)		30.5 (27.5-35)		1.2 (1.0-1.8)	
Hospitalization	Yes	18.5 (14.5-24)	0.264	11.5 (9.5-14.5)	0.920	30 (24-36)	0.568	1.2 (1.1-1.5)	0.516
	No	17 (11-20)		11 (9-17)		30 (20-36)		1.1 (.8-1.7)	
Suicide	Yes	24 (24-26)	0.01	17 (14-20)	0.031	35 (31-46)	0.044	1.7 (1.2-2.2)	0.174
	No	17 (12-20)		11 (9-16)		30 (21-36)		1.1 (.8-1.7)	
Childhood trauma	Yes	17 (13-20)	<0.001	11 (10-15)	<0.001	30 (22-34)	<0.001	1.3 (1.0-1.8)	<0.001
	No	9 (7-17)		9 (6-12)		18 (14-29)		1.0 (.7-1.1)	

Mann-Whitney U test for two-group comparison; Kruskal-Wallis test for three-group comparison; IQR: Interquartile Range; DS-14: Type D Personality Scale; RSES: Rosenberg Self-Esteem Scale; NA: negative affectivity; SI: social inhibition

Table 3. The results of the ROC analysis

	NA	SI	DS-14 total	RSES
Cut-off point	11	8	18	1.08
Sensitivity	76%	78%	85%	57%
Specificity	72%	56%	63%	74%
Positive predictive value	73.08%	63.93%	69.67%	68.67%
Negative predictive value	75%	71.79%	80.77%	63.25%
AUC (area under the curve)	0.786	0.720	0.784	0.659
AUC 95 % confidence interval	0.722-0.840	0.652-0.781	0.721-0.839	0.589-0.724
AUC p value	<0.001	<0.001	<0.001	<0.001

DS-14: Type D Personality Scale; RSES: Rosenberg Self-Esteem Scale; NA: negative affectivity; SI: social inhibition

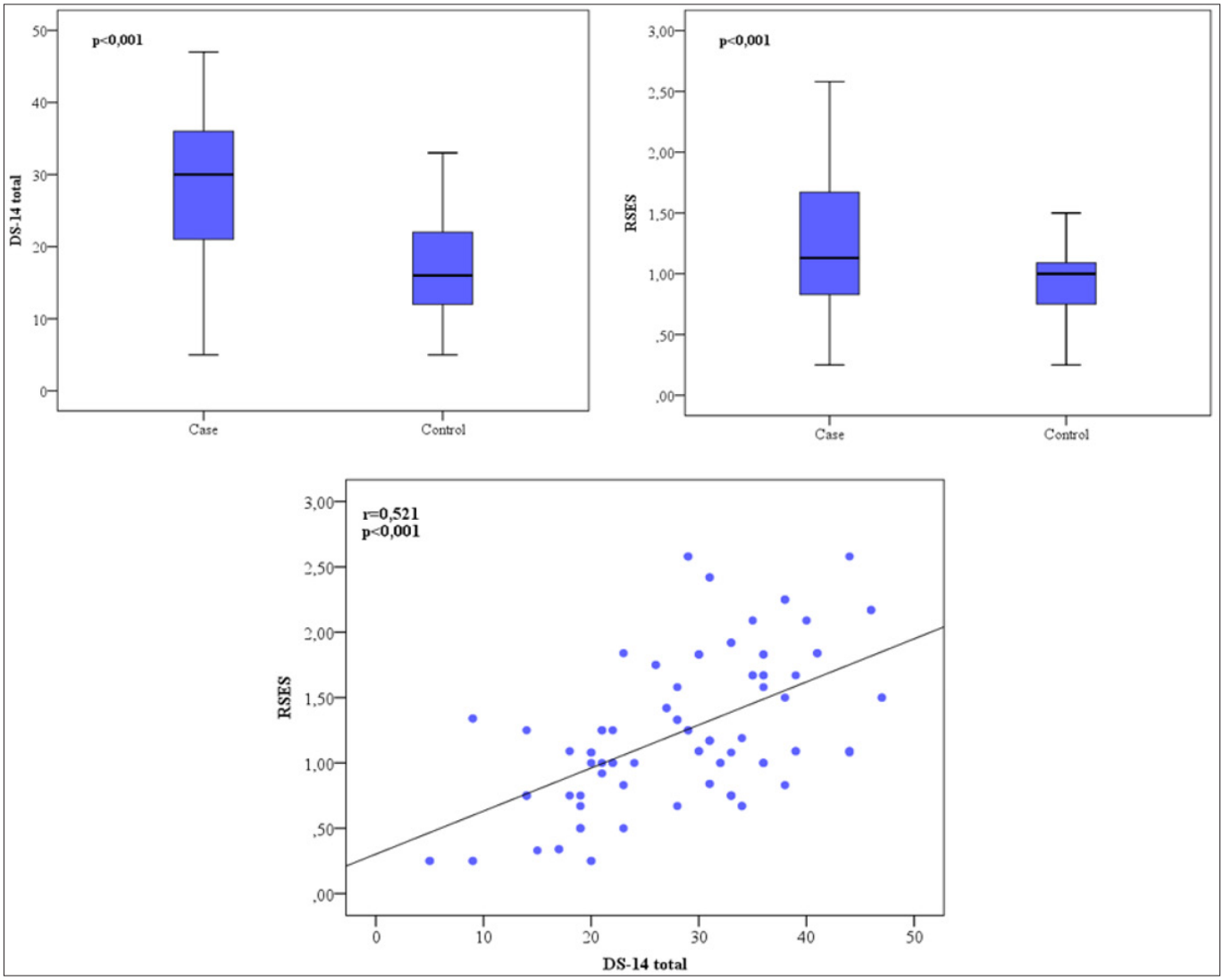

Figure 1. Comparison and correlation of the DS-14 total and RSES scores

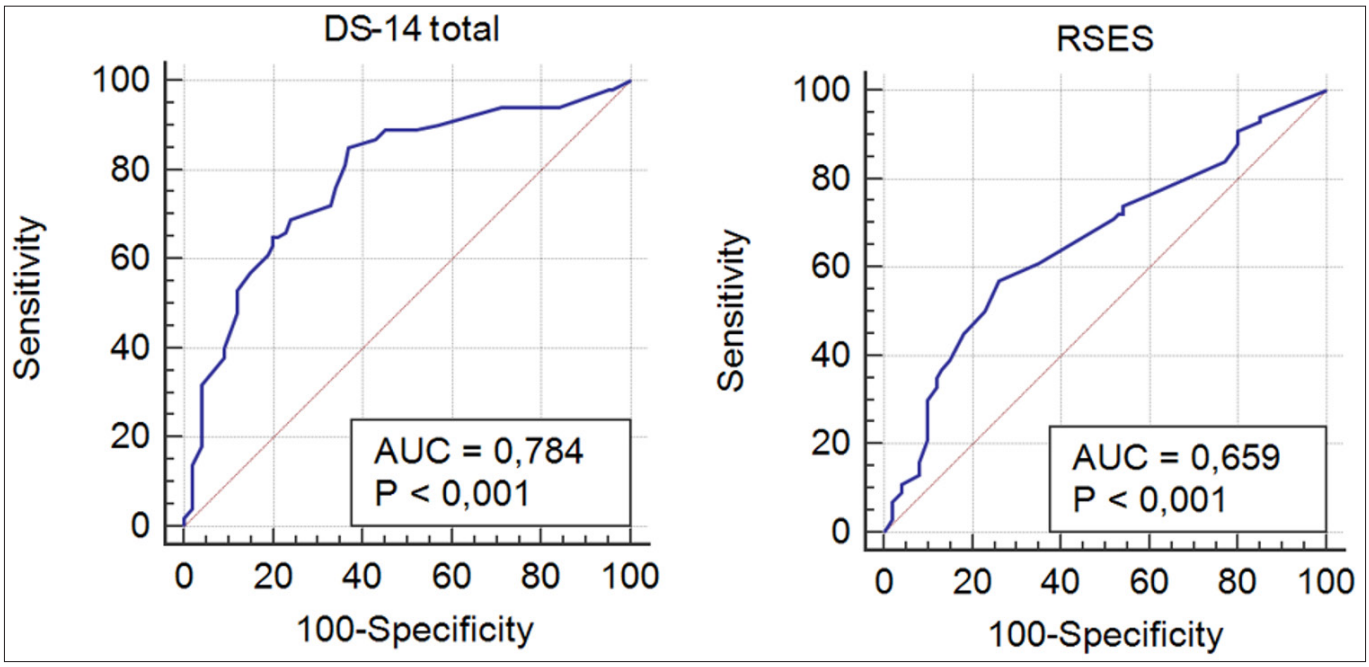

Figure 2. ROC for the DS-14 total and RSES scores

DISCUSSION

The DS-14 total, NA, SI, and RSES scores were significantly higher in the patient group than in the control group, which can be considered the most noteworthy finding of the study. The findings also support our hypothesis that CD patients have prevalent type D personality traits along with low self-esteem.

Individuals with type D personality are generally those who are under chronic stress, prone to negative emotions, such as anxiety and irritability, who cannot find ways to cope with stress, and who often feel socially isolated and lack self-confidence. These people do not share their negative feelings with others for fear of rejection (17). Feeling socially isolated may lead one to feel anxious and suppressed simultaneously, while people with negative affectivity demonstrate more anxiety and physical symptoms (15). Being unable to express negative emotions verbally instead of physically is shown among the causes of conversion disorder (1). Besides, social inhibition is defined as the inhibition tendency in expressing feelings and behaviors due to concerns such as being disliked and not being reciprocated in social relations (12). In our study, we found the SI and NA scores of the patients to be higher than those of the control group. It is already known that anxiety and negative emotions decrease through the symptoms compatible with CD; the same situation may increase one's social acceptance. In case of stress underlying CD, patients may express their social support needs with somatic complaints. When these needs are not met, these individuals may exhibit social withdrawal, introversion, and low cooperation.

In a study, the researchers examined the personality traits of 72 CD patients using the Hacettepe Personality Inventory. As a result, they found the neurotic tendencies and personal adjustment of the patients to be low and attributed low neuroticism to intense use of conversion as a defense mechanism in coping with anxiety (11). Considering the research findings on personality traits in somatoform disorders, patients with somatoform disorder suppress their feelings of inadequacy and exhibit alexithymia, which is defined as difficulty in recognizing and expressing feelings (7). In addition, it was concluded that harm avoidance is prevalent in those adopting somatization excessively (7). These people are also passive, cowardly, and insecure, as well as exhibiting inhibited and shy behaviors in social environments (24). In research utilizing the Temperament and Character Inventory, CD patients were found to have low levels of self-determination and elevated harm avoidance, as well as showing inadequacy in self-management and cooperation (10). Similar studies with Turkish samples compared CD patients with healthy controls and concluded that CD patients had significantly reduced self-directedness (25, 26). Nevertheless, except for such limited findings, the research interest seems to miss type D personality traits in individuals with CD.

One's pessimistic thoughts about the immediate environment can also adversely affect their self-perception. We suppose that one's poor self-perception may cause low self-esteem and frequent psychosomatic symptoms since it is well-known that high self-esteem reduces psychopathological symptoms (27). In the study, the patient group had significantly lower self-esteem than the healthy controls. Both the CD symptoms and the underlying psychiatric reasons may affect one's self-confidence.

In our study, the DS-14 scores increased as the patients' self-esteem decreased. Similarly, previous research reported that type D individuals are dissatisfied with their lives and have low self-esteem (11). Type D personality is closely associated with pessimism, perceived lack of social support, low self-esteem, dissatisfaction, and low quality of life (28). Negative thoughts in type D personality may impair self-esteem and are closely related to how one perceives themselves and events and how they establish healthy relationships with others (11). In addition, it is often mentioned that type D individuals build inadequate rapport with others, contributing to their low self-perception (29).

We discovered the DS-14 total, NA, and SI scores to be significantly higher in CD patients with a previous suicide attempt. Accordingly, type D personality traits may have predicted suicidal behavior in CD. In the literature, a study found major depressive disorder to be associated with suicidal thoughts in CD patients (16). Other studies also determined the rate of type D personality be significantly higher in those with suicidal ideation (17). Therefore, our findings are consistent with what was previously found in the literature.

The DS-14 total, NA, SI, and RSES scores of CD patients with childhood trauma were also significantly higher. Psychosomatic responses are bodily manifestations of psychological factors. Accordingly, adult patients with prevalent psychosomatic symptoms often have a history of childhood trauma (30). In the same context, we found that that a significant part of the patients (45%) had at least one traumatic life event (parental separation or loss, neglect, and abuse (sexual-physical-emotional)), which supports the relevant literature. Individuals having experienced childhood trauma may have learned to avoid trauma by producing physical disorders. Just like in type D personality, this situation may prevent them from cooperating and cause them to withdraw from society, become introverted, and have somatic complaints when facing stress. Traumatic events in the

past may lower one's self-esteem, and such individuals may show symptoms compatible with CD due to their negatively biased cognitive patterns. Traumatic people can also see themselves as worthless and be affected very quickly by any events. Based on our findings, we think that the low self-esteem in the patients may have been because of the adverse effects of self-perception inhibited by past traumas.

Conversion disorder is a disorder that may cause confusion in the diagnosis and fake neurological disorders (1). An auxiliary scale to be used to diagnose CD should have high discriminative power. The results of the ROC analysis on the DS-14 total score by conversion disorder revealed the cut-off point to be 18. At this cut-off point, we calculated the sensitivity to be 85% and the specificity to be 63%. On the other hand, we found the cut-off point to be 1.08 for the RSES score. At this cut-off point, we calculated the sensitivity to be 57% and the specificity to be 74%.

The retrospective nature of this case-control study hinders the generalizability of the results, which can be counted among the limitations to the study. Therefore, conducting experimental and longitudinal studies may help better understand the relationship between self-esteem and type D personality in CD. Another limitation is that the scales used are all based on self-report (we assumed that all participants provided correct and candid responses to the scale items). Finally, just like CD, the fact that type D personality is seen at a higher rate in females (31) can be a disadvantage for the sample group.

CONCLUSION

Overall, we found that CD patients had type D personality traits and low self-esteem. In addition, the DS-14 scores increased as the patients' self-esteem decreased. The finding that CD patients have low self-esteem may enable us to better deal with their psychosomatic symptoms and social problems. Knowing that SI and NA are high in these patients may drive physicians to take measures to mitigate these situations and adopt a different perspective on CD.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Non-interventional Researchs Ethics Committee of Fırat University (Date: 14.01.2021, Decision No: 2021/01-07).

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

Referee Evaluation Process: Externally peer-reviewed.

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Neutrophil-to-lymphocyte ratio in patients with white-coat hypertension

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ABSTRACT

Introduction: White coat hypertension (WCH), a distinct phenotype of hypertension, is defined as elevated office blood pressure (BP) measurements during repeat visits with normal out-of-office BP measurements. The association of WCH with cardiovascular risk remains unclear; however, current data support an association between untreated WCH and the risk for cardiovascular events, cardiovascular mortality, and all-cause mortality. Increased inflammatory processes may explain the underlying pathophysiology of the increased risk for cardiovascular events in WCH; therefore, we evaluated the neutrophil-to-lymphocyte ratio (NLR) of patients with WCH compared with matched normotensive controls.

Material and Method: Forty-five eligible subjects with WCH and 45 age, sex, and BMI matched healthy and normotensive subjects were included in the study. The subjects were assessed by office arterial BP and 24-hour ambulatory BP measurements. An automated blood count analyzer measured the NLR values.

Results: The mean NLR in the patients-with-WCH group was significantly higher than that in the control group (2.67±0.27 vs. 2.46±0.34, p<0.001, Student's t-test); however, NLR was not correlated with BP measurements in either the WCH or control group.

Conclusion: NLR, a marker of inflammation, was increased in patients with WCH compared to the controls. Inflammation is a triggering mechanism for various cardiovascular and cerebrovascular events. Therefore, NLR has value as a potential independent risk factor that deserves further study, particularly in patients with WCH.

Keywords: High blood pressure, systemic hypertension, inflammation, neutrophil-to-lymphocyte ratio, white coat hypertension

INTRODUCTION

Hypertension is a major preventable cause of morbidity and mortality worldwide. Due to the increasing use of out-of-office blood pressure (BP) measurements, several distinct hypertension phenotypes have become apparent. One of these is white coat hypertension (WCH), defined as elevated office BP measurements during repeated visits with normal out-of-office BP measurements as assessed by home and/or 24-hour ambulatory BP monitoring measurements. Therefore, current guidelines recommend using out-of-office BP measurements to diagnose phenotypes, such as WCH (1). A systematic review demonstrated that the overall prevalence of WCH, defined by 24-hour ambulatory BP measurements (ABPM), in the general population ranges from 5% to 65%, and the prevalence increases with age (2-5).

Although hypertension is a well-established risk factor for morbidity and mortality, the association of WCH with cardiovascular risk remains unclear (5, 6). Several previous meta-analyses have reported weak to no associations of WCH with cardiovascular and all-cause mortality (7), especially for treated WCH (4); however, a recent meta-analysis revealed untreated WCH, but not treated WCH, as a risk factor for cardiovascular events, cardiovascular mortality, and all-cause mortality (8). An increase in atherosclerosis possibly causes this increase. Inflammatory processes are increasingly being recognized as playing a central role in the pathogenesis of atherosclerotic diseases and their complications (9). Elevated levels of systemic inflammatory markers have been found to be associated with cardiovascular diseases (10-12), and increased inflammatory processes may explain the underlying pathophysiology of the increased risk for cardiovascular events in WCH. Several previous studies have demonstrated an increase in novel inflammatory markers in WCH and sustained hypertension, such as high-sensitivity c-reactive protein (CRP), soluble CD40 ligand, procalcitonin, and pentraxin-3 (13-16).

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The neutrophil-to-lymphocyte ratio (NLR), routinely determined by complete blood count analysis, has emerged as a novel inflammation marker. It has been documented that NLR may predict inflammatory status for various cardiovascular risk factors, such as hypertension, diabetes mellitus, obesity, and smoking (17-19). Increased NLR is also associated with a poor prognosis of various cancers and ischemic heart disease (20-22). Although it has previously been shown that increased NLR is associated with essential hypertension and its complications, the relationship between NLR and WCH has not been previously studied (23-26). This study aimed to evaluate the NLR in patients with WCH compared with age-, sex-, and body mass index (BMI)-matched normotensive controls.

MATERIAL AND METHOD

Study Setting

This cross-sectional case-control study was conducted in the internal medicine outpatient clinics of the local University Hospital per the Declaration of Helsinki. Approval for this study was obtained from Akdeniz University Medical School Non-Interventional Clinical Researchs Ethics Committee (Date: 08.07.2020, Decision No: 488). All patients provided written informed consent.

Study Design and Participants

This study aimed to evaluate patients with WCH compared to age-, sex-, and body mass index (BMI)-matched normotensive controls. WCH was defined and diagnosed as a measured office BP of greater than 140/90 mmHg with an ambulatory measurement of less than 135/85 mmHg (2). Healthy subjects, recruited from the routine check-up program of the local University Hospital, were used as a control group. Patients with sustained hypertension; diabetes mellitus (fasting glucose >126 mg/dL or hemoglobin A1c >6.5%); a history of smoking or alcohol intake of more than 30 g/day; hyperlipidemia; obesity (BMI ≥30 kg/m^2); cardiac, renal, cerebral, and other systemic diseases; and recent major surgery or illness were excluded from the study. Hypertension was defined as systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg, as recommended by the 2013 ESH/ESC guidelines for the management of arterial hypertension (27). Hyperlipidemia was defined as the presence of at least one of the following conditions: increased plasma triglycerides (>200 mg/dL), total cholesterol (>200 mg/dL), low-density lipoprotein cholesterol (>130 mg/dL), or decreased high-density lipoprotein cholesterol (<40 mg/dL for men and <50 mg/dL for women) (28). Forty-five eligible subjects with WCH and 45 age-, sex-, and BMI-matched healthy and normotensive subjects were included in the final analysis.

Assessment Instruments

The subjects underwent a comprehensive assessment, including medical history, physical examination, and measurement of laboratory variables. Body weight and height were measured with the subjects wearing light clothes without shoes. BMI was calculated as the weight (kg)/height squared (m)^2. All the subjects' resting electrocardiograms were normal. A mercury sphygmomanometer was used to measure arterial BP after the patient had remained in a sitting position for 5 min. For each subject, the average of three readings obtained within 5 min was recorded. For ABPM, a portable non-invasive recorder (SpaceLabs Medical Devices, Inc., Redmond, WA, USA) programed to record BP every 30 min for 24 hours was used for all measurements. This information was then used to calculate the average ambulatory BP over 24 hours for each subject. For laboratory evaluations, blood samples were collected from the antecubital vein without a tourniquet between 08:30 a.m. and 09:00 a.m. after overnight fasting. An automated blood count analyzer measured the total and differential blood cell counts. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. Fasting plasma glucose, creatinine, alanine aminotransferase levels, c-reactive protein and lipid profiles were measured by enzymatic-colorimetric assays (Roche Diagnostic GmbH, Mannheim, Germany).

Statistical Analyses

SPSS statistical software (SPSS for Windows 16.0, Chicago, IL, USA) was used for the analyses. For a type 1 (α) error of 0.05 and a power of 80%, a sample size per group of at least 36 subjects was needed to detect an actual difference. The normality of the distribution was determined by Shapiro–Wilk tests. NLR values between the groups were compared by Student's t-tests and Pearson's correlation coefficients. Data for categorical variables were presented as frequency and percentage, and continuous variables were expressed as mean±SD. Statistical significance was defined as $p<0.05$.

RESULTS

Age, gender distribution, and BMI were similar between the WCH and control groups. Office systolic and diastolic BP measurements were significantly higher in patients with WCH ($p<0.001$, Student's t-test). The metabolic parameters in the study groups were similar as a result of the participant selection process. The mean NLR in the patients-with-WCH group was significantly higher than that in the control group (2.67±0.27 vs. 2.46±0.34, $p<0.001$, Student's t-test). However, NLR was not correlated with BP measurements in the WCH and control groups (Spearman's rho correlation coefficient 0.16 and 0.09, p value 0.14 and 0.42, respectively) (**Table**).

Table. Study group characteristics and laboratory results

Parameters	WCH group (n=45)	Control group (n=45)
Gender (men/women)	45 (21/24)	45 (22/23)
Age (years)	55±3	55±2
BMI (kg/m^2)	23.7±3.1	23.6±3.2
Office systolic BP measurement (mmHg)	146±4.7**	127±5.1
Office diastolic BP measurement (mmHg)	94±5.3***	81±4.6
Fasting plasma glucose (mg/dL)	86.8±9.7	87.1±9.6
Creatinine (mg/dL)	0.9±0.2	0.9±0.2
Alanine aminotransferase (U/L)	25.7±3.5	25.5±3.4
C-reactive protein (mg/dL)	0.23±0.11	0.17±0.07
Total cholesterol (mg/dL)	169.4±21.9	169.9±22.1
LDL cholesterol (mg/dL)	88.3±12.1	88.9±11.6
HDL cholesterol (mg/dL)	48.7±5.4	48.4±5.3
Triglyceride (mg/dL)	129.6±15.8	128.9±16.3
White blood cell (×10^3/mm^3)	6.76±1.72	6.69±1.74
Neutrophil (×10^3/mm^3)	4.74±1.55	4.66±1.62
Lymphocyte (×10^3/mm^3)	1.81±0.19*	1.90±0.21
Neutrophil to lymphocyte ratio	2.67±0.27***	2.46±0.34

WCH: White coat hypertension, BMI: Body mass index, BP: Blood pressure, LDL: low-density lipoprotein; HDL: high-density lipoprotein. *p<0.05, **p<0.01, and ***p<0.001, Student's t-test, WCH group vs. control group.

DISCUSSION

Hypertension is a well-established risk factor for morbidity and mortality, possibly due to its' relation with atherosclerosis. Atherosclerosis is characterized by a complex multifactorial pathophysiology. Inflammatory processes in vessel walls are increasingly recognized as playing a central role in the initiation, progression, and final steps of atherosclerosis (9,29). WCH, a distinct phenotype of hypertension, was recently demonstrated as a risk factor for cardiovascular events, cardiovascular mortality, and all-cause mortality, especially if left untreated (4,8). Several inflammatory markers have been shown to be increased in WCH (14-16), but the relationship between NLR and WCH has not been previously studied. In this matched case-control study, we aimed to evaluate the NLR in patients with WCH compared to age-, sex-, and body mass index (BMI)-matched normotensive controls. We excluded patients with several confounding factors such as sustained hypertension, diabetes mellitus, smoking or alcohol intake, hyperlipidemia, obesity, cardiac, renal, cerebral, and other systemic diseases, and recent major surgery or illness. In this study population, we have demonstrated that NLR was increased in patients with WCH, compared to age-, sex-, and body mass index (BMI)-matched normotensive controls.

There are several studies in the literature on the relationship between inflammation, including NLR, and essential hypertension (23-26). However, there are limited studies about the relationship between systemic inflammation markers and WCH. Ozdogan et al. (14) reported that in patients with WCH, high-sensitivity CRP, a marker of low-grade inflammation, is higher than normotensive patients, whereas in patients with essential hypertension, high-sensitivity CRP levels were even higher than in patients with WCH. Andrikou et al. (30) confirmed higher high-sensitivity CRP levels and demonstrated that WCH is also associated with arterial stiffening compared with normotensive patients. Similarly, several studies have also reported increased soluble CD40 ligand, procalcitonin, and pentraxin-3 levels in WCH compared to normotensive groups (14-16).

Neutrophil-to-lymphocyte ratio is a novel, non-invasive, easily-calculable, and easily obtained marker as a surrogate for inflammation. NLR has been previously studies and in many hemato-oncologic, immunologic and infectious diseases as well as cardiologic disorders, and the increase has been associated with increased morbidity and mortality. It has been well documented that NLR is associated with increased inflammatory status for various cardiovascular risk factors and essential hypertension and its complications (17-19,23-26). Herein, we add to the literature that an increase in NLR is seen in patients with in WCH, compared to controls, as a novel marker of inflammation. Although WCH as a risk factor for cardiovascular events, cardiovascular mortality, and all-cause mortality is still of debate, an increase in NLR as a surrogate for increased inflammation may suggest that patients with WCH are also at risk for cardiological disorders such as ischemic heart disease.

This study has certain limitations. First, the sample size was small, although higher than the calculated sample size; nevertheless, both groups were homogeneous in terms of age, gender, BMI, and laboratory results. Second, due to the case-control study design, the results may not reflect long-term effects. Third, these findings are limited to a homogeneous group of patients, so the results may not apply to all patients with WCH.

CONCLUSION

We have demonstrated that NLR, a marker of systemic inflammation, was increased in patients with WCH compared to controls. Inflammation is a triggering mechanism for various cardiovascular and cerebrovascular events; therefore, NLR has value as a potential independent risk factor that deserves further study, particularly in patients with WCH.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for this study was obtained from Akdeniz University Medical School Non-Interventional Clinical Researchs Ethics Committee (Date: 08.07.2020, Decision No: 488).

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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Is macrocytic erythrocyte a new prognostic parameter in critical COVID-19 disease?

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ABSTRACT

Introduction: In critical COVID- 19 patients, we aimed to examine the relationship mortality between the parameters and the macrocytic/microcytic erythrocyte population that are routinely evaluated in each patient admitted to the intensive care unit.

Material and Method: It is a retrospective cross-sectional study and was conducted in Kastamonu University, Faculty of Medicine, a tertiary hospital intensive care units located in the North of Turkey .198 nonanemic critical COVID-19 patients treated between November 2020 and February 2021 were evaluated. The patients were divided into two groups as survival and non survival. APACHE II score, SAPS II score and routine blood examinations of the patients were evaluated.

Results: Among the groups; APACHE II score ($p<0.001$), SAPS II score ($p<0.001$), CRP ($p<0.001$), ferritin ($p<0.001$), d-dimer ($p<0.001$), platelet distribution width ($p<0.009$), mean platelet volume ($p=0.005$) and large platelet ratio ($p=0.02$) values were higher, platelet counts ($p=0.02$) were lower, and these parameters were statistically significant between the two groups. There was no difference in erythrocyte distribution volume and microcytic erythrocyte percentage between the groups, but according to the cox regression analysis, each unit increase in macrocytic erythrocyte percentage was associated with a 1.203-fold increase in mortality.

Conclusion: Routine procedures performed in every patient admitted to the intensive care unit can provide information about mortality. Macrocytic erythrocyte percentage, which is not generally considered in non-anemic patients, may be a new marker for mortality.

Keywords: Macrocytic erythrocyte population, intensive care unit, COVID-19, complete blood count, mortality

INTRODUCTION

The coronavirus disease emerged in the human population in late December 2019 and spread all over the world, and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (1-2). Although vaccination studies have started in many countries for COVID-19 disease, this disease still continues worldwide with all its severity. In the literature, it has been stated that the intensive care mortality associated with COVID-19 disease is between 40% and 90% (3). Since the beginning of the pandemic; a lot of research has been done to predict the intensive care prognosis and mortality of critical COVID-19 patients. In these studies, some scoring (4), tomography findings (5) and inflammatory markers (6-7) were associated with the intensive care prognosis and mortality of critically ill COVID-19 patients. Acute Physiology and Chronic Health Evaluatioon Score II (APACHE II), Simplified Acute Physiology Score II (SAPS II) and blood tests are used in the routine evaluation of patients in both COVID intensive care unit and non COVID intensive care unit admission. Complete blood count provides vital parameters that guide clinical management, including diagnosis, infection or inflammation, anemia, response to treatment, pathogenesis, and inflammatory process (8).

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The aim of every patient admitted to the ICU is to reduce mortality and morbidity. The markers that guide us in this regard are very important. CBC, which is among the routine tests, has parameters such as lymphocyte count, platelet count, red cell distribution width, etc. that can be associated with mortality in COVID and nonCOVID ICU patients. These parameters are not only correlated with adverse outcomes of COVID-19 disease, but may also be associated with bone marrow suppression due to malignancy or with previously diagnosed anemia.

Macrocytic erythrocyte populations (Macro R) and microcytic erythrocyte populations (Micro R) are routinely studied parameters in the CBC test. These parameters are used to investigate possible causes of anemia (9). However, these parameters are generally not considered in patients who are not anemic.

In critically COVID-19 patients, we evaluated APACHE II , SAPS II, whole blood tests and Macro R/ Micro R, which are routinely used in the evaluation of every patients admitted to the intensive care unit. We aimed to investigate the relationship between this parameters and prognosis or mortality of patients in intensive care units .

MATERIAL AND METHOD

Approval for study was obtained from the Non-interventional Clinical Researchs Ethics Committee of Kastamonu University Faculty of Medicine (Date: 25/02/2021, Decision No: KAEK-2020-143-38). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

It is a retrospective cross-sectional study and was conducted in Kastamonu University, Faculty of Medicine, a tertiary hospital intensive care units located in the North of Turkey. In the study, 236 critical COVID-19 patients treated in the third level COVID intensive care unit between November 2020 and February 2021 were evaluated. For a more objective evaluation of CBC parameters; as inclusion criteria for the study; not having any previous diagnosis of malignancy and anemia defined by the World Health Organization (hemoglobin concentration below 12 g/dL in women and 13 g/dL in men) (10). Thirteen patients with a history of malignancy and 25 patients with anemia were excluded from the study, and 198 patients were included in the study. All of the patients included in the study were critical COVID-19 patients according to the COVID-19 Adult Patient Management Guidelines published by the Ministry of Health of the Republic of Turkey (11). All of the patients included in the study were positive in the real-time polymerase chain reaction (RT-PCR) test performed by the hospital laboratory. Patients who were successfully treated and transferred to the service during the intensive care treatment process were named Survival Group (98 patients) and those who died were named Non- survival Group (100 patients). The medical records and electronic patient data system of the patients included in the study were reviewed retrospectively. Patients; Age, gender, APACHE II scores, SAPS II scores, ferritin, C-reactive protein (CRP), procalcitonin, d-dimer values and 27 parameter CBC values were recorded on the first day of admission to the intensive care unit. . CBC parameters were calculated using an automated hematology analyzer (XN-1000-Hematology-analyzer-Sysmex Corporation, Japan).

Statistical Analysis

The significance in differences between the means of two continuous and normally distributed variables was determined by independent t-test. Non-normal distributed continuous variables were explained by Mann-Whitney U test. Pearson's chi-square test was conducted to determine the relationship in proportions of categorical variables between two groups. The optimal cut-off values of continuous Apache and Saps score were calculated by applying the Receiver Operating Curve (ROC9 analysis. The association of biochemical parameters with survival was determined by Cox regression analysis. Cox regression with forward stepwise method was used to determine the impacts of age, gender, and other important biochemical variables. Relative risk was calculated with hazard risk and 95% confidence interval (CI). $p<0.05$ was considered as statistically significant. All statistical analyses were performed using the SPSS 23.00 (SPSS Inc, Chicago, USA).

RESULTS

A total of 198 patients were included in the study. Of the participants, 67 (33.8%) were female and 131 (66.2%) were male. After intensive care treatment, 98 (49.5%) patients were transferred to the service (Survival Group); 100 (50.5%) patients died during the intensive care treatment process (Non- survival Group).

Sixty-two (63.2%) patients in Survival Group and sixty-nine (69%) patients in Non- survival Group were male. The patients included in the study were between the ages of 40-98 and the mean age of patients was 67.05 ± 0.47. In the data analysis made between the groups; the mean age of the patients in Non- survival Group (68.75 ± 8.98) was higher than the mean age of the surviving patients (65.31 ± 11.79).

The patients in Non- survival Group ; age, APACHE II score, SAPS II score, CRP, ferritin, d-dimer, platelet distribution width (PDW), mean platelet volume (MPV) and large platelet ratio (%P-LCR) were higher on the first day of admission to the intensive care unit, but

platelet(PLT) counts were lower than Survival Group

There were statistically significant differences in these parameters between both groups (Age (P< 0.02), APACHE II (P <0.001), SAPS II (P <0.001), Crp (P <0.001), Ferritin (P<0.001), D -dimer (P <0.001), Plt (P = 0.02), PDW (P = 0.009), MPV (P = 0.005) and % P-LCR (P = 0.002)). Red cell distribution width (% RDW) values were similar between both groups; There was no statistical difference between the groups. ((%RDW (P = 0.56)) (**Table 1**)

Cox regression analysis with stepwise method was used to analyze the risk factors for fatal outcomes in patients with COVID -19. The significant parameters were entered the Cox model to identify independent predictors of death. Model performed well regarding the result of omnibus test. The results of this test explained that our model is significant (X2=60.75, P<0.001) improvement in fit relative to null. Biochemical variables including Saps II (HR 1.110, CI 1.076-1.146, P<0.001), D-dimer (HR 0.873, CI 0.784-0.973, P=0.014), Procalcitonin (HR 0.877, CI 0.773-0.994, P=0.04) , MONO% (HR 0.902, CI 0.845-0.963, P=0.002) and MacroR (%) (HR 1.203, CI 1.058-1.369, P=0.005) were predictive of fatal outcomes. A one-unit increase in MacroR was associated with a 1.203-fold increase in mortality and was considered a major predictor. An increase in Saps II score was also associated with mortality. Each unit increase in Saps II score was associated with a 1.11-fold increase in mortality rate (**Table 2**).

Table 1. Socio-demographics and biochemical characteristics of patients (total N=198)

Variables	Total n=198 M* ± SD	Survival Group n= 98 M ± SD	Non- survival Group n= 100 M ± SD	test	P
Age (years)	67.05 ± 0.47	65.31 ± 11.79	68.75 ± 8.98	t= -2.30	0.02
Gender	198	62/36**	69/31**	X2=0.72	0.39
Apache II	22.41 ± 7.82	16.56 ± 5.31	28.14 ± 5.20	t=--15.48	<0.001
Saps II	30.69 ± 9.54	23.42 ± 5.89	37.81 ± 6.60	t=-16.16	<0.001
Crp (mg/l)	137.22 ± 87.56	114.49 ± 89.01	159.51 ± 80.50	t=--3.73	<0.001
Ferritin (ng/ml)	909.32 ± 454.58	690.28 ± 443.31	1123.98±353.00	t=--7.60	<0.001
D Dimer (mg/l)	2.60 ± 2.04	1.95 ± 1.65	3.23 ± 2.20	t=--4.61	<0.001
Procalcitonin (mg/dl)	1.61 ± 1.88	103.99*	95.10*	Z=-1.10	0.27
WBC (10^3/uL)	10.12 ± 5.35	10.20 ± 5.19	10.04 ± 5.53	t=-0.21	0.83
RBC (10^6/uL)	4.47 ± 0.80	102.67*	96.40*	Z=-0.77	0.44
HGB (g/dL)	14.54 ± 2.14	98.66*	100.32*	Z=-0.20	0.83
HCT (%)	42.17 ± 6.54	100.03*	98.98*	Z=-0.13	0.89
MCV (fL)	85.88 ± 6.82	85.81 ± 7.19	85.95 ± 6.48	t=--0.14	0.88
MCH (pg)	28.89 ± 11.25	95.51*	103.42*	Z=-0.97	0.33
MCHC (g/dL)	33.46 ± 10.16	96.21*	102.72*	Z=-0.79	0.42
PLT (10^3/uL)	205.42 ± 92.01	108.54*	90.65*	Z=-2.19	0.02
RDW-SD (fL)	45.39 ± 6.87	44.86 ± 5.96	45.91 ± 7.66	t=--1.07	0.28
RDW-CV (%)	14.63 ± 2.53	97.11*	101.85*	Z=-0.58	0.56
PDW (fL)	12.11 ± 2.26	11.69 ± 2.00	12.52 ± 2.42	t=--2.62	0.009
MPV (fL)	10.54 ± 0.95	10.35 ± 0.87	10.73 ± 1.00	t=--2.87	0.005
P-LCR (%)	29.10 ± 7.30	27.52 ± 6.65	30.65 ± 7.60	t=--3.08	0.002
NRBC# (10^3/uL)	0.02 ± 0.09	93.35*	105.53*	Z=-1.83	0.06
NRBC% (%)	0.16 ± 0.70	94.21*	104.68*	Z=-1.62	0.10
NEUT# (10^3/uL)	8.46 ± 5.08	8.47 ± 4.96	8.44 ± 5.22	t=0.04	0.96
LYMPH# (10^3/uL)	1.08 ± 0.79	101.20*	97.84*	Z=-0.41	0.68
MONO# (10^3/uL)	0.50 ± 0.34	0.53 ± 0.36	0.48 ± 0.32	t=0.91	0.36
EO# (10^3/uL)	0.04 ± 0.12	102.24*	96.71*	Z=-0.69	0.48
BASO# (10^3/uL)	0.03 ± 0.03	0.03 ± 0.2	0.03 ± 0.02	t=0.21	0.83
NEUT% (%)	81.01 ± 10.97	80.48 ± 11.69	81.51 ± 10.25	t=-0.66	0.51
LYMPH% (%)	12.87 ± 9.22	99.31*	99.69*	Z=-0.05	0.96
MONO% (%)	5.39 ± 3.12	5.51 ± 3.20	5.28 ± 3.05	t=0.51	0.61
EO% (%)	0.41 ± 1.24	102.57*	96.49*	Z=-0.78	0.43
BASO% (%)	0.31 ± 0.22	200.30*	98.72*	Z=-0.19	0.84
MicroR (%)	5.22 ± 6.47	99.88*	99.13*	Z=-0.09	0.92
MacroR (%)	4.09 ± 1.35	96.84*	102.11*	Z=-0.64	0.51

* Median Of Variables, ** Number Of Patients In Terms Of Female Gender t: T test Z: Man whitney u test X2: Chi square test Abbreviations: APACHE II: Acute Physiology and Chronic Health Evaluatioon Score II, SAPS II: Simplified Acute Physiology Score II.CRP: C-reactive protein, WBC: white blood cells, RBC: red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean cell hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: Platelets, RDW: red cell distribution width, PDW: platelet distribution width, MPV: Mean platelet volume, P-LCR: platelet-large cell rate, NRBC: Nucleated red blood cells, NEUT: Neutrophils,LYMPH: Lymphocytes, MONO: Monocytes EO: Eosinophils, BASO: Basophils, MicroR: microcytic erythrocyte populations, MacroR: macrocytic erythrocyte populations

Table 2. Cox regression analysis for prognostic factors

Variables	B	SE	p	HR	95% CI for HR	
					Lower	Upper
Saps II	0.105	0.016	<0.001	1.110	1.076	1.146
D-Dimer	-0.135	0.055	0.014	0.873	0.784	0.973
Procalcitonın	-0.131	0.064	0.040	0.877	0.773	0.994
MONO%(%)	-0.103	0.033	0.002	0.902	0.845	0.963
MacroR(%)	0.185	0.066	0.005	1.203	1.058	1.369

Abbreviations: SAPS II: Simplified Acute Physiology Score II, MONO: Monocytes, MACRO R: macrocytic erythrocyte populations .

The optimal cut-off values of Apache II and Saps II parameters were calculated by the ROC analysis (**Figure 1**). Areas under the curve (AUC) of Apache II and Saps II were 0.928 and 0.944, respectively (**Table 3**). They were the potential mortality biomarkers. The sensitivity and specificity were 0.86 and 0.898 for Apache II , and 0.85 and 0.918 for Saps II . In addition to this, the optimal cut-off values of Apache II and Saps II were 22.50 and 30.50, respectively.

Table 3. Areas under the curve (AUC) of Apache II and Saps II variables

Variables	Area	SE	Asymptotic Sig.	Asymptotic 95% CI	
				Lower	Upper
Apache II	0.928	0.019	<0.001	0.892	0.965
Saps II	0.944	0.016	<0.001	0.912	0.975

Abbreviations: APACHE II: Acute Physiology and Chronic Health Assessment Score II,SAPS II: Simplified Acute Physiology Score II

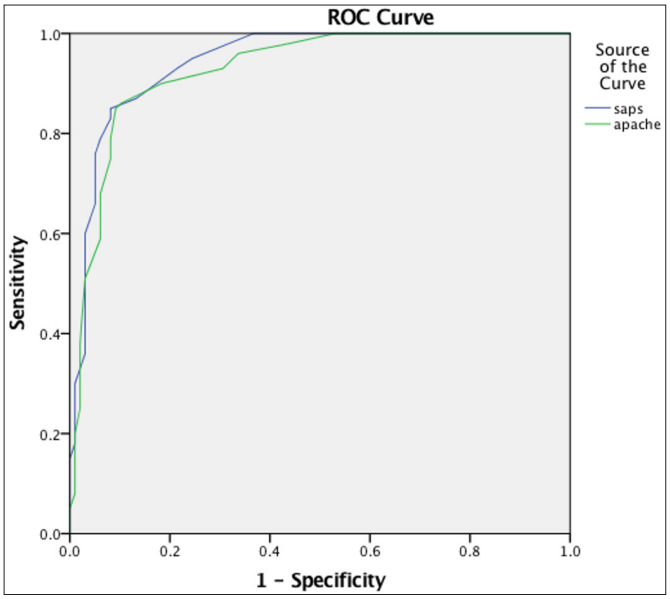

Figure 1. ROC curve of Apache II and Saps II variables

DISCUSSION

Our study revealed that age, APACHE II score, SAPS II score, CRP, ferritin, D-dimer and CBC parameters plt, MPV, PDW, %P-LCR values were statistically significant between surviving and non-surviving patients in critical COVID-19 patients. These findings were similar to previous studies (12-13). Contrary to the findings in the literature, the %RDW values were not statistically different in our study between surviving and non-surviving patients (14). There are few studies on the %Macro R value in the literature. Our study revealed the relationship of increased macro R values with mortality in critical COVID-19 disease, according to the results of the cox regression analysis performed in addition to other studies in the literature. With these results, according to the roc analysis for APACHE II and SAPS II scores; both scores were found to have high sensitivity and specify.

The male patient population is higher among patients treated in intensive care units due to COVID-19 disease. In our study, 66.1% of the total number of patients was male, which was similar to the studies in the literature (12-15). According to the meta-analysis published by Armstrong et al. (16), intensive care mortality in critical COVID-19 disease ranges from 34% to 49.7% . In a study conducted with the participation of more than 17 million COVID-19 patients in England, it was stated that the male gender was 1.6 times more likely to face the risk of mortality compared to the female gender (17). In our study, we found that the ICU mortality was 50.5% and 69% of the patients with mortality were male.

APACHE II and SAPS II scores are important parameters used in the evaluation of prognosis in critically ill patients treated in the intensive care unit. In their study, Xie et al stated that the APACHE II score on the first day of admission to the intensive care unit is an independent risk factor for 28-day mortality in COVID-19 disease. In our study, we found that there was a statistically significant difference in the APACHE II score between patients who survived and died. However, in our study, the APACHE II median values of the patients in both groups were higher than the study by Xie et al. (18). In our study, APACHE II score cut-off value was calculated as 22.5, sensitivity 0.86 and specificity 0.898. This value was found to be consistent with the literature (19). Larcher et al. (20)revealed the relationship between SAPS II scoring and mortality in COVID-19 disease. Duclos et al. (21) found the cut-off value as 31, sensitivity as 1, and specificity as 0.58 for the SAPSII score in their study with critical COVID-19 patients. In our study, the SAPS II score was statistically significant between surviving and non-surviving patients, and the cut-off value was

30.5, the sensitivity 0.85, and the specificity 0.918. In addition, in the cox regression analysis, we found a 1.11 fold increase in mortality with each point increase in the SAPS II score.

CRP and ferritin markers are inflammatory markers; as stated in a meta-analysis including 83 studies in the literature, it is seen that CRP and ferritin values have a significant relationship with mortality in COVID-19 disease (22). Studies have shown that d dimer, which is one of the coagulopathy markers, is associated with mortality at high values in COVID-19 disease (23). In our study, the CRP, ferritin and d dimer values of the patient group who died during the intensive care unit were higher and there was a statistically significant difference between the groups.

Complete blood count examination is an easily accessible and fast test. CBC examination is the most widely used first laboratory examination (24). There are studies in the literature that indicate the importance of CBC parameters about the prognosis of COVID-19 disease. In most of these studies, it was reported that there was a significant difference in neutrophil count, lymphocyte count, and platelet count between surviving and non-surviving patients (25). Ouyang et al. (26) reported in their study that non-survival patients had lower platelet counts. In our study, there was no statistical difference between the lymphocyte count and neutrophil counts among the non-survival patients, while the non-survival patients had lower platelet counts and there was a statistically significant difference between the groups. MPV, PDW and %P LCR; are some parameters related to platelet cells. In a study conducted with COVID-19 patients, the mean % P LCR values were found to be 21.6% in surviving patients and 26.7% in non-survival patients. In our study, the mean % P LCR values of the non-survival patients were higher and there was a statistically significant difference between the groups. In the study of Güçlü et al. (27) investigating the effect of COVID-19 disease on platelet count and indices, they stated that PDW and MPV values were higher in non-survival patients and there was a statistically significant difference. In our study non-survival patients had higher PDW and MPV values, and similar to this study, there was a statistically significant difference between the groups Thrombocytopenia is an expected finding in COVID-19 patients. Accordingly, as the platelet count decreases, there is an increase in platelet release from the bone marrow. This may explain the increase in MPV, PDWand P LCR.

Rdw is a parameter that reflects the degree of heterogeneity of erythrocyte volume used in the differential diagnosis of bone marrow dysfunction, iron deficiency anemia, and hematological system diseases. Proinflammatory state and hypoxia impair erythropoiesis and cause an increase in RDW. The studies have shown that RDW elevation is compatible with mortality in COVID-19 disease and in many disease groups (28). In the meta-analysis published by Lee et al. (29) in 2021; It was stated that RDW elevation was correlated with the negative outcomes of COVID-19 disease. However, the effect of anemia-independent RDW elevation in COVID-19 was not clarified in this study. In the study of Rapp et al. (14) with COVID-19 patients, it was revealed that RDW elevation was associated with mortality and morbidity, independent of anemia. However, patients who were not hospitalized were included in this study. The patients included in our study were patients who were not anemic and were treated in the intensive care unit. There was no statistically significant difference in RDW values between survivors and non-survivors.

%Macro R and %Micro R markers are parameters used to investigate possible causes in the etiology of anemia. Apart from investigating the etiology of anemia, they are not the most important markers for clinicians. There are limited publications in the literature stating that %Macro R and %Micro R values are associated with mortality. One of them was the study conducted by Horne et al (30). This study includes the patient group without COVID-19 disease. As a result of the cox regression analysis performed in our study, each unit increase in % Macro R values in COVID-19 patients was found to be compatible with a 1.2-fold increase in mortality. However, the % micro r value was not associated with mortality. There is an inflammatory state and a hypoxic process in COVID-19 disease (28). Depending on these factors, there is an increase in the release of erythropeitn. The increased secretion of erythropoietin causes the release of immature reticulocyte from the bone marrow into the circulation (31). Macrocytic erythrocytes are thought to be immature macrocytic reticulocytes released from the bone marrow due to the stimulation of increased erythropoietin (30). As it is known, COVID-19 disease is hypoxic respiratory failure disease. Depending on the effect of insufficient oxygen on target cells, there may be more erythrocytes and more oxygen transport reflex in the circulation. We think that the increase in the % Macro R value is associated with mortality.

CONCLUSION

There are many parameters associated with mortality and morbidity in COVID-19 disease. In critical COVID-19 patients who need to be treated in the intensive care unit, APACHE II, SAPS II score, platelet count, PDW, MPV and %P LCR values from CBC parameters can provide information about the prognosis of the patient. Especially in non-anemic patients, the increase in % Macro R value

can be used as a new marker that can be associated with mortality. The small number of participants is a limiting factor for our study. The %Macro R marker, which is used to investigate the etiology of anemia, should be investigated in more participants and different disease groups (especially diseases causing hypoxemia).

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for study was obtained from the Non-interventional Clinical Researchs Ethics Committee of Kastamonu University Faculty of Medicine (Date: 25/02/2021, Decision No: KAEK-2020-143-38).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Clinical and prognostic evaluation of patients admitted to the COVID-19 pandemic unit of the emergency department

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ABSTRACT

Objective: This study aimed to evaluate to prognosis of patients admitted to the COVID-19 isolation unit of the emergency department and present epidemiological data by examining their demographic, clinical, laboratory and lung tomography findings.

Material and Method: A total of 504 patients presenting with COVID-19 symptoms were randomly and retrospectively evaluated based on electronic records obtained from the hospital archive.

Results: Of the patients, 216 (42.9%) were female and 288 (57%) were male. The RT-PCR test was positive in 291 (57.7%) patients, and fever was the most common symptom in 280 (55.5%). A total of 133 patients (26.4%) were admitted to the inpatient ward. The WBC, Troponin-t, CRP, AST, ALT, LDH, D-dimer and ferritin levels were high and lymphocyte count was low in patients who were hospitalized. Sixty-one percent of the patients had a comorbid condition; 19% of had diabetes and 18.8% had hypertension as the most common underlying condition. Totally, 312 (61.9%) had favipiravir, 106 (21%) were favipiravir + hydroxychloroquine, 60 (11.9%) used hydroxychloroquine alone, 26 (5.2%) were followed up without treatment and to 147 of them, antibiotics were prescribed in addition to the medicine; 38.5% of the patients followed up in the intensive care unit were diabetic; 26 patients (5.2%) were followed up in the intensive care unit. The mortality rate was found to be 1.9%. At least one comorbid condition was present in all who were hospitalized and died in the intensive care unit.

Conclusion: The importance of a healthy diet and regular physical activity for metabolic conditions such as diabetes and hypertension as well as for fighting infections is well known. We consider that the mortality and morbidity rates due to the COVID-19 pandemic can be reduced by developing reliable and safe antiviral treatment options and implementing effective and fair vaccination policies.

Keywords: COVID-19, epidemiology, prognosis

INTRODUCTION

Coronavirus 2019 (COVID-19),caused by SARS-COV-2 was first reported in China at the end of 2019 and the World Health Organization (WHO) declared the disease as a pandemic on March 11, 2020. Since then, the number of cases has reached 102 million, and the number of those who died due to the virus has exceeded 2 million. The SARS-COV-2 infection can cause asymptomatic diseases, mild upper respiratory tract infections, respiratory failure, or severe viral pneumonia that may result in death (1-4). Although the real-time reverse transcriptase polymerase chain reaction (RT-PCR) test is the standard method used in the diagnosis of COVID-19, clinical, laboratory and thorax computed tomography (CT) evaluations also provide valuable data (5-8).

In this study, we aimed to analyze the epidemiological data of patients admitted to the COVID-19 isolation unit of the emergency department by evaluating their clinical, laboratory, CT findings, comorbid conditions, and prognoses.

MATERIAL AND METHOD

The study was carried out with the permission of Health Science University, Antalya Training and Research Hospital Clinical Researchs Ethics Committee (Date: 01.04.2021, Decision No: 4-8). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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The study included a total of 504 patients who presented to the emergency department of our hospital with COVID-19 symptoms between June 1, 2020 and March 1, 2021. The patients' demographic data, medical histories, biochemical parameters including laboratory analysis, hemogram, D-dimer, troponin-T, C reactive protein (CRP) and ferritin, thorax (CT) findings, and treatment protocols recorded in the hospital's archive were electronically scanned in a retrospective manner.

Case definitions were made in line with the recommendations in with the current recommendations of the Turkish Ministry of Health COVID-19 Guidelines (9). Patients with positive SARS-COV-2 real-time reverse transcriptase polymerase chain reaction (RT-PCR) test results based on the respiratory tract sample wereaccepted as definitive COVID-19 cases. If the RT-PCR test was negative, but the patient had complaints related to the disease and findings compatible with viral pneumonia on CT scans, they were accepted as possible cases. Patients with a negative result from two consecutive PCR test performed at a 24-hour interval and normal CT findings were considered not to have COVID-19.

In the interpretation of the thoracic CT findings, the presence of ground-glass density of less than 3 cm in three or less foci was classified as mild pneumonia; consolidation or a ground-glass density of more than three foci or greater than 3 cm was classified as moderate pneumonia, and involvement of all lobes in both lungs and at least three lesions larger than 3 cm classified as severe pneumonia (10).

Conforming to the recommendations of the Turkish Ministry of Health COVID-19 Guidelines, the patients were hospitalized based on the following indications: poor prognosis markers; comorbid conditions, such as hypertension (HT), diabetes mellitus, chronic lung disease (lymphocyte number <850\mm2, ferritin >500 ng\mL, D-dimer >1000 ng\mL) or suspicion of the severe form of the disease (9). Medications to be given to the patients, their doses, and treatment options were arranged in line with the recommendations of the same Guidelines (9).

All statistical analyses were performed using IBM SPSS version 25.0 (SPSS Inc., Chicago, Illinois, USA). Tables are presented as Mean±SD for continuous variables, while categorical variables are presented as number (N) and percentage (%). Comparisons between groups were made using the independent t-test and ANOVA test for continuous variables. Among the Post-Hoc tests, Turkey test was utilized. Chi-square test was applied to compare categorical variables. Pearson correlation analysis was used for the relations of continuous variables with each other and $p<0.005$ was considered statistically significant.

RESULTS

Demographic data of patients given in **Table 1**. Among 504 patients who presented to the COVID-19 unit of the emergency department, fever was the most common symptom (n=280, 55.5%). Respectively, weakness and malaise in 232 patients (46%), cough in 120 patients (23.8%), muscle-joint pain in 98 patients (19.4%), shortness of breath in 88 patients (17.4%), sore throat in 76 patients (15%), headache in 76 patients (15%), loss of taste and smell in 43 patients (8.5%), chest and back pain in 37 patients (7.3%), diarrhea in 24 patients (4.8%), anorexia in 12 patients (2.4%), abdominal pain and nausea in 6 patients (1.2%) were observed.

Table 1. Sociodemographic Data of the Patients (N=504)

Demographic Variables	N and/or XOR (min-max)	% and/or Mean ±SD
Gender		
Female	216	42.9
Male	288	57.1
Age (years)	46.00 (18-105)	46.46±17.57
PCR		
Positive	291	57.7
Negative	121	24.0
Possible Case	92	18.3
Tomography (Pneumonia findings)		
Doesn't exist	211	41.8
Mild	227	45.1
Moderate	53	10.5
Severe	13	2.6
Hospital Stay (day)		
1-7	75	60.0
8-14	45	36.0
>14	5	4.0
Treatment Conditions		
Outpatient	371	73.6
Inpatients (ICU + ward)	133	26.4
ICU hospitalization (day)	8.50 (1.00-40.00)	11.34±10.69
LYM	16000.0 (14.60-150000.0)	1786.43±1101.16
Trop T	4.85 (2.00-1280.00)	12.88±63.81
WBC	6535.00 (500.0-50000.0)	7806.84±4520.03
PLT	216.00 (4.12-206000.0)	1057.34±12746.73
CRP	8.90 (0.10-334.00)	33.19±53.27
AST	22.00 (4.00-299.00)	29.16±29.59
ALT	22.00 (1.00-804.00)	33.04±56.55
LDH	204.50 (18.80-2650.00)	235.02±153.65
D-dimer	149.00 (10.80-24000.00)	360.10±1357.69
Ferritin	97.00 (1.00-10000.00)	216.19±663.44
Rate of Death		
Alive	494	98.0
Dead	10	2.0

For treatment, favipiravir was used in 312 of the patients, favipiravir+hydroxychloroquine in 106, hydroxychloroquine alone in 60, while 26 patients were followed up without treatment, and 147 were additionally prescribed antibiotics.

A statistically significant difference was observed between outpatients and hospitalized patients in terms of age. The mean age of the patients admitted to the was higher compared to the outpatients (p<0.001). The remaining parameters that showed statistically significant differences between the outpatient and inpatient groups were lymphocyte (LYM) count, troponin-T, leukocyte (WBC), C- reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic asid dehydrogenase (LDH), D-dimer, and ferritin. The LYM values of the outpatients were significantly higher compared to the hospitalized patients (p<0.001). The troponin-T of the hospitalized patients were higher compared to the outpatients (p=0.023). The WBC values of hospitalized patients were found to be higher than outpatients (p=0.049). The troponin-T, WBC, CRP, AST, ALT, LDH, D-dimer and ferritin values of the hospitalized patients were significantly higher compared to the outpatients (p=0.023, p=0.049, p<0.001, p<0.001, p=0.029, p<0.001, p=0.021, and p=0.002, respectively) (**Table 2**). There was no statistically significant difference in age between the male and female patients and between the patients with different hospitalization days.

Table 2. Comparison of various variables between inpatients and outpatients

Variables	Outpatients (n=371) Mean±SD	Inpatients (n=133) Mean±SD	P
Age	42.21±15.20	57.86±18.44	**<0.001****
LYM	1924.61±1182.23	1413.55±726.42	**<0.001****
Trop-T	6.55±17.96	29.92±117.59	**0.023***
WBC	7554.35±4385.70	8483.19±4813.25	**0.049***
PLT	1325.57±14914.33	338.81±1265.60	0.440
CRP	19.24±34.91	70.58±72.67	**<0.001****
AST	24.23±15.69	42.35±48.32	**<0.001****
ALT	27.94±27.30	46.69±97.80	**0.029***
LDH	218.76±165.76	278.79±103.33	**<0.001****
D-dimer	230.36±619.92	710.20±2375.57	**0.021***
Ferritin	128.26±173.24	451.10±1210.51	**0.002***

Independent T-Test, **p<0.001, *p<0.005
Lymphocyte (LYM) count, troponin-T, leukocyte (WBC), platelet count (PLT), C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic asid dehydrogenase (LDH)

Of the total 504 patients who presented to the emergency department with COVID-19, 96 (19%) were diabetic. Twelve patients were healthcare workers (2.38%) and 15 (3%) were pregnant. Among the inpatients, 63 (47.36%) had diabetes (**Table 3**).

The rate of admission to intensive care was 5.2%. The mortality rate was found to be 1.9%. All patients hospitalized in the intensive care unit had at least one comorbid condition.

Table 3. Comorbidity distributions of 504 patients

Comorbidity	N (%)
Diabetes	96 (19)
Hypertension	95 (18.8)
CVD	53 (10.5)
Chronic lung disease	24 (4.7)
Chronic neurological disease	12 (2.7)
Malignancy	11 (2.6)
Chronic kidney disease	10 (2.4)
Chronic liver disease	8 (2)
Pregnant	15 (3)

CVD: Cardio-Vascular Disease (Coronary Artery Disease, Ischemic Heart Disease, Congestive Heart Failure)

Of the 26 patients that received intensive care, 10 had CVD + Diabetes, four had hypertension, two had CVD + malignancy, two had CVD + Chronic Respiratory disease, one had chronic neurological disease, one had immunosuppressed + CVD, one had chronic neurological disease + chronic respiratory tract disease, one had chronic kidney disease + chronic neurological disease + CVD, and one had malignancy.

Among these 26 patients, 10 (three female and seven male) died, and six had CVD + diabetes, two had chronic neurological disease + chronic respiratory disease, two had CVD + chronic respiratory disease + chronic liver disease.

Lastly, of the 133 people hospitalized in the service, 18 were transferred from the service to intensive care, and 8 were directly admitted to intensive care from the emergency service.

DISCUSSION

There is no specific clinical feature that can reliably distinguish COVID-19 from other respiratory viral infections. COVID-19 has a wide range of manifestations from asymptomatic or mild pneumonia to acute respiratory failure and death.

In our study, the average age of all patients was 46.56 and male patients were in the majority (57%) were male, which is in agreement with the results of previous studies (3,11). WHO has defined common COVID-19 symptoms as fever, fatigue and dry cough. In addition, shortness of breath, myalgia, sore throat and diarrhea have been reported in very few patients (12). In our study, the rate of RT-PCR positivity was found to be 57.7%. Similarly, in another study conducted in Turkey Karakoç et al. (13) reported the rate of PCR positivity as 65.3%. In the current study, 280 patients (55%) presented with fever, 232 (46%) with weakness and malaise, 120 (23%) with dry cough, 98 (19.4%) with muscle-joint pain, 88 (17.4%) with shortness of breath, 76 (15%) with sore throat, 76 (15%) with headache, 43(%8.5) loss of taste and smell, 37(%7.3) with chest and back pain, 24 (4.8%) with diarrhea, 12 (2.4%) with anorexia, and six (1.2%) with

abdominal pain and nausea. This clinical spectrum was similar to most other studies (3,14-16).

According to the COVID-19 Guidelines of the Turkey's Ministry of Health Public Health General Directorate, the disease is classified as uncomplicated, pneumonia and severe pneumonia. Patients without fever, muscle-joint pain, cough, sore throat, nasal congestion, respiratory distress, tachypnea and SpO2 <93%, with no underlying diseases (cardiovascular diseases, diabetes mellitus, hypertension, cancer, chronic lung diseases along with other immunosuppressive conditions), those under 50 years of age, those with normal chest radiography or CT findings, and those without poor prognostic values in blood tests performed at admission (blood lymphocyte count <800/ µl or CRP >40 mg/L or ferritin >500 ng/mL or D-dimer >1000 ng/mL etc.) are defined as uncomplicated diseases, while patients with a respiratory rate of less than 30 per minute are evaluated in the mild pneumonia category, and those with a SpO2 level below 90% in room air, bilateral diffuse pneumonia on chest X-ray or tomography, and poor prognostic values in blood tests at the time of admission are classified as severe pneumonia (17).

The severity of COVID-19 and its mortality course have been shown to be associated with cardiovascular diseases, diabetes, hypertension, chronic lung and kidney diseases, and cancers (14). The fatality rate is higher among those with comorbid conditions. According to the data reported from China, the rate of mortality was 10.5% in patients with cardiovascular diseases, 7.3% in the presence of diabetes, 6.3% in the presence of chronic respiratory failure, 6.0% in the presence of hypertension, and 5.6% in the presence of cancer (18-19). When we evaluated the 504 patients included in our study, those with comorbidities constituted 61% of the whole sample. The most common comorbidities were diabetes (19%) and hypertension (18.8%), followed by CVD (10.5%), chronic lung disease (4.7%), chronic neurological disease (2.7%), and malignancy (2.6%). While the mortality rate was 0.9% among the patients without any comorbidity, it was much higher inthose with comrbidities. In a previous study, the rate of mortality was reported to be 7.4% in patients with diabetes (20). In another study, 32.3% (10/31) of patients that required ICU were diabetic, while the rate of diabetics among those that did not require ICU hospitalization was determined as 9.2% (22). Similarly, in our study 10 of the 26 (38.5%) patients followed up in the intensive care unit were diabetic.

In our study, at least one comorbid condition was present among the 26 patients admitted to the intensive care unit and all 10 patients who died. Of the 10 patients who died, three were female and seven were male, and six had CVD + diabetes, two had chronic neurological disease + chronic respiratory disease + HT, and two had CVD + chronic respiratory disease + chronic liver disease. In a case group consisting of 355 patients who died due to COVID-19 in Italy, the presence of underlying chronic diseases was emphasized in all except 3 cases (21). Similarly, in our study, at least one comorbid condition was found in all patients who died.

Since there is no standard treatment regimen for COVID-19, there are differences between countries. For the treatment planning of symptomatic patients, we followed the guidelines of the Turkish Ministry of Health to decide whether they required inpatient or outpatient treatment. In the treatment of our patients, we used favipiravir in 312 (61.9%), favipiravir + hydroxychloroquine 106 (21%), and hydroxychloroquine alone in 60 (11.9%), while we followed up 26 patients without treatment and prescribed additional antibiotics for 147 patients. Chloroquine is a widely used malaria drug that was shown to have potential broad-spectrum antiviral properties in 2006. It has been reported to successfully block SARS-CoV-2 infections at a low concentration. Favipiravir is a new RNA-dependent RNA polymerase (RdRp) inhibitor. In addition to its anti-influenza activity, favipiravir has the ability to inhibit the replication of flavi-, alpha, filo-, bunya-, arena-, neuro-, and other RNA viruses. Therefore, it may have a potential antiviral effect against SARS-CoV-2, which is an RNA virus(22,23).

All the patients who died in our study had the severe form of the disease and required intensive care. In Turkey, the rate of cases diagnosedwith severe COVID-19 is similar to China, and 6% of the total cases detected are followed up in the ICU (24). Similarly, in our study, the rate of patients followed up in the ICU was 5.2%. According to the WHO statement on March 3, 2020, the global fatality rate due to COVID-19 is 3.4% (25). In our study, the mortality rate was found to be 1.9%. In Turkey, the population over the age of 65 years constitutes about 9% of the total population in contrast to 20% in many European countries. The younger population and treatment capacity not being exceeded may be among the reasons for the lower mortality rate observed in our country (26,27).

The small number of patients and limited treatment options, single-center study and retrospective can be considered as the limitations of our study.

In our study, 47.36% of hospitalized patients had diabetes. This indicates the importance of blood glucose regulation in preventing mortality-morbidity due to COVID-19. The mortality rate among our patients was as low as 1.9%, which shows the importance and success of the Turkish health system infrastructure in combating epidemic diseases.

CONCLUSION

In the current study, diabetes, hypertansion and CVD, were the most commonly observed coöorbidities among the patients with COVID-19 that required hospitalization or intensive care. We consider that these health problems can be managed in most people by a healthy diet and regular physical activity. The challenging process created by the COVID-19 pandemic has once again emphasized the importance of nutrition.We believe that the mortality and morbidity caused by the pandemic can only be reduced with the scientific world developing reliable and safe antiviral treatment options and implementing effective and fair vaccination policies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Health Science University, Antalya Training and Researchs Hospital Clinical Research Ethics Committee (Date: 01.04.2021, Decision No: 4-8).

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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Volumetric study of tracheal diverticulum: a retrospective analysis in computed tomography

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ABSTRACT

Aim: The aim of this study was to retrospectively evaluate the prevalence and morphology (location, diameter, number and volume) of the tracheal diverticulum (TD) on thoracic computed tomography (CT), in a sample of the Turkish population.

Material and Method: The study was conducted over 15 months, from February 2020 to April 2021, and included 3,860 thoracic CT scans, of which 1,891 subjects were men and the rest were women. All of the thoracic CT scans were evaluated retrospectively. All images were reviewed for the presence or absence of the TD in the axial plane, defined as air sacs around the trachea and communication with the trachea. For volume measurement, the TD contour was manually evaluated by a single radiologist, for all patients. Descriptive statistics were reported as mean±standard deviation (SD). A p value of less than 0.05 was considered significant.

Results: The mean age of the patients was 57.87±14.76 (29-84). In this study, the incidence of TD was calculated to be 0.80%. The mean tracheal diverticulum volume (TDV) and maximum transverse diameter (MTD) of TD were 983.62±1 509.80 mm^3 and 8.79±6.19 mm, respectively.

Conclusion: In this study, we demonstrated the volumetric measurement of TD and its prevalence. We believe that the frequency of its use in clinical practice should increase, as volumetric measurements provide healthier results than diameter measurements.

Keywords: Volumetry, tracheal diverticula, prevalence, computed tomography

INTRODUCTION

A tracheal diverticulum (TD) is usually asymptomatic and is detected incidentally during imaging procedures, such as thoracic computed tomography (CT). When TD is symptomatic, it can cause respiratory symptoms such as cough, dyspnea, stridor and hemoptysis (1). It may also present with infection, abscess and rupture (2,3). TD is described as thin-walled air sacs in the paratracheal wall, often in contact with the tracheal lumen (4). TD is divided into 2 subgroups: congenital and acquired (5). Congenital TD is more common in males than females and is located 4 to 5 cm inferior to the vocal cords. Congenital TDs are thought to be caused by a developmental defect in the tracheal cartilage (6), whereas acquired TDs occur due to tracheomalacia or surgical complications. Acquired TD may occur as a result of increased intraluminal pressure due to chronic obstructive pulmonary disease (COPD). They are often located in the posterolateral of the trachea, at the level of the thoracic inlet. In addition, acquired TDs are often larger than congenital TDs (4).

CT is an important tool for the evaluation of the TD and the visualization of other associated pathologies (7,8). CT can be used to assess the location, volume, contour, and wall thickness of the TDs (9). It can also detect communication between the TD and the trachea (5). The TD is often located in the right posterolateral aspect of the trachea and less commonly on the left side (1,4).

The aim of this study was to retrospectively evaluate the prevalence and morphology (location, diameter, number and volume) of the TD on thoracic CT, in a sample of Turkish population.

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MATERIAL AND METHOD

The study was carried out with the permission of Hitit University Non-interventional Researchs Ethics Committee (Date: 28.06.2021, Decision no: 2021-73). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Informed consent was waived due to the retrospective nature of the study, as well as the fact that the assessment utilized anonymous research findings. The study was conducted over a period of fifteen months, from February 2020 to April 2021. Many of the patients had respiratory symptoms, such as chest pain, shortness of breath and cough, while others had no symptoms of respiratory problems but were routinely screened for various diseases, such as pulmonary nodules. Patients who did not have acceptable image quality on the computed tomography scans and medical records were not included in the study.

CT studies were performed using a multidetector 16-row helical CT scanner (Alexion, Toshiba Medical Systems, Nasu, Japan). The CT imaging was performed during a breath-hold at deep inspiration. Spirometric gating was not applied. The scans were obtained from the base of the neck down to the diaphragm. A supine or prone position was chosen. Parameters were 100–120 Kv, the field of view was 350 millimeters (mm), beam collimation was 1×16 mm, gantry rotation time was 0.5 seconds and scan time was 11–13 seconds. Thin-section CT data were reconstructed at a slice thickness of 1 mm with 0.8 mm intervals. Image matrix size was 512×512. We used automatic tube-current modulation at a maximum of 225 mAs for exposure dose reduction. All CT examinations were performed without contrast media.

All of the thoracic CT scans were evaluated retrospectively. All the images were reviewed for the presence or absence of the TD in the axial plane, defined as air sacs around the trachea and communication with the trachea (**Figure 1**). When TDs were observed, the imaging characteristics (emphysema or bronchiectasis), including location (right or left), size (axial diameter and volume) of all cases were noted. Bronchiectasis was defined as present when the diameter of the bronchial lumen was larger than the adjacent pulmonary artery, with no taper in the diameter of the bronchial lumen. Emphysema was defined as small, well or poorly-defined areas of low attenuation, surrounded by normal lungs.

The volume of TDs was analyzed using a Vitrea workstation (Canon Medical Systems Corporation, Otawara, Japan) by a single radiologist (E.G.), with seven years of experience in thoracic radiology. For volume measurement, the TD contour was manually evaluated by the examiner, for all patients. The area in each slice was then recorded and the volumes were measured automatically by the Vitrea post-processing imaging software (Canon Medical Systems Corporation, Otawara, Japan). Finally, the volume was measured by the software and a three-dimensional reconstruction of the organ was generated (**Figure 2**). The tracheal diverticulum volume (TDV) was measured in milliliters (ml) or cubic millimeters (mm^3): 1 ml was considered as 1 000 mm^3. The TD was re-evaluated 5 days after the initial evaluation to assess reproducibility by the same observer.

Statistical Analysis

Statistical analysis was performed using the SPSS v.22 package program (IBM SPSS Statistics, Chicago, IL, USA). The age, gender and radiological characteristics of the participants were noted. Descriptive statistics were reported as mean±standard deviation (SD). The distribution of the data was analyzed using the Kolmogorov-Smirnov test and the relationship between TDV and gender was analyzed using the Mann-Whitney U test. Whether there is a statistically significant difference between the ages of men and women was also examined using the Mann-Whitney U test. The relationship between TDVs and age, and maximum transverse diameter (MTD), was calculated using "Spearman's correlation coefficient test". The "intraclass correlation coefficient (ICC) test" was used to analyze intraobserver reliability, for repeated measurements with a 95% confidence interval. The ICC was indicated as follows: below 0.50: poor; between 0.50 and 0.75: moderate; between 0.75 and 0.90: good; above 0.90: excellent. A p value of less than 0.05 was considered significant.

RESULTS

From February 2020 to April 2021, 3,926 adult patients (> 20 years) underwent thoracic CT examinations at our institution. After excluding 49 patients with diagnostically unacceptable image quality resulting from artifacts, and an additional 17 patients without medical records, the thoracic CT examinations of 3,860 adult patients were finally included in this study (1,891 male, 1,969 female) (**Table 1**).

The mean age of the patients was 57.87±14.76 (29-84). There was no statistically significant difference between the ages of men and women (p=0.091). A total of 46 diverticula were detected in 31 patients (17 male, 14 female). In this study, the incidence of TD was calculated to be 0.80%, all of the TDs were located on the right posterolateral side of the trachea and the prevalence of TD in males and females was 0.89% and 0.71%, respectively. The mean TDV and MTD of TD were 983.62±1509.80 mm^3 and 8.79±6.19 mm, respectively. Descriptive statistics are presented in **Table 2**.

A positive moderate correlation was observed between age and MTD (rho: 0.317; p: 0.032) and volume (rho: 0.327; p: 0.027). There was no statistically significant relationship between gender and mean TDV and MTD (p 0.05).

Intraobserver reliability for repeated measurements yielded an overall ICC of 0.935 (0.921-0.951), with a 95% confidence interval.

Table 1. Flowchart shows study population and patient selection process

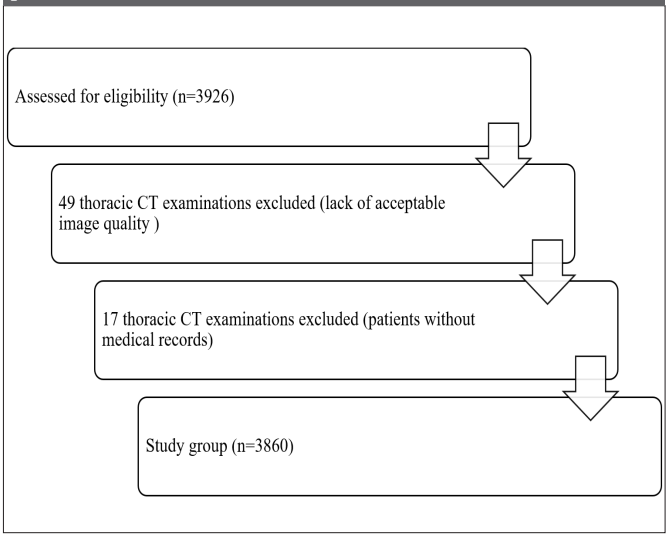

Table 2. Descriptive statistics for age, MTD of TD, Mean TDV and gender distributions of all cases

Variables	Total (n=31)
Age (year old)	57.87±14.76 (29-84)
MTD of TD (mm)	8.79±6.19 (2-30)
Mean TDV (mm³)	983.62±1509.80 (20.95-6401.24)
Gender (M/F)	17/14

Data is expressed as n (number) or the mean± standard deviation (range). F: Female, M: Male, MTD: maximum transverse diameter, TD: Tracheal diverticulum, TD: Tracheal diverticulum volume, ml: milliliters, mm³: cubic millimeters

DISCUSSION

TDs are air spaces lined with ciliary columnar epithelium and connected to the trachea. Despite the fact that autopsy studies report a prevalence rate of 1%, the prevalence of TDs has been reported as high as 8.1% in previous radiological studies. It is divided into two groups: congenital and acquired (4,5). Acquired or congenital diverticula can be distinguished along with the location of the diverticulum, the age of the patient, the size of the diverticulum and the neck of the diverticulum (10). TDs are usually asymptomatic and are detected incidentally in thoracic CT. Possible symptoms include airway obstruction, recurrent lower respiratory tract infections, mass effect dysphagia, hoarseness, gagging and hemoptysis (4).

The associations between underlying lung diseases, such as emphysema or COPD and the prevalence of TDs, are still controversial. Goo et al. (7) and Boyacı et al. (11) reported an association between COPD and paratracheal air cysts (PTACs). In a study conducted by Polat et al. (12) on 301 patients with PTACs, they found no association between COPD and PTACs. In the study conducted by Cheng et al. (13), no association either was found between COPD and PTACs.

The prevalence of TD has been reported to range from 0.75% to 8% in previous studies (1, 11). In some of these studies however, true diverticula were not differentiated

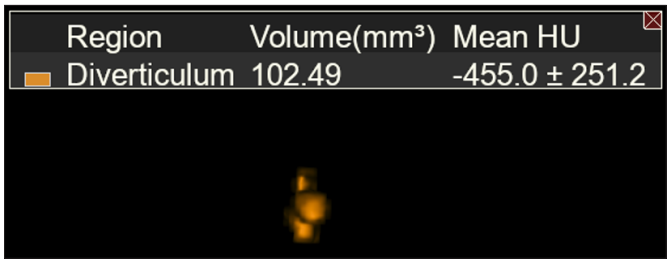

Figure 2. Calculation of TDs volume and three-dimensional reconstructed CT image of its.

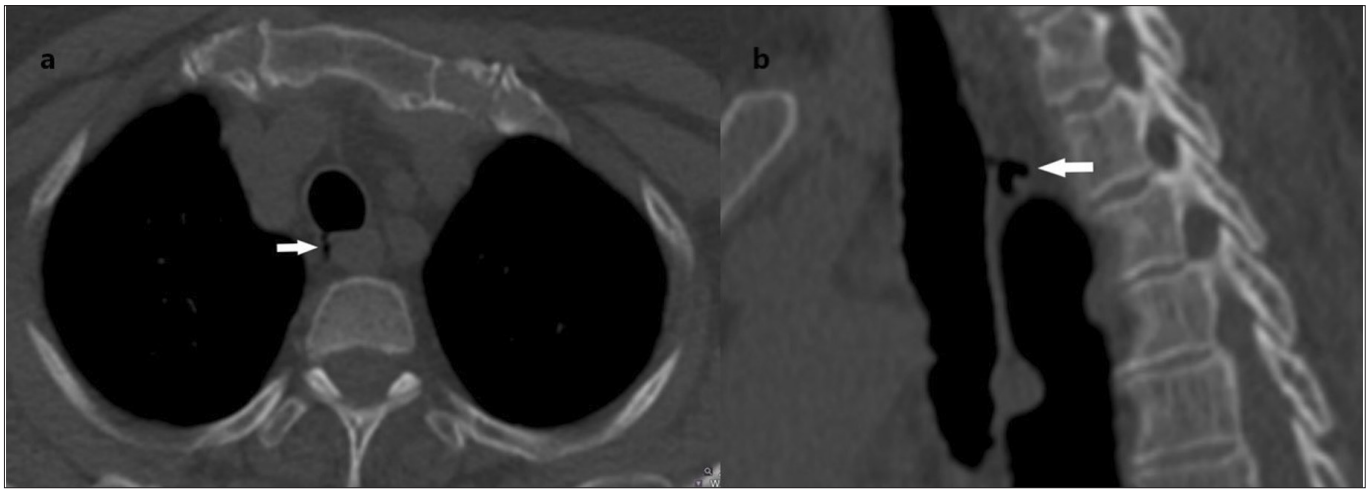

Figure 1. Non-contrast thoracic CT image in the axial **(a)** and sagittal **(b)** plane, showing TD (white arrow) which was located on the right posterolateral side of the trachea.

according to the presence of a tracheal junction, and PTACs were also included in the studies. In our study, PTACs not connected to the trachea were not included in the study, and true diverticula connected to the lumen of the trachea were included in the study. For this reason, we think that the prevalence of TD in our study was lower compared to previous studies. In the study by Pace et al. (5), 124 TDs were detected in 1,679 CT scans and a prevalence of 5.7% was calculated. Kurt et al. (14) in their study, found 412 TDs belonging to 299 patients. In their study, the prevalence of TD was found to be 2.38%, whereas in our study, the prevalence was calculated as 0.8% (males 0.89% and females 0.71%). We think that the high prevalence that was observed in the study of Pace et al. (5) could be due to the difference in sample groups. The difference in prevalence could be due to the presence of CT scans in our patients to screen for COVID-19 pneumonia. Similar to the study by Pace et al., none of the patients in our series TD had a history of trauma, subcutaneous emphysema, nor visible pneumothorax or pneumomediastinum.

According to previous research, TD has a slight female preference (8,11,15). In contrast to previous studies however, in our study, the male gender was more frequently affected than the female gender, as was also reported by Kurt et al. (14). In general, TDs were found on the right side of the trachea, in accordance with previous studies (5,8). This finding may be explained by the fact that both the esophagus and the aortic arch support the left side of the trachea, in terms of resistance to increasing intratracheal stress. TDs are mostly found at the level of the T2 vertebrae on the right posterolateral side of the trachea, which is the transition zone between the intrathoracic and extrathoracic trachea, and this localization also brings the pressure theory to the forefront, as it is a transition zone (16). In our study, TDs were detected in this localization, in all cases.

Boyacı et al. (11) reported the median size of PTACs to be 5.17×6.60×10.95 mm and Kim et al. (8) reported it as 7.5×4.2×11.5 mm. Goo et al. (7) found a mean axial diameter of 10 mm and a mean vertical length of 14 mm. In our study, the mean TDV and MTD of TD were 983.62±1 509.80 mm^3 and 8.79±6.19 mm, respectively. Measurements of diameter may not be accurate due to some variability in the morphology of TD, however volumetric analysis can now provide more standardized evaluations thanks to advances in CT software technology and for this reason, it should be used more widely in practice. Volumetric measurement is important in terms of calculating dimensional size with higher accuracy and predicting potential surgical complications. This is because two-dimensional measurement without three-dimensionality, overlooks the possible difference in volume. In radiological practice, volume measurements are generally obtained by multiplying the diameters, measured in 3 planes by the spherical formula (R1 x R2 x R3 x 0.52). However, for structures with irregular boundaries, errors in volume measurements may occur. In this study, we evaluated the location and volumes of TD, which were detected during thoracic CT examinations in adults. Using the workstation program, the area of each section was calculated separately by the program and volume measurements were performed automatically.

This study has several limitations. First of all, it included only a limited number of patients and was monocentric: future multicenter studies with a larger number of patients will provide more accurate evidence. Second, interobserver reliability could not be assessed because the evaluations were performed by a single radiologist. Finally, our measurements were performed only in vivo and the actual ex vivo volume was not evaluated.

CONCLUSION

We demonstrated with this study the volumetric measurement of TD, as well as its prevalence. We believe that the frequency of its use in clinical practice should increase, as volumetric measurements provide healthier results than diameter measurements.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Non-interventional Researchs Ethics Committee (Date: 28.06.2021, Decision no: 2021-73).

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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Radiological evaluation of spinal canal, dural sac, epidural fat and superior articular process in diagnosis of lumbar spinal stenosis

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ABSTRACT

Aim: The aim of our study; to investigate the location of hypertrophy in the epidural adipose tissue in the lumbar spinal stenosis clinic, to compare the area measurements of the spinal canal and dural sac in patients with a preliminary diagnosis of lumbar spinal stenosis or radiculopathy, and to determine the place of the superior articular process area measurement in the diagnosis of spinal stenosis.

Material and Method: 180 patients aged 50-69 years who underwent Lumbar Magnetic Resonance Imaging were divided into two groups according to the prediagnosis of lumbar spinal stenosis or radiculopathy and retrospectively analyzed. Spinal canal, dural sac, epidural fat, and superior articular process areas were measured. Statistical relationships of the findings were investigated.

Results: There was no difference between the stenosis groups of these patients in terms of age and gender (respectively p=0.078; p=0.564). There is a significant difference in terms of the spinal canal, dural sac, superior articular process, and epidural fat widths between spinal stenosis and radiculopathy (p<0.001; p<0.001; p<0.001; p=0.033, respectively). Superior articular process, spinal canal, dural sac, and epidural fat cross-sectional areas were each found significant for their use as a diagnostic test for diagnosing lumbar spinal stenosis (p<0.001; p<0.001; p<0.001; p=0.034, respectively).

Conclusion: Spinal stenosis is a problem that greatly affects the quality of life of patients. Measuring only the width of the spinal bony canal does not provide sufficient information in the diagnosis of spinal stenosis. In our study, hypertrophy of the superior articular process was the strongest finding in the diagnosis of lumbar spinal stenosis. Hypertrophy of epidural adipose tissue has also been shown to be a risk factor for lumbar spinal stenosis. In radiological evaluations, other structures that narrow the canal should also be carefully examined.

Keywords: Spinal stenosis, dural stenosis, epidural fat, magnetic resonance imaging

INTRODUCTION

The prevalence of lumbar spinal stenosis (LSS) varies between 1.7-13.1% in the population (1). LSS is the most common cause of disability in elderly and middle-aged patients (2). It causes neurogenic intermittent claudication, radicular pain, and sensory and motor disturbance in the lower extremities. Sciatica may also present itself with neurogenic claudication and low back pain complaints (3). Complaints increase with walking (4). However, radiological LSS is not always clinically present (5,6).

Indications for surgery due to LSS are increasing especially in the over 65 age group (7,8). LSS patients benefit from spinal decompression surgery, but non-surgical interventions are preferred primarily (9).

Spinal stenosis is evaluated in three types. The type in which the spinal canal is affected is called central stenosis, the type in which the intervertebral neural foramina are affected is called foraminal stenosis, and the type in which the lateral recesses are affected is called lateral stenosis. (3,10). It is important to determine the location of the LSS in the approach to the patient with neural compression (11).

Spinal stenosis was originally defined as any narrowing of the spinal canal, nerve root canals, or intervertebral foramina (12). It can be congenital, acquired, or mixed (13). Congenital causes are primarily short pedicle structure or facet joint abnormalities. Acquired

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conditions are injuries, bone tumors, hematomas, abscess, metabolic diseases such as acromegaly or achondroplasia, iatrogenic conditions, and degenerative diseases (14). It is also known that the width of the spinal canal is affected by genetic factors (10).

Unfortunately, there is currently no standard for the diagnosis of LSS. Diagnosis is made by clinical signs, physical examination, and radiological confirmation (17,18). In the radiology department, the anterior - posterior diameter measurements of the spinal canal and dural sac; and their cross-sectional area (CSA) measurements are the most frequently used methods. (16).

Unfortunately, there is currently no standard for the diagnosis of LSS. Diagnosis is made by clinical signs, physical examination, and radiological confirmation (17,18). In the radiology department, cross-sectional area (CSA) measurement of the spinal canal and dural sac diameter and anterior-posterior diameter measurement are the most frequently used methods (16).

Magnetic Resonance Imaging (MRI) of the lumbar spinal canal is one of the most frequently used methods to evaluate the morphological structure of the spinal canal and nerve roots after physical examination of people who have difficulty in movement due to complaints such as low back pain and loss of strength in the lower extremities. It is known that MRI provides useful images for back pain and other pathologies of lumbar origin (19). Using MRI, we can easily visualize degenerative changes and spinal canal dimensions (20). MRI is the gold standard for the lumbar spinal canal (10).

In the literature, the ligamentum flavum is mentioned as an important structure that narrows the spinal canal (21). Fluid increase in the facet joint is also a condition that narrows the dural sac (22).

Epidural fat is the adipose tissue that fills the space between the dura mater and the periosteum of the vertebra. (23) It is more prominent at the level of the upper lumbar vertebrae than at the lower lumbar level (24,25).

Back pain is the most frequently reported symptom associated with spinal epidural lipomatosis (SEL) and often presents long before the other symptoms (26). We could not find specific information about epidural adipose tissue hypertrophy or lipomatosis for spinal stenosis in the literature.

Our study aims to investigate the place of hypertrophy in epidural adipose tissue in the clinic of lumbar spinal stenosis, to compare the measurements of the spinal canal and dural sac CSA in patients with a preliminary diagnosis of lumbar spinal stenosis or radiculopathy, and to determine the place of superior articular process CSA measurement in the diagnosis of lumbar spinal stenosis.

MATERIAL AND METHOD

This study was planned as a retrospective study. At all stages, the 1964 Declaration of Helsinki, national research committee standards, and ethical guidelines were meticulously complied with. This study was approved by Ankara Medipol University Faculty of Medicine, Non-interventional Researchs Ethics Committee (Date: 01.07.2021, Decision No: 27).

Study Plan and Patient Selection Criteria

Between January 2020 and December 2020, a total of 896 examinations that underwent lumbar spinal MRI at the imaging center were evaluated retrospectively. Patients aged between 50-69 years were included in the study in order to have the measurements affected by age-related changes as little as possible. Only the first examination of the patients with more than one examination was included in the study. Patients who had undergone surgery, infection, abscess, fracture, hematoma, and malignant mass were not included in the study group. The patients were examined in two groups according to the reasons for their request as those with narrow lumbar spinal canal and spinal stenosis findings and those with suspected radiculopathy. However, we found it necessary to have a complaint of neurogenic claudication in the patient forms in order to include those presenting with suspected spinal stenosis into the group. Thus, 108 lumbar spinal MRIs were accepted in the study.

MRI examinations were performed using 1.5 T (Tesla) (Signa Explorer, GE Healthcare, USA) MRI scanners.

Routine sequences included in the standard protocol were taken in MRI examinations. The retrieved sequences and their properties are given in **Table 1**.

Table 1. Values mean of routine sequences in Lumbar Spinal MRI

	TR (ms)	TE (ms)	FOV	Matrix	ST (mm)	SS (gap) (mm)
AXIAL T2W	7691	105	20×18	352×224	4.0	1.0
SAGITAL T1W	329	15.2	28×28	320×256	4.0	1.0
SAGITAL T2W	3522	111	28×28	320×256	4.0	1.0
SAGITAL STIR	4209	93.6	28×28	320×224	4.0	1.0

TR: Repetition time, TE: Echo time, FOV: Field of view, ST: Slice Thickness, SS: Slice Spacing, STIR: Short tau inversion recovery.

In the evaluation of images, spinal canal CSA (SCCSA), dural sac CSA (DSCSA), epidural fat CSA (EFCSA), and bilateral L5 superior articular process CSA (SAPCSA) measurements were made at the level of L4-5 intervertebral disc (Figure 1-4). Measurements were predominantly made using axial T2W images, and sagittal images were used for the presence of osteophytes or cystic structures and epidural fat boundaries.

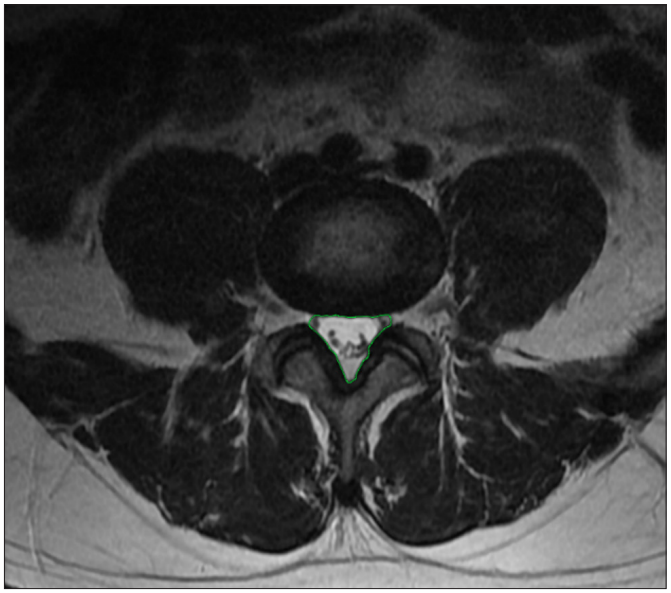

Figure 1. Spinal canal CSA measurement

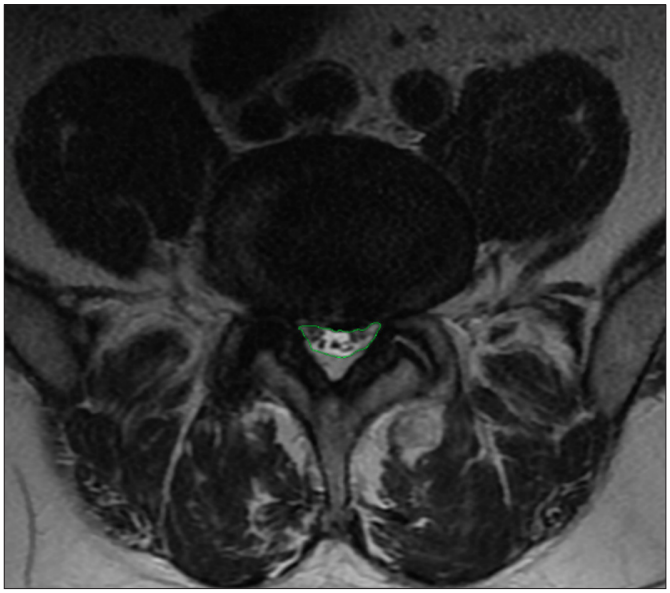

Figure 2. Dural sac CSA measurement

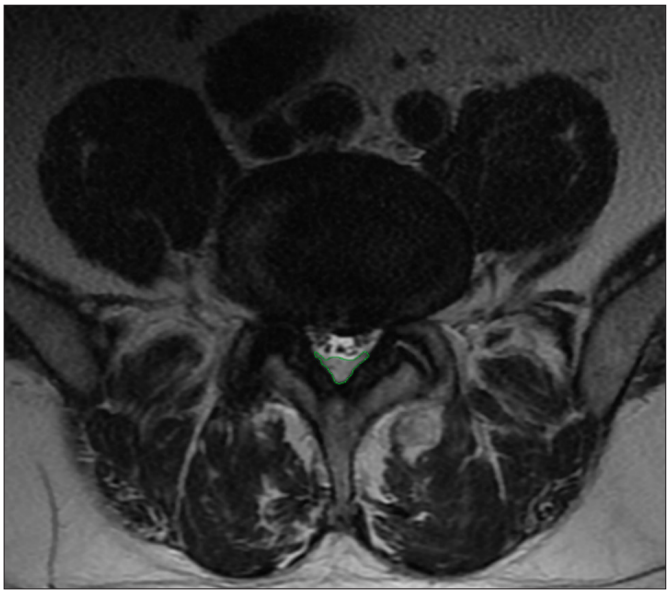

Figure 3. Epidural fat CSA measurement

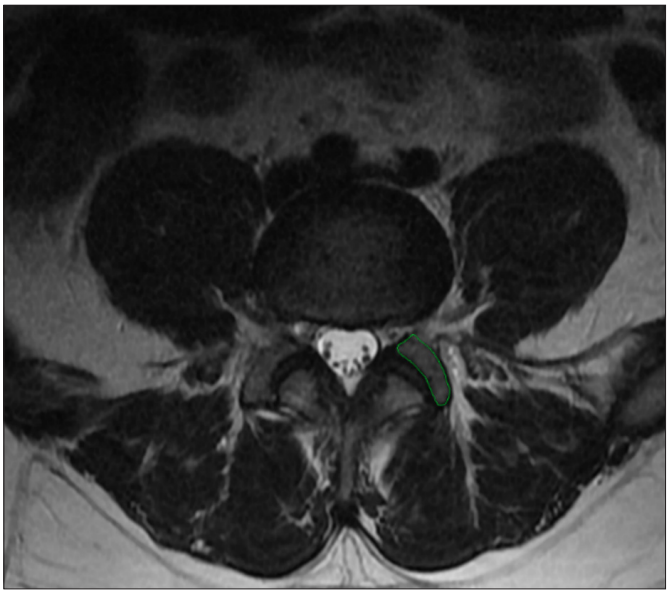

Figure 4. Superior articular process CSA measurement

Images in the collection environment of the center were examined on two separate dates by a radiologist with 22 years of experience using the Picture Archiving and Communication System (PACS). The reliability of the measurements was tested, the intra-observer reliability was found between 0.95-0.98. Results were considered safe and measurements were averaged.

Statistical Analysis

In the study, depending on the assumptions for numerical parameters as descriptive statistics, mean±standard deviation or median (minimum-maximum); Number (n) and percentage (%) for categorical data are given. Student's t test was used if parametric test assumptions were met, and Mann-Whitney U test was used if not, in determining whether there was a difference between groups in terms of numerical variables. ROC (Receiver Operating Characteristic) analysis was performed to test the usability of numerical parameters in estimating stenosis, Area Under Curve (AUC) value and confidence intervals were given, and the cut-off value was found according to Youden index. The analyzes of the study were made in IBM SPSS v22 program. $p<0.05$ was considered statistically significant.

RESULTS

The study consisted of 108 patients, 54 female, and 54 male. The mean age is 57.20±5.191 years. While 54 of the patients had stenosis, 54 did not have stenosis.

There was no difference between the stenosis groups of these patients in terms of age and gender (respectively $p=0.078$; $p=0.564$).

There was no significant difference between men and women in terms of SCCSA, DSCSA, SAPCSA, and EFCSA (p=0.694; p=0.379; p=0.832; p=0.707, respectively).

There is a significant difference in terms of SCCSA, DSCSA, and SAPCSA, and EFCSA between lumbar spinal stenosis and radiculopathy (p<0.001; p<0.001; p<0.001; p=0.033, respectively) (**Table 2**).

SAPCSA, SCCSA, DSCSA, and EFCSA were each found significant for their use as a diagnostic test for diagnosing lumbar spinal stenosis. (p<0.001; p<0.001; p<0.001; p=0.034, respectively) (**Table 3**) (**Figure 5**).

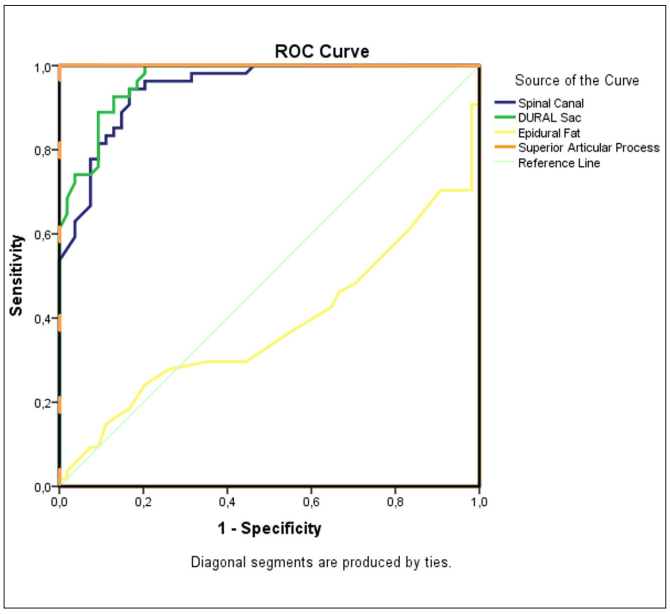

Figure 5. AUC graphs of parameters

Table 2. Comparison in terms of parameters for stenosis

	Spinal Stenosis		P
	Negative (n=54)	Positive (n=54)	
SAP (mm²)	92.93±6.689	119.52±6.068	<0.001[a]
Spinal Canal (mm²)	198.24±40.646	119.98±25.680	<0.001[a]
Dural Sac (mm²)	163.37±41.120	82.87±19.204	<0.001[a]
Epidural Fat (mm²)	14.0(3.0-38.0)	19.0(2.0-42.0)	0.033[b]

[a]:Student's t Test; (Mean±Standard Deviation), [b]: Mann-Whitney U Test; median (minimum-maximum)

DISCUSSION

Jensen et al. (27), in their systemic review of 55 studies, reported that 11% of the general population complies with the clinical diagnostic criteria of LSS, and this rate varies between 25-39% in the clinical population. Radiological findings in spinal stenosis are more extensive than clinical symptoms and signs (3). Some studies have found a relationship between the degree of spinal stenosis and decreased walking distance and leg pain (28,29).

There are also studies in which variations of the spinal canal and its correlation with somatometric parameters in asymptomatic patients have been performed (13). In these studies, it was reported that the spinal canal measurements did not show any change depending on the age or the height of the patient (13).

For the radiological diagnosis of spinal stenosis, there are different opinions about the pathological limits of the anterior-posterior diameter, although they are close to each other. The generally accepted value for absolute stenosis appears to be 11-13 mm. In studies describing dural stenosis as a separate entity, it was determined as normal stenosis above 10 mm, relative stenosis between 8-10 mm, and absolute stenosis below 8 mm (1,10,30-33).

Korse et al. (34) measured the sagittal diameter of the spinal canal at L4-5 level, 10.06 (5-14) mm (5-14) in cauda equina syndrome (CES) and 12.75 (9-20) mm in sciatica, and stated that there was no correlation between MRI findings and CES symptoms.

Pierro et al. (35) described the sagittal diameter of the dural sac as 13.3±2.1 mm at the L4 level and 12.9±2.4 mm at the L5 level, and no difference was found in the dural sac width between males and females. Since Pierro et al. (35) made the measurements from the middle 1/3 of the vertebra, the results may have been different from the literature. However, it is valuable that it indicates a significant difference between the diameter of the dural sac and the diameter of the spinal canal. In the literature review, it is seen that the spinal canal measurement was made at the disc level in some studies and from the corpus level in others, as in this study. In some publications, DSCSA measurement was performed at the levels where the ligamentum flavum is prominent in the figures showing the measurement, while the bone canal was presented as data (1).

Table 3. Identifying diagnostic tests for stenosis

	AUC	95% Confidence Interval	P	Cut-off value	Sensitivity	Specificity
SAP (mm²)	1.00	1.00 – 1.00	<0.001	106.50	1.00	1.00
Spinal Canal (mm²)	0.947	0.909 - 0.984	<0.001	159.50	0.94	0.83
Dural Sac (mm²)	0.965	0.937 – 0.993	<0.001	104.50	0.89	0.91
Epidural Fat (mm²)	0.618	0.510 – 0.727	0.034	23.50	0.30	0.98

Panda et al. (10) measured the sagittal diameter of the spinal canal at L4-5 level as 14.66-16.5 mm in the control group; It was found between 10.92-12.99 mm in the case group. In the same study, he states that there is no difference in age between the discopathy patient group and the control group in the measurements he made at all levels according to age groups.

In the literature, it is said that DSCSA is a more sensitive method for spinal stenosis (16). Most studies that present data on spinal canal width are either on symptomatic patients or as comparative studies. According to the data of this study, a DSCSA less than 75 mm² was determined as absolute spinal stenosis (ASS), and a DSCSA between 75-100 mm² was determined as relative spinal stenosis (RSS). In previous studies, anteroposterior diameter measurement in the sagittal plane was preferred rather than area measurement. Today, we think that area measurement should be preferred, since MR images of patients are made almost entirely on computer systems.

Verbiest says neurogenic intermittent claudication is common in both ASS and RSS (30). Other studies also mention a relationship between the degree of dural stenosis and specific symptoms for stenosis, such as walking ability (4). According to this latest study, the DSCSA limit value is given as 53 mm² for walking ability below 100 m, and 69 mm² for walking ability over 500 m. Walking distances and disability indices were used for such studies (36). In this study of Altinel and Yerli, 70 mm² Shönnström criteria (typo belongs to the authors) were used for DSCSA, and no reference was given as to where these criteria were taken from. In this study, a close relationship was found between the severity of the narrowing and the complaints and findings of the patients (36). Sirvancı et al. (6) examined the results of 63 patients who had undergone surgery for stenosis and found DSCSA at all levels between 18-232 mm² and did not detect a correlation between the Oswestry Disability Index and radiological images. Hurri et al. (37), on the other hand, defends the existence of this relationship. In our study, there is a significant relationship between lumbar spinal stenosis clinic and canal diameter. While the mean SCCSA was 119.98 mm² at the L4-L5 level in patients presenting with spinal stenosis clinic, the mean SCCSA was measured as 198.24 mm² in patients with clinical signs due to discopathy at other levels. DSCSA was 82.87 mm² and 163.37 mm², respectively. Both the spinal canal and dural sac narrowing were statistically significant ($p<0.001$, $p<0.001$). However, since Sirvancı et al.'s study includes only patients with clinical data and the decision to operate, it may not be accurate to compare with our results (6). Jail et al. (38), in their study using disability indexes, found DSCSA between 35-50 mm² in patients with neurogenic claudication and 164 mm² in patients with mild low back pain and reported a significant statistical difference.

Premchandran et al. (39) measured the DSCSA at the level of the vertebral corpuscles of the patients who were taken out of the clinic and did not have fractures or kyphoscoliosis, with the L4 level of 196.36±44.12 in women; 226.57±51.29 in men; L5 level was 187.11±59.76 in women; published as 215.92+-51.35 in men. Unfortunately, there was no information that the data shared in this study was mm², although we interpreted it that way. Rapala et al. also measured the vertebral corpus levels, and they described DSCSA as 267.70 mm² at the L4 level and 303.99 mm² at the L5 level (40). Our findings also support these studies.

In early CT studies, 10 mm for the ASS and 12 mm for the RSS was used as the lower limit of the sagittal diameter of the spinal canal, and the lower limit was 145-150 mm² for the DSCSA. However, in recent studies, the lower limit of 75 mm² is accepted as the lower limit of 100 mm² relative SS, and it has been reported that symptoms may occur below 130 mm² (5,6,29,41,42). Schönström et al. (43) defends 100 mm² as the critical size. In the literature, there are parameters such as normal canal width not less than 145 mm², anterior-posterior diameter greater than 11.5 mm, ligamentum flavum thickness not exceeding 5 mm, and interpeduncular distance above 16 mm in the evaluation of spinal canal stenosis (13).

The old data on DSCSA normal size are as follows: Ulrich et al. (44) argue that stenosis below 145 mm², Hamanishi et al. (45) argue that it can be called stenosis below 100 mm².

Although Danielson et al. obtained significant results with the axial loading technique used in MRI and computed tomography (CT) examinations, this examination has not become widespread in practice. In the same study, they showed that the diameter of the spinal canal increased in flexion and decreased in extension (46). In the axial loading technique, compression is applied to the patients or cadavers from the sole of the foot, so that the vertebrae are exposed to an axial force as when standing. This technique was first described by Schönström and Hansson (47), and in cadaver studies, it was determined that 40-50 mm² difference occurred when force was applied compared to when it was not applied. In addition, it is stated in this study that compression may occur in the nerve roots with diameters below 75 mm².

In another dynamic study, it was reported that the sagittal diameter of the spinal canal increased in extension in 33% of patients and decreased in flexion in most patients (3). In the same study, it is stated that there is no significant relationship between the severity of clinical symptoms and the degree of radiological narrowing.

Lim et al. (16) made measurements from several levels. In this study, bone canal and dural sac diameters were measured separately, and DSCSA measurement was found to be more sensitive for the diagnosis of LSS. In our study, all data were found to be significant.

Another recent consideration for the diagnosis of spinal stenosis is the measurement of SAPCSA (2,48,49). This view is quite logical. Because facet hypertrophy may already be the cause of spinal stenosis alone (50). It is said that this measurement can also be a guide before endoscopic spinal surgery (51). An et al. (48) mention that they examined patients over the age of 60 in their study because there were minimal cartilage changes in the superior articular process before the age of 45. The most definitive result of our study was the significant relationship between SAPSCA measurements and the diagnosis of spinal stenosis ($p<0.001$). The power of this data in diagnosis came as a surprise to us. However, it draws attention to the place of facet joint hypertrophy in the etiology of spinal stenosis. However, it would be appropriate to evaluate it with studies involving more patients. It has been determined that the increase in fluid in the facet joint narrows the dural sac, which becomes evident in axial loading shots (22). Besides the facet joint, the ligamentum flavum is an important structure that narrows the spinal canal (21). We think that it would be more useful to use DSCSA to describe the contractions where the increase in size is prominent in these structures.

Shizas et al. (5) introduced a new classification in which the distribution style of the roots in the neural sac is in the foreground. In this classification, epidural fat is prominent in the posterior in grade C stenosis. This is the first study based on shape in the diagnosis of stenosis. We think that it should be supported by new studies. On the other hand, as suggested by Lim et al. (16), it may be useful to evaluate the presence of CSF obliteration by looking at the shape of the dural sac.

Patients with spinal epidural lipomatosis may be asymptomatic but often present with symptoms secondary to nerve or spinal cord compression (52). Cases, where the EF thickness is over 7 mm, are considered as spinal epidural lipomatosis (26,53).

In studies conducted for EF, measurement was preferred at the thoracic (especially T7) level (54,55). We did not study epidural lipomatosis. Our measurements were made at the L4-5 level.

There is also a study that states that the rate of climbing stairs and feeling well in the elderly increases with the increase in the prominence of epidural fat. However, this association was not found in patients with low back pain in the same study (23). Although it lagged behind other data in our study, there was a significant difference in hypertrophy of epidural adipose tissue between patients with lumbar spinal stenosis clinic and the other group ($p=0.033$). Sions et al. measured the epidural fat thickness as 4.4 mm in their study. Since we measured the area, we could not find the opportunity to compare. In our measurements, the mean EFCSA was 19 mm^2 in patients with lumbar spinal stenosis, and 14 mm^2 in the other group. It may be difficult to evaluate epidural adipose tissue on T2W images taken from the intervertebral disc level for discopathies (**Figure 6**). We recommend using sagittal T1 images for this.

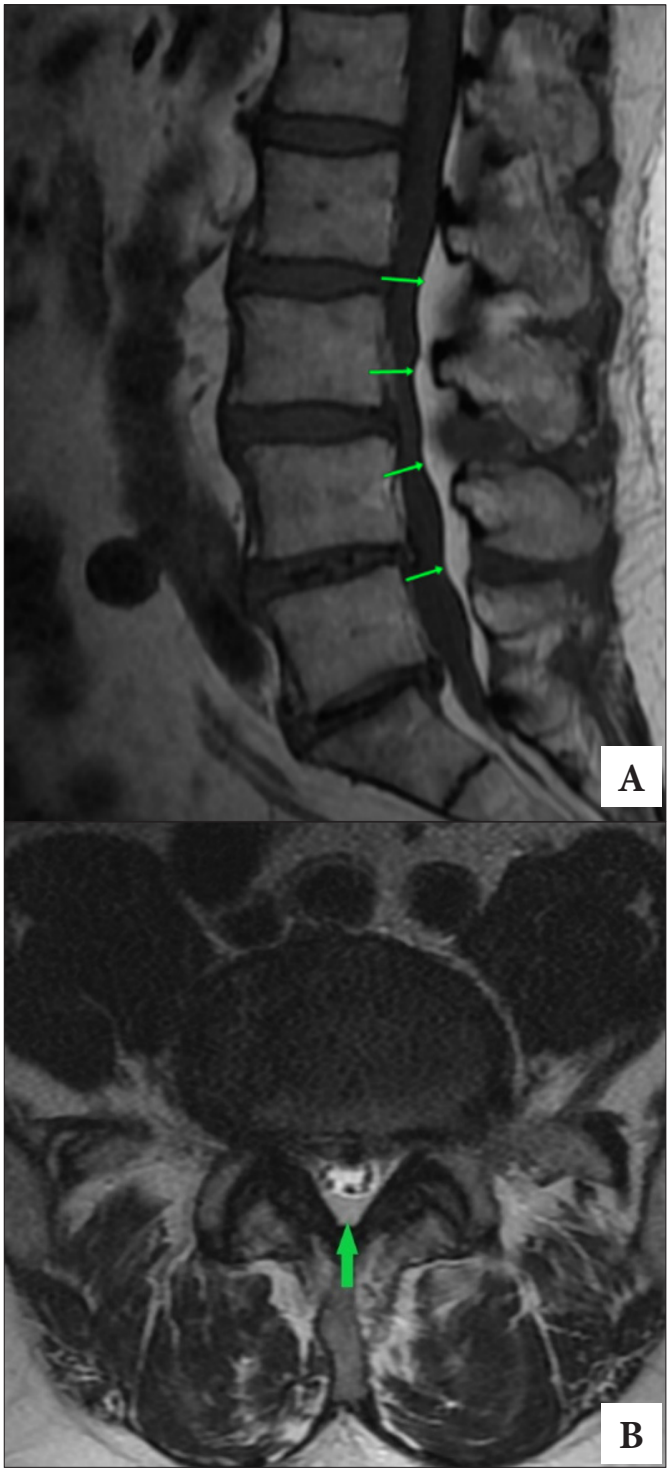

Figure 6. Posterior epidural fat is evident. Images of the same patient (**A**- sagittal T1W, **B**- axial T2W)

In our literature review, we found that epidural fat was not evaluated in detail in studies investigating SCCSA and DSCSA. In our study, we tried to find out whether volume changes in epidural fat tissue contribute to LSS formation. We found 23.50 mm² as the cut-off value.

CONCLUSION

Lumbar spinal stenosis is a problem that greatly affects the quality of life of patients. Measuring spinal bone canal width alone does not provide sufficient information in the diagnosis of spinal stenosis. For the diagnosis of dural stenosis, which is at the forefront of the emergence of symptoms, other structures that narrow the canal should be carefully examined in MRI evaluations. Since hypertrophy in the epidural adipose tissue is a condition that can lead to the finding of dural stenosis, we think that it should be especially evaluated. In addition, we would like to confirm that the diameter of the superior articular process is a valuable finding in the diagnosis of lumbar spinal stenosis.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Ankara Medipol University Faculty of Medicine, Non-interventional Researchs Ethics Committee (Date: 01.07.2021, Decision No: 27).

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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Single-center experience of childhood Hodgkin lymphoma treated without radiotherapy

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ABSTRACT

Aim: Hodgkin lymphoma (HL) constitutes 40% of childhood lymphomas and approximately 6% of all childhood cancers. It is tried to achieve cure with combined treatment modalities consisting of chemotherapy, radiotherapy, monoclonal antibodies, and new treatment agents such as nivolumab. Radiotherapy-related infertility, secondary cancer, thyroid dysfunction, cardiovascular diseases, pulmonary fibrosis, and local skin reactions can be seen in the pediatric age group with a long life expectancy. In this article, pediatric patients diagnosed with Hodgkin lymphoma without the use of radiotherapy in the treatment were evaluated retrospectively and the survival results were reported as a single-center experience.

Material and Method: The patients with Hodgkin Lymphoma in the Pediatrics Hematology-Oncology Center at Erciyes University between January 2010 and December 2019 were included in the study and the data of the patients were evaluated retrospectively.

Results: In 68 pediatric patients with a mean age of 10.7 (±4.6) years, the male/female ratio was 1.3. The most detected finding at the time of diagnosis was cervical lymphadenopathy (83.8%). The most common mixed cellular subtype was identified (48.5%). Stage I-II disease was observed in 38.3% of the patients, and stage III-IV disease was observed in 61.7% of the patients. The median follow-up period of the patients was 61 (range, 8.3-161.6) months. Disease-free survival and overall survival were 85.3% and 94.1%, respectively. Treatment modalities to be used in this disease group, which has a high chance of cure after cytotoxic treatment, should be selected considering treatment-related long-term complications.

Conclusion: Acceptable good results obtained without radiotherapy are satisfactory and the chance of curative success will increase with the addition of new target agents to the treatment.

Keywords: Hodgkin lymphoma, children, radiotherapy

INTRODUCTION

Hodgkin lymphoma (HL) constitutes 40% of childhood lymphomas and approximately 6% of all childhood cancers (1). Hodgkin lymphoma generally shows a bimodal distribution, mostly in young adults and older people. Its incidence peaks at 15-35 years of age and above 50 years of age (2). While the most common subtype in developed countries is the 60-70% nodular sclerosing type, mixed cellular type is most common in developing countries (3). It is among the malignant diseases with the best long-term outcome with 80-90% cure rate after chemotherapy. Relapsed or refractory to treatment is observed in 15-20% of patients (4). It is tried to achieve cure with combined treatment modalities consisting of chemotherapy, radiotherapy, monoclonal antibodies and new treatment agents such as nivolumomab. However, treatment-related side effects should not be ignored. While radiotherapy is an important part of treatment in adults with HL, the risk-benefit ratio in children is discussed. Radiotherapy-related infertility, secondary cancer, thyroid dysfunction, cardiovascular diseases,

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pulmonary fibrosis and local skin reactions can be seen in the pediatric age group with a long life expectancy (2,3,5-7). In this article, pediatric patients diagnosed with Hodgkin lymphoma without the use of radiotherapy in the treatment were evaluated retrospectively and the survival results were reported as a single center experience.

MATERIAL AND METHOD

The study was carried out with the permission of Erciyes University Clinical Researchs Ethics Committee (Date: 11.12.2019, Decision No: 2019/854). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The patients younger than 18 years of age who were diagnosed with Hodgkin lymphoma in the Pediatrics Hematology-Oncology Center at Erciyes University between January 2010 and December 2019 were included in the study and the data of the patients were evaluated retrospectively. The data of the patients included in the study were obtained from the hospital electronic records and patient files.

In the study, demographic data of the patients, Hodgkin lymphoma subtype, presence of B symptoms, spread regions and stage of the disease, laboratory findings at the time of diagnosis, genetic study results, treatments and response to therapy, complications, relapse status, bone marrow transplantation and results, and prognostic factors were evaluated.

Patients Characteristics and Chemotherapy Protocols

The patients were diagnosed with HL by histopathological examination of biopsy samples. Bone marrow aspiration and biopsy, positron emission tomography/computed tomography (PET CT), and symptoms (fever, night sweats and weight loss) was used for staging of all patients. Before starting chemotherapy, the patients were staged according to Cotswolds modification of the Ann Arbor criteria (2).

Cyclophosphamide, vincristine, procarbazine, prednisone (COPP)/ doxorubicin, bleomycin, vinblastin, dacarbazine (ABVD) protocols were used alternately in the initial diagnosis. In early stage (Stage-1A and Stage-2A) HL patients, 2 cycles of ABVD and 2 cycles of COPP protocols were started, and the number of cycles was rearranged to a maximum of 4 for each protocol, according to the response to treatment. In advanced stage (Stage IIB, Stage-3 and Stage-4) HL patients, 3 cycles of ABVD and 3 cycles of COPP were started. According to the response to treatment, 4th cycle ABVD and COPP were given. At least 2 cycles of ifosfamide, carboplatine, etoposide (ICE) protocol were given in refractory or relapsed patients. Brentuximab was added to the treatment of refractory or relapsed patients diagnosed with HL after 2014. Autologous or allogeneic stem cell transplantation was performed in selected cases according to the underlying disease status such as immunodeficiency or relapse and refractory disease. No patient received radiotherapy.

Statistical Analysis

Descriptive statistics and quantitative variables were expressed as mean±standard deviation or median (minimum-maximum) according to whether the distributions were normal or not, using the Shapiro-Wilk test. Nominal variables were expressed as number of cases and percentage (%). The Kaplan-Meier method was used to estimate survival probabilities and the log-rank test for comparisons.

RESULTS

A total of 68 patients, 38 males (56%) and 30 females (44%) diagnosed with HL, were included in the study. Demographic data of the patients are given in **Table 1**. While HL is 3.3 times more common in boys than girls in the 0-5 age group, it is 1.5 times more common in boys in the 5-10 age group. In addition, the male-female ratio in patients older than 10 years is 0.9.

Table 1. Patient characteristics

Variable	Patient (n=68)
Median age (years)	10.7 (±4.6)
Gender (male/female)	38/30
Diagnosis of Patients	
Classical Hodgkin lymphoma (n=63)	
Nodular Sclerosis	27 (39.7%)
Mixed Cellularity	33 (48.5%)
Lymphocyte-rich	2 (2.9%)
Lymphocyte-depleted	1 (1.4%)
Nodular lymphocyte-predominant Hodgkin lymphoma	1 (1.4%)
Unknown subtype	4 (5.8%)
Diagnosis Staging	
Stage I	5 (7.3%)
Stage II	21 (31%)
Stage III	18 (26.3%)
Stage IV	24(35.4)
Disease Status	
Complete remission 1	56 (82.3%)
Relaps/refractory lymphoma	12 (17.6%)

Notes: Values are expressed as n (%).

The majority of our patient group consisted of patients with stage III-IV (61.7%). B symptoms were not observed in 45 patients. **Figure 1** shows the ratio of B symptoms according to the stages of the patients. As lymph node,

the most cervical lymph node involvement was observed (83.8%); in the extra-lymphatic system, the most spread to bone/bone marrow was detected (35.3%) (**Table 2**). Lymph node and non-lymph node involvements are summarized in **Table 2**. Bulky disease was detected in 10 patients (14.7%) and vena cava superior syndrome and/or upper mediastinal syndrome was detected in 8 patients (11.7%) at the time of admission. None of the patients had tumor lysis syndrome.

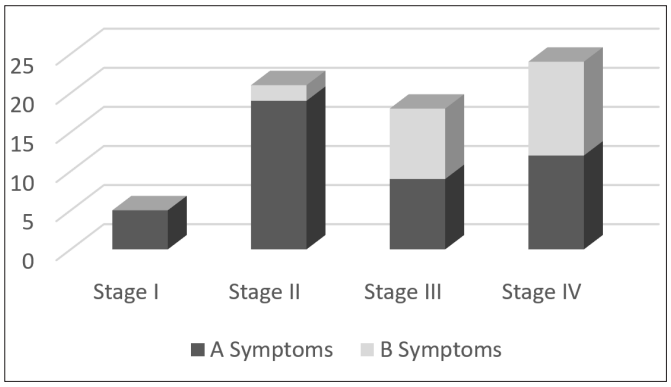

Figure 1. B Symptoms according to the stage of the patients

Table 2. Hodgkin lymphoma involvement sites

Site	Patient
Cervical LN	57 (83.8%)
Mediastinal LN	43 (63.2%)
Axillary LN	23 (33.8%)
Paraaortic LN	22 (32.3%)
Supraclavicular LN	19 (27.9%)
Parailiac LN	10 (14.7%)
Submandibular LN	7 (10.2%)
Inguinal LN	5 (7.3%)
Waldeyer ring	11 (16.1)
Spleen	23 (33.8%)
Bone/bone marrow	24 (35.3%)
Lung	10 (14.7%)
Liver	4 (5.8%)
Intestine	1 (1.4%)

Notes: Values are expressed as n (%).

STAT-6 mutation in 2 patients, CD27 deficiency in 1 patient, F-BAR domain only protein 1 (FCHO1) mutation in 1 patient, Interleukin-2-inducible T-cell Kinase (ITK) mutation in 1 patient, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3CD) mutation in 1 patient, Magnesium transporter 1 (MAGT1) deficiency, X-linked immunodeficiency with magnesium defect, Epstein-Barr virus (EBV) infection, and neoplasia (XMEN syndrome) in 1 patient were found in the genetic analysis of patients with relapse/refractory and reported in several publications (8).

Autologous hematopoietic stem cell transplantation was performed in 9 patients with relapse/refractory, and allogeneic hematopoietic stem cell transplantation was performed in 4 patients with immunodeficiency. One patient who was diagnosed with immunodeficiency and planned for allogeneic stem cell transplantation died due to sepsis. In addition, allogeneic stem cell transplantation is planned for 2 immunodeficiency patients.

The median follow-up period of the patients was 62 (range, 8.3-161.6) months. Disease-free (lymphoma, free) survival (DFS) and overall survival (OS) were 85.3% and 94.1%, respectively (**Figure 2 and 3**). While DFS was 96.2% and OS was 100% in patients with stage I-II, DFS was 76.2% and OS was 90.5% in patients with stage III-IV. One patient died of lymphoma progression, 2 patients died of sepsis after stem cell transplantation, and one patient died of sepsis secondary to immunodeficiency. Lymphoma-related mortality was 1.5% and overall mortality was 5.8%.

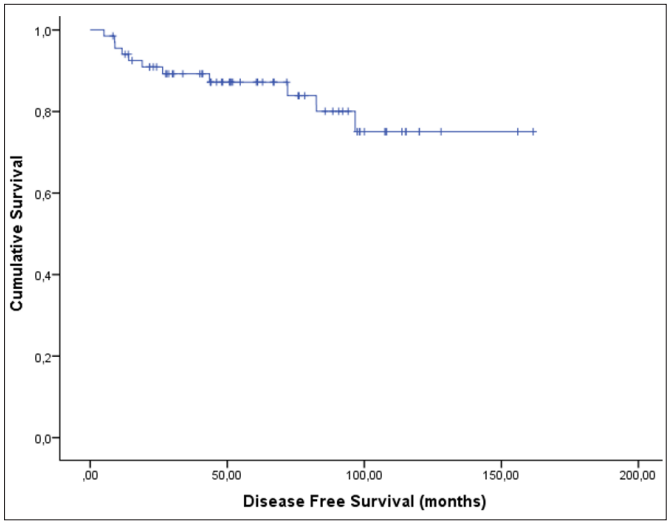

Figure 2. Disease-free survival graph of patients with Hodgkin lymphoma

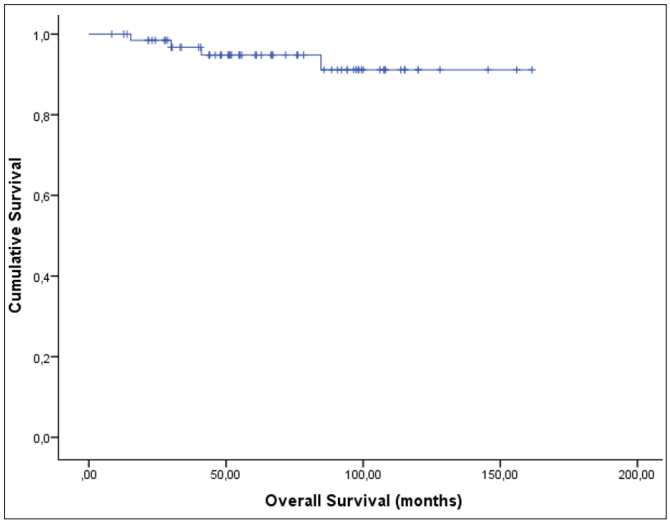

Figure 3. Overall survival graph of patients with Hodgkin lymphoma

DISCUSSION

Hodgkin lymphoma is a malignant disease of unknown specific etiology characterized by enlargement of lymph nodes. Conditions such as genetic diseases, socioeconomic status, positive family history, and Epstein-Barr virus (EBV) infection increase the risk of developing HL (2,9,10). In various studies, the age of incidence in the childhood age group is approximately 6-15.5 years. While Hodgkin lymphoma is more common between 5-10 years of age in developing countries, it has been found to be more common in children over the age of 10 in a study conducted in The United Kingdom (11-13). The mean age of the patients in our study was 10.7 (±4.6) years. 19.1% of the patients were aged 0-5 years, 26.4% were aged 5-10 years, and 54.5% were older than 10 years. The male/female ratio has been reported as 1.3-2.5 (13,14). 56% of our patients were male and 44% were female. The male to female ratio was 1.26, which is consistent with the literature. When we divide the patients into age groups, this ratio is; 3.3 in the 0-5 age group; 1.5 in the 5-10 age group, and 0.9 in patients older than 10 years old.

While nodular sclerosing type HL is observed at a rate of up to 80% in developed countries, mixed cellular type HL is observed at a rate of up to 60% in developing countries (12,13,15). In our study, approximately half of the patients diagnosed with HL (48.5%) were mixed cellular type, while the second most common (39.7%) type was nodular sclerosing type and these findings were comparable with other studies conducted in our country (16,17). The subtypes of the disease vary in relation to the development levels of the countries and the socioeconomic status of the people. Although the mixed cellular type was more common in our study, the incidence of nodular sclerosing type was seriously close to the incidence of the mixed cellular type. Generally, EBV is associated with mixed cellular HL lymphoma and is observed in young children, whereas nodular sclerosing type HL is more common in young adults and adolescents. Tumor cells are infected with EBV in 90% of cases in developing countries and 30% of cases in developed countries (18,19). EBV positivity was detected in tumor cells in 27 of our patients (39.7%).

Positron Emission Tomography (PET CT) is the standard imaging method for staging HL at the time of diagnosis and evaluating the response to treatment. However, it contains ionizing radiation. Nevertheless, in a study comparing magnetic resonance imaging and PET CT, PET CT was shown to be more sensitive in staging at the time of diagnosis and evaluating the response to treatment (20). We used PET CT for staging and evaluation of response to treatment in all of our patients.

Various combination chemotherapy regimens containing vinblastin, vincristine, dacarbazine, procarbazine, bleomycin, etoposide, cyclophosphamide, prednisone, doxorubicin, methotrexate are used in treatment (2). With the increase in survival rates, the focus is on eliminating or reducing the side effects that can be seen in long-term follow-ups. Studies are ongoing to reduce or eliminate anthracyclines due to its cardiotoxic effect, procarbazine for infertility, and radiotherapy due to its multiple side effects . In the studies of Dana Farber and St Jude Consortium, Children's Oncology Group (COG), and the German Society of Pediatric Oncology groups in low-risk patients who received radiotherapy, overall survival and event-free survival were 96.1-100% and 86-95%, respectively. In the studies of COG and the German Society of Pediatric Oncology group, overall survival and event-free survival were 93-98% and 82-94% in patients with medium and high-risk group HL who received radiotherapy (21). In a study using radiotherapy, in which patients with 43.1% early-stage disease and 55.9% advanced-stage disease, 5-year overall survival, and event-free survival were 96.6% and 84.7% respectively (12). In another study in which early-stage 92 patients and advanced-stage 104 patients did not use radiotherapy, 5-year overall survival and event-free survival were found to be 89.6% and 82.1%, respectively (3). Mixed cellular lymphoma was the most common subtype in both studies (3,12). In our study, 38.3% were evaluated as early-stage and 61.7% as advanced-stage. In the early and advanced patient group, 5-year overall survival is 90%.

Autologous hematopoietic stem cell transplantation following high-dose chemotherapy is the standard treatment approach in relapsed and refractory patients. In the study, which included 38 pediatric patients with a diagnosis of HL, 10-year survival and event-free-survival were 71.4% and 67.1%, respectively (22). Allogeneic stem cell transplantation can be performed in relapsed cases after autologous stem cell transplantation or in HL that develops on the basis of disease such as immune deficiency (9,10). In the meta-analysis, overall survival and relapse-free survival after transplantation were 50% (41-58) and 31% (25-37) (21). One of our patients who underwent 10 autologous and 4 allogeneic stem cell transplants died due to post-transplant relapse and 2 died due to sepsis.

Although it varies depending on the stage of the disease in various studies, the total relapse rate has been reported as 11.2-30.4 (11,12,23,24). Relapse is observed in 10% of patients with early stage HL and 25% in those with advanced stage HL after first-line treatment (25,26). The relapse rate was found to be 14.7% in our patients. In relapsed and refractory cases, especially if the age of diagnosis is under 5 years, genetic studies are recommended to investigate diseases predisposing to lymphoma (9,10).

CONCLUSION

Treatment modalities to be used in this disease group, which has a high chance of cure after cytotoxic treatment, should be selected considering treatment-related long-term complications. Acceptable good results obtained without radiotherapy are satisfactory and the chance of curative success will increase with the addition of new target agents to the treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Clinical Researchs Ethics Committee (Date: 11.12.2019, Decision No: 2019/854).

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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Relationship between hope and fatigue levels in cancer patients

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ABSTRACT

Aim: The aim of the present study is; to determine the relationship between fatigue and hope levels in cancer patients.

Material and Method: The study was conducted as a descriptive study. Data were collected using Socio-Demographic Data Qestionnaire, Herth Hope Index, Brief Fatigue Inventory (BFI). The questionnaires were filled outthrough face-to-face interviews.

Results: 213 cancer patients with 50.23% 21-54 years of age were taken into the study. There was no significant relationship between fatigue status of patients and sociodemographic variables. However, a significant correlation was found between the score of hope and the time after diagnosis(KW =2.608; p=0.053). In our study, the difference between mean score of hope level and gender, age,marital status, educational status and employment was not statistically significant.

Conclusions: There was a significant negative correlation between fatigue and hope total scores of the patients. This shows us that the management of fatigue during the illness and treatment positively affects the hope and patients are struggling with their disease. it is suggested that the diagnosis of fatigue and hope levels of the patients from the diagnosis and the planning of the attempts to manage them can be suggested.

Keywords: Cancer, hope, fatique

INTRODUCTION

Among the chronic diseases, cancer is one of the major health problem of today. According to the World Cancer Report 2020, there were 19.3 million new cases and 10 million cancer-related deaths worldwide in 2020 (1). Cancer, a major health problem of modern medicine and human, is perceived as a serious and chronic illness, which causes fear, hopelessness, guilt, helplessness, unbearable pain, fatigue, abandonment, death and evokes the feeling and ideas of death, creates anxiety and chaos in patients and their families (2- 4). Fatigue is the most common symptom in cancer patients. The prevalence of fatigue associated with cancer and its treatments ranges from 40 to 100% (1, 4-5). Fatigue is among the significant symptoms affecting patients. The fatigue experienced during the diagnosis and treatment process had a negative impact on cancer-fighting power and quality of life of the patients (6).

Cancer is perceived as a disappointing disease due to uncertainties, fears, current and future pains experienced by the individuals (7). The uncertainty might threat one's feelings of hope. It may influence deeply the emotional background of the individual (7). Hope is one of the most important factor that enable to cope effectively with cancer-related loss, uncertainty and suffering (8). Loneliness is the most valuable source for overcoming the stress conditions, such as distress and suffering (7-8). In the literature, disease-related factors in cancer patients (cancer diagnosis, disease progression, time since diagnosis), decreasing functional status and increasing symptom burden were stated as important factors affecting the hope level (1,9). Assessment of fatigue, one of the factors that might affect hope in cancer patients, is important in planning effective interventions. By means of these interventions can structure initiative s improving their adaptation to treatment and quality of life by reducing uncertainties about the future, and problems with disease and treatment, increase treatment compliance, and help the patient maintain his/her hope. The aim of the present study is; to determine the relationship between fatigue and hope levels in cancer patients.

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MATERIAL AND METHOD

Ethical Considerations

The study was carried out with the permission of İzmir Katip Çelebi University, Non-Interventional Clinical Investigations Committee of Ethics Committee (Date: 21.01.2016, Decision No: 10), Atatürk Training from the General Secretariat of the Southern Region Public Hospitals Association of İzmir Province (No: 23592379/772.02) and the Research Hospital Oncology Day Care Service. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. That the validity and reliability of scales in Turkey Çınar et al. (10) and Aslan, Sekmen, Kömürcü & Özet (19) written consent (11). Patients who met the study criteria were informed about the study and informed consents were obtained from all volunteers.

Design and Samples

The study was conducted as a descriptive study to examine the relationship between fatigue and hope levels in cancer patients. The study was conducted at the Oncology Outpatient Service of Atatürk Training and Research Hospital, located in a western province of Turkey, between November 2015 - April 2016. Approximately 12,000 patients per year and 60-80 patients per day were admitted to Outpatient Service for treatment. As a selection criterion, patients who were admitted participating in the study, those experienced fatigue within the last 7 days, those over 18 years old, those receiving ambulatory care, those having cancer diagnosis 3 months ago, and those having no perception disorder. Exclusion criterion included patients who had cognitive impairment and mental impairment to disrupt co-operation, and those who did not want to participate in the study. In the G-power statistical program, the required sample size was determined as 210 individuals in a group, at a significance level of 0.05 and 80% power. The sample size was 213.

Measurements

Data were collected using Socio-Demographic Data Questionnaire (Personal Information Form), Herth Hope Index (HHI), Brief Fatigue Inventory (BFI).

Personal Information Form was formed according to the literature by the researchers. The form involved data regarding the age, sex, marital status, educational status, employment, type of cancer, cancer stage, duration of diagnosis and type of treatment of the cases (12-13). There are eight questions in the form.

Brief Fatigue Inventory is a 9-item survey. The form assesses patients' general level of fatigue and its impact on daily activities over the past 24 hours. The BFI uses a scale of 0, indication 'no fatigue' to 10, indicating the greatest interference; 0 point: no fatigue; 1-2: minimal fatigue; 3-4: low fatigue; 5-6: moderate fatigue; 7-8: too much fatigue 9-10: severe fatigue. The original scale was developed by Mendoza et al. (14) and Cronbach α value was found as 0.96. The Turkish Validity and Reliability study of the scale Çınar et al. (2000). Cronbach's alpha value of BFI; It was calculated as 0.97 (10).

Herth Hope Index (HHI) was developed by Dr. Kaye and it is a 12-item Likert-type scale. The 12-item, 4-point Likert-type HHI scale assesses the overall hope level of adults. Each item is rated with a score between 1 (I do not agree) and 4 (I strongly agree) and the total score range is between 12 and 48. The higher the score, the higher hope level. Herth index Cronbach's alpha coefficient was 0.98, in patients with acute disease, 0.96 in patients with chronic disease, and 0.94 in patients in terminal stage (15). The validity and reliability studies of Turkish version of the "Herth Hope Scale" was performed by Aslan, Sekmen, Komurcu and Ozet (11). Cronbach's alpha was 0.75 in the reliability analysis.

There are three subscales within the scope of the scale: "temporary and future", "positive readiness and expectation" and "relations between themselves and their environment". Each sub-dimension consists of 4 questions. Therefore, a participant can score minimum 4 or maximum 16 out of any sub-dimension. The Cronbach alpha values for these three dimensions were 0.77, 0.64 and 0.30, respectively.

Data Collection

Data was collected by a researcher from patients who met the criteria for participating in the study and the study was conducted with those who volunteered to participate in the study. The questionnaires were filled out through face-to-face interviews and the information regarding the diagnosis, duration of diagnosis, metastasis, presence of chronic illness, etc. was filled out by using patients' hospital records. The duration of application for a volunteer was determined as 30-35 minutes.

Data Analysis

The obtained data were analyzed using in IBM SPSS Version 22. Dependent variables; mean fatigue and hope scores of cancer patients in patients. Independent variables; age, gender, educational status, marital status, employment, body mass index, performance status. The socio-demographic characteristics of the sample group were defined by descriptive statistical methods; one-way analysis of variance (Anova), Mann Whitney U test, and Kruskal Wallis test were used to examine the relationship between age, educational status, gender, marital status, employment, cancer type, duration of diagnosis and mean fatigue scores. Pearson correlation analysis was used to examine the relationship between mean hope and fatigue scores of patients, fatigue level and hope. p significance value was determined as <0.005.

RESULTS

When the socio-demographic characteristics of the patients were examined; 66.7% were female, 77.0% were married, 33.3% were male and the majority were between 21 and 54 years old (50.23%). Of the patients, 56.3% were primary school graduates and 42.2% were unemployed. According to the characteristics of the patients regarding the disease and treatment; 41.7% were diagnosed with breast cancer, 55.9% were diagnosed for 12 months or longer, and 93.9% had received chemotherapy treatment (**Table 1**).

There was no significant difference between mean scores of patients and gender (t=0.867, p=0.387), age (t=1.418, p=0.158), marital status (t=1.513, p=0.412), educational status (KW=1.307, p=0.268), employment (KW=0.061, p=0.941), type of cancer (KW=0.905, p=0.513), time since treatment (KW=1.918, p=0.128) and treatment method (U=1.629, p=0.121) (p> 0.05) (**Table 2**).

Table 1. Socio-demographic and disease-related characteristics of patients

Characteristics	Number	%
Gender		
Female	142	66.7
Male	71	33.3
Marital Status		
Married	164	77
Single	49	23.
Age (years)		
21-54	107	50.2
55-80	106	49.8
Educational Status		
Illiterate	6	2.8
Literate	32	15
Primary education	120	56.3
High school	35	16.4
College/Faculty	20	9.4
Employment		
Employed	36	16.9
Retired	87	40.8
Unemployed	90	42.2
Type of cancer		
Breast	89	41.7
Colon	39	18.3
Ovary	19	8.9
Pancreas	11	5.1
Stomach	10	4.6
Rectum	8	3.7
Lung	8	3.7
Connective tissue	5	2.3
Other	24	10.7
Time since diagnosis (months)		
≤3	18	8.4
4-6	32	15.0
7-11	44	20.7
≥12	119	55.9
Treatment method		
Chemotherapy	200	93.9
Chemotherapy and radiotherapy	13	6.1

According to **Table 3**, the difference between mean score of hope level and gender (t=-237; p=0.813), age (t=-121; p=0.225), marital status (t=1,80; p=0.073), educational status (KW =0.553; p=0.531), employment (KW =0.184; p=0.832), and type of cancer (KW=1.089, p=0.372) (p >0.05). Similarly, there was no significant difference between temporary dimension of hope and gender (t= -0.237; p= 0.753), age (t= -1.077; p= 0.378), marital status (t= 1.337; p= 0.165), educational status (F=0.481; p=0.804), and employment (KW=0.016; p=0.934) (p >0.05). There was no significant difference between temporary subdimension of hope and gender (t=- 0.027; p= 0.979), age (t= -1.049; p= 0.189), marital status (t=1.357; p=0.086), educational status (KW =2.022; p= 0.083) and employment (KW=0.328; p=0.586) (p>0.05). There was no significant difference between relations with themselves and those around subdimension of hope and gender (t=- 0.220; p= 0.985), age (t =-0.993; p=0.355), marital status (t= 2.041; p= 0.178), educational status (KW=1.579; p= 0.368), and employment (KW=0.110; p=0.754) (p >0.05). There was a significant difference between time after diagnosis and mean total score of hope (KW =2.608; p=0.053) (p<0.05). Accordingly, the total hope score of patients with diagnosis period of 4-6 months was statistically higher than others. The difference between the time after diagnosis and temporary subdimension of hope (KW =1.670; p=0.175), positive readiness (KW =2.367; p=0.721), and relations with themselves and those around (KW =1.295; p=0.175) (p>0.05). There was a significant difference between treatment method and time after diagnosis and mean total score of hope level (U=- 2.534; p=0.011). While there was no significant association between treatment method and temporary subdimension of hope (U=-1.128; p=0.259), a negative correlation was detected between positive readiness (U= -2.535; p=0.011) and relations with themselves and those around (U=-3.779; p=0.00) (p<0.05) (**Table 3**). When the correlation between total score of brief fatigue inventory and general total score of Hert hope index and subdimensions was analyzed, a statistically negative and significant association was detected (**Table 4**). Although not shown in the table; the mean score of brief fatigue inventory of the patients was 43.22 ± 25.94 and the general score of hope scale was 43.98 ± 4.20. When the subdimensions of hope was examined, mean score of temporary subdimension of hope was 16.66±2.10, mean score of positive readiness subdimension was 14.72±1.10, and mean score of relations with themselves and those around was 12.60±1.01.

Table 2. Comparison of mean fatigue scores according to socio-demographic data and disease-related characteristics of patients

Characteristics	Total Mean Score of Brief Fatigue Inventory			t / KW	p
	n	X	SD		
Gender				t= 0.87	p = .387
Female	142	39.88	23.69		
Male	71	36.94	22.70		
Age (years)				t=1.41	p = .158
21-54	107	41.19	21.88		
55-80	106	36.67	24.35		
Marital status				t=1.51	p = .412
Married	164	40.22	23.39		
Single	49	34.48	22.90		
Educational status				KW=1.31	p = .268
Illiterate	6	54.02	21.45		
Literate	32	35.90	22.69		
Primary education	120	40.47	23.17		
High school	35	37.24	26.10		
University	20	32.65	19.77		
Employment				KW =0.06	p = .941
Employed	36	39.47	22.42		
Retired	87	39.02	24.07		
Unemployed	90	38.05	23.03		
Type of cancer				KW= 0.91	p = .513
Breast	89	37.63	23.96		
Colon	39	41.20	27.13		
Ovary	19	42.37	18.61		
Pancreas	11	40.79	19.43		
Stomach	10	45.40	12.61		
Rectum	8	28.50	24.40		
Lung	8	51.75	27.33		
Connective tissue	5	28.60	18.99		
other	24	38.48	23.14		
Time since diagnosis (months)				KW=1.92	p=.128
≤3	18	28.40	19.87		
4-6	32	35.12	23.26		
7-11	44	42.68	20.88		
≥12	119	40.11	24.37		
Treatment method				U=1.63	p = .121
Chemotherapy	200	38.81	23.83		
Chemotherapy and Radiotherapy	13	45.39	13.21		

Sd: Standard deviation, t: Independent samples t test, Kw: Kruskal-Wallis test

DISCUSSION

Cancer patients suffer from limitations in prognosis and treatment. These limitations can often lead to the disappearance of the joy of life (3). In this study, there was no significant difference between mean fatigue scores and mean score of gender, age, marital, educational and employment status of the patients. Although there was no statistical difference, it was found that mean fatigue scores of female and married patients were higher than males and singles, respectively. The reason for this may be due to the high number of females and married individuals taken into the study. In the literature, it was generally stated that mean fatigue scores of females and married individuals were higher (16). This finding may be derived from the several domestic responsibilities of women because of the traditional family structure in Turkey. Although there was no statistically significant difference in the study, mean fatigue scores of the individuals graduated from primary school. Our findings are consistent with the literature (16-17).

In the study, the difference between mean fatigue score and the cancer type, the time after the diagnosis and mean scores of treatment methods of the patients were not significant. Our finding was consistent with finding Kagure (17). Although there was no statistically significant difference, it was determined that mean fatigue scores of patients with lung cancer were higher. This is due to cough, respiratory distress, and nutritional problems in the patients with lung cancer, which negatively affects the quality of life of the person (16). Cancer-related fatigue

Table 3. Comparison of mean scores of hope levels and socio-demographic and disease-related characteristics of patients

Characteristics	Total score				Sub-dimensions of hope level											
					Temporary dimension of hope				Positive readiness				Relations with themselves and those around			
	n	X	Sd	p	n	X	Sd	p	n	X	SS	p	n	X	Sd	p
Gender																
Female	142	44.17	3.54	.813	142	14.14	1.93	.753	142	14.59	1.60	. 979	142	15.44	0.93	.985
Male	71	44.29	3.81		71	14.23	1.98		71	14.60	1.88		71	15.47	0.87	
Age (years)																
21-54	107	43.89	3.84	. 225	107	14.04	1.97	.378	107	14.47	1.71	.189	107	15.38	0.96	.355
55-80	106	44.50	3.41		106	14.28	1.92		106	14.71	1.69		106	15.50	0.87	
Marital status																
Married	164	44.45	3.55	. 073	164	14.30	1.90	.165	164	14.68	1.68	.086	164	15.51	0.83	.178
Single	49	43.40	3.78		49	13.87	1.82		49	14.31	1.71		49	15.24	1.13	
Educational status																
Illiterate	6	46.02	1.24	.531	6	14.83	1.16	.804	6	15.68	1.51	.083	6	15.50	1.22	.368
Literate	32	43.85	3.51		32	14.33	1.34		32	14.50	1.51		32	15.17	1.09	
Primary school	120	44.41	3.53		120	14.14	2.09		120	14.74	1.57		120	15.54	0.83	
High school	35	43.61	4.48		35	14.35	1.82		35	13.99	2.29		35	15.27	1.04	
University	20	44.06	3.24		20	13.83	1.71		20	14.60	1.47		20	15.63	0.62	
Employment *																
Employed	36	44.48	3.35	.832	34	14.25	1.72	.934	36	14.70	1.65	.754	36	15.53	0.77	.586
Retired	87	44.08	3.53		85	14.14	2.02		87	14.54	1.63		87	15.41	0.92	
Unemployed	88	44.34	3.78		88	14.23	1.93		90	14.61	1.81		90	15.50	0.91	
Type of cancer																
Breast	89	44.16	3.36	.372	89	14.20	1.79	.788	89	14.58	1.56	.416	89	15.39	0.95	.446
Colon	39	43.77	3.99		39	14.10	2.01		39	14.47	2.04		39	15.31	0.97	
Ovary	19	44.00	3.87		19	14.05	1.78		19	14.37	2.01		19	15.58	0.69	
Pancreas	11	41.98	4.21		11	13.18	1.89		11	13.80	1.89		11	15.00	1.34	
Endometrium	10	44.80	3.58		10	14.40	1.58		10	14.80	1.69		10	15.60	0.97	
Rectum	8	46.00	2.88		8	14.50	2.39		8	15.50	0.76		8	16.00	0.00	
Lung	8	44.87	3.64		8	14.37	2.20		8	14.62	1.51		8	15.87	0.35	
Connective tissue	5	44.40	3.91		5	14.80	1.30		5	14.00	1.87		5	16.60	0.89	
Other	24	45.10	3.58		24	14.46	2.19		24	15.12	1.28		24	16.62	0.79	
Time after diagnosis (months)																
≤ 3	18	44.84	3.17	.053		14.72	1.45	.175		14.58	1.56			15.54	0.60	.056
4-6	32	45.67	2.50			14.71	1.65			15.31	0.86			15.66	0.60	
7-11	44	44.09	3.59			14.03	1.59			14.52	1.70			15.53	0.96	
≥12	119	43.76	3.87			14.03	2.075			14.43	1.84			15.34	0.99	
Treatment method																
Chemotherapy	200	43.54	3.15	.011	200	43.54	3.15	.259		38.70	3.35	.011	200	36.60	3.81	.000
Chemotherapy and radiotherapy	13	28.20	3.44		13	24.16	1.06			24.16	1.06		13	24.16	1.06	

*n= 210 (210 patients answered this section) , Sd: Standard deviation

Table 4. Relationship between mean score of brief fatigue inventory and hope and subdimensions of hope

	Total Score of Brief Fatigue Inventory	
Relationship between brief fatigue inventory and hope and subdimensions of hope	r*	p**
Total Score of Herth Hope Index	-.41	<.001
Temporary Subdimension of Hope	-.42	<.001
Total Score of Positive Readiness	-.27	<.001
Relations with themselves and those around	-.23	<.001

* r= Pearson correlation analysis was used as variables met the normal distribution hypothesis. ** Correlation was significant at the level 0.01 (2-tailed).

is associated with the duration of diagnosis and showed elevation after the treatments. Although patients receiving both chemotherapy and radiotherapy may be expected to experience more fatigue, the difference between treatment and mean fatigue scores was not significant (18).

Cancer patients pass the adaptation process to their diseases with various reactions. Positive responses influence the healing process positively (3). In our study, the difference between mean score of hope level and gender, age, marital status, educational status and employment was not statistically significant. In literature, although women are reported to be more disadvantaged than males in situations, such as mood disorders, depression and self-annihilation (19), there are also studies indicating no gender difference in terms of hope (16-17). Our findings are consistent with the literature (20). This suggests that the negative health-related changes reduce the hope level of young patients, and they are easily affected by the negative interactions in their environment (21).

The difference between cancer type and total score of hope level was not significant. This finding might be related to the cancer type and stage of the individuals. It is noteworthy that the five-year survival rates of cancer types are relatively high. Five-year survival rate is 87% for breast cancer, 59% for bowel cancer, and 65% for colon cancers (22-23). Moreover Costa et al. (24) found that patients with breast cancer had higher total hope scores, and it was stated that high hope levels helped individuals to flow the changes in their values and caused the questioning of the meaning of life by providing internal self-motivation. However, the data regarding the cancer stages were not obtained from the patient records. This is the limitation of our study. There was a significant difference between the time after the diagnosis and mean total hope score and the subdimension of positive readiness ($p < 0.05$). However, it was determined that total and subdimension scores of hope were lower as the duration of diagnosis was prolonged. It might be concluded that the hope of the cancer patients decreases as the duration of diagnosis and treatment increases. According to the literature, fear and uncertainty caused by cancer, long treatment period and uncontrollable side effects constituted a risk factor in terms of hopelessness, future anxiety and negative thoughts, depression and hopelessness (25-26). Likewise, in the same study, it was found that the scores of temporary subdimension of hope in patients with duration of diagnosis ≤3 months, mean scores of positive readiness and relationship with themselves and their environment in patients with duration of diagnosis between 4-6 months were found to be higher. This shows us that patients maintain positive relationships with their environment to maintain their hope during their disease. The scores of positive readiness involving fatigue score and hope subdimensions, and the subdimension scores of relationship with themselves and their environment were found to be higher. The fatigue levels of patients were low (mean=43.229) and general hope score was high (mean=432). As patients' fatigue reduce, hope increases. In the literature, while a similar association was found between fatigue and hope (26). In addition, we found a weak negative correlation between subdimensions of positive readiness and relationship between themselves and their environment. The result shows that individuals who are struggling with cancer have confidence to the treatments and maintain their hopes for recovery. In addition, new therapies related to cancer and treatment cause the maintenance of hope feeling for recovery in individuals. This shows us that the management of fatigue during the illness and treatment positively affects the hope and patients are struggling with their disease (27).

Study Limitations

The study was done in a single hospital, the number of samples, and the fact that the sample of the patient group was not able to reach the cancer stage data was a limitation.

CONCLUSION

The study was a significant negative correlation between fatigue and hope subdimensions. It was determined that the duration of diagnosis and the treatment method affected the hope and fatigue scores. At the end of this study, it is recommended to perform the fatigue assessment of patients as from the diagnosis, to inform the patients regarding fatigue management, to structure attempts for increasing the hope levels and to plan interventions for developing the coping-skills of patients with disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İzmir Katip Çelebi University, Non-Interventional Clinical Investigations Committee of Ethics Committee (Date: 21.01.2016, Decision No: 10).

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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The effect of COVID-19 pandemic on inguinal hernia emergencies

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ABSTRACT

Introduction: COVID-19 has spread all over the world and caused significant changes in healthcare practices. This is why many expert associations have published new guidelines on COVID-19 management. This study aims to investigate whether the COVID-19 pandemic has an effect on Inguinal hernia (IH) emergencies.

Material and Method: A total of 63 patients diagnosed with strangulated/incarcerated inguinal hernia who presented to the emergency surgery department of our hospital between April 2020 and January 2021 during the pandemic (DP) and between April 2019 and January 2020 before the pandemic (BP) were retrospectively analyzed and compared.

Results:There was no statistically significant difference between both groups in terms of demographic characteristics. The comparison of the number of admissions, admission time, anesthesia type, hospital stay, postoperative complications, ASA score, hernia, WBC, and CRP averages showed no statistically significant difference between the groups. Moreover, there was no statistically significant difference between the two groups in terms of distributions of hernia types, hernia repair types, mesh use, and additional resection requirement. The comparison of the patients who underwent organ resection by admission time in both groups showed no statistically significant difference. It was observed that the number of patients who required small bowel resection were especially high on the 4th day. In DP, small bowel resection was performed on 4 (66.7%) patients and omentectomy was performed in 2 (33.3%) patients. In BP, only one right hemicolectomy was performed. The comparison of the patients with an admission time of 4th day revealed a statistically significant difference (p=0.03).

Conclusion: We observed that morbidity increased as the admission time was delayed. Anticipating that the fear of COVID-19 infection will hold back the emergency response during the pandemic period, patients should be informed not to delay early diagnosis and treatment.

Keywords: COVID -19 pandemic ,emergency, inguinal hernia

INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. In December 2019, the World Health Organization declared a covid 19 epidemic. Most COVID-19 patients battle a mild or moderate prognosis, with up to 5-10% going to have a severe, potentially trying course (4). The tide of death is approaching fast, the queue is easy to mess with and it's about close-up events and concerns at close proximity (5).

The COVID-19 pandemic has taken the whole world under its influence in a short time, and unexpected and unprecedented radical changes in health practices have affected all countries. During the COVID-19 pandemic, surgical units gave priority to emergency procedures and oncological cases and postponed elective cases (6).

Abdominal hernia surgery is one of the most performed surgeries with a rate of 100 to 500 cases per 100,000 per year (7). While mortality rates are around 0.5% in elective hernia surgery, it is around 5% in emergency cases (8). In the cases of incarceration or strangulation, although the final treatment is often surgical, a decision must be made based on the patient's condition (age, accompanying diseases, Computed Tomography finding, SIRS, etc.). If there is a high probability of strangulation, the decision should an urgent surgery.

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The goals of emergency hernia surgery can be divided into three parts: saving lives (gangrenous bowel/tissue resection); restoring GI continuity (if bowel resection was performed); repairing the abdominal wall. These goals can be achieved in the same operation, yet a phased approach may also be required. Mortality and morbidity in the emergency department increase 10-20 times compared to elective surgery, and bowel infarction is the major risk factor for this (9).

Inguinal hernia (IH) is a common pathology that has the risk of developing significant complications in the case of strangulation. Strangulated inguinal hernias are a surgical emergency and are associated with high morbidity and mortality (10,11). Delayed diagnosis of patients with incarcerated/strangulated inguinal hernia and not performing surgery in the first 24 hours are associated with increased bowel resection. Therefore, early diagnosis of these patients and timing of treatment gain importance.

This study aims to investigate the effect of the pandemic on inguinal hernia emergencies.

MATERIAL AND METHOD

The study was approved by the Hitit University Non-interventional Researchs Ethics Committee (Date: 30.04.2021, Decision No:2021-66). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Strangulated/ incarcerated inguinal hernia patients presented to the emergency department and operated during the April 2019-December 2019 pre-pandemic (BP) period and the April 2020-December 2020 pandemic period (DP) were retrospectively analyzed.

Patients presented to the emergency department of the Hitit University Erol Olçok Training and Research Hospital General Surgery Clinic and operated for incarcerated/ strangulated inguinal hernia between the specified dates were retrospectively searched from the hospital system. The data of 63 patients were retrieved. The patients were divided into two groups as BP and DP. There were 35 patients in the BP group and 28 patients in the DP group. The patients' gender, age at admission, comorbidities, ASA scores, presence of ileus symptoms, number of days from incarceration to admission, presence of air-fluid level on erect abdominal radiograph (EAR), white blood cell count (WBC), C-reactive peptide (CRP) level, albumin (Alb) level, hernia type (indirect, direct, femoral), type of surgery performed (anterior repair, McVay, Plug Mesh), mesh use, type of anesthesia used, whether resection was performed, operative time, presence of perioperative morbidity, local complications during hospitalization and length of stay were found from the Hospital Information Management System and included in the study.

Numerical variables such as age, time to admission, WBC, CRP, operative time, length of stay were reported using the mean±standard deviation and median in parentheses. Correlations between numerical measurements were assessed with Pearson or Spearman correlation coefficient in accordance with the data distribution. Categorical variables such as gender, number of comorbidities, ASA score, presence of ileus symptoms, presence of air-fluid level, type of hernia and type of surgery, presence of mesh, anesthesia type, resection type, perioperative morbidity, and local complications were reported as numbers and percentages in parentheses. Normally distributed data were evaluated using the Shapiro-Wilk test. Statistical differences between age, time to admission, CRP, operative time, and length of stay were evaluated with the Mann-Whitney-U test. Ratio comparisons and correlation analyses of categorical variables according to study groups were performed using the Chi-square and Fisher's exact tests. The level of statistical significance was set at $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics for Windows software (version 26; IBM Corp., Armonk, N.Y., USA).

RESULTS

Of the 63 patients included in the study, 50 were male (79.4%), with a mean age of 68.21±17.89 years. The median age was 74 years, the youngest patient was 23 years old, and the oldest patient was 94 years old.

There were 32 patients (50.8%) who had no disease in the entire group, 23 (36.5%) of the patients were evaluated as ASA2, 27 (42.9%) as ASA3, and 13 (20.6%) as ASA4.

The patients presented to the hospital 2±0.89 days after the onset of symptoms, 39 patients (61.9%) had ileus symptoms at admission, but only 30 (47.6%) had air-fluid levels on EAR. The mean and standard deviations of WBC and CRP of the patients at admission were 9.23±3.40 109/L, 20.17±45.03 mg/dL , respectively.

The majority of the patients (41 patients (65.1%)) were evaluated to have an indirect hernia. While 53 (84.1%) hernias were repaired with the anterior approach, 6 (9.5%) were repaired with McVay repair, and 4 (6.3%) with Mesh Plug technique. The rate of mesh use was calculated as 85.7%, with 54 patients. Omentectomy was added to the repair of 7 patients (11.1%), small bowel resection was performed on 8 (12.7%) patients, primary repair of the colon was performed on 1 (1.6%) patient, and right hemicolectomy was performed on 1 (1.6%). Most of the operations (76.2% (46)) were performed under spinal anesthesia, with a mean operative time of 80.40±25 minutes.

In the perioperative period, acute renal failure (ARF) was observed in 2 patients, postoperative ileus in 1 patient, cerebrovascular disease (CVD) in 1 patient, thrombocytopenia in 1 patient, and COVID-19 positivity in 2 patients. Among all patients, 1 seroma, 1 hematoma, and 1 intra-abdominal hematoma were observed. The patients were discharged after a mean length of hospital stay of 4.05±2.64 days postoperatively (**Table 1**).

Table 1. Whole group

	Whole Group (Median/Percent) (n=63)
Gender	
Male	50 (79.4%)
Female	13 (20.6%)
Age (years)	68.21±17.89 (74)
Number of Comorbidities	
0	32 (50.8%)
1	12 (19.0%)
2	12 (19.0%)
3	7 (11.1%)
ASA	
2	23 (36.5%)
3	27 (42.9%)
4	13 (20.6%)
Ileus Symptoms	39 (61.9%)
Time to Admission (Days)	2±0.89 (2)
Air Fluid Level in EAR	30 (47.6%)
WBC	9.23±3.40 (9.08)
CRP	20.17±45.03 (3.50)
Type of Hernia	
Indirect	41 (65.1%)
Direct	9 (14.3%)
Femoral	13 (20.6%)
Type of Surgery	
Anterior	53 (84.1%)
McVay	6 (9.5%)
Plug Mesh	4 (6.3%)
Presence of Mesh	54 (85.7%)
Type of Anesthesia	
Spinal	48 (76.2%)
General	15 (23.8%)
Type of Resection	
Omentectomy	7 (11.1%)
Small Bowel Resection	8 (12.7%)
Primary Repair of Colon	1 (1.6%)
Right Hemicolectomy	1 (1.6%)
Postoperative Complications	
ARF	2 (3.2%)
Ileus	1 (1.6%)
CVD	1 (1.6%)
Thrombocytopenia	1 (1.6%)
COVID-19	2 (3.2%)
Local Complications	
Intraabdominal Hematoma	1 (1.6%)
Hematoma	1 (1.6%)
Seroma	1 (1.6%)
Length of Hospital Stay (days)	4.05±2.64 (3)

The patients were divided into two groups as BP and DP. The comparison of the distribution of gender, age, number of comorbidities, and ASA scores in the two groups showed no statistically significant difference (**Table 1**).

The mean time to admission was 1.77±0.64 days in the BP group and 2.29±1.08 days in the DP group, with no significant difference between the two periods (p=0.066). Ileus symptoms were present in 60% (21) of the patients presented BP and in 64.3% (18) of the patients presented DP, with no statistically significant difference (p=0.798). Air-fluid levels were observed in 19 (54.3%) patients in the pre-COVID-19 period and in 11 (39.9%) patients in the post-COVID-19 period, but there was no significant difference (p=0.312).

The comparison of the mean WBC and CRP values of the patients in both groups showed no statistically significant difference (**Table 2**). Moreover, no statistically significant difference was observed between the two groups in terms of distributions of hernia types, hernia repair types, mesh use, and additional resection requirement (**Table 2**).

The comparison of the anesthesia type in the BP and DP groups showed that the rate of preferring spinal anesthesia was 71.4% in BP, whereas this rate increased to 82.1% in the post-COVID-19 period, though no statistically significant difference was found (p=0.383). The mean operative times were 80.00±28.64 minutes (75) and 80.89±20.04 (75) minutes in BP and DP, respectively, with no significant difference (p=0.487).The distribution of postoperative and local complications between the two periods was not statistically significant (p=0.189, p=0.168, respectively).The mean length of stay was 3.74±2.79 (3) days in the BP group and 4.43±2.45 (4) days in the DP group, with no statistically significant difference (p=0.068)(**Table 2**).

The difference between the patients who underwent organ resection by admission time was not statistically significant (**Table 3**).It was observed that the number of patients who required small bowel resection were especially high on the 4th day. In DP, small bowel resection was performed on 4 (66.7%) patients and omentectomy was performed in 2 (33.3%) patients. In BP, only one right hemicolectomy was performed. The comparison of the patients with an admission time of 4th day revealed a statistically significant difference (p=0.03)(**Table 4**).

Table 2. Comparison of patients before and after COVID-19

	Whole Group (Median/Percent) (n=63)		
	BP-group 1 (n=35)	**DP- group 2 (n=28)**	**P-value**
Gender			
Male	30 (85.7%)	20 (71.4%)	p=0.215
Female	5 (14.3%)	8 (28.6%)	
Age (years)	66.83±19.89 (74)	69.93±15.20 (72.5)	p=0.729
Number of Comorbidities			
0	17 (48.6%)	15 (53.6%)	p=0.760
1	7 (20.0%)	5 (17.9%)	
2	8 (22.9%)	4 (14.3%)	
3	3 (8.6%)	4 (14.3%)	
ASA Score			
2	14 (40%)	9 (32.1%)	p=0.807
3	14 (40%)	13 (46.4%)	
4	7 (20%)	6 (21.4%)	
Ileus Symptoms	21 (60%)	18 (64.3%)	p=0.798
Time to Admission (Days)	1.77±0.64 (2)	2.29±1.08 (2)	p=0.066
Air Fluid Level in EAR	19 (54.3%)	11 (39.9%)	p=0.312
WBC (109/L)	8.87±2.94 (9.09)	9.68±3.91 (9.01)	p=0.352
CRP (mg/dL)	19.81±43.75 (3.13)	20.63±47.37 (4.30)	p=0.352
Type of Hernia			
Indirect	24 (68.6%)	17 (60.7%)	p=0.736
Direct	5 (14.3%)	9 (14.3%)	
Femoral	6 (17.1%)	13 (20.6%)	
Type of Surgery			
Anterior	30 (85.7%)	23 (82.1%)	p=0.928
McVay	3 (8.6%)	6 (9.5%)	
Plug Mesh	2 (5.7%)	4 (6.3%)	
Presence of Mesh	32 (91.4%)	22 (78.6%)	p=0.170
Type of Anesthesia			
Spinal	25 (71.4%)	23 (82.1%)	p=0.383
General	10 (28.6%)	5 (17.9%)	
Type of Resection			
Omentectomy	3 (8.6%)	4 (14.3%)	p=0.704
Small Bowel Resection	4 (11.4%)	4 (14.3%)	
Primary Repair of Colon	1 (2.9%)	0 (0%)	
Right Hemicolectomy	1 (2.9%)	0 (0%)	
Operative Time (min)	80.00±28.64 (75)	80.89±20.04 (75)	p=0.487
Perioperative Morbidity			
ARF	2 (5.7%)	0 (0%)	p = 0.189
Ileus	0 (0%)	1 (3.6%)	
CVD	0 (0%)	1 (3.6%)	
Thrombocytopenia	1 (2.9%)	0 (0%)	
COVID-19	0 (0%)	2 (7.1%)	
Local Complications			
Intraabdominal Hematoma	0 (0%)	1 (3.6%)	p=0.168
Hematoma		1 (3.6%)	
Seroma		1 (3.6%)	
Length of Hospital Stay (days)	3.74±2.79 (3)	4.43±2.45 (4)	p=0.068

Table 3. Comparison of Resection Rates by Days

Admission Day	Resection	Whole Group (n=63)		Statistical Significance
		Pre-COVID-19 (n=35)	Time of COVID-19 (n=28)	
1	No	10 (90.9%)	7 (100%)	p=1
	Yes	1 (9.1%)	0 (0%)	
2	No	16 (72.7%)	11 (91.7%)	p=0.378
	Yes	6 (27.3%)	1 (8.3%)	
3	No	0 (0%)	2 (66.7%)	p=1
	Yes	1 (100%)	1 (33.3%)	
4	No	0 (0%)	0 (0%)	*
	Yes	1 (100%)	6 (100%)	

Table 4. Comparison of Resection Types on Day 4

Admission Day	Type of Resection	Whole Group (n=7)		Statistical Significance
		Pre-COVID-19 (n=1)	Time of COVID-19 (n=6)	
4	Omentectomy	0 (0%)	2 (33.3%)	(p=0.03)
	Small Bowel Resection	0 (0%)	4 (66.7%)	
	Right Hemicolectomy	1 (100%)	0 (0%)	

DISCUSSION

Patients presenting with acute inguinal hernia (IH) remain a common surgical emergency. The popular "watch and wait" policy for IH increases the immediate presentation of such hernias. The management of such patients is associated with significant morbidity, and indeed mortality (12). It is known that especially age > 65 years, prolonged symptom duration, delay in admission, diagnosis and surgery, time from admission to surgery, strangulation more than 24 hours, symptom duration of 3 days or longer, femoral hernia, female gender, ASA class III and IV, and ileus increase mortality and morbidity in patients (11). The COVID-19 pandemic has taken the whole world under its influence in a short time, and unexpected and unprecedented radical changes in health practices have affected all countries. During the COVID-19 pandemic, surgical units gave priority to emergency procedures and oncological cases and postponed elective cases (6). Emergency IH is considered one of the most common emergency surgeries performed in any surgical department because of the high risk of morbidity and mortality associated with delayed repair. On the other hand, elective hernia repair for uncomplicated hernias has been postponed at our institution and in many countries of the world to reduce the risk of viral transmission to operating room personnel and other patients. Therefore, we anticipated an increase in the number of hernia-related complications following the cancellation of elective procedures. However, in DP, the number of emergency applications has decreased in general and a significant decrease has been observed in emergency IHs. However, a study based on German data reported that the decrease in elective inguinal hernia repairs was almost 70% in the weeks when the pandemic reached its peak, however, contrary to expectations, the rate of emergency inguinal hernia surgery did not increase (13). In this study, the number of patients admitted to our institution with the diagnosis of emergency IH in DP and BP was not statistically significant between both groups. While the number of applications to hospital emergencies decreased all over the world, it is seen that the number of applications for inguinal hernia emergencies was not affected in the Germany study. While our study was compatible with the German study, it was not compatible with other studies.

During the pandemic period, patients were concerned about the risk of COVID-19 infection, and the rate of admissions to hospital emergency departments decreased (14,15). Therefore, the admission time and the duration of strangulation were expected to increase emergency IHs. However, in general, this study found no statistically significant difference in terms of admission time to the emergency departments s of hospitals in DP and BP. Nevertheless, when the admission times were evaluated separately, there was a significant difference between the two groups in terms of patients who had small bowel resection on the 4th day (p=0.03).We think that this result is probably caused by late admission to the hospital due to the risk of COVID-19 infection .

In this study, the number of patients admitted to our institution with the diagnosis of emergency IH in DP and BP was not statistically significant between both groups. In DP, the number of emergency admissions has generally decreased in the world, and there has been a significant decrease in emergency IHs (14). There was no significant difference between the groups in terms of age, hernia type, admission time, and ileus presentation, which affect the mortality of emergency IHs. There was no statistically significant difference between the two groups in terms of the length of hospital stay, local complications, requirement for organ resection, ASA score, selection of anesthesia type, and operative time.

As it is known, SARS-CoV-2 is a virus transmitted by aerosol droplets. Although it has been isolated from many body fluids such as feces, urine and blood, no definite evidence has yet been revealed in terms of direct transmission from the peritoneal cavity. However, it is a fact that has been revealed a long time ago that viruses of similar nature can be transmitted through surgical smoke (16,17).In the guideline published by the European Hernia Society for hernia surgery in adult patients during the COVID-19 pandemic, it is recommended to decide on the use of laparoscopy in emergency cases by considering the balance between risks and benefits.In addition, in the guide published by China in February, the concern that laparoscopy in COVID (+) patients may cause additional lung damage to the existing viral damage through increased intra-abdominal pressure was brought to the agenda. Although this idea remained more of a theoretical hypothesis, it has become a new topic of discussion (18). In this study, direct open surgery was performed in all patients with DP and spinal anesthesia was performed. An important factor in this choice was the concern that pneumoperitoneum desufflation created during laparoscopic surgery might increase viral aerosolization. Moreover, the fact that laparoscopy made general anesthesia inevitable came to the fore as an important factor.In this study, there was no significant difference in terms of anesthesia choice in PE and DP, and the patients were operated under spinal anesthesia in general. Since laparoscopy was used less in both periods, spinal surgery was preferred, especially in DP, to minimize the risk of transmission during intubation.

All patients waiting for elective surgery are important and should be assured. Most of the time, delay in elective

hernia repair may not cause an increase in the risk of complications; nevertheless, patients should be informed about possible complications in case of delay, and the conditions that require admission to the emergency department should be explained thoroughly to ensure that early diagnosis and treatment are not delayed. The role of not delaying emergency services during the pandemic, the correct management of elective surgical procedures in the pre-pandemic period, and rapid diagnosis and use of treatment protocols by surgeons during the pandemic period are important (18,19).

Although there were curfew restrictions during the pandemic period in our country, we think that treatments have been effectively administered without delays and disruptions in the case of surgical emergencies. Pandemic-related morbidity and mortality rates of hernia surgery can be reduced by taking patient-hospital oriented preventive measures to reduce viral transmission. Early diagnosis and treatment undertake a key role.

CONCLUSION

During the pandemic period, elective surgical procedures should be managed accurately, patients should be informed about possible complications in case of delay, and the conditions that require admission to the emergency department should be explained thoroughly to patients. We observed that morbidity increased as the admission time was delayed. Anticipating that the fear of COVID-19 infection will hold back the emergency response during the pandemic period, patients should be informed not to delay early diagnosis and treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Hitit University Non-interventional Researchs Ethics Committee (Date: 30.04.2021, Decision No: 2021-66).

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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Original Article

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Hypofractionated radiotherapy results of patients with malign glioma aged 60 and over

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ABSTRACT

Aim: The aim of this study is to examine the treatment results of 25 malign glioma patients, aged >60 who underwent hypofractionated radiotherapy, respectively.

Material and Method: Total excision was applied to 14 of the patients, subtotal excision was applied to 5 and biopsy was applied only to 3. Three patients were evaluated as inoperable. Pathological diagnosis is compatible with glioblastoma in 22 patients, gliosarcoma in 2 patients and anaplastic astrocytoma in 1 patient. Hypofractionated radiotherapy was applied to all patients in the dose range 2.66-3.4 Gy x 10-15 fractions. Ten patients were treated concurrent temozolomide with radiotherapy and then 4-6 cures of adjuvant temozolomide. Only concurrent temozolomide treatment was applied to 3 patients, while only adjuvant temozolomide was applied to 4 patients.

Results: The median age is 72 years. (min 60–max 86) . The rate of patients with comorbid disease is 44%. The median follow-up period of the patients is 5 months (min 1-max 22). 6-months, 1-year, 18-months overall survival were 47%, 20%, 10%, respectively. Median survival in patients aged >70 is 3 months and 8 months in patients <70 (p=0.025). Median survival is 10 months in patients receiving both concurrent and adjuvant temozolomide treatment, and median survival is 3 months (p=0.007) in patients who do not receive it.

Conclusion: Overall survival is statistically better in patients under the age of 70 and patients receiving both concurrent and adjuvant temozolomide therapy with hypofractionated radiotherapy.

Keywords: Malignant glioma, hypofractionated dose, chemotherapy, elderly

INTRODUCTION

High-grade malignant gliomas are 50% of all brain tumors in adults. Glioblastoma multiforme (WHO grade 4) constitutes 75% of high-grade malignant gliomas, and most of these patients are over 60 years of age (1). The prognosis for patients with glioblastoma is generally poor; age, Karnofsky performance status (KPS), type of surgery are important prognostic factors. Anaplastic gliomas (WHO grade 3, anaplastic astrocytoma, anaplastic oligoastrocytoma, anaplastic oligodendroglioma) constitute approximately 25% of high-grade malignant gliomas in adults. In these patients, besides age, surgical method and KPS, molecular genetic factors are also important in prognosis.The standard treatment approach in patients with high-grade malignant glioma is 60 Gy (2 Gy x 30 fractions) adjuvant radiotherapy (RT) after surgical excision and concomitant temozolomide, followed by 6 cycles of adjuvant temozolomide.With standard treatment approaches, overall survival in these patients is 16-17 months (2,3).However, this treatment approach may cause problems due to toxic effects, especially in patients over 60 years of age and/or with poor performance status.

Various hypofractionated radiotherapy and chemotherapy approaches have come to the fore in these patients to reduce treatment-related toxicity and shorten the treatment period (4-13). The use of chemotherapy alone in this patient group has also been investigated as another treatment option. (14,15).

The aim of this study is to evaluate the treatment results in patients with malignant glioma aged 60 years and older who received hypofractionated radiotherapy, respectively.

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MATERIAL AND METHOD

The study was carried out with the permission of University of Health Science Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Researchs Hospital Clinical Research Ethics Committee (Date: 09.06.2021, Decision No: 2021/06-1223). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

Twenty-five patients who underwent hypofractionated radiotherapy between February 2012 and December 2020 with the diagnosis of high-grade malignant glioma in our clinic were included in the study. Twenty-two patients had been operated at baseline. The operation type was gross total excision in 14 patients, subtotal excision in 5 patients, and biopsy only in 3 patients. Three patients were initially considered inoperable. Histopathological examination results were consistent with glioblastoma in 22 patients, gliosarcoma in 2 patients, and anaplastic oligoastrocytoma in 1 patient. The most common hypofractionated dose schedule was 2.66 Gy x 15 fractions (15 patients). (**Table 1**). In addition, 3 Gy x 15 fractions (fx) radiotherapy was applied to 7 patients, 3.4 Gy x 10 fx to 1 patient, 3 Gy x 13 fx to 1 patient, and 3 Gy x 16 fx hypofractionated radiotherapy to 1 patient. Ten patients were treated with temozolomide concomitantly with radiotherapy and then adjuvant 4-6 courses of temozolomide. While 3 patients were treated with only temozolomide concurrently with radiotherapy, only adjuvant temozolomide therapy was applied to 4 patients. Patients were followed up with brain MRI every 3-6 months after radiotherapy.

Table 1. Radiotherapy and chemotherapy schemes applied to patients

	No	%
Radiotherapy		
2.66Gy x 15 fraction	15	60
3Gy x 15 fraction	7	28
3Gy x 13 fraction	1	4
3.4Gy x 10 fraction	1	4
3Gy x 16 fraction	1	4
Chemotherapy		
Concomitant	3	12
Adjuvant	4	16
Concomitant+adjuvant	10	40

Statistical Analysis

SPSS version 22 was used for statistical analysis. Kaplan-Meier method was used in the overall survival analysis of the patients. Patient, tumor, and treatment-related variables were evaluated by univariate analysis. $p<0.05$ was considered statistically significant.

RESULTS

Table 2 shows the general characteristics of the patients. Fourteen of the patients were male and 11 were female. The number of patients with comorbid disease was 11 (44%), the most common comorbid disease was hypertension (45%). Comorbid diseases other than hypertension are diabetes mellitus and cerebrovascular disease. A lower lip epidermoid carcinoma was detected in one patient as second primary cancer. The median age of the patients was 72 (min 60-max 86). The average time between operation and RT is 40 days (min 15-max 75).

Table 2. General characteristics of the patients

	No	%
Gender		
Male	14	56
Female	11	44
Surgery		
Gross total excision	14	56
Subtotal excision	5	20
Biopsy only	3	12
Inoperable	3	12
Comorbid Disease		
Yes	11	44
No	14	56
Tumor Diameter		
≥5 cm	9	
<5 cm	12	

The median follow-up period is 5 months (min 1-max 22). Overall survival at 6 months, 1 year, and 18 months was 47%, 20%, and 10%, respectively (**Figure 1**).

In univariate analysis, the median survival was 3 months in patients aged 70 years and older, and 8 months in patients younger than 70 years (p=0.025) (**Figure 2**). Median survival was 2 months in patients receiving concomitant temozolomide therapy with RT, 1 month in patients receiving only adjuvant temozolomide therapy, and 10 months in patients receiving both concomitant and adjuvant temozolomide therapy (p=0.065). Median survival was 10 months in patients who received both concomitant and adjuvant temozolomide treatment, and 3 months in patients who did not receive it (P=0.007) (**Figure 3**). In addition, a significant difference was found in the Spearman correlation test between the performance status of the patients and the tolerance of concomitant and adjuvant temozolomide therapy(p=0.05). Median survival was 4 months in patients with a tumor size of 5 cm and above, and 5 months in patients with a tumor size of less than 5 cm, and no statistical difference was found (p=0.36). The median survival was 3 months in patients with an operation-radiotherapy interval of 35 days or more, and 5 months in patients with less than 35 days (p=0.98). The prognostic significance of the IDH mutation could not be evaluated because the number of patients who were examined was insufficient.

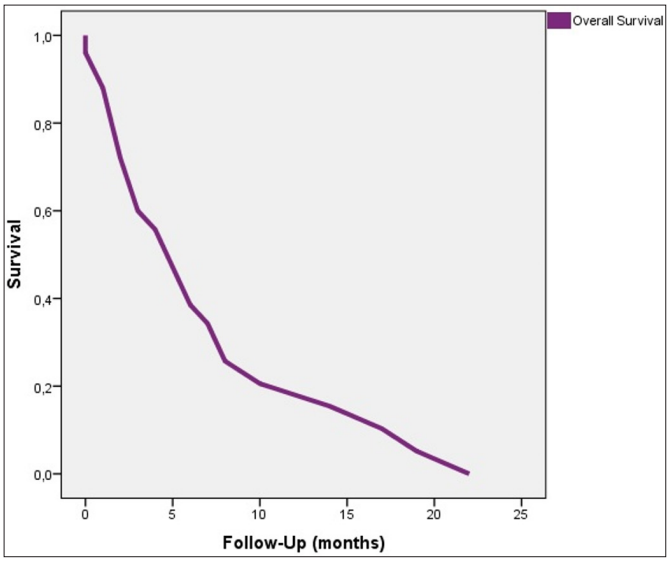

Figure 1. Overall survival of patients

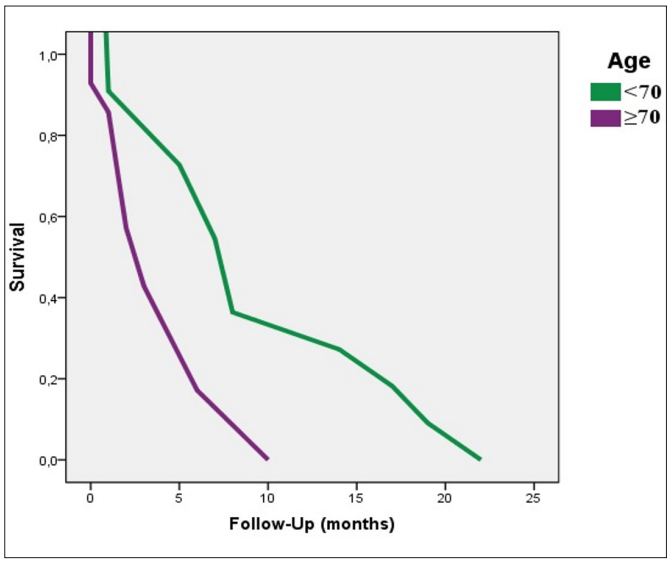

Figure 2. Survival in patients aged 70 years and older versus under 70 years of age

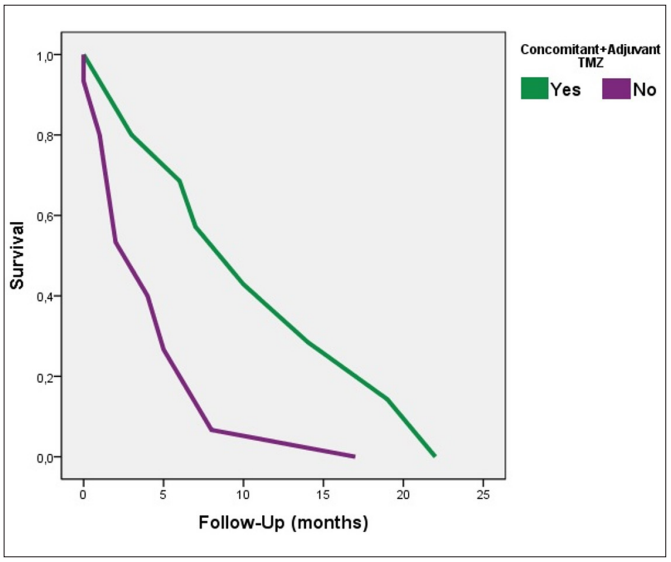

Figure 3. Survival in patients receiving concomitant and adjuvant temozolomide and patients not taking it

DISCUSSION

The standard treatment approach in patients with high-grade malignant glioma is 60 Gy adjuvant radiotherapy and concomitant temozolomide after surgical resection, followed by adjuvant temozolomide therapy. However, shorter-term hypofractionated radiotherapy options have come to the fore due to the difficulties of tolerating and completing standard treatment in patients over 65 years of age and /or with poor performance status.

In Nordic randomized phase 3 study, survival outcomes of 342 patients with glioblastoma who received the standard radiotherapy regimen, temozolomide only, and hypofractionated radiotherapy alone were compared. In this study, it was reported that the survival results were similar compared to standard radiotherapy in the patient groups treated with both temozolomide and hypofractionated radiotherapy. The median survival was shown as 8.3, 7.5 and 6 months, respectively (11).

In the study of Minniti et al. (10), the median overall survival and progression-free survival of patients receiving standard RT (60 Gy) and hypofractionated RT as well as concomitant and adjuvant temozolomide were retrospectively compared. Survival outcomes were similar in both groups (12 and 5.6 months, 12.5 and 6.7 months, respectively). In the study of Kimberly et al. (16), when the survival results of 4498 patients who received standard radiotherapy (93.4%) and hypofractionated radiotherapy (6.6%) were compared, it was reported that the survival results of patients who received standard treatment were better. However, in this study, the fact that chemotherapy and surgical treatment were less applicable due to the higher incidence of comorbid diseases due to the older age of the patients receiving hypofractionated treatment.

In our study, hypofractionated radiotherapy was applied to all patients (2.66-3.4 x10-15 fx) in the dose range, and 10 patients were treated with temozolomide concomitantly with radiotherapy and then adjuvant 4-6 cycles. While 3 patients were treated only with temozolomide concurrently with radiotherapy, only adjuvant temozolomide therapy was applied to 4 patients. The median overall survival of patients is 5 months. However, due to the small number of patients in our study, the inclusion of patients who underwent both biopsy and inoperable in the survival analysis calculation may have caused the analysis to be performed with a heterogeneous group. This should be considered when evaluating the survival analysis results.

In the study of Perry et al. (4), treatment results of 562 patients who received concomitant- adjuvant temozolomide (50%) treatment in addition to hypofractionated radiotherapy (2.66 Gy x 15 fx) and

hypofractionated radiotherapy were compared. It was observed that survival results were better in patients who were also treated with temozolomide (median survival 9.3 months-7.6 months). In our study, 40% of the patients who received hypofractionated radiotherapy were administered concomitant and adjuvant temozolomide, and the median survival in these patients was significantly better than the patients who received only adjuvant or only concomitant temozolomide with RT. In addition to the efficacy of the treatment, this may be associated with the better performance status of the patients who can be treated with both concurrent and adjuvant temozolomide treatment compared to the patients who receive only concurrent or only adjuvant temozolomide treatment. While temozolomide treatment was ongoing, treatment-related thrombocytopenia was detected in 2 of the patients, and pulmonary thromboembolism was observed in 1 patient.

In parallel with the type of surgery performed in patients with high-grade malignant glioma, overall and disease-free survival times are prolonged in patients who underwent total excision compared to patients who underwent subtotal excision and biopsy (17,18). In our study, 14 patients underwent total excision, 5 subtotal excision, and 3 patients only biopsy. Gross total excision was performed in most patients. If there is no comorbid disease that will prevent the operation, standard surgical approaches can be applied in elderly patients aged 60 and over, as in younger patients.

One study compared the treatment outcomes of patients who received combined chemoradiotherapy (89.6%) with those who received only radiotherapy (10.4%) in 1479 patients with high-grade malignant glioma who had only undergone biopsy for different reasons. (13). In this study, it was observed that there was a significant survival advantage in the patient group receiving chemoradiotherapy compared to the group receiving only radiotherapy (median 9.2 months-5.6 months). In our study, the number of patients who only underwent biopsy was 3, and only radiotherapy was applied to 1 patient, simultaneous chemotherapy with radiotherapy was applied to 1 patient, and both adjuvant and concurrent chemotherapy was applied to 1 patient. The average survival of these patients is 6 months.

In our study, 3 patients were initially considered to be inoperable and were treated as high-grade malignant glioma according to clinical and radiological findings. In the treatment of these patients, only hypofractionated radiotherapy was applied to 1 patient, and concurrent and adjuvant chemotherapy with RT was applied to 1 patient. In one patient, RT followed by adjuvant temozolomide treatment was given, and the treatment was terminated after the second cycle of temozolomide due to side effects. The average survival of these 3 patients is 6 months.

In patients with high-grade malignant glioma, performance status is an important prognostic factor in addition to age. In our study, the ECOG performance score was at the median level of 2.

CONCLUSION

Overall survival was statistically better in patients receiving both concomitant and adjuvant chemotherapy with hypofractionated radiotherapy. The better performance status of this group of patients facilitated the full administration of both radiotherapy and chemotherapy protocols. In patients under 70 years of age, overall survival is statistically better than the older group.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Science Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Clinical Researchs Ethics Committee (Date: 09.06.2021, Decision No: 2021/06-1223).

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

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Pain catastrophizing in migraine patients and associated factors

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ABSTRACT

Aim: We carried out this study to examine the level of pain catastrophizing (PC) among migraine patients and factors possibly affecting PC.

Material and Method: The sample of this study comprised 120 patients who applied to the neurology clinic of a state hospital and were diagnosed with migraine by the International Headache Society's (IHS) criteria between April 2017 – March 2019. Then, we collected the data from those meeting the inclusion criteria using a socio-demographic information form, the Pain Catastrophizing Scale (PCS), and the Barrat Impulsiveness Scale Short Form (BIS-11-SF). Data analysis was performed on the SPSS 25.0 statistics software. To explore the relationships between the variables, we ran one-way ANOVA and multiple regression analyses and calculated Pearson's correlation coefficients.

Results: We divided the participants into three groups: Group 1 included 30 patients who got full benefit from the treatment during a migraine attack (25%); Group 2 included 25 patients who were unable to obtain any benefit from the treatment at all (20.8%); Group 3 included 65 patients with partial benefit from the treatment (54.17%). The PCS scores were higher in Group 1 and Group 3, while Group 2 had significantly higher PCS total and Rumination scores than Group 1. The number of attacks and impulsiveness levels of the patients explained 18.6% of the variance in PC.

Conclusion: The cognitive capacity of individuals is essential in identifying the prognosis of migraine. Catastrophizing pain is likely to lower treatment response in migraine patients. Besides, the increased number of attacks and impulsiveness levels of patients influence their PC levels. Finally, migraine is a disorder with a psychiatric aspect; therefore, performing appropriate mental evaluations and offering necessary psychiatric support may enhance the chance of success in migraine treatment.

Keywords: Migraine, pain catastrophizing, clinical aspects, impulsiveness

INTRODUCTION

The International Headache Society (IHS) defines migraine as a type of primary headache. While migraine prevalence varies by society, it is estimated between 12.1-16.4% worldwide and more common among females (1,2). In migraine, a headache occurs in attacks, settles on one side, has a throbbing effect, and can continue for up to 72 hours. The pain can be accompanied by some neurologic, autonomic, and gastrointestinal system symptoms (3). The severity of clinical symptoms generally follows a mild to severe course. Headache and other accompanying symptoms adversely impact quality of life among individuals. According to the Global Disease Burden study, migraine ranks the seventh among all diseases that cause disability and the first among neurological disorders (4,5).

The primary symptom affecting the functionality of migraine patients is a headache. The perception of pain depends on bio-psycho-social factors, which determine the prognosis of the disease (6). In parallel with the pain, the concept of pain catastrophizing (PC), which is defined as having negative cognition and emotions about pain, is indicated as a risk in chronicization of migraine symptoms (7). People catastrophizing pain are trapped in constant pain and thoughts about pain. They cannot end their exaggerated negative thinking about pain and believe they have nothing to do to perish the pain (8). Previously, the presence of PC in migraine patients was found to be correlated with the increased number of and prolonged attacks, reduced treatment response, increased number of medical consultations, and impaired quality of life (9,10).

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According to Goli et al. (11) PC contributes to developing depressive and anxious symptoms in migraine patients. Many studies suggested that PC adversely affects clinic outcomes in migraine; however, few attempted to examine how or why PC occurs.

The research foci are often on the impacts of the psychiatric aspects of migraine and accompanying mental complaints on its prognosis. Substantial evidence showed that the most common accompanying psychiatric disorders to migraine are depression and anxiety disorder (12,13). Moreover, some studies demonstrated that migraine patients have cognitive impairments (14) and more frequently experience negative affections such as embarrassment, anger, and guilt (15). The research exploring the personality traits of migraine patients reports that neurotic and impulsive traits are more common and adversely affect the prognosis of the disorder (16,17). On the other hand, impulsiveness is a thought or behavior pattern that one may externalize without anticipating its outcomes, often leading them into an undesirable situation. It is not solely an indicator of pathology but is shown as a reason for developing many psychopathologies (18). Accordingly, the relevant literature previously suggested that impulsiveness could be a variable influencing PC. Yet, no study has attempted to investigate the relationship between PC in migraine patients and impulsiveness

Ultimately, we carried out the study with migraine patients having obtained benefits from relevant treatment at varying degrees. The main purpose of the study was to examine PC and impulsiveness levels in migraine patients. We also sought answers to how clinical aspects of migraine and impulsiveness affect PC. As it causes chronicization of migraine, understanding the underlying reasons for PC occurrence and development and, thus, creating an appropriate treatment plan will likely increase treatment success.

MATERIAL AND METHOD

The sample of the present research, which was a cross-sectional and descriptive study, comprised the patients who applied to the neurology clinic of a state hospital and were diagnosed with migraine based on the IHS criteria between April 2017-March 2019. KTO Karatay University, Non-Pharmaceutical and Non-Medical Device Studies Ethics Committee granted the relevant approval to our study (Date: 30.03.2017, Decision No: 2017/002). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. We carried out the study with only voluntary individuals diagnosed with migraine and aged between 18-65. Yet, we had to exclude those who had a psychiatric history, had a different, pain-related physical disorder, and had a physical or mental disorder adversely influencing filling out a questionnaire form. Moreover, we did not consider the data of those leaving their forms missing. Eventually, we recruited a total of 120 patients with ongoing polyclinic follow-ups. All of the patients were on prophylactic migraine treatment and received additional treatments in times of attacks. We grouped the participants into three groups depending on their responses to the therapies: Group 1 included those whose complaints were entirely relieved in an attack period, while Group 2 was composed of those without recovering at all and with continuing persistent pains. Finally, Group 3 comprised the patients whose complaints were partially relieved. We informed the participants about the study, obtained their consent to participate in the study, and asked them to fill out a questionnaire booklet covering a socio-demographic information form, the Pain Catastrophizing Scale (PCS), and the Barrat Impulsiveness Scale Short Form (BIS-11-SF).

Socio-demographic Information Form: We prepared the form to include open-ended questions to the participants about their age, educational attainment, occupation, marital status, clinical aspects of migraine attacks, general health condition, and family history.

Pain Catastrophizing Scale (PCS): Sullivan et al. (19) developed the scale to identify the catastrophizing levels of individuals with pain symptoms. The self-report instrument is a Likert-type 13-item scale with three subscales: Rumination, Magnification, and Helplessness. Ugurlu et al. (20) carried out its Turkish validity and reliability study. In their study, the reliability of the Turkish version of the scale ranged between 0.73-0.93. Also, the researchers calculated its Cronbach's alpha value to be 0.95 and the internal consistency coefficient to be 0.83. Analyses pertinent to construct validity and internal consistency of the scale suggested that the scale is a valid and reliable data collection tool in the Turkish context.

Barrat Impulsiveness Scale Short Form (BIS-11- KF): The scale developed to measure individuals' impulsiveness levels was revised by Patton et al. (21). The Likert-type instrument is based on self-report and has 30 items within three subscales: Non-planning Impulsiveness, Motor Impulsiveness, and Attentional Impulsiveness. Tamam et al. (22) adapted the scale into Turkish and found Cronbach's alpha values to range between 0.64-0.82 and high internal consistency coefficient. Analyses pertinent to construct validity and internal consistency of the scale suggested that the scale is a valid and reliable data collection tool in the Turkish context.

Statistical Analysis

We analyzed the data using Statistical Package for Social Sciences (SPSS) for Windows 25.0 (SPSS Inc., Chicago, II, USA). In the study, we utilized parametric analyses since Skewness and Kurtosis values revealed the data to show a

normal distribution. Then, we ran a one-way ANOVA to compare the scores of the groups on the scales. Next, we calculated Pearson's correlation coefficients to uncover the relationship between the variables. Finally, we performed a multiple regression analysis to determine the predictive value of pain catastrophizing. Multicollinearity assumption was detected to be met considering Durbin Watson, Tolerance, and VIF values. In all statistical analyses, we accepted $p<0.05$ to be statistically significant.

RESULTS

We carried out the study with a total of eligible 120 patients diagnosed with migraine and divided them into three groups: Group 1 included 30 patients who got full benefit from the treatment during a migraine attack (25%); Group 2 included 25 patients who were unable to obtain any benefit from the treatment at all (20.8%); Group 3 included 65 patients with partial benefit from the treatment (54.17%). The results revealed that sex (p=.597), educational attainment (p=.293), age (p=.392), accompanying aura (p=.673), number of attacks (p=.225), disease duration (p=.673) did not have an impact on treatment response. In this study, the only variable causing a difference in treatment response was marital status; the results of the Tukey test suggested that married and single individuals did not differ by treatment response, while divorced patients had significantly worse responses to the treatment (p=.028). At the same time, we could not find any significant differences between married and divorced patients (p=.233). Evaluating the patients in 3 groups based on their treatment responses allowed us to explore possible variables that might influence PC and impulsiveness. The descriptive characteristics of the patients are outlined in **Table 1**.

On the other hand, we determined that the patients in Group 2 catastrophized pain more. In this group, the mean PCS total score was 28.44±13.79, while the participants obtained a mean score of 12.72±6.15 on the Rumination subscale, 6.36±3.32 on the Magnification subscale, and 9.36±5.41 on the Rumination subscale. The patients in Group 1 had the PCS total and subscale scores, while their PCS total and Rumination scores were significantly lower than those of Group 2 (p=.030 and p=.003, respectively). We could not reach significant differences in the scores of Group 3. When it comes to the BIS-11-SF, Group 2 had the highest scores, while other groups did not show any significant difference in their scores. Yet, there was a significant difference between Group 2 and Group 1 by Non-planning scores (Group 2: 27.64±9.22; Group 1: 22.90±5.89; p=.035). **Table 2** present the scores of the groups on the scales.

We then analyzed the variables that might be associated with PC and impulsiveness. Accordingly, we found out that the mean BIS-11-SF total score (number of attacks (r=.351) and impulsiveness level (r=.256)) was positively correlated with PC. Nevertheless, there was no significant relationship between the number of attacks and disease duration and impulsiveness. **Table 3** presents the correlation table.

Table 1. Demographic data of patients and clinical features of migraine

	Group 1 (n=30)	Group 2 (n=25)	Group 3 (n=65)
Age	39±8.31	32.24±11.42	36.06 ±11.35
Sex			
Female	28	20	57
Male	2	5	8
Marital Status			
Single	5	9	18
Married	25	16	47
Education			
Uneducated	1	0	0
Primary education	14	19	32
High school	9	9	20
University	6	6	13
Aura			
Yes	2	2	6
No	28	23	59
Disease duration (year)	12.33±10.19	7.96±8.25	11.12±8.36
Number of attacks (in a year)	4.67±3.75	8.64±3.86	6.57±4.09

Table 2. PCS and BIS–11–SF scale scores of the patients

	Group 1 (1) (n= 30)	Group 2 (2) (n= 25)	Group 3 (3) (n= 65)	p	F
PCS					
Total	19.03±14.09	28.44±13.79	23.2±13.06	p (1-2); .030*	3.32
Helplessness	9.27±7.04	12.72±6.15	10.69±6.16	.140	1.99
Magnification	4.70±3.43	6.36±3.32	4.74±3.57	.118	2.18
Rumination	5.23±4.38	9.36±5.41	7.51±4.32	p (1-2); .003*	5.65
BIS – 11 – SF					
Total	49.70±12.31	57.84±14.85	53.83±11.92	.063	2.83
Attentional imp.	10.50±3.32	10.48±2.37	11.34±3.34	.345	1.07
Motor imp.	15.97±4.84	18.64±5.1	17.29±4.1	.095	2.41
Non-planning imp.	22.90±5.89	27.64±9.22	24.60±6.4	p (1-2); .035*	3.23

p<.05; * Tukey HSD, BIS-11-SF; Barrat Impulsiveness Scale Short Form, imp.; impulsiveness, PCS; Pain Catastrophizing Scale.

Tablo 3. The relationship between the number of attacks and the duration of the disease with pain catastrophizing and impulsivity

	1	2	3	4
PCS total (1)	1			
BIS-11-SF total (2)	.351**	1		
Number of attacks (3)	.256**	.097	1	
Disease duration (4)	.170	-.009	.070	1

*p< .05, **p< .01, BIS-11-SF; Barrat Impulsiveness Scale Short Form, PCS; Pain Catastrophizing Scale.

Finally, we performed a multiple regression analysis to explore the predictive values of impulsiveness, demographic variables, and clinical aspects on PC. In this context, we first sought to satisfy the multicollinearity assumption of the analysis. Accordingly, we computed the Durbin-Watson value to be 2.104 and the corrected R2 value to be 0.186, which indicated no multicollinearity in the model. The results of the multiple regression analysis uncovered that sex, educational attainment, aura, number of attacks, disease duration, and impulsiveness were the factors predicting PC. However, we discovered that sex, educational attainment, aura, and disease duration did not have a significant contribution to the variance, while the number of attacks and impulsiveness explained 18.6% of the variance in PC. In other words, a change of 1 unit in the number of attacks caused a change of 01.9 points in the PCS total score, while a change of 1 unit in the BIS-11-SF total score caused a change of 0.37 points on the PCS. The regression model is demonstrated in **Table 4**.

Table 4. Multiple regression: factors affecting catastrophizing pain

	Model 1		
	B	S.E.	β
Sex	2.272	3.649	.055
Education	-2.132	1.099	-.186
BIS – 11 – SF	.398	.093	.373**
Aura	1.046	4.377	.021
Number of attacks	.647	.295	.189**
Disease duration	.184	.136	.119
R		.476	
R2		.227	
Adj.R2		.186	
R2 change		.227	

*p<.05, **p<.01, BIS-11-SF; Barrat Impulsiveness Scale Short Form, Adj.R2; Adjusted R2

DISCUSSION

We concluded that the level of treatment response changed with varying modes of perception of pain in migraine patients. Accordingly, the patients who did not respond to treatment at all catastrophized their pains more and had more negative ruminative thoughts about pain. Besides, two important factors leading the patients to catastrophize their pains were found to be impulsiveness level and the number of attacks.

Despite available research, there are still areas in the dark about the etiology and treatment of migraine. Differentiations in treatment types and responses are believed to be linked with such uncertainty. Studies on the factors causing migraine to become chronic yielded different results, which might be attributed to the study location, patients' characteristics, and methodological issues. Wiendels et al. (23) demonstrated that sex does not influence treatment response, while Scher et al. (24) reported that female sex is a risk factor for the chronicization of headache. In addition, Seferoğlu (25) demonstrated that age and sex do not alter the clinic course of migraine, yet individuals with lower educational attainment experience elevated chronicization. Many studies determined that being married enhances adherence to therapy and treatment success (26,27). In this study, among the demographic variables, we found only being divorced to be a factor to deteriorate treatment. Divorce is a process that requires adaptation to a changing situation, which may be challenging to maintain mental health. Therefore, being divorced may be a variable that can trigger headaches and make it harder to respond to the treatment. From this perspective, the results of our study seem to be in parallel to the literature.

Pain catastrophizing is a cognitive error that disrupts patients' pain perception and clinic progress in pain (28). According to Shim et al. (29) those with poor ability to recognize and express their emotions tend to catastrophize their headache. Bond et al. (9) asserted that an increased number of and prolonged attacks and higher pain sensitivity increase PC level in migraine. Gil-Martinez et al. (30) determined that PC intensifies pain in chronic migraine patients and that individuals with disrupted functionality due to pain are likely to catastrophize it more. Alvarez-Astorga et al. (31) reported that the presence of PC increases the severity of a headache and facilitates the occurrence of mental complaints such as depression and anxiety. Ultimately, previous research suggests that migraine and PC have a reciprocal relationship where PC is a factor that disrupts the clinic course of migraine. In parallel to the literature, we found that PC levels were higher in the patient group with worse treatment response, and this group significantly differed from the group fully responding to the treatment. In disorders characterized by persistent pain, like migraine, individuals can have a negative cognition about pain, which may worsen the pain, ruin treatment response, and, thus, lead the patients to catastrophize pain more.

The relationship between migraine and PC is highly scrutinized in the literature. Yet, studies are limited on the reasons for PC occurrence. Some studies claim

that the primary issue in catastrophizing pain is about attention. The inability to distract the focus away from pain may aggregate pain perception and sensitivity to pain (32). According to Borsook et al. (33) the structural and functional changes led by migraine may cause PC. In brain imaging procedures in migraine patients, individuals catastrophizing pain have diminished gray substance under the somatosensory cortex, medial and dorsolateral prefrontal cortex, medial temporal cortex, and frontal cortex (34,35). Mathur et al. (36) demonstrated that insula activity is often distinctively impaired in migraine patients with PC. As imaging procedures are not longitudinal, the question of whether PC occurs because of the structural impairments in the brain or structural-functional impairments remains unanswered. A noteworthy finding of our study was that two variables predicted PC occurrence at the rate of 18.6%: the number of attacks and impulsiveness. The literature hosts some studies on the number of attacks and PC levels among patients (9,37); nevertheless, these studies did not address the impact of the number of attacks on PC. An increased number of attacks may cause individuals to be subject to more pain, foster negative opinions about pain, and adversely affect quality of life. Increasing unpleasant thoughts about pain can also disrupt the pain perception and lead to pain catastrophizing. Again, we did not encounter any study exploring the impacts of impulsiveness on PC in migraine patients

Studies that examined impulsiveness in migraine patients yielded varying results. While some reported migraine patients are more impulsive (38), others suggested impulsiveness along with pain is less common (3). According to Sanchez et al. (40) what determines impulsiveness in migraine patients is the pain becoming chronic. Prolonged pain deteriorates quality of life and represses personality traits, such as novelty seeking, pleasure, and impulsiveness in people. In this study, the impulsiveness scores of the patients did not differ significantly, but only Group 2 had significantly higher scores on the Non-planning Impulsiveness subscale. The regression analysis identified impulsiveness as a factor affecting PC, which may be explained by the desire to get rid of the pain in the shortest time possible and the inability to tolerate migraine pain.

CONCLUSION

Pain management is essential in identifying treatment responses in migraine patients. Migraine patients showing non-response to the treatment during pain attacks are likely to catastrophize their pain more. Besides, the number of attacks and impulsiveness affect PC in migraine patients. Identifying factors that might alter pain perception and lead to PC may be critical for managing migraine. Therefore, psychiatric examinations in addition to neurological treatments, as well as additional pharmacologic or psychotherapeutic treatments, will facilitate pain management and improve quality of life among patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out following the principles of the Helsinki Declaration and with the approval of the KTO Karatay University Non-Pharmaceutical and Non-Medical Device Studies Ethics Committee (Date: 30.03.2017, Decision No: 2017/002).

Informed Consent: Verbal and written informed consent was obtained from all participated in this study.

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Original Article

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The effect of body mass index on osteoporosis and fracture risk in patients with type 2 diabetes mellitus

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ABSTRACT

Aim: Type 2 diabetes mellitus (DM), osteoporosis and obesity are increasingly common diseases due to the increase in the elderly population and the change in eating habits. However, the relationship between diabetes, obesity and osteoporosis has not been fully clarified in studies in the literature. In our study, it was investigated whether the combination of diabetes and obesity caused osteoporosis and increased fracture risk.

Material and Method: Our study was carried out with a total of 95 patients between the ages of 40 and 80, including 24 males and 71 females, who were tested due to the preliminary diagnosis of osteoporosis and diagnosed with type 2 DM in the physical medicine and rehabilitation outpatient clinic. The demographic characteristics, body mass index (BMI), bone mineral density (BMD) measurements, X-ray imaging, and fracture risk assessment scale (FRAX) calculations of patients were performed. Groups were compared in terms of BMD and FRAX according to the BMI classification.

Results: BMD and FRAX hip values were higher in the obese group according to the BMI classification compared to the pre-obese/normal group ($p<0.05$). In the obese group, the number of patients with a history of low-energy fractures and with identified fractures on X-ray scanning was higher compared to the pre-obese/normal group; however, there was no significant relationship between the groups ($p>0.05$).

Conclusion: As a result of our study, it was observed that type 2 DM and obesity were associated with increased fracture risk despite high BMD values.

Keywords: Bone mineral density, osteoporosis, obesity, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (DM) is an increasingly widespread metabolic disease that is significantly associated with morbidity and mortality (1). Type 2 DM is characterized by hyperglycemia caused by defects in insulin secretion, efficacy, or both (2). As a result of chronic hyperglycemia and insufficient glycemic control, diabetic complications, including macrovascular (acute coronary syndrome, stroke, claudication intermittence) and microvascular (retinopathy, neuropathy, nephropathy) diseases occur (3).

In addition to macro or microvascular long-term complications, patients with type 2 DM may develop various skeletal system disorders, including osteoporosis and fractures (4). Osteoporosis is the most important metabolic bone disease that develops in type 2 DM patients (4, 5). Patients with type 2 DM show an increased risk of fractures due to impaired bone quality (6). Obesity is the most important risk factor for type 2 DM (7). Obesity, which is common in type 2 DM, has been found to be associated with high bone mineral density (BMD) (8). However, in some studies, BMD was observed to be low (9) or normal in patients with type 2 DM despite high body weight (10, 11).

Therefore, there is no clear conclusion about the relationship between type 2 DM patients and BMI and BMD in the literature. In this study, it is aimed to investigate whether BMI has a positive or negative effect on vertebral-hip bone mineral density and fragility of bone in patients with type 2 DM and to draw attention to the mutual potential interactions of these common diseases.

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MATERIAL AND METHOD

Using the hospital automation system, patient data to be used in our study were obtained within the scope of patient information security. In this retrospective study, a total of 95 patients between the ages of 40 and 80 who were diagnosed with type 2 DM and whose BMD values were measured, who applied to the Physical Medicine and Rehabilitation outpatient clinic of Amasya University Hospital between October 2019 and February 2020, were included. The demographic characteristics of the patients are shown in **Table 1**. Patients who have previously received or were receiving osteoporosis treatment were not included in our study. Ethics committee approval for the study was received from the Amasya University Non-interventional Clinical Researchs Ethics Committee (Date: 03.09.2020, Decision No: 9/104). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Table 1. Demographic features type 2 DM patients

	Total 95	
Age	60.589	±8.20
Gender		
Male	24	25.3%
Famale	71	74.7%
BMI		
BMI <30 (Pre-obese/Normal)	26	27.4%
BMI ≥ 30 (Obese)	69	72.6%

Blood tests (Hemogram, biochemistry, electrolyte levels, vitamin levels) and thoracolumbar spine X-rays are routinely requested from patients who apply to our outpatient clinic for osteoporosis screening. Weight and height are measured and BMI is calculated with the TANITA MC-180MATM (Tanita Corporation, Japan) device. L1-L4 posteroanterior lumbar spine, femur total and femoral neck BMD measurements are then performed on the dual-energy Hologic DXA device. BMD values are calculated by T scores defined according to peak young adult bone density values. According to the World Health Organization (WHO) classification, patients with a T score ≤ -2.5 are considered osteoporotic, those with a score between -2.5 and -1 are considered osteopenic, and those with a score of ≥ -1 are considered normal (12). Fracture risk assessment scale (FRAX) is a fracture assessment tool developed by WHO. FRAX is a scale that can calculate the ten-year fracture risk in terms of hip or major fractures (hip, wrist, humerus or spine fractures) (12). In this scale age, BMI, low-energy fracture history, family history of hip fractures, current smoking, alcohol use (alcohol 3 or more units/day), and other secondary osteoporosis causes are used to calculate. Also, femoral neck T scores are included in the calculation (13) **Figure 1** shows the routine evaluation diagram of the patients who applied to our outpatient clinic in terms of osteoporosis. The patients included in our study were divided into two groups according to their BMI as "pre-obese/normal" for those with a BMI < 30 (n =26), and "obese" for those with a BMI ≥ 30 (n = 69) (14).

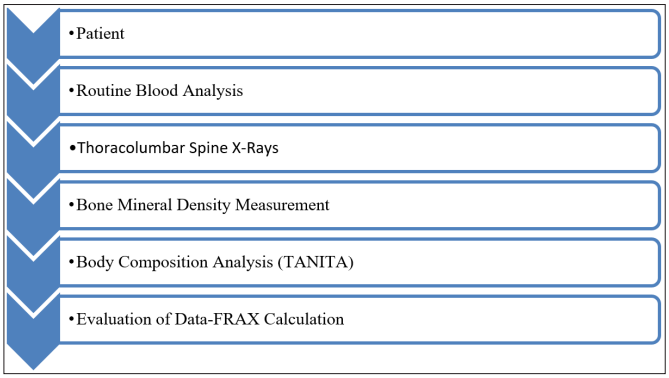

Figure 1. Routine evaluation diagram of patients in terms of osteoporosis

SSPS® version 21.0 statistical package program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. In the statistical analysis, after evaluating whether the data showed normal distribution or not, using the Kolmogorov Smirnov test, independent samples t-test was applied to the parameters showing normal distribution, while the Mann Whitney U test was used for the parameters that did not show normal distribution. P <0.05 values were considered statistically significant.

RESULTS

It was determined that 25.3% of the type 2 DM patients included in the study were male, 74.7% were female, and the general average age was 60.589 (± 8.20). When the demographic characteristics are examined according to the BMI classification in **Table 2**, the average age of the patients was found to be 60.44 (±9.00) in the obese and 60.96 (±5.68) in the pre-obese/normal. In addition, according to the BMI grouping, the mean duration of disease diagnosis was 12.36 (±8.37) years in the obese group, while it was 14.80(±8.47) years in the pre-obese/normal group, and the p-value between the duration of diagnosis and the BMI groups was found to be 0.215. There was no significant difference between the two groups in terms of smoking.

Table 2. Demographic characteristics according to BMI classification

	BMI < 30 (Pre-obese/Normal)	BMI ≥ 30 (Obese)	P value
Age (year)	60.96 (±5.68)	60.44 (±9.00)	.743
Gender (n: female/male)	16/10	55/14	.087
DM time (year)	14.80(±8.47)	12.36 (±8.37)	.215
Smoking rate	11.5%	7.2%	.574

FRAX and BMD values according to the BMI classification are shown in **Table 3**. When the groups were compared in terms of FRAX and BMD values, it was noted that the averages of these values were higher in the obese group. When FRAX and BMD values are examined according to BMI classification in **Table 3**, FRAX hip, L1-L4 total T score, femoral neck T score, femur total T score and femur total g/cm² values were found to be significantly higher in the obese group ($p<0.05$).

Table 3. FRAX and BMD values according to BMI classification

	BMI <30 (Pre-obese/Normal)		BMI ≥ 30 (Obese)		p value
	Mean	Std. Deviation	Mean	Std. Deviation	
FRAX major	6.353	1.875	6.518	3.931	.341
FRAX hip	0.542	0.404	0.629	1.203	.018*
L1-L4 total T score	-0.073	1.578	0.727	1.761	.047*
L1-L4 total g/cm²	1.142	0.202	1.238	0.222	.052
Femoral neck T score	-0.719	0.753	0.215	1.919	.019*
Femoral neck g/cm²	0.917	0.120	1.018	0.237	.060
Femur total T score	-0.396	0.944	0.755	1.925	.001*
Femur total g/cm²	0.979	0.132	1.108	0.239	.006*

FRAX: Fracture risk assessment scale, BMI: Body mass index, BMD: Bone mineral density, *p-value: Mann Whitney U Test ($p<0.05$)

In addition, while the history of low-energy fracture was 8.69% in the obese group, there was no fracture history in the pre-obese/normal group. While fractures were seen in the obese group with a rate of 17.39%, in the pre-obese/normal group, fractures were detected in 3.84% in the X-ray scan. Accordingly, although the low-energy fracture history and the number of fractures detected in the X-ray scan were higher in the obese group, no significant difference was observed between the two groups ($p> 0.05$).

DISCUSSION

In this study, obese and pre-obese/normal patient groups diagnosed with type 2 DM with similar lifestyles (smoking and alcohol consumption), average age and duration of diagnosis were examined. There was a significant difference between the groups in terms of BMD values (L1-L4 total T score, femoral neck T score, femur total T score, and femur total g/cm²) and FRAX hip fracture. Obesity is a common finding in patients with type 2 DM. Obesity is thought to improve bone quality and mass by increasing the tension on the skeleton, thus protecting it from osteoporosis(15). However, in the study conducted by Nielson et al. (16) on 5918 obese and elderly men, it was found that obesity was not protective against fracture angle. Again, in the study of Tanaka et al. (17) 1614 on female patients in the postmenopausal period, obesity was associated with an increased incidence of vertebral fractures. Similarly, higher BMD values were found in the patient group with a BMI ≥ 30 in our study. However, in this group, it was observed that the number of patients with a history of fracture, fracture on X-ray and the mean FRAX major and FRAX hip values were higher than the patient group with a BMI <30. Low-energy fracture history, family history of hip fractures, current smoking may be the reason for the high fracture risk according to the FRAX score despite the high BMD values in the obese group in our study.

Although DXA is accepted as the gold standard analysis in measuring BMD (18), it is known that type 2 DM has effects on the bone that cannot be measured by the DXA device (15). Despite high BMD values, there is a high risk of fractures in patients with type 2 DM. Although fragility of bone increases in type 2 DM, FRAX underestimates the risk of bone fracture (19). Obesity, which is common in patients diagnosed with type 2 DM, can be shown as one of the reasons for these effects (20). Type 2 DM and obesity have been found to cause deterioration in bone quality by affecting bone remodeling (20). The practical way to adjust the fracture risk in these patients is to reduce the standard deviation of 0.5 in the BMD T score (21).

Although type 2 DM affects bone metabolism with many mechanisms, its results are controversial. A study conducted in Brazil showed that blood glucose control is not an independent risk factor for vertebral fracture(22). However, an in vivo study by Garcia et al. (23), it was found that high blood glucose levels decrease bone mineral quality by affecting the biomineralization in osteoblasts. Serum levels of osteocalcin, which has an important role in binding calcium and hydroxyapatite crystals to bone, were found to be low in patients with type 2 DM (24).

Melton et al. (25), evaluated bone structure and strength with quantitative computed tomography in their study consisting of diabetic patients and nondiabetic control groups. In this study, it was shown that despite higher BMD values in patients with type 2 DM, this increase was entirely due to trabecular bone. In another cross-sectional study, higher trabecular volume was found in patients with postmenopausal type 2 DM compared to the control group, but cortical bone strength was found to be impaired (26). The mechanisms behind these changes in type 2 DM are mainly related to obesity, diabetic complications, age, duration of diabetes, comorbidities and medications (27). In our study, although BMD values were higher in the obese patient group with a diagnosis of type 2 DM, the average FRAX and the number of patients with fractures were found to be higher than in the pre-obese/normal group.

CONCLUSION

In our study, when the obese and pre-obese/normal groups diagnosed with type 2 DM were evaluated in terms of BMD values (L1-L4 total T score, femoral neck T score, femur total T score and femur total g/cm²), a significant difference was found between the groups. However, when the patient groups are examined, fracture risk in the obese patient group is higher than in the pre-obese/normal group. More studies are needed to understand the relationship between diabetes, obesity, and fracture.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Amasya University Non-interventional Clinical Research Ethics Committee (Date: 03.09.2020, Decision No: 9/104).

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 approved the final version.

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Original Article

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Hypoechoic nodule structure increases non-diagnostic rate of thyroid fine needle aspiration biopsy

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ABSTRACT

Aim: Thyroid fine needle aspiration biopsy (FNAB) performed with ultrasonography is an effective method in diagnosing thyroid cancer. Nevertheless, some of them have non-diagnostic results. The aim is to investigate the factors that affect non-diagnostic result.

Material and Method: FNABs of 361 nodules of 361 patients were analyzed retrospectively. The patients were divided into two groups as fine needle aspiration biopsy result with and without non-diagnostic. The groups were compared according to demographic, clinical and sonographic data.

Results: Non-diagnostic rate was 14.4% in all biopsies. There was no difference in terms of age, gender, previous thyroid surgery history, smoking history, aspirin use, Hashimoto's thyroiditis, toxic nodule. Sonographic findings of the thyroid glands and nodules were similar. Only the echogenicity of the nodule was found to affect the non-diagnostic result (p=0.015). In the post hoc analysis, the difference was found to be caused by hypoechoic nodules (32.7% versus 15.5). It was observed that the significant relationship found in univariate logistic regression analysis (p=0.009, OR: 3.227, CI: 1.334-7.803) continued in multivariate analysis (p=0.024, OR: 3.175, CI: 1.163-8.668).

Conclusion: Only hypoechoic echogenicity increases the risk of non-diagnostic rate. Other factors do not increase the non-diagnostic rate.

Keywords: Thyroid fine needle aspiration biopsy, ultrasonography, non diagnostic, hypoechogenicity

INTRODUCTION

The frequency of thyroid cancer continues to increase with each passing year. With the proliferation of ultrasonography (USG), nodular thyroid disease has been detected more frequently. The most appropriate method for making the necessary surgical decision and avoiding unnecessary surgery and related complications is fine needle aspiration biopsy (FNAB) (1,2). FNAB is an easy, inexpensive and effective diagnostic method. It is more effective when performed with USG. It has a 95% negative, 97-99% positive predictive value. FNAB indication is based on the sonographic features of the thyroid nodule. FNAB should be performed in the case of hypoechogenicity, irregular edge structure, microcalcification, ratio of anterior-posterior diameter to transverse diameter> 1, and central vascularity (3).

FNAB assessment is done according to the Bethesda system and the results are given in 6 categories; benign, non-diagnostic (ND), indeterminate atypia (AUS)/ indeterminate follicular lesion (AUFL), suspicious for follicular neoplasia or follicular neoplasia (FN), suspicious cytology for malignancy and malignancy (4). In the best series, the ND rate is 2-16% (5,6). Fifty five-74% of biopsies are reported as benign, 2-5% as malignant, 2-18% as AUS/AUFL, 2-25% as FN and 1-6% as suspicious for malignancy (6).

In order to FNAB to be sufficient, there must be at least 6 groups of follicular cells containing at least 10 well-preserved epithelial cells in one area. It is reported as ND, which does not meet these criteria. It is recommended to repeat FNAB, and if ND comes again, surgery or close follow-up is recommended. While the estimated malignancy risk of the nodule with a biopsy ND is 1-4%, the actual risk of malignancy of this nodule is 9-32% (5). A nodule whose biopsy is 2 times ND has a 25% risk of being malignant (7). Therefore, surgical and medical follow-up decision should be made carefully in nodules whose biopsy is ND.

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Studies investigating the factors affecting FNAB adequacy can be found in the literature. Different causes such as advanced patient age, nodule heterogeneity (8), aspirin (9), radioactive iodine therapy (10) were found to be related to ND. In this study, it was aimed to investigate the factors affecting ND biopsy results in a large sample group.

MATERIAL AND METHOD

Ethical Approval

The study was carried out with the permission of Amasya University Non-interventional Clinical Researchs Ethics Committee (Date: 02.05.2019, Decision No: 05-26). All procedures were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The subjects have given their written informed consent. This study does not contain animals.

Patient Selection

Between January 1, 2018 and January 1, 2019, 361 patients who underwent FNAB were included. These biopsies were performed by an endocrinologist experienced in terms of USG and FNAB. One FNAB result of each patient was included in the study. Consent was obtained from the participants before and after the FNAB. Demographic, laboratory, ultrasound and FNAB data of the participants were obtained from the hospital information processing system. The age, gender, smoking status, aspirin usage, previous thyroid surgery was recorded. According to thyroid autoantibodies and USG, which patients had Hashimoto's thyroiditis (HT) was detected. Thyroid scanning was performed by the endocrinologist with thyroid ultrasonography device (Aloka, Mollsfeld, Meerbusch, Germany). Thyroid gland and nodule volumes were calculated according to the formula of anterior-posterior diameter x transverse diameter x longitudinal diameter x 0.52 (11). Whether the nodule is thyrotoxic in hyperthyroid patients was recorded based on thyroid scintigraphy. Biopsy Indications and Results

FNAB was performed according to the size (> 1 cm), content (solid), echogenicity (hypoechoic), edge structure (irregular), vascularity (central), calcification (microcalcification), anterior-posterior diameter/transverse diameter ratio (> 1) of the thyroid nodules (12). Local anesthesia was not applied before biopsy. FNAB was made by aspiration method by 22-gauge needle. During aspiration, the USG probe was turned from the transverse position to the longitudinal position, the needle was inserted into the nodule at a right angle to the neck, the needle was moved 5-6 times to the anterior-posterior and lateral sides. The material was spread to the slides by the endocrinologist at the bedside. Slides were delivered to the pathology department after being dried in the air. FNAB results were categorized by experienced cytopathologists according to the Bethesda system (4).

Statistical Analysis

Statistical analyzes were made in IBM SPSS for Windows Version 22.0. Numerical variables were summarized with mean±standard deviation. Categorical variables were indicated by number and percentage. The chi-square test or Fisher's exact test was used to determine whether there was any difference between the groups in terms of categorical variables. The Kolmogorov Smirnov test, standard deviation/mean ratio, skewness/kurtosis measures, histogram and detrended normal graphics were used to determine whether the numeric variables showed normal distribution, and the homogeneity of the variance was examined by the Levene test. Differences between two independent groups in terms of numerical variables; In the case of parametric test assumptions, t-test was used for independent groups. In the absence of parametric test assumptions, Mann Whitney U test was used. Post hoc analysis was used to compare more than 2 groups. Univariate and multivariate logistic regression analyses were applied to determinate independent predictors affecting ND result. The significance level was taken as $p < 0.05$.

RESULTS

The results of the FNABs are as shown in **Table 1**. ND rate was 14.4% among all biopsies. The most reported category was benign, with a rate of 67.6% (**Table 1**).

All participants were divided into two groups according to whether the FNAB result was ND or not. Data on age, advanced age (>65), gender, previous history of thyroid surgery, smoking status, use of aspirin, presence of HT are shown in **Table 2.** No significant difference was found between the two groups (**Table 2**).

Thyroid USG features of all participants are given in **Table 3.** There was a significant difference in nodule echogenicity between the groups (p=0.015). Post hoc analysis was performed to determine which subtype caused the difference. The difference was caused by hypoechoic subgroup by applying Bonferoni correction and adjusted residual (**Table 3**).

Table 1. FNAB results

Total, n	361
Non-diagnostic, n (%)	52 (14.4)
Benign, n (%)	244 (67.6)
AUS/AUFL[a], n (%)	50 (13.9)
Follicular neoplasia, n (%)	4 (1.1)
Malignant, n (%)	1 (0.2)
Suspicious for malignancy, n (%)	10 (2.8)

[a]: AUS/AUFL: unspecified atypia/indeterminate follicular lesion

Table 2. Baseline clinical characteristics of patients and comparisons of two groups

Parameters	Total (n=361)	Non-ND[a] group (n=309)	ND[a] group (n=52)	p
Age, mean±std dev	53.03±11.78	52.66±11.96	54.79±10.35	0.227
Age >65, n (%)	58 (16)	49 (15.9)	9 (17.3)	0.792
Gender, n (%)				0.417
Male	75 (20.8)	62 (20.1)	17 (25)	
Female	286 (79.2)	247 (79.9)	39 (75)	
Previous thyroid surgery, n (%)	25 (6.9)	22 (7.1)	3 (12)	0.784
Smoking, n (%)	60 (16.6)	48 (15.5)	12 (23.1)	0.176
Aspirin, n (%)	42 (11.6)	37 (12)	5 (9.6)	0.624
HT[b], n (%)	92 (25.5)	83 (26.8)	9 (17.3)	0.184
TSH, mean±std dev	1.84±7.94	1.89±8.56	1.59±1.71	0.896

[a]: ND: non-diagnostic, [b]: HT: Hashimoto's thyroiditis

Table 3. Baseline sonographic characteristics of patients and comparisons of two groups

Parameter	Total (n=361)	Non-ND[a] group (n=309)	ND[a] group (n=52)	P
Right thyroid volume, mL	20.403±15.016	20.429±15.272	20.250±13.525	0.697
Left thyroid volume, mL	18.580±14.020	18.381±14.290	19.760±12.354	0.202
Istmus, mm	5.71 ± 3.99	5.64 ± 4	6.14 ± 3.95	0.348
Parenchym				0.891
Homogeneous	21 (5.8)	17 (5.5)	4 (7.7)	
Mild heter[b]	181 (50.1)	157 (50.8)	24 (46.2)	
Moderate heter[b]	113 (31.3)	96 (31.1)	17 (32.7)	
Advanced heter[b]	46 (12.7)	39 (12.6)	7 (13.5)	
Nodule noc, n (%)				0.950
Solitary	89 (28.1)	76 (24.6)	13 (25)	
MNGd	272 (71.9)	233 (75.4)	39 (75)	
Nodule size, n (%)				0.332
< 10 mm	15 (4.1)	11 (3.6)	4 (7.7)	
10.01-39.99 mm	312 (86.4)	270 (87.4)	42 (80.8)	
> 40 mm	37 (9.5)	28 (9)	6 (11.5)	
A-P diame/T diamf				0.142
<1	345 (95.6)	293 (94.9)	52 (100)	
>1	16 (4.4)	16 (5.1)	0 (0)	
Content,n (%)				0.113
Solid	158 (43.7)	130 (42.1)	28 (53.8)	
Mixed	203(56.3)	179 (57.9)	24 (46.2)	
Echogenity,n(%)				0.015
Isoechoic	91 (25.2)	82 (26.5)	9 (17.3)	
Hypoechoic	65 (18)	48 (15.5)	17 (32.7)	
Hyperechoic	13 (3.6)	10 (3.2)	3 (5.8)	
Spongiform	192(53.2)	169 (54.8)	23 (53.2)	
Edge, n (%)				0.393
Smooth	270 (74.8)	230 (74.4)	40 (76.9)	
Irregular	61 (16.9)	53 (17.2)	8 (15.4)	
Undefined	30 (8.3)	26 (8.4)	4 (7.7)	
Halo, n (%)				0.284
Absent	325 (90)	275 (89)	50 (96.2)	
Present	36 (10)	34 (11)	2 (3.8)	
Calcification,n (%)				0.885
Absent	303 (83.9)	261 (84.5)	42 (80.8)	
Microcalcification	10 (2.7)	9 (2.9)	1 (1.9)	
Macrocalcification	28 (7.7)	22 (7.1)	6 (11.5)	
Micro+macrocalc	7 (1.9)	6 (1.9)	1 (1.9)	
Eggshell calc.	13 (3.8)	11 (3.6)	2 (3.8)	
Vascularity, n (%)				0.365
Absent	224 (62)	194 (62.8)	30 (57.7)	
Peripheral	108 (29.9)	89 (28.8)	19 (36.5)	
Central	3 (0.8)	2 (0.6)	1 (1.9)	
Periph+central	26 (7.3)	24 (7.8)	2 (3.8)	
Toxic nodule, n (%)	50 (13.9)	41 (13.3)	9 (17.3)	0.435

[a]: ND: non-diagnostic, [b]: heter: heterogeneity, c: no:number, calc: calcification, d:MNG: multinodular goiter, e:A-P diam: antero-posterior diameter, f:T diam: transvers diameter

Table 4. Univariate and multivariate regression analysis

Parameter	Univariate			Multivariate		
	P	OR[a]	95 % CI	P	OR	95 % CI[b]
Nodule echogenicity	0.009*	3.227	1.334-7.803	0.024*	3.175	1.163-8.668
Nodule size (>1-3.99)	0.037*	0.303	0.098-0.930	0.077	0.311	0.085-1.137

[a]:OR: odds ratio, [b]:CI: confidence interval, *Univariate and multivariate analyzes were performed on other parameters, since the results were not significant, they were not shown in the Table 4.

In univariate logistic regression analysis, nodule echogenicity and size were found to have a significant effect on the ND result (p=0.009 and p=0.037). In the multivariate analysis, while the effect of echogenicity on FNAB remained (p=0.024, OR: 3.175, CI: 1.163-8.668), the effect of the nodule size was not detected (p=0.077, OR: 0.311, CI: 0.085-1.137) (**Table 4**).

DISCUSSION

In this study, the fine needle aspiration biopsy results performed by the endocrinologist in 1 year were investigated and the factors affecting the ND result were investigated. According to the results of the analysis, it was found that only hypoechogenity was increasing the likelihood of ND results.

In the study, 14.4% of total biopsies were determined as ND. This rate is similar to the literature since the probability of obtaining ND results is 2-16% (5,6). Results in other categories (except for those malignant and suspected of malignancy) are similar to the literature (6). The experience of the endocrinologist performing the biopsies provided the ND rate to be similar to the literature, and the experience is an important factor affecting the success of the biopsy (13).

Patients were divided into two groups as those with and without ND FNABs, and they were compared according to age, gender, previous history of thyroid surgery, smoking, presence of HT, thyroid USG findings.

Inci and his colleagues (8) retrospectively examined the results of FNABs performed by a radiologist (not endocrinologist) of 270 individuals in 2 years, ND rate was 31.9% and they found that advanced patient age is a factor that increases the risk of ND. Based on this study, we compared the groups in terms of age. The average age of the first study was 46 and 53 in our study. In the first study, the number of geriatric patients was not specified. In our case, there are roughly 16% geriatric patients. Contrary to the first study, we did not find a significant difference between the groups in terms of both the average age and the distribution of geriatric population, and we can say that age is not a risk factor for ND result. ND rate of the first study was markedly high, which may have led to such a conclusion about age.

The gender distribution was also similar in terms of the two groups. As in many studies, although the number of women who underwent FNAB in our center was significantly higher than men, the effect of gender on ND outcome was not detected. For example, in the study of Ozel et al. (14), gender distribution in ND and non-ND groups is similar.

Postoperative recurrent nodular thyroid disease is seen in 2-42% in different series in patients without total thyroidectomy (15). Onal et al. (16) found that, subtotal thyroid surgery increases the risk of ND outcome by causing fibrosis and scar formation. We also considered our patients' history of thyroid surgery. The patients who had previous thyroid surgery were 6.9% and consisted of recurrent MNG cases with FNAB indication in the residual thyroid gland. There was no significant difference in the distribution of these patients into groups (7.1% versus 12%, p=0.784). The conclusion drawn from this is that, unlike the previous study, having a previous thyroid surgery does not increase the risk of ND. However, since the number of patients with operated recurrent nodular goiter in our study was lower compared to other studies (123 patients versus 25 in total), more participatory studies are needed to support this result.

Since smoking exposure has been shown to worsen Graves' ophthalmopathy, studies have been conducted to demonstrate the relationship between smoking and thyroid. Although these studies have shown that smoking increases the risk of nodule formation and thyroid cancer (17,18), there are also studies showing that it is ineffective (19,20). In the literature, as we have determined, there are no studies investigating the effect of cigarette exposure on ND biopsy results. For this reason, we also considered the smoking history of the patients. When the distribution of the patients who ex-smokers and still smokers was compared between the two groups, no significant relationship was found (p=0.176, table 2). However, we did not elaborate on cigarette exposure (duration, amount, passive smoking, etc.). Therefore, more detailed studies to support this finding will help enlighten the issue.

HT is the most common inflammatory thyroid disease. The main diagnosis is made histopathologically, but in daily practice, HT is diagnosed according to thyroid autoantibodies and USG findings (18). According to the studies, the success of FNAB decreases in the presence of HT and it increases the diagnosis ND and AUS/AUFL

(21,22). In our study, while HT was detected in 25.5% of all patients, we could say that HT did not increase the risk of ND since there was no significant difference between the groups in terms of HT.

Toxic nodules previously thought to be protective for thyroid cancer, more FNABs are being performed today and it has been found to carry a risk of malignancy as much as non-toxic nodular goiter (23). Baser et al. (24) examined FNAB results of toxic and non-toxic nodules and showed that they had similar ND ratios. In our study, toxic nodules with FNAB were also included because they had sonographically risky features. The distribution of toxic nodules was similar between the groups, and it was determined that the toxic nodule had no effect on the ND result.

Studies investigating the efficacy/adequacy of thyroid FNAB were mostly based on sonographic findings. In our study, we compared both the general thyroid USG findings of the patients and the features of the nodules undergoing FNAB. There was no difference between the two groups in terms of thyroid gland size, parenchymal structure and number of nodules. The nodule size, the ratio of anterior-posterior diameter to transverse diameter, nodule content, echogenicity, margin arrangement, vascularity and calcification content were compared and only the nodule echogenicity was effective on the ND result. This difference was also shown by post hoc analysis that it was caused by hypoechoic echogenicity (table 3). In both univariate and multivariate logistic regression analyzes, hypoechogenity has been shown to affect the ND biopsy result. So, it was shown in our study that only hypoechoic echogenicity contributed to the ND result. Inci et al. (8) stated that heterogeneous echogenicity leads to more ND results, which may result from the hemorrhagic and necrotic content of the nodule. Ozel et al. (14) concluded that needle size is effective in ND result, and sonographic features of nodule have no effect. Moon and colleagues (25) found hypoechogenity as an independent factor for ND, as in our study. There are many other studies showing that more ND results are obtained in hypoechoic nodules (26,27). The possible reason is that due to the hypoechoic nodules being more fibrotic, sufficient material cannot be taken with fine needle aspiration (26). While cystic content increased the risk of ND in some studies (13,28), it was shown that it did not pose any risk in some (27). In our study, cystic content did not increase the risk of ND. No relation was found between size, vascularity, calcification (especially macrocalcification) and ND, as in many other studies (8,13,27).

Consequently, according to our study, the only factor that adversely affects the FNAB adequacy is the nodule hypoechogenity. Other demographic, clinical and sonographic features do not affect biopsy adequacy.

Our study provided homogeneity, covering a shorter period of time (1 year), by the same expert (1 endocrinologist), using the same USG, the same needle (22 gauge) and the same technique (aspiration). Although it covers 1 year, the number of patients (361) undergoing FNAB is quite high. We believe that this is a detailed study since it includes both demographic, clinical and USG findings.

Our study had limitations, too. We included only 1 biopsy result of 1 nodule of each patient, we did not include the results of re-biopsy. For this reason, we were unable to answer the question of whether the rate of ND will change with re-biopsy. The sample size of the study is also one of the factors limiting the study (361 biopsies performed in 1 year by 1 endocrinologist were examined.). Considering how common thyroid nodules are, analyzes with larger numbers of subjects are needed.

In some studies, the thickness of the needle used for FNAB was also investigated. The general recommendation is to make FNAB with a fine needle.12,28 We performed all biopsies with a 22- gauge needle and we did not compare different needles, so we could not comment on this.

CONCLUSION

Sonographic imaging and fine needle aspiration biopsy have an important role in the evaluation of thyroid nodules. The most important factor affecting biopsy adequacy is the echogenicity of the nodule. Performing the biopsy with USG, reduces non-diagnostic results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Amasya University Non-interventional Clinical Researchs Ethics Committee (Date: 02.05.2019, Decision No: 05-26).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author daclared (design, execution, and analysis) that all stages of the work were done by the author herself.

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Original Article

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The role of ECG as a mortality predictor in COVID-19 patients treated in the intensive care unit

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ABSTRACT

Background: COVID-19 infection has reached serious morbidity and mortality rates all over the world for a short time. Many studies have investigated the relationship of COVID-19 disease with mortality and morbidity. In this study, we wanted to elucidate the relationship between rhythm character (sinus rhythm vs atrial fibrillation) and QTc and frontal plane QRS-T angle, which parameters are reflecting the depolarization-repolarization kinetics, and mortality, which were not emphasized enough in previous studies.

Material and Method: 259 patients admitted to the intensive care unit due to COVID-19 infection between 01.04.2020 and 01.12.2020 was included in the study. The demographic characteristics of the patients, clinical backgrounds, laboratory values at the time of admission to the intensive care unit, and 12 derivation ECG records were obtained from the patient files. Rhythm, PR distance, QRS duration and morphology, QT interval, T wave morphology, presence of atrioventricular (AV) block, QRS axis, presence of ventricular premature contraction, frontal plane QRS-T (f(QRS-T)) angle degree were determined on 12 derivation ECG records. Patient groups were classified as "deceased patient" and "survivors" and the relationship of these parameters with survival was tried to be elucidated.

Results: the frequency of atrial fibrillation was significantly higher in the "deceased patient" group. For patients in sinus rhythm, there was no difference between the two groups in terms of PR distance and 1st degree AV block. Severe AV block was not observed in either group. There was no difference between the two groups in terms of QRS morphology, T wave inversion, and ventricular premature contractions. QRS duration, corrected QTc duration, and frotal plane QRS-T angle values were found to be statistically significantly higher in in the "deceased patient" group.

Conclusion: Atrial fibrillation, prolonged QTc duration and increased frontal plane QRS-T angle can be considered as mortality predictors in COVID-19 infection whose mortality rate is high all over the world.

Keywords: COVID-19, ECG, AF, QRS-T angle, mortality

INTRODUCTION

COVID-19 disease, caused by the severe acute respiratory syndrome Coronavirus 2 (SARS-Cov-2) virus, continues to affect the whole world for more than a year. The rate of spread of the infection is very high, as the number of infected patients increases, morbidity and mortality numbers also increase (1,2). Pneumonia caused by the COVID-19 disease creates respiratory stress, and the need for non-invasive or invasive mechanical ventilation support may arise. With the treatment administered in the intensive care unit, clinical recovery occurs as well as mortality.

Electrocardiography (ECG) is one of the most frequently used diagnostic tests in clinical practice. Many studies have shown the prognostic significance of ECG findings in many diseases, especially in cardiovascular system diseases. Information on COVID-19 infection is newly accumulating, and information on the clinical course and mortality of COVID-19 is emerging with the studies. In the early stages of Covid 19 infection, ECG findings - especially QTc value - were used to determine treatment options and side effects of treatment (3). In addition, studies related to Covid 19 mortality and ECG findings are also published. In addition, studies related to Covid 19 mortality and ECG findings have begun to be published. Our aim in this study is to reveal the relationship between in-hospital mortality and data and parameters to be obtained from 12-lead ECGs of patients who have been followed up in intensive care with COVID-19 infection.

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MATERIAL AND METHOD

259 patients admitted to the intensive care unit due to COVID-19 infection between 01.04.2020 and 01.12.2020 were included in the study. The demographic characteristics of the patients, clinical backgrounds, laboratory values at the time of admission to the intensive care unit, and 12 derivation ECG records were obtained from the patient files. Rhythm, PR distance, QRS duration and morphology, QT interval, T wave morphology, presence of atriventricular (AV) block, QRS axis, presence of ventricular premature contraction, frontal plane QRS-T (f(QRS-T)) angle degree were determined on 12 derivation ECG records. Patient groups were classified as "deceased patient" and "survivors" and the relationship of these parameters with survival was tried to be elucidated. This retrospective study was carried out with the permission of Keçiören Trainig and Research Hospital Clinical Researchs Ethics Committee (Date: 22.12.2020, Decision No: 15/2206). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

12 leads ECG recordings were performed at a speed of 25mm/sec and with a height of 10 mm/mV. In 12 derivations ECG, recordings with irregular R-R distance without P waves were accepted as atrial fibrillation (AF). Recordings longer than 120 milliseconds were defined as Right Bundle Branch Block or Left Bundle Branch Block according to their morphology. Other wide QRS morphology that does not fit this classification was collected under the interventricular conduction defect (IVCD). The corrected QT (QTc) distance is calculated according to the Bazett formula. All ECG records had automatically reported QRS axis and T axis data. The angle f(QRS-T) was calculated from these angles as the distance between the QRS axis and the T axis.

Statistical Analysis

SPSS for Windows version 23.0 (SPSS Inc., IL, USA) was used for statistical analysis. Continuous variables were expressed as means± standard deviations and categorical variables were expressed as percentages. Continuous data were compared using Student's t test. Categorical data were compared using the chi-square test. With ch square test, Pearson's correlation coefficient was used for correlation analysis. A value of $p< 0.05$ was considered statistically significant.

RESULTS

When the patient groups were compared, the mean age was higher in the "deceased patient" group (71.15±10.75 vs. 63.09±12.69; $p<0.001$). When co-morbidities were taken into account, heart failure and history of cerebrovascular events were statistically significantly higher in the "deceased patient" group, while there was no statistically significant difference in terms of hypertension, coronary artery disease, diabetes mellitus, malignancy, chronic obstructive pulmonary disease and gender (**Table 1**).

When ECG parameters were examined, the frequency of atrial fibrillation was significantly higher in the "deceased patient" group. For patients in sinus rhythm, there was no difference between the two groups in terms of PR distance and 1st degree AV block. Severe AV block was not observed in either group. There was no difference between the two groups in terms of QRS morphology, T wave inversion, and ventricular premature contractions. QRS duration, corrected QTc duration, and frotal plane QRS-T angle values were found to be statistically significantly higher in in the "deceased patient" group (**Table 2**).

Tablo 1. Demographic, clinical and laboratory characteristics of the study patients

	Died n=101	Survived n=158	P value
Age	71.15±10.75	63.09±12.69	<0.001
Gender			0.171
Female	33 (32.7%)	65 (41.1%)	
Male	68 (67.3%)	93 (58.9%)	
Hypertension	44 (43.6%)	81 (51.3%)	0.226
Diabetes mellitus	23 (22.8%)	38 (24.1%)	0.813
Coronary artery disease	21 (20.8%)	28 (17.7%)	0.538
Heart failure	9 (8.9%)	5 (3.2%)	0.046
Chronic obstructive pulmonary disease	16 (15.8%)	21 (13.3%)	0.567
Malignancy	6 (5.9%)	12 (7.6%)	0.610
Cerebrovascular event	8 (7.9%)	4 (2.5%)	0.044
Glucose (mg/dL)	189.55±96.22	175.69±99.48	0.270
Creatinine (mg/dL)	1.22±0.44	1.01±0.31	<0.001
GFR (mL/min)	61.51±23.11	80.53±78.19	0.018
Albumine (mg/dL)	3.05±0.46	3.36±0.61	<0.001
AST (IU/L)	69.89±93.02	43.57±39.29	0.002
ALT (IU/L)	51.61±77.61	37.54±42.01	0.061
GGT (IU/L)	69.44±83.69	64.10±83.19	0.616
LDH (IU/L)	639.64±428.56	391.51±185.71	<0.001
CRP (mg/dL)	141.93±99.01	109.52±99.40	0.011
Ferritin (ng/mL)	791.42±522.57	465.60±459.42	<0.001
D-dimer (mg/L)	6.85±15.19	2.93±9.33	0.012
Troponin (ng/L)	532.45±2459.57	33.79±177.63	0.012
Lactate (mmol/L)	3.43±2.50	1.21±0.81	<0.001
Sat O_2 (%)	74.07±8.78	85.79±7.82	<0.001
WBC ($\times10^3/\mu L$)	12.4899±6.2614	8652.6±3866.1	<0.001
Neutrophile ($\times10^3/\mu L$)	10.8151±5.8133	7008.4±3917.7	<0.001
Lymphocyte ($\times10^3/\mu L$)	992.2±1671.9	1139.1±616.7	0.317
Platelet	241144±113290	257284±98665	0.227
Hgb (g/dL)	13.37±2.18	13.34±1.65	0.903

(ALT: alanine aminotransferase, AST: aspartate transaminase, CRP: C-reactive protein, GFR: Glomerular Filtration rate, GGT: gamma-glutamyl transpeptidase, Hgb: hemoglobin, LDH: lactic acid dehydrogenase, WBC: white blood cell)

Table 2. ECG findings and characteristics of the study patients

	Died n=101	Survived n=158	P value
Heart rate (b.p.m)	102.68±25.04	90.77±19.14	<0.001
Rythm			<0.001
Sinus Rythm	83 (82.2%)	157 (99.4%)	
Atrial Fibrillation	18 (17.8%)	1 (0.6%)	
PR interval (msec)	152.70±24.10	154.31±28.48	0.796
First degree AV block	3 (3.6%)	8 (5.1%)	0.602
QRS (msec)	95.77±16.53	92.04±13.83	0.051
QRS morphology			0.489
Normal	77 (76.2%)	129 (81.6%)	
IVCD	15 (14.9%)	22 (13.9%)	
LBBB	6 (5.9%)	4 (2.5%)	
RBBB	3 (3.0%)	3 (1.9%)	
Ventricular premature contraction	14 (13.9%)	16 (10.1%)	0.360
Corrected QT (msec)	426.75±35.50	416.97±25.66	0.011
T wave inversion	14 (13.9%)	11 (7.0%)	0.067
Abnormal QRS axis	50 (49.5%)	50 (31.6%)	0.004
Frontal Plane QRS-T angle (0)	64.96±52.69	37.90±34.43	<0.001

IVCD: interventricular conduction disease, LBBB: left bundle brunch block, RBBB: right bıundle brunch block

As an acute phase reactant, albumin levels were significantly lower in the "deceased patient" group, and ferritin and C-reactive protein (CRP) levels were significantly higher in the "deceased patient" group. There was a statistically significant difference between the two groups in terms of creatinine, glomerule filtration rate (GFR), aspartate transaminase (AST), lactic acid dehydrogenase (LDH), troponin, d-dimer, lactate and Sat O_2, white blood count (WBC), neutrophil count (**Table 1**).

DISCUSSION

COVID-19 infection rapidly affected the whole world, reaching high mortality rates with the increase in the number of infected patients and the respiratory stress it caused. The fact that the infection is caused by a newly emerging virus limits the diagnosis and treatment options, and as the knowledge about the disease increases, knowledge about the clinical course and causes of mortality increases.

The 12 derivation ECG is one of the most frequently used tests in emergency services, outpatient clinic visits, inpatient wards and intensive care units. Until today, the relationship between the clinical course and mortality of many diseases, especially cardiovascular diseases, and ECG findings has been revealed. Some studies on COVID-19 disease and ECG have been published, and the number of studies demonstrating its relationship with mortality is small, and different parameters were used in each study (6-10). In this study, we wanted to reveal the relationship between ECG parameters and COVID -19 and emphasize the importance of rhythm character and the frontal plane QRS-t angle, which reflects especially the depolarization-repolarization state.

It is emphasized in the updated atrial fibrillation guidelines that the mortality due to all causes is 1.5-3.5 times higher in patients diagnosed with atrial fibrillation, and the presence of AF in addition to those with cardiovascular disease increases mortality and morbidity (11). Among the 259 patients included in the study, 19 patients had a diagnosis of atrial fibrillation. The clinical course of 18 of these patients ended with mortality. In studies conducted with COVID-19 infection, the relationship between AF and mortality has not been emphasized enough, and considering the results of this study, COVID-19 patients with AF may be considered to be at higher risk for mortality.

QT distance is one of the reflections of the ventricular depolarization and repolarization process on the ECG. The relationship between corrected QT (QTc) duration and ventricular arrhythmias and mortality has been demonstrated (12,13). With prolonged QTc, the frequency of ventricular arrhythmias and mortality rate increases especially in cardiovascular diseases. Prolonged QTc duration has been reported to be associated with prognosis in COVID-19 infection (14,15). In our study, the QTc duration was found to be significantly longer in the "deceased patient" group. Considering the results of the study, the prolonged QTc duration may be considered as a mortality predictor in patients diagnosed with COVID-19. In addition, the number of patients with an abnormal QRS axis other than 0-90 degrees is higher in the "deceased patient" group. There was no significant difference between the two groups in terms of the frequency of ventricular premature contraction (VPC). It was concluded that the frequency of VPC does not have a clear effect on mortality in the study population. There was no significant difference between the two groups in terms of PR duration and the frequency of first degree atrioventricular block in the study population. 2nd and 3rd degree atrioventricular block, which is associated with cardiovascular system morbidity and mortality, was not observed in the rhythm records of both groups.

The frontal plane QRS-T angle f (QRS-T), is another ECG parameter that reflects the ventricular deoplarization-repolarization process (16). In previous studies, increased f (QRS-T) angle was found to be associated with mortality in the general population and cardiovascular disease (17-19). In our study, higher f(QRS-T) angle values were observed in the "deceased patient" group compared to the surviving patients. Increased f(QRS-T) angle was thought to be associated with an increased risk of mortality in patients with a diagnosis of COVID-19.

Considering the demographic characteristics and laboratory results of the study population, there was a higher mean age in the deceased patient group. In previous studies, it was stated that mortality in covid 19 patients increased with increasing age (20,21). Heart failure and history of cerebrovascular disease were statistically significantly higher in the deceased patient group. However, there was no significant difference between the two groups in terms of diseases with high mortality rates such as hypertension, diabetes mellitus, coronary artery disease, chronic obstructive pulmonary disease, malignancy and gender. In addition, as acute phase reactants, low albumin, high CRP and ferritin were statistically significant in the deceased patient group, same as results of previous studies (22,23). In our study, as in previous studies, troponin and d-dimer levels were higher in the patient group who died, and their relationship with mortality was also revealed in this study (24,25).

CONCLUSION

Atrial fibrillation, prolonged QTc duration and increased frontal plane QRS-T angle can be considered as mortality predictors in COVID-19 infection, whose mortality rate is high all over the world. We think that patients with these parameters can be evaluated as high-risk and closely monitored from the time of diagnosis, and positive effects on mortality rates can be achieved.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Keçiören Trainig and Research Hospital Clinical Researchs Ethics Committee (Date: 22.12.2020, Decision No: 15/2206).

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

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Evaluation of the diagnostic efficiency of systemic immune-inflammation index in prostate biopsy

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ABSTRACT

Aim: This study aimed to evaluate the diagnostic efficacy of systemic immune-inflammation index (SII) in patients with prostate cancer (PCa) who have undergone prostate biopsy and have a prostate-specific antigen (PSA) value of 4–10 ng/ml.

Material and Method: In this study we included patients with a PSA value of 4–10 ng/mL, who underwent transrectal ultrasound-guided 12-core prostate biopsy between January 2010 and March 2021. The patients were divided into two groups as those with and without cancer. Those with cancer were divided into two groups as low grade (grade 1 and 2) and high grade (grade 3–5) according to the International Society of Urologic Pathologists (ISUP) grades. PSA, neutrophil-lymphocyte ratio (NLR), prostate volume, platelet-to-lymphocyte ratio (PLR) and SII were compared between the groups.

Results: The mean age of the 182 patients included in the study was 63.4±8.0 years, and the mean PSA value was 6.69±2.45 ng/mL. Benign prostate disease were detected in 142 (78%) of the patients and PCa in 40 (22%) patients. PSA, PLR, NLR and SII did not differ in those with cancer. Low ISUP grade cancer was detected in 80% of the patients and high ISUP grade cancer in 20%. PSA and PLR values were significantly higher in patients with high ISUP grade PCa compared to those with low ISUP grade ($p < 0.05$). There was no difference in SII values between the two groups. PSA, PLR and SII values of patients with high ISUP grade PCa were significantly higher than those without cancer ($p<0.05$).

Conclusions: SII and PLR, which can be easily calculated from peripheral blood, can be an effective predictor of pre-biopsy high-grade ISUP PCa.

Keywords: Prostate cancer, diagnosis, systemic immune-inflammation index, platelet-to-lymphocyte ratio, biopsy

INTRODUCTION

Prostate cancer (PCa) is the most common cancer in men following lung cancer. Mortality rates vary worldwide, with the highest being in the African descent and lowest in Asian (1). The use of serum prostate-specific antigen (PSA) has become widespread due to the increasing elderly population. Accordingly, prostate biopsy rates have increased significantly. Although the PSA value to predict cancer remains unknown, 4 ng/mL is used as a cut-off value for prostate biopsy (2). Since PSA can increase in benign prostate diseases, it is not an ideal biomarker for PCa (3). PCa was detected in approximately 25% of prostate biopsies performed with a cut-off value of 4.0 ng/mL; however, approximately 50% of the detected cancers were clinically insignificant (4). PSA density and free PSA/total PSA are used in the differentiation of cancer and benign diseases; however, their sensitivity and specificity are low (5). Therefore, there is a need for effective biomarkers to detect clinically important cancer.

Inflammation is an important predictor of tumour invasion and progression (6) and plays a role in PCa development (7). Therefore, inflammation parameters, such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are used as tumour markers. Systemic immune-inflammation index (SII) is a new inflammatory marker calculated from lymphocytes, neutrophils and platelets and is used in cancer diagnosis and prognosis (8). In this study, we aimed to evaluate the effectiveness of NLR, PLR and SII in distinguishing PCa and clinically important cancer in patients with a PSA value of 4–10 ng/mL and who underwent biopsy for PCa screening.

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MATERIAL AND METHOD

The study was carried out with the permission of Harran University Clinical Researchs Ethics Committee (Date: 12.04.2021, Decision No: HRU/21.08.07). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The data of patients who underwent prostate biopsy for prostate cancer screening between January 2010 and March 2021 were retrospectively reviewed. Patients with a total PSA value of 4–10 ng/mL were included in the study. Patients who had previously undergone prostate biopsy and those with suspected cancer during the rectal examination were not included in the study. Before the biopsy, urine culture and complete blood count were performed. Appropriate treatment was applied to patients with infection in the analysis results, and biopsy was performed after their cultures were negative. All patients received antibiotic prophylaxis before the biopsy. All patients underwent a 12-core prostate biopsy using an 18 gauge Tru-Cut needle accompanied by transrectal ultrasound. From the complete blood count results, NLR, PLR and SII (neutrophil count x platelet count / lymphocyte count) were calculated. Prostate volume was calculated according to the formula 0.52× transverse × anteroposterior × transverse × longitudinal diameters. The patients were divided into two groups those with and those without PCa. Those with PCa were divided into two groups low ISUP grade (grade 1 and 2) and high ISUP grade (grade 3–5) according to the International Society of Urological Pathology 2014. Age, PSA, prostate volume, lymphocyte, neutrophil, platelet, NLR, PLR and SII were compared between the groups.

Statistical Analysis

Mean, lowest and highest median values, standard deviation, ratio values and frequency were used in the descriptive statistics of the data. Kolmogorov–Smirnov test was used to measure the distribution of variables. Quantitative independent data was analyzed using independent samples t-test and Mann–Whitney U test. SPSS 27.0 was used for statistical analyses.

RESULTS

The mean age of the 182 men included in the study was 63.4±8.0 years, and the mean PSA value was 6.69±2.45 ng/mL (**Table 1**).

Benign prostate diseases were detected in 142 (78%) patients and PCa in 40 (22%) patients. There was no significant difference (p>0.05) in age, prostate volume, PSA value, lymphocyte value, neutrophil value, NLR, PLR and SII ratio between the groups with and without PCa. Platelet value was significantly higher (p<0.05) in the group with PCa than in the group without PCa (**Table 2**).

Low ISUP grade cancers were detected in 80% of the patients and high ISUP grade cancers in 20% of the patients. Patients' age, lymphocyte value, neutrophil value, NLR ratio and SII ratio did not differ between the low ISUP grades and high ISUP grades groups (p> 0.05). PSA value, platelet value and PLR were significantly higher (p<0.05) in the group with high ISUP grades compared to the group with low ISUP grades (**Table 3**). PSA, PLR and SII were significantly higher in patients with high ISUP grade PCa compared with those without cancer (p<0.05).

Table 1. Characteristics of the patients

		Min-Max	Median	Mean±sd/n-%
Age		44.0-78.0	63.0	63.4±8.0
PSA		4.00-10.00	6.294	6.69±2.45
Lymphocyte		0.40-4.40	1.90	2.05±0.79
Neutrophil		1.50-45.00	5.25	5.69±3.75
Platelet		111.0-446.0	267.5	267.8±66.0
NLR		0.71-15.52	2.64	3.16±2.24
PLR		47.1-757.5	129.6	148.6±82.9
SII		160.3-4851.7	698.6	843.2±656.4
ISUP grade	I		23	57.5%
	II		9	22.5%
	III		3	7.5%
	IV		3	7.5%
	V		2	5.0%

NLR: Neutrophil lymhocyte ratio, PLR: Platelet lymphocyte ratio, SII: Systemic inflammation index

Table 2. Comparison of patients according to biopsy results

	No cancer n:142		Cancer n:40		p
	Mean±sd	Median	Mean±sd	Median	
Age	63.3±8.0	63.0	63.4±83.3	66.0	0.986[t]
PSA	6.61±2.62	6.24	6.93±1.91	6.50	0.226[m]
Prostate volume (mL)	50.8±29.2	48.00	47.7±16.9	46.00	0.08[m]
Lymphocyte	2.00±0.67	1.90	2.19±0.85	2.10	0.080[m]
Neutrophil	5.72±4.21	5.10	5.62±2.01	5.60	0.416[m]
Platelet	253.1±62.0	255.5	308.9±59.9	296.5	***0.000***[m]
NLR	3.11±1.91	2.72	3.29±3.00	2.27	0.253[m]
PLR	138.4±52.9	126.0	177.2±132.2	144.8	0.146[m]
SII	780.4±498.0	714.6	1019.3±958.9	694.3	0.376[m]

[t]: t test, [m]: Mann-Whitney u test, statistically significant results are in bold italics (p<0,05), NLR: Neutrophil lymhocyte ratio, PLR: Platelet lymphocyte ratio, SII: Systemic inflammation index

Table 3. Comparison of patients according to ISUP grades

	ISUP Grade I-II		ISUP Grade III-IV-V		p
	Mean±sd	Median	Mean±sd	Median	
Age	62.5±8.0	64.5	66.9±8.9	67.0	0.249[m]
PSA	6.55±1.74	6.10	8.44±1.93	9.20	***0.016***[m]
Lymphocyte	2.23±0.77	2.20	2.04±1.17	1.75	0.278[m]
Neutrophil	5.71±1.92	5.60	5.26±2.43	5.40	0.735[m]
Platelet	293.3±52.1	286.0	371.0±49.5	368.5	***0.001***[m]
NLR	3.16±2.72	2.29	3.78±4.15	2.10	0.685[m]
PLR	159.5±121.6	130.0	248.0±157.6	222.7	***0.046***[m]
SII	934.1±800.6	667.3	1359.9±1455.4	823.0	0.279[m]

[m]Mann-whitney u test, statistically significant results are in bold italics (p<0.05), NLR: neutrophil lymhocyte ratio, PLR: platelet lymphocyte ratio, SII: systemic inflammation index

DISCUSSION

Only half of the patients with PCa develop symptomatic disease throughout their lives (4). With the widespread use of PSA, a significant increase is observed in the diagnosis of clinically insignificant PCa (9,10). While increased diagnosis leads to overtreatment, its impact on patients' overall quality of life is still uncertain (11). In patients with ISUP grade 1–2 PCa, the mortality rate is 7% in 15 years of follow-up, which is quite low (12). In addition, studies have shown that 10% of patients with clinically insignificant cancer develop severe depression as a side effect (13). Therefore, it is important to detect clinically significant PCa by avoiding overdiagnosis in patients with PSA values of <10 ng/ml and no evidence of disease on rectal examination. In this study, cancer was detected in 22% of the patients who underwent prostate biopsy. Low ISUP grade (grade 1; 57.5%, grade 2; 22.5%) PCa was detected in 80% of these patients. These rates were similar to previous studies (4).

Inflammation is an important predictor of tumour invasion, progression and metastasis; additionally, it plays a role in PCa development (7,14,15). Inflammatory markers, such as lymphocyte count, NLR, PLR and lymphocyte-to-monocyte ratio, are used in determining the diagnosis and prognosis of cancer due to their affordability and availability (16).

SII is calculated from the combination of NLR and the number of platelets, and studies emphasise that it may be a better marker than NLR (17). Recent studies have shown that high SII values in urological cancers are associated with poor prognosis (8,18). Görgel et al. (19) reported that preoperative high SII value could be an independent prognostic factor in patients with muscle-invasive bladder tumour who underwent radical cystectomy, and cancer-specific survival was low in patients with SII>843. Another study evaluating patients who underwent nephroureterectomy for upper urinary tract ureteral cancers showed that high SII value is associated with high pathological stage and poor prognosis (20).

Studies examining the role of SII in PCa have been published in the recent years. Stangl-Kremser et al. (21) reported that SII may be a predictor of overall survival in patients with castration-resistant PCa. In the study by Rajwa et al. (22), in which the preoperative SII values of patients who underwent salvage radical prostatectomy were examined, it was shown that high SII value was a predictor for lymph node involvement and non-organ-confined disease. Another study published recently has investigated the effect of SII in cancer detection in patients who have undergone prostate biopsy. This study could not show an effect of SII in the distinction between PCa and benign prostate disease (23). In their study evaluating patients who underwent fission prostate biopsy, Sönmez et al. reported similar SII between benign prostatic diseases and low ISUP grades (grade 1 and 2) PCa. However, it was observed that SII was significantly higher in patients with high ISUP grades (grade 3–5) PCa (24). It has been reported that SII can be a predictor in detecting clinically important PCa. Although the SII value in patients with PCa in our study was higher than benign prostatic diseases, this difference was not statistically significant. Regarding the patients with PCa, there was no statistical difference between those with low ISUP grades and high ISUP grades. However, in patients with high ISUP grade PCa, the SII value was statistically significantly higher than benign prostatic diseases. Therefore, we think that SII can be a predictor in detecting clinically important PCa.

Patients with cancer usually have a hypercoagulable state. Platelets are an effective parameter in the progression of cancer and the prothrombotic state of the cancer patient. (25). Li et al. (15) reported that PLR value increases in patients with PCa and is an independent predictor of 3-year mortality. Adhyatma et al. (26) reported that PLR is a promising predictor of cancer in patients with suspected PCa. On the contrary, Murray et al. (23) found that PLR was similar between those with cancer and those with a benign prostate disease in patients who underwent prostate biopsy. In another study of metastatic PCa patients receiving hormonal therapy, NLR and PLR could not be associated with disease-specific survival, but platelet count was shown to be an independent prognostic factor (27). While the platelet value was significantly higher in patients with PCa in our study, PLR did not differ. However, in patients with high ISUP grades PCa, PLR and platelet values were significantly higher than those with both low ISUP grades PCa and benign prostate disease.

Our study includes some limitations. The first is the low number of patients. The reason for this is that patients with PSA >10 and patients with suspected cancer on rectal examination were excluded from the study. Another limitation is that the study is retrospective.

CONCLUSION

In PCa screening, ideal biomarkers that can differentiate benign and malignant tumours and detect clinically important cancer are needed to avoid unnecessary biopsies and overtreatment, especially in men with a PSA value in the gray zone (4–10 ng/mL) and no evidence of rectal examination. In our study, we showed that SII and PLR were higher in patients with higher ISUP grades than those with benign pathology. We think that SII and PLR, which can be easily calculated from peripheral blood, can be used as predictors of high ISUP grade PCa before performing a biopsy. For this, prospective studies with high numbers are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Harran University Clinical Researchs Ethics Committee (Date: 12.04.2021, Decision No: HRU/21.08.07).

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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Analysis of *Helicobacter pylori* positiveness and upper gastrointestinal system endoscopy results of Yozgat region in Turkey

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ABSTRACT

Aims: The aim of this study is to analyze the endoscopy and pathology results of patients who underwent endoscopy in Yozgat region, to determine the frequency of *Helicobacter pylori* (*H. pylori*) positivity and to compare the results with the data in the literature.

Material and Method: Among the patients who applied to our clinic for any reason between 01/01/2019 and 31/12/2019, 772 patients who underwent upper gastrointestinal system endoscopy were included in the study and analyzed retrospectively.

Results: The mean age of the patients was 50±16.78 (16–89). 61.3% (n=473) of the patients were female; 38.7% (n=299) were male. When gastroscopic diagnoses were evaluated, the rate of patients reported as "normal upper gastrointestinal endoscopy" was found to be 5.2% (n= 40). The most frequently reported diagnosis was “nonerosive gastritis” with a rate of 59.2% (n=457). Biopsy was obtained in 646 of 772 patients. The most common diagnosis was reported as “chronic active gastritis” with 90.6% (n=585). 626 of the patients had *H. pylori* pathological evaluation results. While 18.7% (n=117) of the patients were found to be *H. pylori* negative, 81.3% (n=509) were found to be *H. pylori* positive. Stomach cancer was found to be 1.3% (n=9) and esophageal cancer was found to be 0.1% (n=1).

Conclusion: In the Yozgat region, the frequency of *H. pylori* positivity was found to be 81.3%, and it is often mildly (+) positive. In addition, the frequency of *H. pylori* positivity was similar between men and women, consistent with the literature. The most common diagnosis was reported as "chronic active gastritis". While stomach cancer was detected at a rate of 1.3%, esophageal cancer was found at a rate of 0.1%.

Keywords: Upper gastrointestinal endoscopy, *Helicobacter pylori*, histopathology, gastric cancer, esophageal cancer

INTRODUCTION

Upper gastrointestinal endoscopy (Esophago-gastroduodenoscopy) is the most reliable diagnostic method widely used in the diagnosis of esophageal, stomach and proximal duodenal diseases. The advantage of endoscopy is to see the inner surface of the organ directly, to perform pathological sampling from the lesions, and to apply endoscopic treatment when necessary. For treatment purposes, interventional procedures such as excision of the polyp, control of upper and lower gastrointestinal system bleeding (esophageal varices bleeding, sclerotherapy, coagulation), and removal of foreign bodies can be performed (1).

The most common symptoms of upper gastrointestinal system diseases can be detected as dysphagia, dyspepsia, nausea and vomiting, retrosternal burning, epigastric pain, bleeding, and weight loss. These symptoms may indicate a benign or malignant condition. Therefore, patients with these symptoms should be evaluated with endoscopy (2,3).

H. pylori is a frequently encountered gram-negative pathogenic bacterium that affects almost half of the world population (4). *H. pylori* is implicated as an important pathogen in some diseases; chronic gastritis, peptic ulcer, nonulcer dyspepsia, gastric carcinoma and MALT lymphoma are the main ones (5). It is estimated that 50-90% of the world population is infected with *H. pylori* (6,7).

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H. pylori is a human pathogen that is transmitted from person to person and causes chronic active gastritis (of varying severity) in all colonized subjects. *H. pylori* gastritis is considered an infectious disease regardless of the symptoms of the individual and the stage-complications of the disease (8). In 1994, when the World Health Organization International Agency for Research on Cancer concluded that *H. pylori* is a definite carcinogen in humans, attention was focused on this bacterium (9). The need for endoscopy is increasing due to increasing gastrointestinal malignancies, increased frequency of *H. pylori* and widespread screening programs.

The gold standard for the diagnosis of *H. pylori* is the histopathological examination of the samples taken by gastroscopic biopsy and the demonstration of the bacterium by culture (10,11).

In this study, we aimed to retrospectively determine and present the frequency of esophageal, stomach and proximal duodenum diseases and the frequency of *H. pylori* in endoscopic biopsy samples, and its relationship with age and gender in patients admitted to a tertiary hospital and undergoing upper gastrointestinal system endoscopy.

MATERIAL AND METHOD

The study was carried out with the permission of Yozgat Bozok University Clinical Research Ethics Committee (Date: 14.04.2021, Decision No: 2017-KAEK-189_2021.04.14_04). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In our study, 772 patients who underwent upper gastrointestinal endoscopy for any reason in the endoscopy unit of a tertiary hospital between 01/01/2019 and 31/12/2019 were retrospectively analyzed. The patients' endoscopy reports, tissue biopsy pathology reports and other information were obtained from hospital records.

It was observed that all endoscopic procedures were performed under pharyngeal local anesthesia and sedated anesthesia (propofol i.v.) after 8 hours of fasting. The age, gender and esophagus, stomach and proximal duodenum findings in the endoscopy reports of the patients were determined.

Biopsy preparations taken for *H. pylori* and histopathological diagnosis were stained with hematoxylin-eosin and modified giemsa and examined under light microscopy, and reported as (+) mild, (++) moderate, (+++) severe positive according to the bacterial density in the preparations. It was observed that PNL activation, Hp activity, chronic inflammation, intestinal metaplasia, presence of atrophy were graded according to Sydney classification (**Table 1**) (12). It was observed that more advanced staining and techniques were used in malignant cases. Pathological data were obtained from these reports.

Statistical Analysis

SPSS version 20.0 (Statistical Package for Social Sciences) program was used for statistical analysis of the findings. Descriptive statistics were used in the analysis of the data. While continuous variables were expressed as mean±standard deviation (SD), nominal variables were expressed as percentages. Chi-square test and Mann-Whitney U test were used to evaluate the relationship between groups. P value <0.05 was considered statistically significant.

RESULTS

When the retrospective files were scanned, it was determined that 772 patients underwent upper gastrointestinal endoscopy between 01/01/2019 and 31/12/2019. The mean age of the patients was 50±16.78 (16–89). 61.3% (n=473) of the patients were female; 38.7% (n=299) were male.

When gastroscopic diagnoses were evaluated, the rate of patients reported as "normal upper gastrointestinal endoscopy" was found to be 5.2% (n=40). The most

Table 1. Sydney Classification (11)

Feature	Definition	Garding guidelines
Chronic inflammation	Increased lymphocytes and plasma cells in the lamina propria	Mild, moderate, or severe increase in density
Activity	Neutrophilic infiltrates of the laminapropria, pits, or surface epithelium	Mild: < 1/3 of pit and surface infiltrated Moderate: 1/3 - 2/3 of pit and surface infiltrated Severe: > 2/3 of pit and surface infiltrated
Atrophy	Loss of specialized glands from either antrum or corpus	Mild: mild loss Moderate: moderate loss Severe: severe loss
Intestinal metaplasia	Intestinal metaplasia of the epithelium	Mild: < 1/3 of mucosa involved Moderate: 1/3 - 2/3 of mucosa involved Severe: > 2/3 of mucosa involved
Helicobacter pylori	Density of *H. pylori* overlying epithelium	Mild: scattered organism covering < 1/3 of surface Moderate: intermediate numbers Severe: large cluster or a continuous layer over > 2/3 of surface

frequently reported diagnosis was "nonerosive gastritis" with a rate of 59.2% (n=457). Gastric ulcer rate was 6% (n=46); duodenal ulcer rate was found to be 2.3% (n=17). A mass lesion suggesting malignancy in the stomach was found to be 1.3% (n=10). Esophagitis was reported as 23.4% (n=181), varicose veins in the esophagus 0.8% (n=6) and a mass lesion suggesting malignancy in the esophagus was reported as 0.1% (n=1) (**Table 2**).

Table 2. Diagnoses, patient numbers and rates reported in upper gastrointestinal endoscopy

Endoscopic Diagnoses	Number of patients (n)	Patient ratio (%)
Normal Endoscopy	40	5.2
Nonerosive Gastritis	457	59.2
Pangastritis	44	5.7
Erosive Gastritis	42	5.4
Alkaline Reflu Gastritis	49	6.3
Gastric Ulcer	46	6
Gastric Malignant Mass Lesion	10	1.3
Gastric Polyp	14	1.8
Bulbit	33	4.3
Duodenal Ulcer	17	2.2
Esophagitis	181	23.4
Lower Esophageal Sphincter Slackness	178	23
Hiatal Hernia	11	1.4
Esophageal Varicose	6	0.8
Esophageal Diverticulum	1	0.1
Esophageal Malignant Mass Lesion	1	0.1
İnlet Patch	10	1.3

When the pathological diagnoses were examined, it was determined that biopsy was taken in 646 of 772 patients. The most common diagnosis was reported as "chronic active gastritis" with 90.6% (n=585). It was observed that chronic atrophic gastritis was reported with a rate of 4.8% (n=31). Gastric adenocarcinoma rate was 1.2% (n=8), neuroendocrine tumor rate was 0.1% (n=1), and esophageal squamous cell cancer rate was 0.1% (n=1) (**Table 3**).

Table 3. Reported pathological diagnoses, patient numbers and rates

Endoscopic Diagnoses	Number of patients (n)	Patient ratio (%)
Chronic Active Gastritis	585	90.6
Chronic Atrophic Gastritis	31	4.8
Stomach Adenocarcinoma	8	1.2
Gastric Neuroendocrine Tumor	1	0.1
Hyperplastic adenoma	8	1.2
Tubular Adenoma	2	0.2
Tubulovillous Adenoma	1	0.1
Xanthelasma	1	0.1
Esophageal Columnar Metaplasia	2	0.2
Squamous Cell Carcinoma of the Esophagus	1	0.1
Celiac Disease	2	0.2

626 of the patients had *H. pylori* pathological evaluation results. While 18.7% (n=117) of the patients were found to be *H. pylori* negative, 81.3% (n=509) were found to be *H. pylori* positive. The rates of positivity degrees according to Sydney Classification in *H. pylori* positive patients are given in **Table 4**.

Table 4. Rates of *Helicobacter pylori* positivity according to Sydney classification

Degree of Positivity	Number of patients (n)	Rate of patients (%)
Helicobacter pylori +1	379	74.5
Helicobacter pylori +2	103	20.2
Helicobacter pylori +3	27	5.3

When the relationship between *H. pylori* status and gender was examined, it was found that there was no significant relationship (p=0.595). When the relationship between *H. pylori* status and age was examined, it was found that there was no significant relationship (p=0.195).

When metaplasia was examined, metaplasia was found in 8.6% (n=53) of 615 patients. When atrophy was examined, it was found that 16.3% (n=100) of 613 patients had atrophy.

DISCUSSION

In addition to the use of upper gastrointestinal endoscopy for diagnostic purposes in esophagus, stomach and proximal duodenal diseases, its therapeutic use is becoming widespread with the development of therapeutic endoscopes.

Esophageal cancer is the 8th most common cancer in the world and ranks 6th in cancer-related deaths (13). Esophageal cancers are more common in developing countries and these cancers are usually detected at an advanced stage (inoperable period). Esophageal cancer is more common in males. According to 2013 statistical data in Turkey, the incidence of esophageal cancer is 1.3 per hundred thousand in women and 2.1 per hundred thousand in men (14).

In studies conducted in our country, the incidence of esophageal cancer shows regional differences. Esophageal cancer was detected in 4.3% of the patients who underwent endoscopy in the study conducted in the Van basin in the Eastern Anatolia Region. 61% of the patients were women and 39% were men, and esophageal cancer was 1.5 times more common in women than in men. The mean age was determined as 54.7 (15). In a study conducted in the Harran Region, the incidence of esophageal cancer was found to be 0.2% in a series of 5286 patients (16). In a study evaluating 6912 upper gastrointestinal endoscopy performed in the Elazig region, esophageal cancer was reported as 0.1%.

In a study conducted in the Western Black Sea Region, the rate of esophageal cancer was reported as 0.06% in 7703 patients who underwent upper gastrointestinal endoscopy (17). In a study conducted in Aydın Region, upper gastrointestinal malignancy was detected with a rate of 2.71% in endoscopic examinations (18). In our study, the detection rate of esophageal cancer in the Yozgat Region was found to be 0.1% (n=1).

While gastric cancer is the fourth most common type of cancer in the world, it ranks third in cancer-related deaths (19). Gastric cancer is the most common type of cancer in women after breast and colorectal cancers in our country. In men, it is the most common type of cancer after lung cancer. In general, it occurs twice as often in men than in women. The average age of occurrence is between 60-70 years (20,21). The incidence of gastric cancer in our country varies between 0.5-4% (17). In a study involving 504 gastric cancers in Erzurum Region, 36.1% of gastric cancer patients were female and 63.9% male, and the mean age of the patients was 62.4 years (22). In a study conducted in the Elazig Region, gastric cancer was detected with a rate of 1.3% in upper gastrointestinal endoscopy (17). In a study conducted in the Harran Region, the frequency of gastric cancer was found to be 0.5% in a series of 5286 patients (16). Gastric cancer in the Van Region was found 1.6 times more frequently in men than in women. The mean age of the cases was 54.8, and it was most common between the ages of 51-60 (39%) (15). In our study, the incidence of gastric cancer was 1.3% (n=9) in 772 patients who underwent upper gastrointestinal endoscopy. The findings are similar to the literature. Gastric adenocarcinoma was detected in 8 patients, and gastric neuroendocrine tumor was detected in 1 patient.

There are various diagnostic methods used in the detection of *H. pylori* infection. These methods are divided into invasive methods that require endoscopic gastric mucosal biopsy and noninvasive methods that do not require endoscopy. Invasive tests; culture, histopathological test, rapid urease test and PCR. Noninvasive tests consist of Urea breath test, serological tests and *Helicobacter pylori* antigen in stool (10,11).

In studies conducted in different regions of Turkey and on different dates, it has been reported that the frequency of *H. pylori* is found to be between 43-88.6%, the frequency varies between regions and age groups, and tends to decrease with age in recent years (23-37). Özardalı et al. (28) in 1998, Uyanıkoğlu et al. (24) in 2012, in their study covering the Şanlıurfa region and including cases with different endoscopic diagnoses; reported *H. pylori* positivity as 89.8% and 71%, respectively. Konakçı et al. (23) found *H. pylori* positivity in 50.5% of 218 patients who underwent endoscopic biopsy due to dyspepsia complaint in 2010. In studies conducted in Konya region, while Kesli et al. (29) found *H. pylori* positive in 36.6% of 168 patients who applied with dyspepsia complaints and underwent endoscopic gastric biopsy in 2010, Korkmaz et al. (30) found *H. pylori* positive in 45.4% of 198 dyspepsia patients who underwent endoscopy in 2012. In studies conducted between 2003 and 2008, the prevalence of *H. pylori* antigen was reported as 70.1% (30) in Sivas, 64% - 44.2% (32, 33) in Konya, and 58.4% (34) in Kayseri. The most comprehensive study on epidemiological studies on *H. pylori* in adults in our country is the TURHEP study conducted in 2003. In this study, the general prevalence of *H. pylori* was found to be 82.5% using the urea breath test in 5.549 adults over the age of 18, and the prevalence was found to be 84% in men and 81% in women (35). Again, in the study of Çiftel et al. (36) involving 653 patients in Erzurum region, *H. pylori* positivity was found to be 57.7% and it was reported as mild (+) positive in 58.7% of the patients with *H. pylori*. When the literature is examined in terms of positivity and clinical and laboratory results, not many studies have been found. Only Dağlı et al. (37) found a relationship between the degree of *H. pylori* positivity and mast cell density, which is known to be the primary trigger of inflammation. In our study, *H. pylori* was examined histopathologically in patients whose biopsy was taken. In addition to the presence of *H. pylori*, its density was also examined. In our study, *H. pylori* was found to be positive in 81.3% of the patients who underwent endoscopy by histopathological examination. The degree of *H. pylori* positivity was mildly (+) positive with a rate of 74.5% in line with the literature, and the frequency of *H. pylori* positivity between women and men was similar as in the literature (36).

The difference in the frequency of *H. pylori* positivity in studies may be due to the presence of various risk factors in the studied population, such as environmental reasons, hygiene, low education level, regional backwardness, previous gastrointestinal endoscopy, and less eradication. Megraud et al. (38) showed that with the increase in the incidence of atrophic gastritis in advanced ages, *H. pylori* lost its ecological home and its frequency decreased. Low education level, regional backwardness, hygiene and less eradication may be the main factors due to the high *H. pylori* positivity rates in our region.

Approximately one million people worldwide die from diseases associated with *H. pylori* every year (39). Considering that *H. pylori* is the main etiological factor especially in gastric cancer and its associated other diseases and its frequency in the general population, it is estimated that *H. pylori* infection will remain on the

agenda as the leading health problem in developing countries for the next 50 years (11, 39). For this reason, it becomes necessary to monitor the changes in the frequency of *H. pylori* and to make its eradication. Expecting a reduction in the incidence of gastric cancer with the eradication of *H. pylori* seems to be the most effective and cost-effective method.

Gastric polyps are detected incidentally during the endoscopy procedure. In a study involving 13 thousand patients, the prevalence of gastric polyps was found to be 1.2% (40). In a study conducted in the Kırıkkale region of our country, the frequency of gastric polyps was found to be 1.5% (41). In our study, it was found to be 1.8%.

In studies conducted in our country, while the frequency of gastric ulcer was 10% and duodenal ulcer frequency was found to be 11% in Şanlıurfa region, the frequency of gastric ulcer was 9% and duodenal ulcer frequency was found to be 15% in Aydın region (42,43). In our study, the frequency of gastric ulcer was 6% and the frequency of duodenal ulcer was 2.2% in the Yozgat region.

CONCLUSION

The frequency of *H. pylori* was found to be 81.3% in Yozgat region and it was often mildly positive (+). In addition, the prevalence of *H. pylori* was similar between men and women, consistent with the literature. The most common diagnosis was reported as "chronic active gastritis". Gastric ulcer frequency was 6%, duodenal ulcer frequency was 2.2%, gastric poly frequency was 1.8%, gastric cancer was 1.3%, and esophageal cancer was 0.1%. As a result, with this study, we have presented the upper gastrointestinal endoscopy data of our region to the literature and it is the first study conducted in the Yozgat region.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Yozgat Bozok University Clinical Researchs Ethics Committee (Date: 14.04.2021, Decision No: 2017-KAEK-189_2021.04.14_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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Distribution of clinical isolates obtained from sterile body fluids: a four-year retrospective data

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ABSTRACT

Aim: Infections of sterile body fluids (SBFs) require rapid and accurate diagnosis and treatment, since their morbidities and mortalities are high. To achieve this goal, definite epidemiologic data is absolutely required, since empiric and preemptive treatments are mainly based on this. The aim of this study was to evaluate infectious agents isolated from SBFs, susceptibility results and molecular analysis (PCR) data, retrospectively.

Material and Method: Clinical samples of SBFs (Cerebrospinal, pleural, peritoneal, pericardial and synovial fluids) obtained from January 2017 to December 2020 in Atatürk City Hospital (tertiary center) were included. Identification of bacterial and fungal agents and antibiotic susceptibility were done by conventional and automated system (BD Phoenix™, Becton Dickinson Co., Sparks, MD, USA). Löwenstein-Jensen media and BACTEC Mycobacteria Growth Indicator Tube 960 (Becton Dickinson Co., Sparks, MD, USA) were used for mycobacterial analysis. Bosphore Viral Meningitis Panel Multiplex PCR Kit (Anatolia Geneworks, İstanbul, Turkey) were applied to detect HSV-1, HSV-2, VZV, Enterovirus and/or Parechovirus.

Results: A total of 221 (9.74%) organisms were detected among 2269 samples. Particularly common gram negative bacterial agents covered the top of the list (*Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp. and *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex). *Staphylococcus aureus* was the most frequent gram positive strain, followed by enterococci. Most of the *A. baumannii* isolates were multidrug resistant, *Pseudomonas* spp. showed over than 20% resistance rate to ceftazidime, cefepime and piperacillin-tazobactam. All enterococci were vancomycin-susceptible, one *S. aureus* strain was methicillin-resistant. All *Mycobacterium tuberculosis* complex isolates were found to be susceptible to first-line anti-tuberculosis drugs.

Conclusions: Continuous laboratory surveillance even in local phase is important to guide clinicians. Even though our data did not show significant changes, improvements on laboratory capabilities and clinical awareness must be done. Isolation rates might be underestimated due to requirement of improvements in our laboratory, especially about sampling, anaerobe transportation and usage of blood culture vials.

Keywords: Meningitis, pleuritis, infection, sterile body fluids

INTRODUCTION

Body fluids from sterile sites such as cerebrospinal (CSF), pleural, peritoneal, pericardial and synovial fluids are defined as sterile body fluids (SBFs). The crucial point from microbiologic view is that any growth on culture of these fluids is accepted as causative agent, except any suspicion of contamination. These samples are obtained via invasive procedures and preanalytic transportation to laboratories has great importance. Exact identification of microorganisms to at least genus level (preferably species level) directly guides clinicians to the appropriate treatment, even in the shortage of susceptibility tests. Generally, a wide spectrum of microbiological analysis for SBFs is required, including bacterial, fungal, viral and mycobacterial agents (1-3).

Meningitis, pericarditis, pleural infection (either complicated parapneumonic effusion or empyema) and septic arthritis cover major forms of SBFs infections. The most frequent causative agents of these infections are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenza*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* complex, *Candida* spp. and viruses (2,3). Many factors including

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infection site, age, immune status and underlying pathologies have major effect on species epidemiology of etiologic agent. Diagnosis depends on several methods such as culture, PCR and serology (4-8).

Infections of SBFs require rapid and accurate diagnosis and treatment, since their morbidities and mortalities are high. To achieve this goal, definite epidemiologic data is absolutely required, since empiric and preemptive treatments are mainly based on this. Furthermore, due to epidemiologic variations between geographic locations, local data of infections are crucial, since they create a base for national management guidelines. The aim of this study was to evaluate infectious agents isolated from SBFs, susceptibility results and molecular analysis (PCR) data, retrospectively.

MATERIAL AND METHOD

The study was carried out with the permission of Balıkesir University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 11.11.2020, Decision No: 2020/202). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Clinical samples of SBFs (CSF, pleural, peritoneal, pericardial and synovial fluids) obtained from January 2017 to December 2020 in Atatürk City Hospital (tertiary center) were included in the study. Identification of bacterial and fungal agents were done by conventional and automated system (BD Phoenix™, Becton Dickinson Co., Sparks, MD, USA). Antibiotic susceptibility tests were also performed by the same system and evaluated according to "The European Committee on Antimicrobial Susceptibility Testing (EUCAST)" criteria (9). Antifungal susceptibility tests were not applied.

Mycobacterial analysis were performed by Löwenstein-Jensen (LJ) media (RTA Laboratories, Kocaeli, Turkey) and BACTEC "Mycobacteria Growth Indicator Tube" (MGIT) 960 (Becton Dickinson Co., Sparks, MD, USA) automated system. Susceptibility to the first-line antituberculosis drugs (Isoniazid-INH, rifampicin-RIF, ethambutol-ETM, streptomycin-SM) were also analyzed with the same system according to manufacturer's guidelines.

In patients with suspicion of viral meningitis, Bosphore Viral Meningitis Panel Multiplex PCR Kit (Anatolia Geneworks, İstanbul, Turkey) were applied to detect Herpes simplex virus-1 (HSV-1), Herpes Simplex Virus-2 (HSV-2), Varicella-Zoster Virus (VZV), Enterovirus (Coxsackie A ve B, Echovirus, Poliovirus and Enterovirus 68 – 71) and/or Parechovirus nuclear material.

Statistical Analysis

Retrospective definitive analysis was done. n (numbers) and ratios (%) were shared. SPSS 22.0 (SPSS, IBM, Chicago, IL, USA) programme was used.

RESULTS

The majority of sterile samples were pleural fluid (n=1105), followed by peritoneal fluid (n=540), CSF (n=440), synovial (n=183) and pericardial fluid (n=1), respectively. A total of 221 (9.74%) agents were observed (157/540 in peritoneal fluid; 41/1105 in pleural fluid; 15/440 in CSF; 8/183 in synovial fluid and no isolation in pericardial fluid). **Table 1** shows distribution of etiologic agents and ratios of isolated microorganisms. Bacterial strains had a dominance, particularly common gram negative bacterial agents covered the top of the list (*Escherichia coli*, *Pseudomonas* spp., *Klebsiella spp*. and *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex). *Staphylococcus aureus* was the most frequent gram positive strain, followed by enteroccocci.

Most of the *A. baumannii* isolates were multidrug-resistant (MDR) showing carbapenem resistance (90%) and amikacin resistance (60%), thus, colistin seemed to be the only therapeutic option (data not shown). On the other hand, *Pseudomonas* spp. showed over than 20% resistance rate to ceftazidime, cefepime and piperacillin-tazobactam. All enterococci were vancomycin-susceptible, however one *S. aureus* strain was methicillin-resistant (MRSA). Cephalosporin, fluoroquinolone and co-trimoxazole resistance were in seriously threatening levels for *E. coli* (over than 30%). **Table 2** shows antibiotic susceptibility results of *E. coli*, *K. pneumoniae* and *Pseudomonas* spp. All *Mycobacterium tuberculosis* complex isolates detected in SBFs were found to be susceptible to first-line anti-tuberculosis drugs.

DISCUSSION

Infections of SBFs are one of the most frequent infections in developing countries. The microorganism spectrum show extensive variation due to several factors such as antibiotic administrations, methodological differences and patient factors (underlying disease, surgeries, etc). In overall, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *Citrobacter* spp and *Acinetobacter* spp. are the most frequently isolated species among gram negative bacteria, while *S. aureus*, *Streptococcus pneumoniae* and enterococci are among gram positive ones. On the other hand, the question of the most common causative agent remains unanswered, since several studies indicated different results (10,11).

Many experts and stewardship programs published different guidelines to manage and treat SBF infections, especially focusing on meningitis (12,13). All these guides mainly depend on intensive antimicrobial therapies according to the causative agent. Thus, awareness of epidemiology even in local and/or national level directly affects the empiric and preemptive treatments, since epidemiological variations are severe.

Table 1. Infectious agents isolated from SBFs and frequency (%)

Types of infectious agents n (%)	Microorganism	Overall n (%)	Peritoneal fluid n (%)	Pleural fluid n (%)	CSF n (%)	Synovial fluid n (%)
Bacterial Agents (n=234; 94.4%)						
	E. coli	115 (52.03)	108 (48.87)	4 (1.81)	2 (0.90)	1 (0.45)
	Pseudomonas spp.	25 (11.31)	16 (7.23)	8 (3.62)	None	1 (0.45)
	Klebsiella spp.	17 (7.69)	13 (5.88)	2 (0.90)	2 (0.90)	None
	A. baumannii	10 (4.52)	3 (1.36)	3 (1.36)	4 (1.81)	None
	S. aureus	9 (4.07)	2 (0.90)	4 (1.81)	None	3 (1.36)
	Enterococcus spp.	8 (3.62)	3 (1.36)	2 (0.90)	1 (0.45)	2 (0.90)
	S. maltophilia	6 (2.71)	1 (0.45)	5 (2.26)	None	None
	S. pneumoniae	4 (1.81)	None	3 (1.36)	1 (0.45)	None
	Group B Streptococci	4 (1.81)	4 (1.81)	None	None	None
	Proteus spp.	3 (1.36)	1 (0.45)	2 (0.90)	None	None
	Citrobacter spp.	2 (0.90)	2 (0.90)	None	None	None
	Enterobacter spp.	2 (0.90)	2 (0.90)	None	None	None
	Serratia spp.	2 (0.90)	1 (0.45)	None	1 (0.45)	None
***Mycobacterium* spp. (n=6; 2.4%)**						
	M. tuberculosis complex	6 (2.71)	None	5 (2.26)	None	1 (0.45)
Fungal Agents (n=6; 2.4%)						
	C. albicans	3 (1.36)	1 (0.45)	1 (0.45)	1 (0.45)	None
	C. parapsilosis complex	2 (0.90)	None	1 (0.45)	1 (0.45)	None
	C. tropicalis	1 (0.45)	None	1 (0.45)	None	None
Viral Agents (n=2; 0.8%)						
	HSV-1	2 (0.90)	None	None	2 (0.90)	None
Total		**221 (100.0)**	**157 (71.04)**	**41 (18.55)**	**15 (6.79)**	**8 (3.62)**

Table 2. Resistance rates of particular strains (%) (Modified from references 15,18,19)

Antibiotic	This Study			CAESAR 2020[b] (R-Rate-%)			EU/EEA Country Range (R-Rate-%) (EARS-Net; 2015-2019)			SENTRY (R-Rate-%) (20-Year BSIs Surveillance)	
	E. coli (n=115)	*Klebsiella* spp. (n=17)	*Pseudomonas* spp. (n=52)	*E. coli*	*K. pneumoniae*	*Pseudomonas* spp.	*E. coli*	*K. pneumoniae*	*Pseudomonas* spp.	*Enterobacterales*	*Pseudomonas* spp.
AK	None	17.6	7.7	2.0	27.0	14.0	ID	ID	ID	1.3	7.6
AG[c]	11.3	23.5	NA	26.0	45.0	21.0	4.7-24.4	3.5-57.3	0.3-48.9	11.0	17.4
MEM	2.6	29.4	36.5	3.0	39.0	38.0	0.0-1.6	0.0-58.3	0.0-55.4	1.0	16.3
IMP	1.7	29.4	36.5							1.7	20.0
ERT	11.3	35.3	51.9	9.0	51.0	ID	ID	ID	ID	ID	ID
CRO	31.3	35.3	48.1	53.0	73.0	ID	6.2-38.6	4.3-75.7	ID	15.7	ID
CAZ	28.7	35.3	42.3	47.0	70.0	28.0			3.5-52.2	ID	17.4
FEP	29.6	35.3	32.7	ID	ID	31.0	ID	ID	ID	7.7	10.1
AMC[a]	45.2	41.2	NA	61.0	75.0	ID	ID	ID	ID	ID	ID
TZP	6.1	35.3	NA	22.0	60.0	34.0	ID	ID	2.3-52.8	5.6	14.9
TMP-SXT	37.4	29.4	NA	ID	ID	ID	ID	ID	ID	25.3	ID
FQ	24.3	35.3	NA	52.0	65.0	35.0	11.3-43.5	4.3-66.9	4.5-52.2	17.5	23.0

AK: Amikacin; AG: Aminoglycoside; MEM: Meropenem; IMP: Imipenem; ERT: Ertapenem; CRO: Ceftriaxone; CAZ: Ceftazidime; FEP: Cefepime; AMC: Amoxicillin-clavulanic acid; TZP: Piperacillin-tazobactam; TMP-SXT: Trimethoprim-sulfamethoxazole; FQ: Fluoroquinolones; ID: Insufficient Data; NA: Not applicable
[a]Aminopenicillin susceptibility of *Enterobacterales* is for intravenous administration. [b]Data of Turkey. [c]Indicates gentamicin/netilmicin/tobramycin.

Some reports indicated that gram positive bacteria including *S. pneumoniae* and staphylococci showed a dominance, however, there are also studies stating gram negative superiority, like this study (10,11,14). According to Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) report of Turkey, which included CSF and blood culture (BC) data, even though it was followed by *Acinetobacter* spp., *S. pneumoniae* had a few times more CSF isolation counts than the other strains. On the other hand, BC data seems to be different, since gram negative ones counted with a huge superiority (15). Contrary results were observed in a ten-year BC study from Turkey, since coagulase-negative staphylococci (CoNS) took the largest counts (16). The Turkish National Antimicrobial Resistance Surveillance System (UAMDSS) focuses on antimicrobial resistance (AMR) of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. pneumoniae*, *Enterococcus faecium*/*faecalis* and *A. baumannii* that were isolated from CSF and BCs. These strains were particularly under surveillance, since they were the most frequently isolated ones and they showed the most problematic resistance patterns (17). Of note, it is obvious that BC and other SBF data should be separated, because their microbiologic cultivation and management procedures are also different.

International antimicrobial stewardship programs supported by global organizations like World Health Organization (WHO), The Centers for Disease Control and Prevention (CDC) and The European Centre for Disease Prevention and Control (ECDC), and national authorities (Public Health Directorate of Turkey) specifically endorse a continuous surveillance on AMR, especially for particular species and infections. In this study, despite of only a limited data of pathogens were shared (**Table 2**), it can be observed that AMR is a serious issue for common species. The CAESAR, The SENTRY Antimicrobial Surveillance Program (20-Year bloodstream infections-BSIs data) and The European Antimicrobial Resistance Surveillance Network (EARS-Net) data were also shared in **Table 2** (15,18,19). Although our sample size is much smaller and our data was based on samples other than BCs, our rates showed a slightly lower resistance profile, which might be explained with this sample differences. On the other hand, regarding EU/EEA Country Ranges in the EARS-Net data, it is obvious that our facility is in the "high-threat" zone (19). MDR rates of *Acinetobacter* spp., β-lactam resistance of *Pseudomonas* spp., cephalosporin, fluoroquinolone and co-trimoxazole resistance of *E. coli* were major observations. On the other hand, it is very promising that there was not any vancomycin-resistant enterococci (VRE) and MDR-tuberculosis cases, and there was only one MRSA strain, while UAMDSS and CAESAR reports stated opposite results (15,17).

Recently, there are some reports that strongly recommended cultivation of SBFs with BC vials. Nowadays, there is a microbiological consensus that this method is very beneficial especially for reducing time to detection and increased growth ability of fastidious microorganisms (20,21). In our facility, this technique is also recommended by our laboratory; however it is rarely preferred by clinicians. Thus, this might be a limitation of this study, since our isolation rates might have been underestimated. However, internationally-suggested methods are applied in our laboratory, so we believe this should be a minor effect. Another limitation is that anaerobic microbiological analysis cannot be performed in our laboratory due to physical issues. We believe anaerobes could also be causative agents in our infection profiles, but they were not able to be detected by our laboratory. Thirdly, we could not reach to any information of empiric and preemptive treatments applied to the patients and time of sampling. These patients generally required such kind of therapy, and time of sampling (before or after treatment) directly affects the isolation of the agent, especially for bacterial and fungal infections. If so, isolation rates might have also been underestimated.

CONCLUSION

Continuous laboratory surveillance even in local phase is important to guide clinicians. Data of this study did not seem to show any significant change in epidemiology, but it revealed that clinic and laboratory communication requires extensive improvement, especially about sampling, anaerobe transportation and usage of BC vials. Our laboratory also needs to focus on anaerobic cultivation methods. On the other hand, it is promising that any potential contaminant strain (E.g.; CoNS, gram positive rods) was not observed at any stage of cultivation. More data should be collected to modify national guidelines.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Balıkesir University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 11.11.2020, Decision No: 2020/202).

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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Silicosis and methylated arginines/L-arginines: case-control adapted a cross-sectional design

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ABSTRACT

Aim: Silicosis has long been recognized as an important occupational lung disease that is included in the group of pneumoconiosis. As already well-known silicosis is a progressive pneumoconiosis characterized by fibrosis in the lungs. Also, chronic exposure to silica may cause chronic obstructive pulmonary disease, emphysema, lung cancer, and pulmonary fibrosis. Asymmetric dimethyl arginine (ADMA), symmetric dimethyl arginine (SDMA), and L-NMMA (NG-mono-methylated-L-arginine) are the products of protein arginine methyltransferase (PRMT) enzymes. The aim of this study is to investigate the relationship between silicosis and arginine metabolites in silica exposed and non-exposed workers.

Material and Method: 180 male subjects (90 non-exposed workers (age matched-control) and 90 workers diagnosed with silicosis occupational physician based on radiological and clinical findings and exposure history-(silica-exposed) were included in this study. The serum levels arginine, ADMA, SDMA, and L-NMMA were determined using enzyme-linked immunosorbent assay.

Results: ADMA, SDMA, L-NMMA values were significantly higher in the silica-exposed group compared to the control group. The positive correlations were observed between methylated arginine parameters such as ADMA and SDMA, ADMA and L-NMMA levels ($r=0.43$, $r=0.60$; $p<0.01$). The negative correlations were found between SDMA and arginine/ADMA, L-NMMA, and arginine/ADMA, arginine and SDMA/ADMA, arginine/ADMA and SDMA/ADMA levels, respectively ($r=-0.22$; $r=-0.22$, $r=-0.34$, $r=-0.29$; $p<0.01$). The strongest positive correlation was found between arginine and arginine/ADMA ratio, and the strongest negative relationship between ADMA and arginine/ADMA ratio, respectively ($r=0.87$; $r=-0.48$; $p<0.01$).

Conclusion: The results could provide additional insight into understanding the disease and the potential for developing biomarkers.

Keywords: Silicosis, arginine, Asymmetric dimethyl arginine, symmetric dimethyl arginine, NG-mono-methylated-L-arginine

INTRODUCTION

Silicosis has long been recognized as an important occupational lung disease that is included in the group of pneumoconiosis. The risk increases due to the concentration of dust, the percentage of silica in the dust, and the duration of total exposure. The total burden of silica in the body is mainly directly related to the risk of pneumoconiosis. Silicon dioxide has two forms as amorphous and crystalline. The amorphous form does not seem to cause severe pulmonary disease. Histologically, it can be defined as a diffuse interstitial fibro nodular lung disease. In the post-mortem examinations of silicotic lungs, the tissue is characterized with hilar and parenchymal lymphadenopathies and silicotic nodules, generally located in the upper lobes of the lung. Crystalline silica particles can be seen in a polarized light microscope (1).

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Asymmetric dimethyl arginine (ADMA), symmetric dimethyl arginine (SDMA) and L-NMMA (NG-mono-methylated-L-arginine) are the products of protein arginine methyltransferase (PRMT) enzymes (2). As nitric oxide (NO) has reactive oxygen species (ROS) scavenging functions and promotes the activation of endogenous anti-oxidant defense systems, ADMA can contribute ROS imbalance by reducing NO bioavailability and invoke superoxide production (3,4). The mechanism underlying between silicosis and inflammation is still unknown, but several studies provide many results supporting this link. Many cellular mechanisms can contribute to the inflammation progress in silicosis. The inflammatory cytokines (interleukin (IL)-8, IL-6), arachidonic acid metabolites (leukotrienes, prostaglandins), transcription factors ((nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) and activator protein-1), and chemokines such as macrophage inflammatory protein (MIP)-2, MIP-1α, MIP-1β and monocyte chemoattractant proteins are involved in the inflammatory process. There are also some experimental studies which have shown to decrease inflammation and production of MIP-2, preventing the subsequent pulmonary fibrosis and silicosis (1,5) ADMA, SDMA and L-NMMA are first extracted in human urine in 1970's (6). Since then, there have been many publications on these metabolites and their relationship with various diseases. Most of them has been highlighted that ADMA contributed in endothelial dysmorphology and can be selected as a screening marker. It has been found to be elevated in the serum in cases of renal insufficiency, cardiovascular diseases and atherosclerosis, renal failure, hyperglycemia and hyperhomocysteinemia (2,4,6–8). The studies evaluating the relationship between arginine metabolites and pulmonary diseases, different hypotheses have been suggested (11,12). However, there is lack of knowledge about the relationship silicosis and arginine metabolites. We aim to identify an expanding role of serum ADMA, SDMA and L-NMMA levels in silica exposed and non-exposed workers.

MATERIAL AND METHOD

This study was ethically approved by the Non-interventional Clinical Researchs Ethics Committee of the Bozok University Medical Faculty (Date: 17.10.2016, Decision No:17/04). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Groups

180 male subjects (90 non-exposed workers (age matched-control) and 90 workers diagnosed with silicosis occupational physician based on radiological and clinical findings and exposure history-(silica-exposed) were included in this study (13). Serum samples taken as a result of routine examinations were collected from denim bleaching workers diagnosed with silicosis and people in the control group who did not show any symptoms or radiological findings despite working in the same environment. Control group and study group consist of non-smoker or ex-smoker (more than 10 years) workers. In the study, consent forms containing questions about the socio demographic variables, medical history and workplace conditions of both control and exposed groups were filled and strictly adhered to the principles of the Declaration of Helsinki. The workers with chronic diseases (coronary heart disease, hypertension, diabetes, etc.), and acute infections were excluded in the study. The patients were selected among Category 2 patients classified according to ILO pneumoconiosis classification. Oral and written informed consent was granted by all women involved in the study. Serum samples for arginine, ADMA, SDMA, and L-NMMA levels were collected as biological material from the individuals. At the end of the shift week, first morning voiding venous blood were collected into tubes (BD Vacutainer, USA), than centrifuging the samples at 3500×g for 10 min at 4°C, serum were separated and stored at −80°C further until transfer samples. The samples were transferred in boxes in ice molds to Yozgat Bozok University Science and Technology Application and Research Center (BILTEM) for measuring arginine, ADMA, SDMA and L-NMMA levels.

Laboratory Analysis

Within the scope of the study, the analysis and preliminary preparation processes of the samples that were thawed in accordance with the commercial kit procedure were performed. The serum arginine, ADMA, SDMA and L-NMMA levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions. The reading of the samples placed on the microplates was done with a BMG LABTECH ClarioStar model ELISA device with a wavelength of 450 nm. Control materials used for optimization and for validity of the ELISA methods. Control samples were used for verification. The analysis was done in 5 points according to the calibration curves created against the standard measurements in the ELISA device. Each kit was read at least 10 times and regression analysis was performed by taking the averages.

Statistical Analysis

Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 25.0) program was used for the analysis of all parameters. The data were evaluated in terms of compliance with normal distribution using the Kolmogorov-Smirnov test. Descriptive statistics were

presented with mean, standard deviation, median and minimum-maximum values. Since it was determined that the parameters were not compatible with the normal distribution, the Mann Whitney U test was used to evaluate the status of two independent variables relative to each other. The relationship between variables was determined by Spearman Correlation Analysis. P values of <0.05 and <0.01 were considered as statistically significant.

RESULTS

The 90 non-exposed workers and 90 silica-exposed workers included in the study were available for measuring arginine, ADMA, SDMA, and L-NMMA levels. **Table 1** shows the differences in methylated arginine between the study groups. Arginine, arginine /ADMA and SDMA/ADMA values were higher in the silica-exposed group, but this difference was not statistically significant (p>0.05). Also, arginine levels acquired high in exposed groups than control as 84.88±89.16µmol/L and 73.79±44.72 µmol/L (p>0.05). However, ADMA, SDMA, L-NMMA values were significantly higher in the silica-exposed group compared to the control group. The age and body mass index (BMI) were found not different statistically between the groups (p>0.05). The working experience control and silica-exposed groups were observed similar 6.21±3.31 years and 5.38±3.52 years, respectively (p>0.05). On the other hand, median of arginine/ADMA and SDMA/ADMA ratios different groups (control and silica-exposed) were found as 495.94 and 460.49; 1.36 and 1.38, respectively; but these differences were not statistically significant (p>0.05).

The Spearman correlations methylated arginine levels showed in **Table 2**. The positive correlations were observed between methylated arginine parameters such as ADMA and SDMA, ADMA and L-NMMA levels (r=0.43, r=0.60; p<0.01). The negative correlations were found between SDMA and arginine/ADMA, L-NMMA and arginine/ADMA, arginine and SDMA/ADMA, arginine/ADMA and SDMA/ADMA levels, respectively (r= -0.22; r= -0.22, r= -0.34, r= -0.29, p<0.01). The strongest positive correlation was found between arginine and arginine/ADMA ratio, and the strongest negative relationship between ADMA and arginine/ADMA ratio, respectively

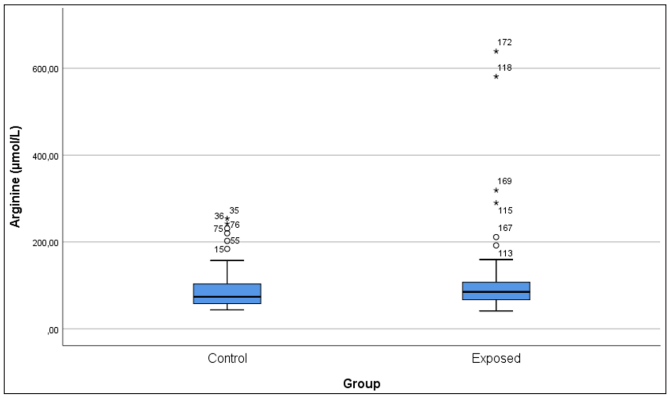

Figure 1. The relationships arginine levels between control and silica-exposed groups.

Table 2. The Spearman correlations methylated arginine levels.

	Arginine	ADMA	SDMA	L-NMMA	Arginine/ADMA	SDMA/ADMA
Arginine	1	-0.04	-0.03	0.05	.87**	-.34**
ADMA		1	.43**	.60**	-.48**	-0.08
SDMA			1	-0.04	-.22**	-0.02
L-NMMA				1	-.22**	-0.08
Arginine/ADMA					1	-.29**
SDMA/ADMA						1

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level. n=180. ADMA: Asymmetric dimethyl arginine, SDMA: symmetric dimethyl arginine, L-NMMA: NG-mono-methylated-L-arginine

Table 1. The relationships main parameters between control-exposed groups.

	Group"	Mean	Median	Std. Deviation	Minimum	Maximum	p
Arginine (µmol/L)	0	88.98	73.79	44.72	43.50	254.10	0.133
	1	105.50	84.88	89.16	41.00	639.10	
ADMA (µmol/L)	0	0.16	0.16	0.03	0.11	0.28	0.001**
	1	0.19	0.18	0.04	0.12	0.30	
SDMA(µmol/L)	0	0.21	0.21	0.05	0.10	0.35	0.001**
	1	0.24	0.24	0.04	0.15	0.38	
L-NMMA (µmol/L)	0	0.02	0.02	0.01	0.01	0.05	0.001**
	1	0.03	0.03	0.01	0.02	0.06	
Arginine/ADMA ratio	0	560.74	495.94	288.06	197.09	1694.00	0.522
	1	587.65	460.49	472.15	176.10	3363.68	
SDMA/ADMA ratio	0	1.36	1.36	0.29	0.79	2.17	0.756
	1	1.37	1.38	0.34	0.61	2.21	

"0: Control (n=90). 1: Exposed (n=90); ** p<0.01. ADMA: Asymmetric dimethyl arginine, SDMA: symmetric dimethyl arginine, L-NMMA: NG-mono-methylated-L-arginine

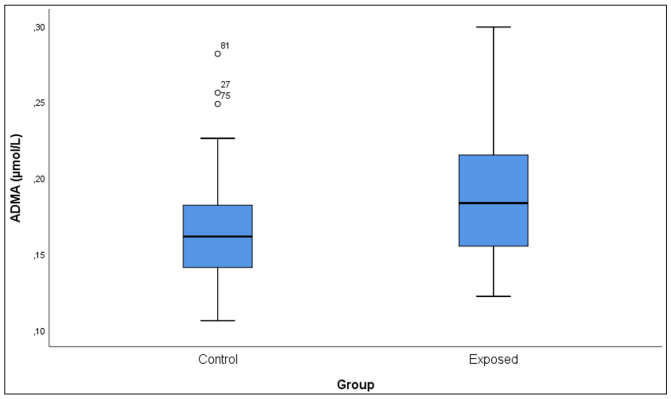

Figure 2. The relationships ADMA levels between control and silica-exposed groups.

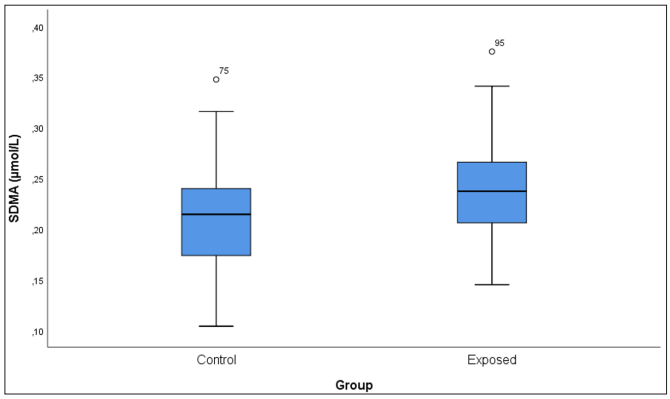

Figure 3. The relationships SDMA levels between control and silica-exposed groups.

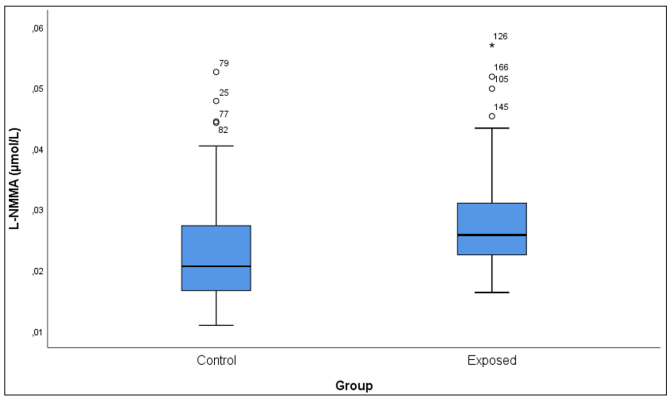

Figure 4. The relationships L-NMMA levels between control and silica-exposed groups.

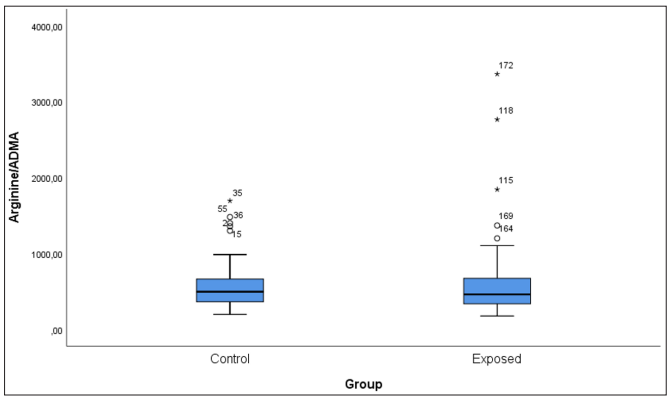

Figure 5. The relationships arginine/ADMA ratios between control and silica-exposed groups.

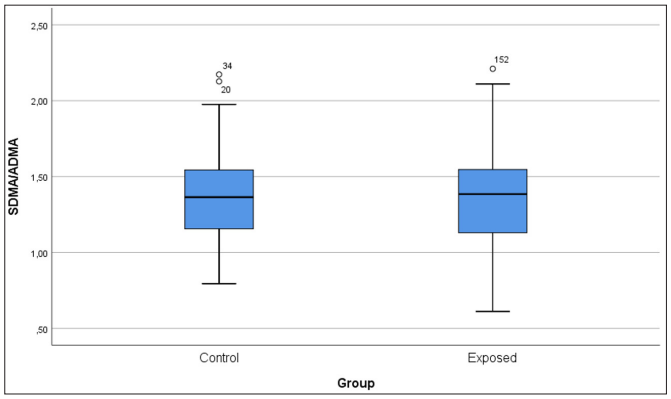

Figure 6. The relationships SDMA/ADMA ratios between control and silica-exposed groups.

(r=0.87; r= -0.48; p<0.01).

DISCUSSION

The arginine and NO pathway for many diseases and mechanism is well known. ADMA, SDMA, L-NMMA values are significantly higher in the many acute and chronic diseases especially based on hypoxemic mechanism and endothelial dysfunctions (7,9,14,15). Despite, such extensive and comprehensive studies, the role of arginine pathway in the pathogenesis of silicosis has not been studied in detail. In our study, ADMA, SDMA, L-NMMA values were significantly higher in the silica-exposed group compared to the control group. In contrast to these diseases in which the mechanism of endothelial dysfunction is predominant (16,17), our study provide information regarding the relationship between the toxicity of silica stored in the pulmonary parenchyma and serum ADMA, SDMA and L-NMMA levels. The results not only indicate a correlation between ADMA, SDMA and L-NMMA, and silicosis, but also provide a discussion understanding of the role of the arginine in the silicosis.

The studies evaluating the relationship between arginine metabolites and pulmonary diseases, different hypotheses have been suggested. Airway diseases such as asthma, chronic obstructive pulmonary disease, increased arginase activity in the bronchies may contribute to bronchi obstruction and hyper responsiveness of the airways by reducing of bronchodilatory NO levels and airway remodeling mechanisms. In several animal studies of silicosis, arginase activity and arginase pathway has been also increased in the parenchyma and alveolar macrophages (16,18-20). Wells et al. (11) performed an experimental study by using continuous subcutaneous ADMA infusion to lung fibroblasts and showed altered collagen deposition in the lungs. They state ADMA is major metabolites by inhibiting arginine metabolism in epithelial cells and report that NO is effective on inflammation pathways, as well. Kitowska et al. (21) hypothesize that l-arginine

metabolism is changed in pulmonary fibrosis by affecting extra cellular matrix synthesis.

As already well known silicosis is a progressive pneumoconiosis characterized by fibrosis in the lungs. Also, chronic exposure to silica may cause chronic obstructive pulmonary disease, emphysema, lung cancer, and pulmonary fibrosis (22). It has complex pathophysiological mechanisms and involves many systematic, cellular and molecular arrangements. Crystalline silica entering the airways, triggers a number of inflammatory processes (23,24). The repeated process of defense circle, the macrophages induces activation of the reactive oxygen radicals and inflammatory reactions, which is linked with pulmonary interstitial fibrosis mechanism (25,26). On the other hands, the role of arginine pathway in the pathogenesis of pneumoconiosis has not been studied in detail. Xue et al. (27) studied distinct metabolic features in the silicosis. They stated sphingolipid and arginine and proline metabolism were the dominant metabolic pathway in silicosis and arginine was positively correlated with the stage of silicosis. Increased levels of some inflammatory markers IL-8 and IL-6, tumor necrosis factor, and IL-1β, -were elevated in fibrotic lung tissue of silica-induced lung damage compared with normal lung tissue. (clear form of abbreviations should be stated where they are first used.) In the early pathogenesis of silicosis, the enhancement of arginine metabolism and is closely related to the severity of the fibrosis (28,29) In the end stage, the formation of silicotic micro nodules is the pathological manifestation of the production of collagen bres and pulmonary interstitial fibrosis mediated by amino acid metabolism, which is consistent with the recent studies that arginine pathway and extracellular matrix synthesis were the dominant mechanism in silicosis (27).

ADMA is similar to arginine in terms of molecular structure and it competes with arginine for NOS binding, thus blocking the production of NO from arginine by NOS directly. Since, ADMA competes with arginine for NOS, the bioavailability of NO depends on the equation between the two, the so-named arginine/ADMA ratio (30). Keller et al. (31) stated that the arginine/ADNA ratio is a more sensitive risk marker in cardiac diseases. In our study arginine/ADMA and SDMA/ADMA ratios were not significantly different exposed versus non exposed groups. Also in correlation analysis we found positive correlation between arginine and arginine/ADMA ratio. Molnar et al. (14) assessed L-arginine, arginine/ADMA ratio in stroke patients. They stated that arginine levels may tend to both increase and decrease and different results can be found in different stages of silicosis.

Arginine, ADMA, SDMA, L-NMMA studies show improvement (32,33). An increasing number of studies have been reported on the use them in the early diagnosis, treatment and follow-up of diseases. Major promising improvement has been made in research on ADMA-lowering therapies in cancer, cardiovascular diseases and so on. However, further human studies are needed in order to treat human diseases related to elevated ADMA/SDMA levels (34–36). Furthermore, the studies that L-arginine may have a predictive role in monitoring silicosis and many other diseases, as well (27,37). Several studies stated that ADMA and oxidative stress biomarkers as a screening biomarker for pulmonary arterial hypertension and silicosis, COPD respectively (38–40). Pacheco et al. (41) stated macrophages polarize to M2 phenotype in response to arginase, and in this case they are involved in the inflammation resolution and tissue repairs. However, different results regarding these studies are also reported (17). Further evaluations with larger study groups are necessary.

Strengths and Limitations

Some strengths and limitations should be discussed. The case and control group matched in terms of age and working year were included in the study are the strengths of this study, as it reduces confounding factors. On the other hand, despite the absence of environmental silica measurements, the absence of an evaluation of the disease stages of silicosis patients with a relatively small number of cases are main limitations in our study. Finally, given the cross-sectional design, the study has limited power to explain the potential metabolic biomarkers for disease progression and survival, which are clinically significant.

CONCLUSION

In conclusion, the diagnosis and screening of silicosis basically depend on a history of silica exposure and radiological findings. Recent studies on pathophysiological mechanisms and markers to understand the nature of silicosis have a great contribution to better understanding silicosis. Our small study showed the relationship between silicosis and arginine pathway. The results could provide additional insight into understanding the disease and potential for developing biomarkers. We believe that the role of ADMA, SDMA and arginine in the diagnosis, screening, treatment and follow-up of silicosis will be better understood with future studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was ethically approved by the Non-interventional Clinical Researchs Ethics Committee of Bozok University Medical Faculty (Date: 17.10.2016, Decision No:17/04).

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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Impact of quality of life on nutritional risk and malnutrition: a cross-sectional study in elderly patients

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ABSTRACT

Objectives: To investigate prevalence of malnutrition and nutritional risk, related factors, and the association between nutritional status and quality of life in the elderly.

Material and Method: Cross-sectional study. Participants; elderly (≥65 years) patients without severe hearing and vision impairment and dependency on a bed or wheel chair who admitted internal medicine outpatient clinics. Measurements; the mini nutritional assessment questionnaire, Charlson comorbidity index, the World Health Organization quality of life (WHOQOL-OLD) questionnaire. Intervention; none.

Results: The study included 532 patients with a mean age of 70.8±5.4 years. The median Mini Nutritional Assessment score was 26, and the total prevalence of nutritional risk and malnutrition was 26.7%. There were significant differences between the nutritional groups regarding the educational, living, and marital status, smoking history, presence of any systemic disease, Charlson Comorbidity Index, and polypharmacy ($p<0.05$). WHOQOL-OLD and its subdomain scores had a significant relationship with having nutritional risk or malnutrition ($p<0.001$). Total WHOQOL-OLD score and the Charlson Comorbidity Index were significant independent risk factors for developing nutritional risk and malnutrition.

Conclusion: Having nutritional risk or malnutrition in the elderly were significantly associated with the Charlson Comorbidity Index and the WHOQOL-OLD total and its subdomain scores.

Keywords: Elderly, nutritional status, malnutrition, health-related quality of life, comorbidity.

INTRODUCTION

The aging population has emerged as a new demographic trend in recent years. There is an inverse relationship between increased life expectancy and quality of life, most probably due to chronic diseases and disability (1,2). Besides, a substantial proportion of community-dwelling elderly lacks an adequate nutrient intake leading to an increased risk for malnutrition (1,3,4). Food is not only required for physiological well-being, but it also contributes to social, cultural, and psychological quality of life (4). So, aging and increased risk of malnutrition lead to a low quality of life as well as physical, mental, and social disabilities (5).

As a significant and common public health problem in older adults, the prevalence of malnutrition and associated factors show substantial variations based on the nutritional screening tools and the studied population's characteristics (4,5). In previous studies, the rate of malnutrition ranged from 20 to 30% in clinical settings and from 2 to 8% in older adults in community-residences (2). Several physiological, socio-economic, and neuropsychological health-related factors influence the nutritional status, including depression, social isolation, and frailty status. Thus, the real-time knowledge about the prevalence of malnutrition in a specified population alerts the physicians dealing with older adults.

The assessment of nutritional risk is a strong determinant of interventions to prevent malnutrition and manage different aspects of quality of life, including its physical and mental components (1). Identifying people with malnutrition or nutritional risk includes some inherent difficulties (3). Besides the use of a diverse set of instruments for the evaluation of malnutrition and its risk, there is still a lack of a gold standard for the optimal definition of malnutrition. Nevertheless, the Mini Nutritional Assessment (MNA) has been recommended for its higher efficiency to predict malnutrition in the elderly (6,7).

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In this study, we aimed to determine the prevalence of malnutrition and associated factors and analyze the association between the nutritional status of the older adults indicated by the MNA and their quality of life indicated by the World Health Organization Quality of Life (WHOQOL-OLD) questionnaire.

MATERIAL AND METHOD

The study was carried out with the permission of Erzurum Regional Training and Researh Hospital Clinical Researchs Ethics Committee (Date: 06.01.2020, Decision No: 2020/01-06). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

The MNA questionnaire was applied to all consecutive patients who were 65 or older and admitted to the Internal Medicine Outpatient Clinics after obtaining their consent between 01.01.2020 and 01.07.2020. Patients who had communication problems such as severe hearing (n=235) and vision impairment (n=192) or were dependent on bed or wheelchair (n=59) were not evaluated. A total of 532 patients were included in the study.

Several sociodemographic and clinical variables, including age, sex, body mass index (BMI), educational and marital status, living status (alone, with relatives, or residential care center), history of smoking, comorbidities, and drugs used, were evaluated. The patients were classified based on BMI values as underweight (BMI < 18.5 kg/m2), normal (18.5–24.9 kg/m2), or overweight (BMI ≥ 25 kg/m2). Patients using five or more drugs were grouped as polypharmacy. The Charlson Comorbidity Index (CCI) was calculated for each patient using the website www.mdcalc.com/charlson-comorbidity-index (8).

Questionnaires and Scales

Nutritional status was evaluated using the MNA questionnaire that was translated and validated in the Turkish language (9). MNA includes 18 items for the anthropometric measurements, dietary intake, global health assessment, and self-assessment of health and nutrition. The patients were categorized as satisfactory nutritional status (scores ≥24), nutritional risk (scores between 17 and 23.5), and malnutrition (scores <17) (1).

The WHOQLD-OLD questionnaire was applied to the patients to assess their quality of life. The WHOQLD-OLD contains six different subdomains: sensory abilities, autonomy, past, present, future activities, social participation, death and dying, and intimacy (1). Higher scores represent a higher self-rated quality of life. The 30-item Geriatric Depression Scale (GDS) was used to evaluate depression. A GDS score >10 was regarded as depression (10, 11). Cognitive ability was assessed using the Mini-Mental State Examination (MMSE), commonly used to monitor dementia and cognitive states (12, 13). The MMSE score ranges from 0 (impaired) to 30 (normal). Scores below 24 were regarded as cognitive impairment (14). The Hendrich II Fall Risk Model (HIIFRM) was used to analyze the factors associated with fall risk. The HIIFRM score ranges from 0 to 16, where scores ≥5 were considered a high risk for falls (15).

Anthropometric Measurements

The anthropometric evaluation included body circumference (calf, arm) and triceps skinfold thickness. All measurements were carried out in the morning after an overnight fast of at least 8 hours. The BMI values were calculated as the bodyweight divided by the square of the height (kg/m2). The calf and arm circumferences were measured using a non-elastic tape measure. For calf measurements, the tape measure was placed around the calf without compressing the subcutaneous tissue and was moved along the calf's length to obtain the maximal circumference while the patient was lying down, and the leg was angled 90° at the knee. Calf circumference values were recorded as the average of the measurements from two trials for each leg, which were averaged again to get the overall measurement (16). Forearm circumference (mid-upper arm circumference) was measured at the midpoint between the acromion and olecranon bones while the arm was angled 90° at the elbow (17). For the measurement of triceps skinfold thickness, while the patients were standing and the arms were free at both sides, the arms were angled 90°, and the midpoint between the shoulder and the acromion was marked with a pencil. The skinfold was measured at the marked point. The anthropometric measurements were performed twice by experienced nurses and averaged later.

Biochemical analyses included hemoglobin (g/dL), fasting blood glucose (mg/dL), HbA1c (%), total cholesterol (mg/dL), low density lipoprotein (LDL) cholesterol (mg/dL), triglyceride (mg/dL), albumin (mg/dL), ferritin (ng/mL), vitamins B12 (pg/mL) and D (ng/mL) and B9 (folic acid) (ng/mL). Glomerular filtration rate (mL/min) was calculated using the Modification of Diet in Renal Disease (MDRD) formula (18).

Statistical Analysis

The primary outcome of the study was the prevalence of nutritional risk and malnutrition. The analysis of the factors associated with nutritional risk and malnutrition and the association between nutritional risk and the quality of life were the secondary outcomes.

Descriptive statistics were given as mean±standard deviation or median [interquartile range-IQR] for the continuous variables depending on their distribution.

Numbers and percentages were used for categorical variables. The Kolmogorov-Smirnov test was used to check normal distribution. The One-Way ANOVA test was used to compare more than two independent groups when the numerical variables had a normal distribution. For variables without normal distribution, the Kruskal-Wallis test was applied.

For the analyses in which non-parametric tests were used, the differences between the groups were evaluated with the Tukey test when data was homogeneous based on its distribution. The Games-Howell test was used in situations where data was not homogeneous. The Dwass-Steel-Critchlow-Fligner test was used to evaluate the differences between the groups. To compare the differences between categorical variables, Pearson Chi-Square and Fisher-Freeman-Halton tests were used in 2x2 tables. Spearman correlation coefficients were calculated to analyze the relationships between numerical variables. Univariate and multivariate logistic regression analyses were performed to analyze the demographic and clinical factors that impact nutritional risk and malnutrition. Statistically significant demographic and clinical characteristics in the univariate analysis were included in the multivariate analysis. For statistical analysis and figures, Jamovi (version 1.6.3, retrieved from https://www.jamovi.org) and JASP (version 0.13.1, retrieved from https://jasp-stats.org) software were used. The significance level (p-value cutoff) was set at 0.05 in all statistical analyses.

RESULTS

There were 532 patients with a mean age of 70.8±5.4 years. The female to male ratio was 1.1. The majority of the patients lived with their relatives. Although hypertension was the most significant comorbid disease (358 patients, 67.3%), there was no comorbidity in 249 patients (46.8%). The median CCI score was 4. The demographic and clinical characteristics of the study group were given in **Table 1**.

Table 1. Demographic and clinical characteristics of the study groups

	Overall (n=532)	Groups			p-values
		Satisfactory nutritional status (n=390)	Nutritional risk (n=83)	Malnutrition (n=59)	
Age (year)†	70.8±5.4	70.7±5.4	70.9±6.0	71.4±4.4	0.514
Sex‡					0.938
Male	253 (47.6)	187 (47.9)	38 (45.8)	28 (47.5)	
Female	279 (52.4)	203 (52.1)	45 (54.2)	31 (52.5)	
Educational status‡					**<0.001**
Illiterate	125 (23.5)	69 (17.7)[a]	28 (33.7)[b]	28 (47.5)[b]	
Primary	307 (57.7)	230 (59.0)[a]	46 (55.4)[a]	31 (52.5)[a]	
Secondary-college	76 (14.3)	67 (17.2)[a]	9 (10.8)[a]	0 (0.0)[b]	
University	24 (4.5)	24 (6.2)[a]	0 (0.0)[b]	0 (0.0)[a,b]	
Living status‡					**<0.001**
Alone	136 (25.6)	88 (22.6)[a]	33 (39.8)[b]	15 (25.4)[a,b]	
With relatives	344 (64.7)	291 (74.6)[a]	45 (54.2)[b]	8 (13.6)[c]	
Residential care center	52 (9.8)	11 (2.8)[a]	5 (6.0)[a]	36 (61.0)[b]	
Marital status‡					**<0.001**
Single	38 (7.1)	28 (7.2)[a]	5 (6.0)[a]	5 (8.5)[a]	
Married	333 (62.6)	289 (74.1)[a]	40 (48.2)[b]	4 (6.8)[c]	
Divorced/widowed	161 (30.3)	73 (18.7)[a]	38 (45.8)[b]	50 (84.7)[c]	
Smoking‡	208 (39.1)	145 (37.2)[a]	30 (36.1)[a]	33 (55.9)[b]	0.019
Comorbidity‡					
Hypertension	358 (67.3)	239 (61.3)[a]	62 (74.7)[b]	57 (96.6)[c]	**<0.001**
Diabetes mellitus	210 (39.5)	149 (38.2)	37 (44.6)	24 (40.7)	0.548
Any systemic disease	283 (53.2)	172 (44.1)[a]	54 (65.1)[b]	57 (96.6)[c]	**<0.001**
Systemic disease‡					**<0.001**
Absent	249 (46.8)	218 (55.9)[a]	29 (34.9)[b]	2 (3.4)[c]	
Coronary artery disease	84 (15.8)	66 (16.9)[a]	14 (16.9)[a,b]	4 (6.8)[b]	
Chronic heart failure	17 (3.2)	10 (2.6)[a]	5 (6.0)[a]	2 (3.4)[a]	
Chronic renal failure	40 (7.5)	34 (8.7)[a]	4 (4.8)[a]	2 (3.4)[a]	
Cerebrovascular accident	66 (12.4)	18 (4.6)[a]	11 (13.3)[b]	37 (62.7)[c]	
Chronic liver failure	10 (1.9)	7 (1.8)[a]	1 (1.2)[a]	2 (3.4)[a]	
Malignancy	23 (4.3)	6 (1.5)[a]	11 (13.3)[b]	6 (10.2)[b]	
Chronic obstructive pulmonary disease	43 (8.1)	31 (7.9)[a]	8 (9.6)[a]	4 (6.8)[a]	
Charlson comorbidity index§	4.0 [2.0-14.0]	4.0 [2.0-10.0][a]	5.0 [3.0-13.0][b]	8.0 [4.0-14.0][c]	**<0.001**
Polypharmacy‡	215 (40.4)	121 (31.0)[a]	42 (50.6)[b]	52 (88.1)[c]	**<0.001**
Mini Nutritional Assessment§	26.0 [6.0-29.0]	26.0 [19.0-29.0][a]	19.0 [11.0-26.0][b]	9.0 [6.0-17.0][c]	**<0.001**

†mean±standard deviation,‡n (%),§median (IQR), Each subscript letter denotes a subset of group categories whose column proportions do not differ significantly from each other at the 0.05 level.

The median MNA score was 26 [6-29]. Based on the MNA scores, 83 patients (15.6%) had nutritional risk, and 59 patients (11.1%) had malnutrition; 390 patients (73.3%) had satisfactory nutritional status. The combined prevalence of malnutrition (nutritional risk and malnutrition) was 26.7%. The comparison of different nutritional status (satisfactory nutritional status, nutritional risk, and malnutrition) for various variables was given in **Table 1**. Significant differences were found between the groups regarding their education, living and marital status, smoking history, systemic diseases, CCI scores, and polypharmacy status. Malnutrition was more common in patients with low education, those living in residential care centers, and those who were divorced or widowed (p<0.001 for each). Smoking was significantly associated with malnutrition (p=0.019). Hypertension and any systemic disease were more common in patients with nutritional risk and malnutrition (p<0.001 for both). Cerebrovascular accident was the most common systemic disease (62.7%) in the patients with malnutrition (p<0.001). The CCI score was significantly higher in malnourished patients (p<0.001). Polypharmacy was more frequent in the patients with malnutrition (88.1%) than in the patients with nutritional risk (50.6%) and satisfactory nutritional status (31.0%).

Outcomes of the questionnaires, anthropometric measurements, and laboratory analyses were summarized in **Table 2**. The median total WHOQOL-OLD scores were 27.0, 36.0, and 56.0 in patients with malnutrition, nutritional risk, and satisfactory nutritional status, respectively; the differences between the groups were significant (p<0.001 for all). The WHOQOL-OLD and its domain scores were significantly associated with nutritional risk and malnutrition. There were significant differences between the groups regarding all subdomain scores of the WHOQOL-OLD (p<0.001). The rates of

Table 2. Questionnaires, anthropometric measurements and laboratory values of the study groups

	Overall (n=532)	Groups			p values
		Satisfactory nutritional status (n=390)	Nutritional risk (n=83)	Malnutrition (n=59)	
Questionnaires					
WHOQOL-OLD total score[β]	50.0 [22.0-94.0]	56.0 [29.0-94.0][a]	36.0 [27.0-68.0][b]	27.0 [22.0-41.0][c]	**<0.001**
Sensory abilities[β]	9.0 [4.0-17.0]	10.0 [5.0-17.0][a]	7.0 [4.0-13.0][b]	5.0 [4.0-8.0][c]	**<0.001**
Autonomy[β]	9.0 [3.0-17.0]	10.0 [3.0-17.0][a]	6.0 [4.0-12.0][b]	5.0 [3.0-6.0][c]	**<0.001**
Past, present, and future activities[β]	8.0 [3.0-17.0]	9.0 [5.0-17.0][a]	6.0 [4.0-12.0][b]	5.0 [3.0-8.0][c]	**<0.001**
Social participation[β]	8.0 [3.0-17.0]	9.0 [4.0-17.0][a]	6.0 [4.0-13.0][b]	4.0 [3.0-7.0][c]	**<0.001**
Death and dying[β]	8.0 [3.0-16.0]	9.0 [4.0-16.0][a]	5.0 [3.0-11.0][b]	4.0 [3.0-7.0][c]	**<0.001**
Intimacy[β]	8.0 [3.0-16.0]	9.0 [4.0-16.0][a]	6.0 [4.0-13.0][b]	4.0 [3.0-6.0][c]	**<0.001**
Geriatric depression scale[β]	8.5 [0.0-21.0]	8.0 [0.0-20.0][a]	9.0 [5.0-21.0][b]	17.0 [6.0-20.0][c]	**<0.001**
Depression[‡]	142 (26.7)	50 (12.8)[a]	36 (43.4)[b]	56 (94.9)[c]	**<0.001**
Mini Mental State Examination[β]	26.0 [5.0-29.0]	26.0 [6.0-29.0][a]	25.0 [7.0-29.0][b]	9.0 [5.0-27.0][c]	**<0.001**
Dementia[‡]	102 (19.2)	24 (6.2)[a]	27 (32.5)[b]	51 (86.4)[c]	**<0.001**
Hendrich II fall risk model[β]	3.0 [2.0- 9.0]	3.0 [2.0- 4.0][a]	8.0 [3.0- 11.0][b]	10.0 [8.5- 11.0][c]	**<0.001**
Fall risk[‡]	203 (38.2)	97 (24.9)[a]	50 (60.2)[b]	56 (94.9)[c]	**<0.001**
Anthropometric measurements					
Body mass index (kg/m2)[†]	23.2±3.5	25.1±1.5[a]	19.7±1.6[b]	16.1±1.0[c]	**<0.001**
Body mass index (kg/m2)[‡]					
<18.5	67 (12.6)	0 (0.0)[a]	67 (47.2)[b]	0 (0.0)[c]	**<0.001**
18.5–24.9	247 (46.4)	174 (44.6)[a]	73 (51.4)[b]	174 (44.6)[c]	
≥ 25	218 (41.0)	216 (55.4)[a]	2 (1.4)[b]	216 (55.4)[b]	
Triceps skin fold thickness (mm)[β]	16.0 [7.0-26.0]	17.0 [9.0-26.0][a]	13.0 [9.0-18.0][b]	9.0 [7.0-16.4][c]	**<0.001**
Calf circumference (cm)[†]	35.47±5.54	38.13±3.12[a]	30.66±2.81[b]	24.66±2.23[c]	**<0.001**
Forearm circumference (cm)[†]	25.89±5.58	28.33±3.68[a]	22.01±2.98[b]	15.19±1.9[c]	**<0.001**
Laboratory analyses[β]					
Hemoglobin (g/dL)	13.1 [8.4-18.1]	13.8 [10.9-18.1][a]	11.1 [8.4-17.2][b]	10.2 [8.4-13.1][c]	**<0.001**
Fasting blood glucose (mg/dL)	86.0 [48.0-268.0]	87.0 [71.0-268.0][a]	82.0 [58.0-185.0][b]	71.0 [48.0-245.0][c]	**<0.001**
Creatinine (mg/dL)	0.8 [0.3-3.1]	0.8 [0.6-2.8][a],b	0.9 [0.4-2.9][a]	0.7 [0.3-3.1][b]	**0.007**
Albumin (mg/dL)	4.6 [3.1-5.4]	4.7 [3.5-5.4][a]	3.7 [3.4-4.6][b]	3.2 [3.1-3.6][c]	**<0.001**
Total cholesterol (mg/dL)	185.0 [41.0-321.0]	196.0 [88.0-321.0][a]	106.0 [78.0-263.0][b]	85.0 [41.0-198.0][c]	**<0.001**
LDL (mg/dL)	142.0 [32.0-247.0]	149.0 [89.0-247.0][a]	99.0 [56.0-174.0][b]	79.0 [32.0-146.0][c]	**<0.001**
Triglyceride (mg/dL)	163.0 [41.0-326.0]	174.0 [59.0-326.0][a]	101.0 [67.0-236.0][b]	86.0 [41.0-168.0][c]	**<0.001**
Ferritin	22.0 [1.8-124.0]	28.1 [6.8-124.0][a]	8.4 [1.8-56.1][b]	5.6 [1.9-17.0][c]	**<0.001**
Folic asit	6.5 [1.6-10.1]	6.9 [3.9-10.1][a]	3.7 [1.9-7.6][b]	2.4 [1.6-6.1][c]	**<0.001**
Vitamin D	15.6 [2.1-41.1]	17.0 [5.8-41.1][a]	6.2 [2.8-21.3][b]	3.7 [2.1-16.4][c]	**<0.001**
Vitamin B12	248.5 [85.0-413.0]	258.0 [169.0-413.0][a]	174.0 [94.0-325.0][b]	136.0 [85.0-186.0][c]	**<0.001**
HbA1C (%)	5.7 [4.1-13.2]	5.7 [4.9-11.3][a]	5.2 [4.4-10.9][b]	4.6 [4.1-13.2][c]	**<0.001**
Glomerular filtration rate (ml/min)	69.0 [16.0-89.0]	74.0 [18.0-89.0][a]	51.0 [18.0-78.0][b]	45.0 [16.0-88.0][b]	**<0.001**

[†]mean±standard deviation,[‡]n (%),[β]median (IQR), Each subscript letter denotes a subset of group categories whose column proportions do not differ significantly from each other at the 0.05 level.

depression, dementia, and fall risk were significantly higher in patients with nutritional risk and malnutrition (**Table 2**). We also detected significant differences between the groups in terms of the anthropometric measurements, including BMI, BMI category, triceps skinfold thickness, the calf and forearm circumferences, and laboratory parameters (**Table 2**).

There were significant correlations between the MNA score and total WHOQOL-OLD and subdomain scores (**Table 3**). Increased MNA scores (indicating a satisfactory nutritional status) were associated with increased scores in WHOQOL-OLD and its subdomain (indicating a higher quality of life). Besides, a positive correlation was found between the MNA and MMSE scores (r=0.545, p<0.001) while there were negative correlations between the MNA score and the GDS and HIIFRM scores (r=-0.462, r<0.001 and r=-0.380, p<0.001, respectively).

Univariate and multivariate logistic regression analyses of the variables for nutritional risk and malnutrition were given in **Table 4**. Most of the independent variables were significantly associated with nutritional risk according to the univariate analysis (**Table 4**). Multivariate logistic regression analysis showed that total WHOQOL-OLD score (OR=1.40, CI 95%:1.30-1.52, p<0.001) and CCI score (OR=1.73, CI 95%:1.34-2.23, p<0.001) were the significant independent risk factors for the development of nutritional risk and malnutrition.

Table 3. Correlation of Mini Nutritional Risk Assessment with patient reported outcomes and WHOQOL-OLD score

	Mini nutritional assessment	
	r	p
Geriatric Depression Scale	-0.462	**<0.001**
Mini Mental State Examination	0.545	**<0.001**
Hendrich II fall risk model	-0.380	**<0.001**
WHOQOL-OLD total score	0.693	**<0.001**
Sensory abilities	0.673	**<0.001**
Autonomy	0.683	**<0.001**
Past, present, and future activities	0.654	**<0.001**
Social participation	0.666	**<0.001**
Death and dying	0.677	**<0.001**
Intimacy	0.641	**<0.001**

DISCUSSIONS

In the present study, we showed significant associations between malnutrition risk and quality of life. Besides, meaningful relationships between the MNA score and depression, cognitive impairment, and a higher fall risk were also detected in older adults. As one of the first studies focusing on evaluating the association between quality of life and nutritional risk in Turkish elderly patients, the overall prevalence of nutritional risk and malnutrition was relatively high. Nutritional status and quality of life in elderly patients were regarded as the indicators for each other.

Depending on the study groups' characteristics and the country of the study, reported prevalence of nutritional risk and malnutrition shows significant variation. The combined rates of nutritional risk and malnutrition (based on the MNA scores) range from 14.3% to 82.6% (1,4,5,7,19–22). Several factors have been speculated to explain these variations. Living in institutions with menus for three meals that may lack essential nutrients was regarded as an underlying factor (22). Extended family support was suggested as another factor; for example, living in crowded families prevents loneliness and social isolation (4). In our study, the combined malnutrition risk was the highest among the patients living in residential care centers and the lowest in

Table 4. Univariate and multivariate logistic regression analyses of the variables for the development of nutritional risk and malnutrition

	Univariate		Multivariate	
	Crude OR [95%CI]	crude p value	Adjusted OR [95%CI]	crude p value
Age	1.02 [0.98-1.05]	0.397	-	-
Sex: Female vs. male	1.06 [0.72-1.56]	0.764	-	-
Educational status: Ref. literate (primary+secondary-college+university) vs. illiterate	3.03 [1.98-4.64]	**<0.001**	0.68 [0.32-1.43]	0.308
Living status: Ref. relatives				
Alone	2.99 [1.90-4.73]	**<0.001**	0.58 [0.14-2.37]	0.447
Residential care center	0.15 [0.07-0.31]	**<0.001**	1.14 [0.38-3.42]	0.813
Marital status: Ref. married with single+divorced/widowed	6.37 [4.18-9.72]	**<0.001**	2.13 [0.53-8.49]	0.285
Smoking: Present vs. absent	1.35 [0.91-1.99]	0.134	-	-
Comorbidity: Present vs. absent	0.06 [0.01-0.24]	**<0.001**	2.15 [0.13-35.43]	0.592
Hypertension: Present vs. absent	0.31 [0.19-0.50]	**<0.001**	1.44 [0.62-3.37]	0.400
Charlson comorbidity score	0.56 [0.50-0.62]	**<0.001**	1.73 [1.34-2.23]	**<0.001**
WHOQOL-OLD total score	1.24 [1.19-1.29]	**<0.001**	1.40 [1.30-1.52]	**<0.001**
Depression: Present vs. absent	0.08 [0.05-0.13]	**<0.001**	1.17 [0.40-3.42]	0.770
Dementia: Present vs. absent	0.05 [0.03-0.09]	**<0.001**	0.53 [0.15-1.86]	0.322
Fall risk: Present vs. absent	0.11 [0.07-0.17]	**<0.001**	1.88 [0.76-4.61]	0.171

OR: odds ratio, CI: confidence interval

patients living with relatives. Evaluation of these factors in a homogenous manner may not help reach a definitive conclusion; therefore, the complexity of the problem may necessitate prospective large-scale studies.

The general health status of elderly patients is a critical variable that predicts the nutrition risk. Coexisting diseases and their related indexes such as the CCI and the use of multiple medications (polypharmacy) might be used as the indicators for this purpose (3, 7). Polypharmacy may have a negative impact on nutritional status due to drug-food interactions or gastrointestinal complaints (3,7). Our results were consistent with these studies. The presence of multiple comorbidities has been speculated as a significant risk factor for malnourishment in elderly patients (23). Although the relationship between comorbidities and malnutrition remains obscure, it has been thought that these comorbidities may have a negative impact on the nutritional status of the patients. In association with polypharmacy, comorbidities, as reflected by the CCI in the present study, may be regarded as indicators for developing malnutrition. Therefore, those who provide care for elderly patients are recommended to pay attention to the presence or absence of such factors because of their close association with malnutrition.

The educational, marital, and living status are thought to impact the nutritional quality of older adults significantly (1,7). Living alone or widowed older men are more vulnerable to nutritional risk because of difficulty buying and preparing food (1). In this study, we found that elderly illiterate, divorced, or widowed people living alone or in residential care centers were the riskiest group for malnutrition (4). The lower level of education was significantly associated with malnutrition (23). Sex discrepancy in nutrition is regarded as a complex and poorly understood issue (3). Some researchers reported that female sex is an independent risk factor for developing poor nutritional status, but we did not find any association between sex and nutrition (3,5,6). These controversial results might be affected by multiple unidentified confounders among the patients' demographic and clinical characteristics (4). Therefore, reciprocal associations of several demographic and clinical features should be considered while evaluating these findings.

The association between the presence or severity of depression and nutritional status was also investigated. There was a significant negative correlation between the GDS and MNA scores in the present study. In the BRIGHT trial, patients with more depressive symptoms were at moderate or high risk of malnutrition (3). In previous studies, nutritional disorders and malnutrition are regarded as modifiable risk factors for preventing and progression of age-related cognitive impairment (24-26). It was also mentioned that there was a relationship between malnutrition and the severity of cognitive impairment and that good nutritional status is associated with normal cognition. In this study, we showed a significant positive correlation between the MMSE and MNA scores. Poor nutritional status has been speculated as a significant factor for falls, especially in elderly patients, based on community-based studies (27). As opposed to our findings, Adly et al. (27) demonstrated a significant association between malnutrition and fall risk assessment scores using various scales. In light of this evidence, we may conclude that poor nutritional status in the elderly may reflect many underlying problems, such as impaired motor function, psychological abnormalities, and cognitive and functional impairment. So, efforts should be made to improve nutritional status considering these outcomes, especially in the elderly.

The strongest associations between nutritional risk and the physical health and sensory abilities subdomains of the WHOQOL-OLD scale has been reported in previous studies (1,3,5). Physical health status may be regarded as a critical factor for routine daily activities such as eating and walking; however, close associations between the other domains are also possible (1). Sensory abilities such as sight, touch, smell, and taste may also impact both malnutrition and the quality of life. Our study showed that the total WHOQOL-OLD score and all subdomain scores were significantly correlated with the risk of malnutrition, as opposed to findings in other studies (3). Some studies have also reported similar findings (1). Using several scales for quality of life, other studies have shown that mental and physical components had a critical impact on nutritional status (7,19). Therefore, we may conclude that older adults' nutritional status may be directly related to all aspects of the quality of life, and the elderly individuals with nutritional risk are more likely to score lower on all or some domains of quality of life scales.

Considering the study's limitations, there may be controversial cause-and-effect relationships between the primary outcomes of the risk of malnutrition and the quality of life. Besides, the inclusion of only the patients admitted to the outpatient clinics was regarded as another limitation. Causality of the associated variables were lacked due to the cross-sectional design of the study. It is better to assess the likelihood of adverse causality and temporal relationships between quality of life, malnutrition risk, and other relevant variables. On the other hand, the present study had several important strengths, including the use of MMSE, GDS, and HIIFRM.

CONCLUSION

The prevalence of nutritional risk and malnutrition was high among elderly patients. The study also showed how various demographic and clinical factors were associated with malnutrition or nutritional risk. Nutritional risk and malnutrition were associated with the total WHOQOL-OLD score and the CCI score. Besides, there were significant correlations between all subdomains of the WHOQOL-OLD questionnaire and malnutrition. Having poor quality of life and higher comorbidities were the main determinants of malnutrition and nutritional risk in the multivariate analysis. The present findings contribute to the understanding of nutritional risk and its potential determinants. Results also underline the association between quality of life and nutritional status. These findings may help prevent nutritional risk and determine appropriate interventions, especially for the elderly population.

Clinical Implications

The combined prevalence of nutritional risk and malnutrition was 26.7% in the elderly patients.

There were significant correlations between all subdomains of the WHOQOL-OLD questionnaire and malnutrition and nutritional risk assessed with MNA.

Rates of depression, dementia, and fall risk were significantly higher in patients with nutritional risk and malnutrition.

Nutritional risk and malnutrition were significantly associated with the WHOQOL-OLD and CCI scores. Having poor quality of life and higher comorbidities were the main determinants of malnutrition and nutritional risk in the multivariate analysis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erzurum Regional Training and Researh Hospital Clinical Researchs Ethics Committee (Date: 06.01.2020, Decision No: 2020/01-06).

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

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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A reliable source of information on botulinum toxin injection used in the treatment of spasticity: YouTube

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ABSTRACT

Aim: The aim of the study is to assess the quality and reliability of YouTube videos on botulinum toxin injection for the treatment of spasticity.

Material and Method: In this cross-sectional study, a search of YouTube videos was made on 15 September 2020, using the keywords "botulinum toxin and spasticity", "botulinum toxin treatment for spasticity", "spasticity management with botulinum toxin" "treating spasticity with botox". Two experienced reviewers on botulinum toxin injection on spasticity reviewed the first 200 videos. The quality of the videos was evaluated according to the Global Quality Scale (GQS) and three groups were formed: high quality, intermediate quality and low quality. The DISCERN tool was used to evaluate the reliability of the videos.

Results: Of the 77 videos analyzed according to GQS, 36 (46.8%) were high quality, 31 (40.2%) were intermediate quality and 10 (13.0%) were low quality. Most of the videos (44.15%, n=34) uploaded by health professionals (physiatrist, orthopedist etc.) and the majority of the health professional groups had produced high quality videos (67.64%). When the parameters of the videos were compared according to quality levels, no significant differences were found in the number of view, comment, likes, dislikes, video length, days since upload ($p>0.05$). Significant differences were only found between the groups in respect of the DISCERN scores ($p<0.001$).

Conclusion: YouTube can be considered as a reliable source for botulinum toxin injection in spasticity. The importance of the video source should be explained to the patient and healthcare professional using YouTube.

Keywords: YouTube, botulinum toxin, spasticity

INTRODUCTION

Spasticity is a motor disorder characterized by the velocity-dependent increase in tonic stretching reflexes and increased tendon responses due to the hyperexcitability of stretching reflexes (1). It is one of the complications that occur as a result of upper motor neuron lesion. Although the pathophysiology of spasticity is complex, it occurs as a result of the hyperexcitability of spinal motor neurons in the damage of the inhibitory system, which starts from the cortex and extends to the spinal cord and ensures the control of spinal reflexes and maintains the normal tone in the muscles (2). Although the beneficial effects of spasticity such as standing upright positioning and protecting bone mass are known, it has negative effects on functional recovery in many cases. It should be treated if it restricts functional movements, affects daily life activities and hygiene care, and causes contractures (3).

Posture and positioning, stretching and strengthening exercises and orthosis are used in the non-pharmacological treatment of spasticity. In addition, superficial and deep heaters, cryotherapy and some electrotherapy agents are other non-pharmacological treatment options. Pharmacologically, oral anti-spastic agents, intrathecal applications and motor point and nerve blocks are used in the treatment (4). Botulinum toxin is a potent neurotoxin produced by clostridium botulinum. It binds to presynaptic cholinergic nerve endings at the neuromuscular junction and blocks muscle contraction by preventing acetylcholine release into the intersynaptic area. Many studies have shown that botulinum toxin administration is effective and safe in spasticity treatment (5).

YouTube is an internet platform that is frequently used to obtain health-related information. Although there is information on the diagnosis and treatment of many

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diseases on this platform, the accuracy of the videos or the educational quality of the videos are questioned, as anyone can easily upload a video and there is no quality control mechanism. Therefore, there are studies in the literature questioning the reliability of YouTube videos in different disease groups (6-9).

The objective of this study is to evaluate the quality and reliability of YouTube videos on botulinum toxin injection for the treatment of spasticity to determine if it is a reliable resource for the people.

MATERIAL AND METOD

Ethics Statement

Ethics Committee approval was not required for this study because videos are publicly available on YouTube and there were no human or animal participants in the study. Similarly, Ethics Committee approval was not obtained in other studies that evaluated YouTube videos (13-15). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

A search was made of videos on YouTube (www.youtube.com) on 15 September 2020, using the keywords "botulinum toxin and spasticity", "botulinum toxin treatment for spasticity", "spasticity management with botulinum toxin" "treating spasticity with botox". It has been shown that many YouTube users watched the first 60-200 videos after searching (10). In present study, two experienced reviewers on botulinum toxin injection on spasticity reviewed the first 200 videos. The reviewers who evaluated the quality of the videos did not have any information about the video's number of likes, dislikes, views and comments in order not to affect the assessment. In cases where there was disagreement between reviewers in terms of evaluation and scoring, a consensus was achieved by conducting detailed literature research and discussion. Non-English videos, duplicate videos, videos about botulinum toxin injection in different diseases (bruxism, wrinkles, headache etc.) and off-topic videos were excluded in the study. After the exclusion, a total of 77 videos were analyzed.

Video Assessment

The source of the video, number of likes and dislikes, number of comments, number of views, video length and upload day to YouTube of all videos were recorded. Sources of the video were divided into five categories: 1.health professionals, 2 independent users, 3. independent users with health, 4.PhD/anatomist/physiotherapist, 5.TV/magazine media. The target audiences of the videos were divided into four categories: 1.patients, 2.health professionals, 3.both patients and health professionals, 4.everybody. Videos containing general information about the indications and complications of botulinum toxin injection were included in the first group, videos with detailed information such as the location, dosage, anatomy of the injection and which patients should be applied were included in the second group, and videos that provided both general and detailed information were included in the third group. Videos containing superficial information not teaching botulinum toxin application were included in the fourth group.

The quality of all videos was evaluated with the Global Quality Scale (GQS). The GQS is a 5-point Likert scale developed for internet research, with scores ranging from 1 to 5.A score of 1 or 2 indicates a low-quality video, 3 points indicate intermediate quality, and a score of 4 or 5, high quality (11). To evaluate the reliability of the videos DISCERN tool that consisting of 5 yes-no questions and each question is one point (maximum 5 points), was used (12). This GQS and DISCERN tool is used in studies where many YouTube videos are examined in educational terms.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS for Windows 22.0 software. Inter-observer agreement was calculated as the kappa score. The conformity of numerical variables to normal distribution was assessed with the Shapiro Wilk test. In the comparisons of 3 or more groups of variables not showing normal distribution, the Kruskal-Wallis and Dunn tests were applied. A value of $p<0.05$ was considered statistically significant.

RESULTS

Of the 200 videos examined by the researchers, 91 duplicated videos, 28 off-topic videos, and 4 non-English languages videos were excluded from the study. Thus analysis was made of 77 videos. The mean GQS score of the videos was 3.65 ± 1.04, the mean DISCERN score was 3.50 ± 1.20. Video length, days since upload, likes, dislikes, number of views, number of comments data are shown in **Table 1**. In terms of target audience, more than half of the videos (53.2%, n = 41) were in the patient group, 14 (18.2%) were in the health professionals group, 10 (13.0%) were both patients and health professional group, and 12 (15.6%) were in the study population group.

When the 77 videos were analyzed according to GQS, 36 (46.8%) were high quality, 31 (40.2%) were intermediate quality and 10 (13.0%) were low quality. Most of the videos (44.15%, n=34) were uploaded by health professionals (physiatrist, orthopedist etc.), whereas 9.09% (n=7) were uploaded by independent users, 19.48% (n=15) were uploaded by independent users with an accompanying health professional, 12.98% (n=10) were uploaded by PhD/anatomist/physiotherapist and

Table 1. General features of the videos

	n	Mean	Std. Deviation	Minimum	Maximum
Video duration (second)	77	386.45	497.04	38.00	4213.00
Days since upload_(date)	77	1731.61	1080.49	56.00	3812.00
Likes	77	56.94	126.89	.00	903.00
Dislikes	77	4.26	13.90	.00	116.00
Number of comments	63	5.71	11.64	.00	72.00
View	77	3238.56	32.004	2.00	229494,00

14.28% (n=11) were uploaded by TV/magazine media. While the majority of the health professional groups had produced high quality videos (67.64%), the rate of high quality videos in the other groups was between 28.6% and 36.4% (**Table 2**). There was no statistically significant difference between the groups in terms of likes, dislikes, number of views, video length, days since upload, number of comments, comments per day, or views per day.

When the parameters of the videos were compared according to the quality levels, no significant differences were found in the likes, dislikes, number of views, video length, days since upload, number of comments, comments per day, or views per day ($p>0.05$). Significant differences were only found between the groups in respect of the DISCERN scores and ($p<0.001$). The kappa score, which shows the interreviewer agreement, was determined as 0.85.

Table 2. Categorization of the videos according to sources, n (%)

	Low quality	Intermediate quality	High quality	Total
Health professionals	3 (8.8)	8 (23.5)	23 (67.64)	34
Independent users	1 (14.3)	4 (57.1)	2 (28.6)	7
Independent users with health	3 (20.0)	8 (53.3)	4 (26.7)	15
PhD/anatomist/ physiotherapi st	1 (10,0)	6 (60,0)	3 (30,0)	10
TV/magazine media	2 (18.2)	5 (45.5)	4 (36.4)	11

n number, % percentage

DISCUSSION

Botulinum toxin injection is a method commonly used in the treatment of spasticity in upper motor neuron injury diseases such as stroke, spinal cord injury, traumatic brain injury, and multiple sclerosis. Although YouTube has been used for many different purposes since its creation, in recent years it has been used by both healthcare professionals and patients for medical information. The aim of the study was to evaluate the quality and reliability of botulinum toxin injection videos on spasticity on YouTube. It was observed that some of the videos evaluated were addressed to healthcare professionals, some to the patient population, and some to both groups. When the video quality was examined, it was seen that 87% of the videos were high and intermediate quality. When evaluated as a video source, it was observed that health professionals had a higher reliability.

The videos investigated in present study had over 1 million views, with an average 13238 views per video, demonstrating that botulinum toxin videos are widely available on YouTube. Moreover, the videos garnered total 439 comments and 4381 likes from users, reflecting both the active input and support form YouTube users. That's why the videos on such a frequently used platform should have the correct information and quality. The high rate of high quality video shows that people have reached the right information on this subject. There are conflicting results in studies evaluating the quality of YouTube videos in the literature. While some studies have concluded that the quality of the videos on the related subject is high (16,17), others have concluded that the video quality is low (18). In the present study, 36 (46.9%) were high quality, 31 (40.2%) were intermediate quality and 10 (13.0%) were low quality according to the GQS.

Botulinum toxin injections are also used for aesthetic and non-aesthetic (cervical dystonia, headache, sialorrhea, temporomandibular joint disorders, bruxism) purposes other than spasticity (19). However, there are only two studies evaluating the quality of YouTube videos in the literature on this subject. First, Wong et al examined botulinum toxin injection videos used for wrinkles and stated that these videos had high-quality content and YouTube was a useful resource for patients (20). In the other study, Gaş et al. (21) evaluated botulinum toxin injection videos for bruxism and concluded that YouTube is a reliable source in this field. Similarly, in this study, it was concluded that YouTube videos on spasticity are a reliable source for both patients and healthcare professionals.

When examined as a video source in present study; although the video quality was better in the health professionals group, it was observed that there was no difference between the groups in terms of views, likes, dislikes, number of comments. This means that YouTube users are interested in videos regardless of source. Similarly, previous studies assessing the quality of videos on YouTube have demonstrated that the videos produced

by healthcare professionals were of higher quality (6). The fact that nearly half of the videos examined in present study were uploaded by qualified health professional supports that they are both reliable and valuable.

The study has several limitations. First, as in all studies YouTube videos are evaluated, the results of the study depend on the day of the research, due to the dynamic structure of YouTube. Second, the scales used in the study are subjective and therefore results depend on the person evaluating it. Third, we evaluated only English-language videos but botulinum toxin injection is also popular in non-English speaking countries.

CONCLUSION

YouTube contains reliable and educational information about botulinum toxin injection used in the treatment of spasticity. In particular, the qualities of videos uploaded by the health professional are higher than others. Therefore health professionals should be encouraged to make and upload more videos for educational purposes.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics Committee approval was not required for this study because videos are publicly available on YouTube and there were no human or animal participants in the study. Similarly, Ethics Committee approval was not obtained in other studies that evaluated YouTube videos (13-15).

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 25-hydroxy vitamin D levels in COVID-19 positive patients

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ABSTRACT

Aim: With this study, the aim was to evaluate the serum 25-hydroxy vitamin D levels in COVID-19 positive patients.

Material and Method: The study retrospectively screened the archive records for patient data from 732 patients, attending the pandemic clinic in our hospital from March 2020 to February 2021 aged over 18 years with COVID-19 positivity and serum 25-hydroxy vitamin D levels examined, with data from 360 control patients between the same dates in similar age group and without COVID-19 positivity. COVID-19 positive patients and control group patients were divided into three groups according to serum 25-hydroxy vitamin D levels (deficiency, insufficiency, and normal level).

Results: Of the 732 COVID-19 positive patients included in the study, 66.4% were female (n=486) and 33.6% were male (n=246). The mean serum 25(OH)D level in the COVID-19 positive patient group was calculated as 14.2± 11.7 ng/ml. For the 360 patients included in the COVID-19 negative control group, 70% were female (n=252) and 30% were male (n=108). Mean serum 25(OH)D level in the COVID-19 negative control group was 27.6±12.1 ng/l. There was a significant difference between the groups (p=0.012).

Conclusion: High rates of vitamin D deficiency and insufficiency are seen in COVID-19 positive patients. Sufficient levels of replacement for patients with low vitamin D levels will provide significant reductions in musculoskeletal system symptoms and complaints of patients. We think vitamin D is a protective vitamin for COVID-19.

Keywords: COVID-19, pandemic, 25-hydroxy vitamin D, prognosis

INTRODUCTION

The coronavirus family is a positive strand RNA virus family which is responsible for 5-10% of all acute upper respiratory tract infections in adults (1). Research after reports of viral pneumonia cases in Wuhan city in China in December 2019 identified a new coronavirus vector. Initially called '2019-nCoV' the virus was later called 'SARS-CoV-2' due to similarity to the virus causing severe acute respiratory syndrome (SARS). The disease spread rapidly around the world and a pandemic was declared by the World Health Organization in March 2020 (2). 'Coronavirus disease 2019 (COVID-19)', causing a broad clinical spectrum from asymptomatic cases to cases with acute severe respiratory distress requiring intensive care, has infected many people since December 2019 to date and caused the death of many people (3).

Patients with SARS-CoV-2 infection generally have mild clinical progression, but may also develop severe complications resulting in mortality (4,5). Among the risk factors for severe COVID-19 infection are advanced age, obesity, smoking habit, chronic renal failure, and comorbid diseases like chronic lung disease, chronic liver disease, cardiovascular diseases, hypertension, malignancy and diabetes mellitus (6,7). As most of these risk factors are preventable and controllable diseases, they are important to lessen the severity of COVID-19. A study by Dong et al. (8) assessed 731 COVID-19 positive patients with 1412 COVID-19 suspected cases. They identified that more than 90% of cases were asymptomatic, mild or moderately symptomatic cases.

Vitamin D is a steroid with hormone-like functions playing a role in bone growth and mineral metabolism (9). At the same time, vitamin D has a direct effect on calcium and phosphorus metabolism (10). Vitamin D receptors were identified in many tissues and this situation shows that vitamin D deficiency plays a role in many diseases (11).

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Currently, vitamin D deficiency and insufficiency are important problems and are thought to play a role in the etiology of many chronic diseases (12). In many studies, vitamin D insufficiency was shown to be associated with viral and bacterial infections, cardiovascular diseases, multiple sclerosis, psychiatric disorders like depression, diabetes and autoimmune diseases and even breast and colon cancer (13-16). Patients with chronic disease who do not receive sufficient amounts of vitamin D in foods or who have disrupted vitamin D metabolism due to the chronic disease process may have vitamin D deficiency. Additionally, medications used for chronic diseases may disrupt vitamin D metabolism. Some studies showed that vitamin D deficiency in patients with chronic disease may shorten the life expectancy of the patient and increase mortality and cause severe complications (17). They proposed that the reason for this in situations with vitamin D insufficiency is due to the loss of the positive effects of vitamin D in the body of anti-inflammatory, antioxidant and anti-ischemic properties (18).

Serum 25-hydroxy vitamin D (25(OH)D) measurements are a test used to show vitamin D levels of individuals. Serum 25(OH)D levels below 20 ng/mL are vitamin D deficiency, 21-30 ng/mL are vitamin D insufficiency and levels above 30 ng/mL are accepted as normal vitamin D levels (19). Low 25(OH)D levels may cause widespread body pain, muscle and skeletal weakness, bone fractures and bone mineralization inadequacy in patients (20,21).

There are several studies showing the protective effects of vitamin D against community-acquired pneumonia, interstitial pneumonia and influenza A infections. Again, these studies showed regulatory effects on monocyte activation and IL-6 in the cytokine storm (22,23). Several other studies showed the active form of vitamin D of 1,25(OH)2D3 increased ACE2 expression in the lungs and had positive effects on lung injury (24,25).

Cytokine storm is a significant problem in COVID-19 patients. It is possible to lessen the severity of disease by keeping vitamin D at normal levels to reduce the synthesis of proinflammatory cytokines in COVID-19 patients (26). A study by Ilie et al. (27) identified a negative correlation between mean vitamin D levels of patients and COVID-19 case numbers in some European countries.

With this study, the aim was to evaluate the serum 25-hydroxy vitamin D levels in COVID-19 positive patients.

MATERIAL AND METHOD

This study was approved by Ordu University, Clinical Research and Ethics Committee (Date: 15/04/2021, Decision No: 93). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study retrospectively screened archive records of 732 patients, aged over 18 years with COVID-19 positivity attending the pandemic clinic in our hospital from March 2020 to February 2021, with 360 patients without COVID-19 positivity comprising normal individuals in a similar age group between the same dates. Patient data (age, gender, serum 25(OH)D levels) were retrospectively obtained from file records. Our study did not include patients receiving vitamin D and immunosuppressive medication treatment, with bone metabolism diseases, with cancer, pregnant or breastfeeding patients, with osteoporosis and osteomalacia diagnosis, with primary hyperparathyroidism, with chronic renal failure, patients who drank alcohol or smoked and with inadequate or missing records. Our hospital studied serum 25(OH)D levels with an Abbott Architecture i2000-SR autoanalyzer. Patients with serum 25(OH)D levels below 20 ng/mL were placed in the vitamin D deficiency group, those with 21-30 ng/mL were in the vitamin D insufficiency group and patients with values above 30 ng/mL were included in the normal vitamin D group. Groups were analyzed for the presence of statistically significant differences. Additionally, data for the COVID-19 positive patient group were compared with data for the COVID-19 negative control group. Vitamin D levels of COVID-19 positive patients were examined in age groups. Serum 25(OH)D levels of patients were statistically assessed according to age group.

Statistical Analysis

All data were uploaded to IBM SPSS Statistics 22.0 (SPSS Inc., Chicago, Illinois) program. For assessment of data, number, percentage, mean and standard deviation were used as descriptive statistics. Distribution of data was assessed with the Kolmogorov-Smirnov test. Comparisons of groups used the student T test. Correlation analyses used the Pearson and Spearman tests. P values smaller than 0.05 were accepted as significant.

RESULTS

Of the 732 COVID-19 positive patients included in the study, 66.4% were women (n=486) and 33.6% (n=246) were men. Mean age of patients was 45.2±11.9 years (men 47.7±10.4 years, women 46.5±10.1 years). The mean serum 25(OH)S levels in the COVID-19 positive patient group were 14.2±11.7 ng/mL. For the 360 patients included in the COVID-19 negative control group, 70% were women (n=252) and 30% were men (n=108). The mean age of controls was 45.8±10.6 years (men 44.5±11.4 years, women 46.6±12.7 years). The mean serum 25(OH) D levels for the COVID-19 negative control group patients were 27.6±12.1 ng/mL. When statistical comparison was made between gender and serum 25(OH)D levels in the COVID-19 positive patient group and COVID-19

negative control group patients, there was a significant difference between the groups (p=0.012) (**Table 1**).

Table 1. Statistical comparison of gender and serum 25(OH) D levels in COVID-19 positive patient group and COVID-19 negative control group

Serum 25(OH)D levels	COVID-19 positive patient group (n=732)		COVID-19 negative control group (n=360)		p value
	Female (n=486)	Male (n=246)	Female (n=252)	Male (n=108)	
	n/%	n/%	n/%	n/%	
Deficiency (<20 ng/ml)	234/48.2	138/56.2	69/27.3	18/16.7	0.012
Insufficiency (21-30 ng/ml)	141/29.0	72/29.2	96/38.1	42/38.8	
Normal (>30 ng/ml)	111/22.8	36/14.6	87/34.5	48/44.5	

n: number of patients, %: percent, 25(OH)D: 25-hydroxy vitamin D

Additionally, COVID-19 positive patients were divided into age groups and serum 25(OH)D levels were assessed according to age group (**Table 2**). The serum 25(OH)D level was identified to be lowest in the 51-65-year age interval.

Table 2. Classification of COVID-19 positive patients according to age group and assessment of serum 25(OH) D levels in age groups.

Age interval (year)	Mean serum 25(OH) D level (ng/ml)	Patient number and percentage n (%)
18-35	17.70±13.04	138 (18.9)
36-50	16.78±17.42	213 (29.1)
51-65	12.36±11.01	240 (32.8)
>66	13.45±10.21	141 (19.2)

n: number of patients, %: percent, 25(OH)D: 25-hydroxy vitamin D

DISCUSSION

The correlation between vitamin D levels and viral infections was reported in some studies. A study in India identified that after 6-months follow-up, patients with vitamin D deficiency had more upper respiratory tract infections compared to normal healthy individuals (28). The majority of these upper respiratory tract infections comprised viral infections. Among these, the most frequent vectors are rhinovirus, coronavirus, respiratory syncytial virus (RSV), adenovirus, influenza and parainfluenza. A study by Banajeh et al. (29) identified that vitamin D deficiency in patients with upper respiratory tract infections was associated with reduced neutrophil counts in circulation and hypoxemia. A study by Yao et al. (30) reported H7N9 pneumonia in two patients with advanced degree of vitamin D deficiency. In our study, 50.8% of COVID-19 positive patients had vitamin D deficiency, 29% had vitamin D insufficiency and 20.2% had normal vitamin D levels. Generally, vitamin D levels were identified to be lower in the COVID-19 positive patient group compared to the COVID-19 negative control group. Among the COVID-19 positive patients, 34 required respiratory support. The vitamin D levels of these patients were severely low and below 3 ng/mL. Our small-scale study results show that COVID-19 patients have vitamin D deficiency; however, there is a need for more studies including higher patient numbers.

Though there are several studies assessing the relationship between vitamin D deficiency and infections, studies in recent times researched the protective effect of vitamin D supplementation on infections. For this reason, administering sufficient amounts of vitamin D supplementation to patients with low vitamin D levels is thought to provide a protective effect against infections (31). For this reason, we think that vitamin D replacement at appropriate doses for patients with vitamin D deficiency identified will be protective against several other infections, led by COVID-19 infection. However, a study by Arihiro et al. (32) showed that in spite of the significantly low incidence of upper respiratory tract infections in the patient group with vitamin D deficiency, there was no difference in influenza incidence. Urashima et al. (33) reported that vitamin D support in the winter months may reduce influenza A incidence. Similarly, a study by Camargo et al. (34) showed vitamin D supplementation significantly reduced acute respiratory tract infection risk in patients with vitamin D deficiency. In our study, we began oral vitamin D supplementation at appropriate doses for patients who were COVID-19 positive and with vitamin D deficiency/insufficiency identified.

To date, a variety of studies were performed researching vitamin D levels in healthy children and adults in Turkey. A study by Uçar et al. (35) identified 51.8% vitamin D deficiency and 20.7% vitamin D insufficiency. Erol et al. (36) found 25(OH)D levels were 10.6±6.5 ng/mL and 92.2% of patients had vitamin D levels below 20 ng/mL. In our study, mean serum 25(OH)D levels were 14.2±11.7 ng/mL in COVID-19 positive patients, and 50.8% of patients had vitamin D levels below 20 ng/mL (vitamin D deficient), 29% had vitamin D levels from 21-30 ng/mL (vitamin D insufficient) and 20.2% had normal vitamin D levels (>30 ng/mL). In Turkey, inadequate nutrition, lifestyle variations and environmental factors play roles among reasons for the low vitamin D levels.

A review by Silva and Furlanetto (37) proposed the need to carefully assess reductions in 25(OH)D measured during the acute phase response. If vitamin D acts like a negative acute phase reactant, vitamin D supplementation will not provide any benefit. However, we think there is a need for more studies to strengthen this view. Ebadi and Montano-Loza (38) proposed the need to measure vitamin D levels in COVID-19 patients in their article, and recommended 50,000 IU vitamin D supplementation (total 100,000 IU) twice per week at time of diagnosis for patients with vitamin D below 20 ng/mL.

CONCLUSION

Vitamin D deficiency and insufficiency are observed at high rates in COVID-19 positive patients. For this reason, sufficient levels of replacement for patients with low vitamin D levels will provide clear reductions in musculoskeletal system symptoms and complaints. Additionally, vitamin D was shown to be a significant determinant of mortality due to antiinflammatory, antioxidant and antiischemic properties. For this reason, we think vitamin D is a protective vitamin for COVID-19

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Ordu University, Clinical Research and Ethics Committee (Date: 15/04/2021, Decision No: 93).

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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Investigation of the effect of watercress via nuclear factor-E2-related factor-2 pathway on 7,12-dimethylbenz[a] anthracene-induced oxidative damage in rat liver

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ABSTRACT

Aim: Nuclear factor E2-related factor-2 (Nrf2) regulates many cytoprotective antioxidants, and detoxifying enzymes, has been a hopeful approach for chemoprevention of cancer. We researched the chemopreventive impact of watercress on the DMBA-induced oxidative damage in the rat liver via the Nrf2/ARE signalling pathway in the present study.

Material and Method: Female Wistar albino rats have categorized into three groups. The control was the first group, rats in the second and third groups were administered 7,12-dimethylbenz[a]anthracene (DMBA) (20 mg/kg b.w., i.p.) and rats in the third group were given daily doses of watercress (250 mg/kg b.w. Nasturtium officinale R.Br. for 4 weeks by oral gavage). Antioxidant and phase II detoxification enzyme activities, Nrf2 transcription factor level in liver, and serum ALT were determined. Also, histopathological analysis of the liver was performed.

Results: We observed that watercress induces DNA-binding of Nrf2. It was related to increased enzyme activities of phase II detoxifying and the antioxidant. Our results also demonstrated that watercress ameliorated liver injury.

Conclusion: Our data ensured considerable evidence that the dietary watercress ameliorates DMBA induced liver toxicity via regulating the activation of the Nrf2/ARE pathway which increases the expression of cytoprotective enzymes.

Keywords: Watercress, Nrf2, DMBA, xenobiotic enzymes, hepatoprotective effect

INTRODUCTION

Cancer incidence of the world and its mortality is associated with lifestyle and environmental agents like food and chemicals. Sustained exposure to chemicals directly or indirectly leads to increase cancer risk by participating in all aspects of carcinogenesis (1). Polycyclic aromatic hydrocarbons (PAHs) are xenobiotic compounds that are environmental contaminants. PAHs are lipophilic and metabolized by xenobiotic-metabolizing enzymes (XMEs) (2). Firstly, enzymes of cytochrome P 450 family 1 that includes CYP1A1, CYP1A2, and CYP1B1 oxidize lipophilic compounds and generates reactive metabolites that interact with protein and DNA. Secondly, highly reactive compounds are converted to more hydrophilic species and/or inactivated by enzymes of phase II detoxification such as glutathione-S-transferase (GST), UDP -glucuronosyltransferases, heme oxygenases-1 (HO-1), NAD(P)H: quinine oxidoreductase 1 (NQO1), and sulfotransferases (3).

DMBA is one of the PAHs that is mainly found in automobile exhaust, cigarette smoke, grilled foods (4) which leads to the generation of reactive oxygen species (ROS), causing oxidative damage and decreased antioxidant defense enzymes, and increases carcinogenicity and toxicity (5). DMBA causes liver toxicity through the released carcinogenic metabolites as a result of xenobiotic metabolism (6). Normally Nrf2, is sensitive to redox changes, exists in an inactive form with a cytosolic inhibitor called Kelch-like epichlorohydrin-associated protein 1 (Keap1). Oxidative stress generating factors including electrophiles, xenobiotics, heavy metals, UV radiation, and physiological stress lead to cleavage of Nrf2 from the Nrf2-Keap1 complex. Nrf2 translocates to

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the nucleus and binds to the antioxidant response element (ARE). This causes the transcription of genes of phase II enzymes including NQO1 and GST (7). Excessive ROS generation due to the xenobiotic metabolism of DMBA causes oxidative stress, liver damage, and carcinogenesis. The Nrf2/ARE binding results in the induction of detoxification enzymes, antioxidative stress proteins, and other defense mechanisms (8).

Watercress belongs to the Cruciferae, also called Brassicaceae, family and is one of the perennial freshwater macrohydrophytes that has ecological and economic importance. It has been used by humans for many years both for food and medical purposes (9). This plant has some medicinal properties such as a diuretic (10), antihypertensive (11), antidiabetic (12), antihyperlipidemic (13), antiasthmatic, antituberculosis (14), and anticarcinogenic (15). Furthermore, it contains vitamins (Vitamin A and C), phenolic compounds, minerals, and glucosinolates (16). Glucosinolates are converted to isothiocyanates (ITC) non-enzymatically by physical factors or enzymatically by myrosinase during food preparation, cooking, and chewing (17). Phenethylisothiocyanate (PEITC) in watercress, is an ITC, has been extensively investigated as the most promising chemopreventive compound (18).

In this study, we hypothesized that watercress-mediated Nrf2 activation is a novel hepatoprotective pathway. The goal of this study was to evaluate the chemopreventive effect of watercress grown in the Malatya region of Turkey on oxidative liver injury induced by DMBA via the Nrf2/ARE pathway.

MATERIAL AND METHOD

Animals and Experimental Design

All experimental procedures of animals were approved by the Local Ethics Committee of the Animal Experiments of İnönü University (Date: 20/02/2015, Decision No: 2015/A-25). Twenty-seven female Wistar albino rats (weighing 150–250 g) accommodated in a room kept at 22°C with a 12-hr light/dark cycle and had free access to food. Rats were separated randomly into three groups (9 rats per group): Rats in group I served as control and were injected intraperitoneally with corn oil. Rats in group II (DMBA) and group III (DMBA+watercress) were injected intraperitoneally with DMBA (20 mg/kg body-weight in corn oil) (Sigma-Aldrich, USA) (19). Rats in group III were given daily doses of watercress (Nasturtium officinale R.Br) (250 mg/kg b.w) for 4 weeks by oral gavage before intraperitoneal injection of DMBA (20 mg/kg body-weight in corn oil). After 30 days, under xylazine/ketamine anesthesia blood of rats in all groups was collected and then liver tissues of the rats were resected, washed in phosphate buffer, and rapidly frozen at -80°C.

Plant Material and Extraction

Nasturtium officinale R.Br were obtained from the pond in Arguvan country, city of Malatya, Turkey. A voucher specimen (No: 1001) was deposited into a herbarium at the Faculty of Pharmacy of İnönü University in Malatya, Turkey (**Figure 1**). Samples were freeze-dried and were powdered. The powdered sample was extracted with 70% methanol by heating at 70°C for 30 minutes and filtered. This procedure was carried out two more times and then centrifuged at 4000 rpm for 10 min. The solvent was removed with a rotary evaporator (Heidolph Laborota 4011-digital) at 90 rpm, and 45°C for 2 h and dried.

Figure 1. The aerial parts of Nasturtium officinale R.Br. were collected from the pond in Arguvan country, city of Malatya, Turkey (Fresh plant and a voucher specimen (No: 1001)).

Determination of PEITC Content of the Watercress

The PEITC content of the watercress was determined by HPLC. PEITC standards were prepared with acetonitrile at concentrations of 0.312, 0.625, 1.25, 2.5, 5, and 10 ppm. 100 µL of the cyclocondensation working reagent (10 mM 1,2 benzene dithiol isopropanol and pH 8.5 phosphate buffer) was mixed and incubated at 65°C for 2 h. For the determination of PEITC amount is needed the conversion of glucosinolates to isothiocyanates and derivatization of the isothiocyanates by a cyclocondensation reaction. 2 mg of dry watercress powder was solved in 2 mL deionized water and added 1 mL myrosinase. It was incubated at 37°C for 2 h, and centrifugated at 875 g for 15 min. It was derivatized by cyclocondensation reaction before HPLC analysis (20). For the chromatographic separation method in HPLC (Agilent 1100, Germany), a C18 column (150×4.6 mm i.d., 5 µm-ACE columns) and a mobile phase (Methanol-water (90:10, v/v) were used. It flowed at 1.1 mL/min and was measured at 365 nm. The amount of PEITC was calculated according to the calibration curve of PEITC standards. The correlation coefficient was found to be 0,999 for PEITC (**Figure 2**). The amount of PEITC in watercress was expressed as µg PEITC per mg of watercress.

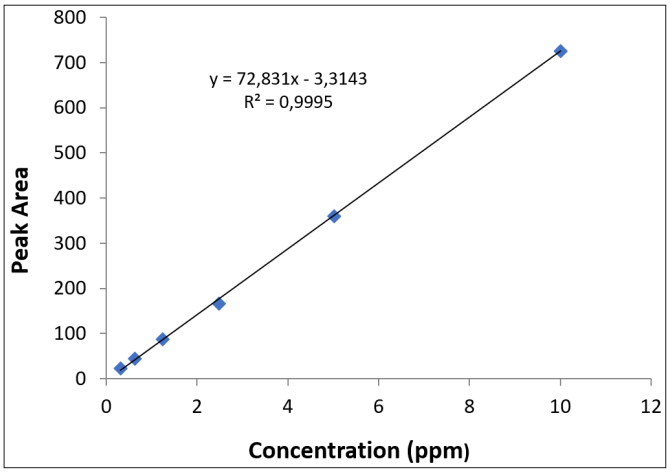

Figure 2. Calibration curve of standard solutions of PEITC.

Biochemical Analysis

Determination of protein content of liver tissues: The protein levels in the liver tissues were measured by the method of Bradford using BSA as the standard (21). Protein levels were expressed as milligram protein.

Determination of alanine aminotransferase (ALT): Rat ALT ELISA kit (Elabscience) was used to measure ALT level in the rat serum, according to the manufacturers' instructions. ALT level was expressed as ng/mL.

Determination of superoxide dismutase (SOD) activity: The SOD activity was measured using the Nitroblue tetrazolium (NBT) assay as described by Beauchamp and Fridovich (22). NBT-formazan was spectrophotometrically determined at 560 nm. SOD activity was expressed as U/mg protein.

Determination of glutathione peroxidase (GPx) activity: GPx activity was determined by the method of Lawrence and Burk (23). The absorbance at 340 nm was recorded for 1 min. GPx activity was expressed as U/mg protein.

Determination of catalase (CAT) activity: CAT activity was determined by the method of Luck (24). The absorption was detected spectrophotometrically at 240 nm for 1 min. CAT activity was expressed as U/mg protein.

Determination of malondialdehyde (MDA): Amount of MDA was measured using the method of Mihara and Uchiyama (25). The absorption at 532 nm was recorded using 1,1,3,3-tetramethoxypropane as the standard. MDA content was expressed as nanomoles per milligram protein.

Determination of GST activity: GST activity assay kit (Cayman) was used to determine the activity of GST in the rat liver, according to the manufacturers' instructions. The absorption was monitored at 340 nm. GST activity was expressed as nmol/min/mg protein.

Determination of NQO1 activity: NQO1 activity assay kit (Abcam) was used to determine the activity of NQO1 in rat liver, according to the manufacturers' instructions. The absorbance was detected at 440 nm. NQO1 activity was expressed as U/mg protein.

Determination of Nrf2-DNA binding activity: To measure the DNA binding activity of Nrf2 in liver nuclear extracts Nrf2 Transcription Factor Assay Kit (Cayman) was used.

Histological Analysis

The liver tissues were fixed in 10% formalin solution for 24 h. Following embedded in paraffin, sections of the liver tissue were cut at 5 µm and stained with hematoxylin-eosin (H-E) and Masson's trichrome methods. These samples were examined under light microscopy (Leica Micros Imaging Solutions Ltd., Cambridge, UK). The microscopic score of each tissue was calculated as the sum of the scores given to each criterion. Scores were given as absent (0), slight (1), moderate (2), and severe (3) for each criterion. The maximum score was 9.

Statistical Analysis

The IBM SPSS software version 22 was used for data analysis. Whether the data showed normal distribution was analyzed according to the Kolmogorov-Smirnov Test. For the data that had normal distribution, the One-Way ANOVA test was used for statistical analyses. The Post-hoc LSD Test was used for the comparisons among the groups. For the data that didn't have a normal distribution, the Kruskal-Wallis test and the Post-hoc Mann-Whitney U test were used. Arithmetic mean ± Standard Deviation (SD) was used for the biochemical data. Differences were considered significant when p values were less than 0.05 and 0,0001 for the biochemical and histological data, respectively.

RESULTS

Histological Findings

Histopathological analysis results were given in **Table 1**. The liver tissues of the control group did not present any histopathological alterations and significant damage was shown in the liver tissue of the DMBA-treated group ($p < 0.0001$). Liver tissues of the DMBA+watercress (WS) group showed markedly reduced damage defined by less amount of mononuclear cell infiltration, hemorrhage, eosinophilic cells with a pyknotic nucleus ($p < 0.0001$). Histopathological images of the control, DMBA, and DMBA+WS groups are shown in **Figures 3**, **4**, and **5**, respectively. In the control group, the normal parenchymal structure was observed in liver tissue (**Figure 3 (A,B)**). There is no histological alteration of the central vein and surrounding hepatocytes in the portal areas. The liver tissue of the DMBA group (**Figure 4 (A-E)**) showed significantly histopathological damage. Liver necrosis (**Figure 4 (A,E)**), hepatocytes with a pyknotic nucleus and eosinophilic cytoplasm (**Figure 4 (A,B)**), hemorrhage (**Figure 4 (A,B**)), mononuclear cell infiltration (**Figure 4 (C)**), vascular congestion (**Figure 4 (C,E)**), and sinusoidal dilatation (**Figure 4 (D)**) were observed. The liver tissue of the DMBA+WS group presented a decrease in histopathological damage. In liver tissue of the DMBA+WS group, a small amount of mononuclear cell infiltration (black arrows), hemorrhage (**Figure 5 (A)**), eosinophilic cells with a pyknotic nucleus (black arrows) were observed (**Figure 5 (B)**).

Biochemical Findings

Alterations in serum ALT level related to liver function are represented in **Figure 6**. It was observed that serum ALT level increased as a result of the application of DMBA, and then decreased in the DMBA+WS group ($p < 0.05$). The DNA binding activity level of the Nrf2 transcription factor was decreased significantly ($p < 0.05$) in the DMBA group, and a significant increase ($p < 0.05$) was observed in the DMBA+WS group (**Figure 7**). Our results showed decreased activities of NQO1 and GST in the DMBA group . The administration of watercress significantly modulated the activities of NQO1 and GST (**Figure 8**). Furthermore, activities of SOD, GPx, and CAT were decreased in the DMBA group compared to the control and increased in the DMBA+WS group (**Table 2**). MDA levels were significantly elevated in the DMBA group and decreased by watercress administration (**Table 2**).

Table 1. The mean histopathological damage score of all groups. Lowercase letters a, b, c in the same column show the differences between groups. $p<0.0001$.

Group	Histopathological damage score (Mean±SE)
Control	0.39 ± 0.59^{a}
DMBA	2.11 ± 0.88^{b}
DMBA+watercress	1.30 ± 0.80^{c}

Lowercase letters a, b, c in the same column show the differences between groups. $p<0.0001$

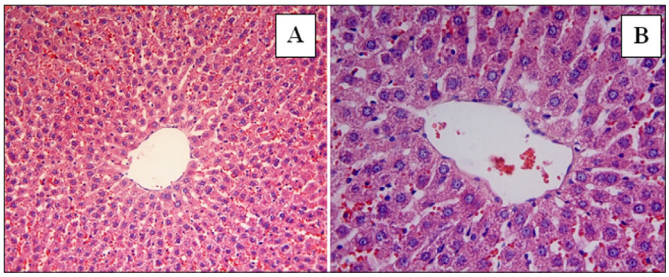

Figure 3. (A,B) Liver specimen from the control group showing a normal appearance. In the control group, liver tissue was normal histological and hepatocytes arranged in the form of cords around the vena centralis were observed. A: H-E; X20, B: H-E; X 40.

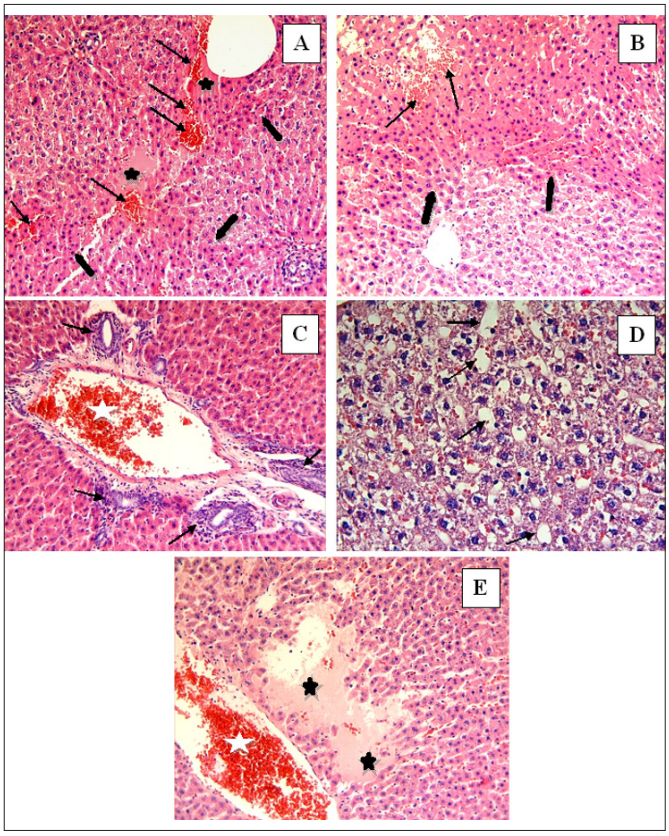

Figure 4. (A-E) Liver specimen from the DMBA-treated group. In the DMBA-treated group, necrosis of the liver tissue (black star) (A, E), hepatocytes with eosinophilic stained pyknotic nuclei (thick black arrows) (A, B), haemorrhage (thin black arrows) (A, B), mononuclear cell infiltration (thin black arrows)) (C), vascular congestion (white star) (C, E) and sinusoidal dilatation (thin black arrows) (D) were observed. A, B, C, E: H-E; X20, D: H-E; X 40.

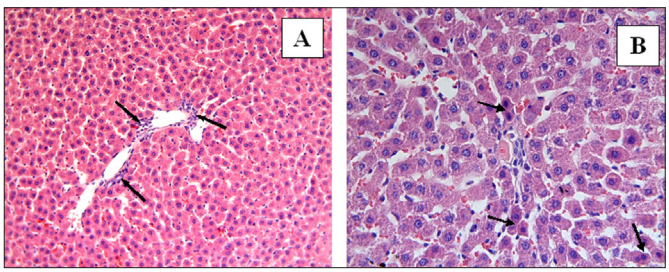

Figure 5. (A,B) Liver specimen from the DMBA+watercress group. In the DMBA+watercress group, mononuclear cell infiltration (black arrows) (A), haemorrhage (black arrows) (A), and hepatocytes with eosinophilic stained pyknotic nuclei (black arrows) (B) were observed. A: H-E; X 20, B: H-E; X 40.

Table 2. Effects of DMBA and DMBA +Watercress treatments on antioxidant parameters in rat liver tissues. Data are presented as mean±SD

Group (n)	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)
Control (9)	7550.4±582.04	38.398±11.7	130.12±2.74	134.97±9.42
DMBA (9)	6642.2±521.69[a]	19.272±6.02[a]	108.23±4.59[a]	212.46±8.57[a]
DMBA+Watercress (9)	7425.4±262.54[b]	67.691±14.9[a,b]	118.66±3.92[a,b]	152.98±3.07[a,b]

[a]p <0.05 compared to the control group; bp < 0.05 compared to the DMBA group.

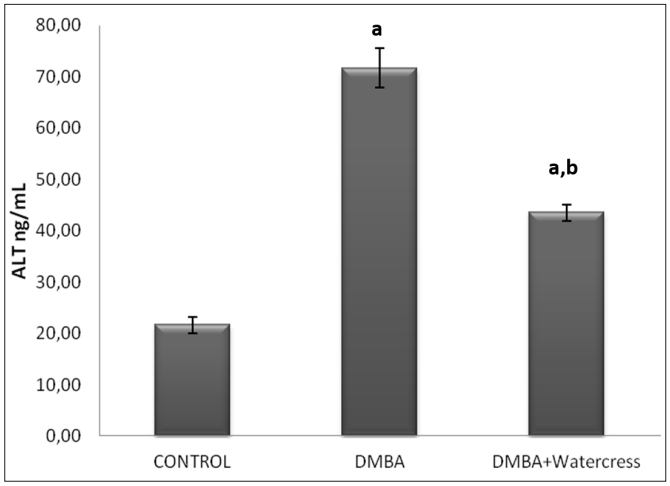

Figure 6. Serum ALT levels in all groups. Data are presented as mean±SD. [a]p<0.05 versus control; [b]p<0.05 versus DMBA

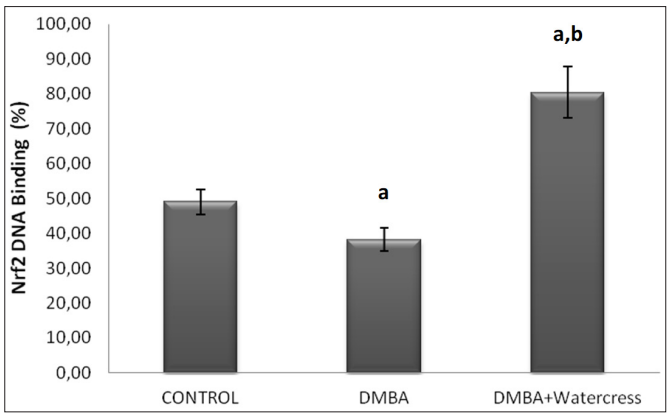

Figure 7. DNA binding activity level (%) of Nrf2 transcription factor in all groups. Data are presented as mean±SD. [a]p<0.05 versus control; [b]p<0.05 versus DMBA

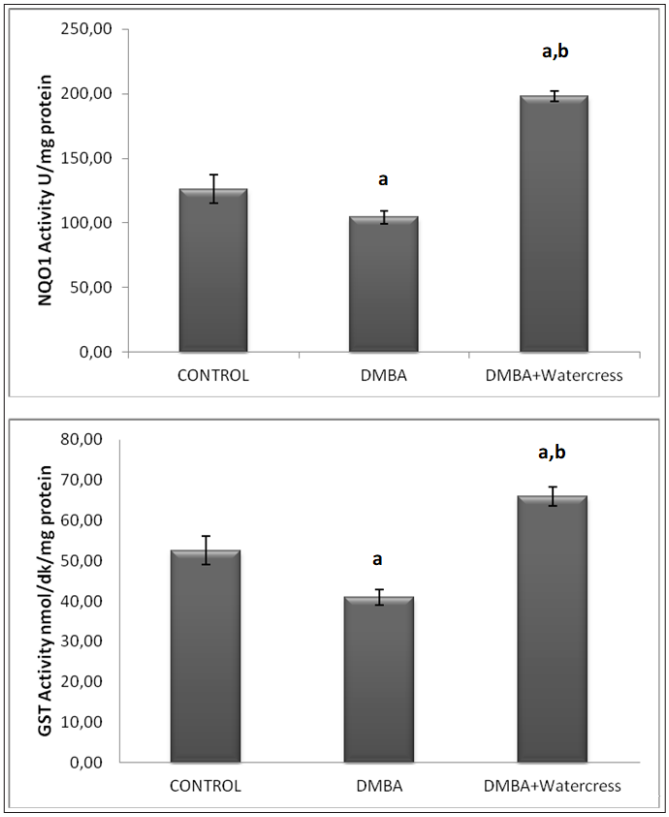

Figure 7. GST and NQO1 activities in all groups. Data are presented as mean±SD. [a]p<0.05 versus control; [b]p<0.05 versus DMBA

DISCUSSION

The present study investigated both histopathological and biochemically whether watercress has a protective effect in DMBA-induced liver injury by Nrf2/ARE signalling pathway. DMBA is a potent carcinogen and causes liver damage via reactive metabolites and oxidative stress (26). In this study, we used histopathological findings and serum ALT level to evaluate the severity of DMBA-induced liver damage. We observed that there was a significant increase in ALT level in the DMBA group in comparison to the control group. Administration of watercress caused a significant decrease in ALT level compared to the DMBA group. Moreover, the histopathological analysis showed normal parenchymal structure in the control group, whereas DMBA showed significant histopathological damage in the liver tissue. Reduction in histopathological damage was observed in the watercress treated group and a small amount of mononuclear cell infiltration, haemorrhage, eosinophilic stained pyknotic nucleus cells were observed. Taken together, histopathological data and serum ALT level of our study, we can suggest that watercress ameliorated DMBA-induced liver damage. In parallel with our results, Azarmehr et al. (27) reported that watercress has a hepatoprotective effect against acetaminophen-induced liver damage. Additionally, Doustimotlagh et al. (28) showed that Nasturtium officinale R. Br and quercetin combination protected against cyclophosphamide-induced hepatotoxicity in rats.

Overproduction of ROS triggers serious damage to various cells associated with increased MDA level that is an important marker of lipid peroxidation and oxidative damage. In our study, the highest tissue MDA level was observed in the DMBA group. MDA level was significantly lower in the watercress group when compared with the DMBA group (29). Activities of

SOD, CAT, GPx were significantly decreased in DMBA-treated rats when compared to control group and administration of watercress enhanced the activities of SOD, CAT, GPx. The alteration in antioxidant status increases the risk of oxidative damage to nucleic acids, proteins, lipids, and small intracellular molecules (30). Many experimental studies reported that enhancement of oxidative stress is contributed to DMBA-induced cancer (31). In this study, administration of watercress significantly reduced DMBA-induced elevation lipid peroxidation and also decreased the activities of liver enzymatic antioxidants. In line with our results, Sadeghi et al. (32) reported that hydroalcoholic extract of watercress ameliorated oxidative stress and liver injury in bile duct ligation-induced cholestatic rats by preventing the hepatic protein oxidation and enhancing the activity of the antioxidant enzymes.

It has been reported that up-regulation of Keap1 and down-regulation of Nrf2 expression increased cellular ROS (33). Nrf2-mediated antioxidant signalling pathways are critical for the prevention of oxidative stress-induced cell injury (34). It has been showed that various dietary phytochemicals target Nrf2/Keap1 signalling and induce the expression of antioxidant and phase II enzymes to inhibit the development of DMBA-induced carcinogenesis model (35). Our results revealed that the binding percentage of the Nrf2 transcription factor had a statistically significant decrease in the DMBA group comparison to the control group. In the watercress group, the percentage of Nrf2/ARE binding significantly was activated compared to the DMBA group. These results can be associated with sulfur-containing glucosinolate derivatives, such as PEITC. These natural isothiocyanates reduce Nrf2 degradation which results in the translocation to the nucleus of Nrf2 (36). Previous studies reported that dietary isothiocyanates are an important cancer chemoprevention compound for the Nrf2/ARE system, which induces the activation and expression of phase II enzymes (37). Saravan et al. (38) were reported that thymoquinone protected liver tissue from DMBA toxicity by regulating phase I and phase II detoxification enzymes. In present study, there was a statistically significant decrease in phase II enzymes (NQO1 and GST) activities in the DMBA group compared to the control group. Administration of watercress significantly increased the activities of these enzymes compared to the DMBA group. According to our study, it can be suggested that there is a good correlation between the protective effect of watercress and supporting the DMBA detoxification by the Nrf2 signalling pathway. Induction of phase II detoxifying enzymes by Nrf2 assists to eliminate toxic reactive intermediates generated via xenobiotic metabolism (39).

There are two major limitations for our study. First, we were only be able to measure ALT to evaluate the level of liver damage. Changes in ALT level were supported with histopathological analysis, but we could not measure other liver function parameters due to financial conditions. Another limitation is that we did not design and conduct a study on the molecular mechanism about which way watercress affects the Nrf2/ARE pathway. This topic has been planned for our future research.

Overall, this experimental study suggests that watercress ameliorated oxidative stress and liver injury through the regulation of antioxidant and phase II enzymes by the activation Nrf2/ARE signalling pathway in DMBA-induced liver damage in rats.

CONCLUSION

The study revealed that the watercress plant is effective through the activation of Nrf2/ARE signalling resulting in increased antioxidant and phase II detoxification enzyme activities which are important for the chemoprevention of liver damage caused by DMBA. Our data support that activation of Nrf2 is a promising strategy for chemoprevention. In this context, we think that watercress functions as an Nrf2 activator and effect of its PEITC content on the molecular mechanism should be investigated with further studies

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Local Ethics Committee of the Animal Experiments of İnönü University (Date: 20/02/2015, Decision No: 2015/A-25).

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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Transplantation for ultra high-risk neuroblastoma patients: effect of tandem autologous stem cell transplantation

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ABSTRACT

Aim: Neuroblastoma is a tumor with a high mortality rate originating from primitive sympathetic ganglion cells. In recent years, the definition of "ultra high-risk" has been used, in which the response to treatment is added in addition to the disease characteristics. In this study, the results of tandem high-dose Iodine 131 (^{131}I) metaiodobenzylguanidine (MIBG) and tandem autologous hematopoietic stem cell transplantation (HSCT) in patients who were diagnosed as refractory after first-line treatments and considered with ultra-high risk nöroblastom (NBL) in Erciyes University Medical Faculty Pediatric Bone Marrow Transplant Center were shared.

Material and Method: Eleven patients who underwent tandem MIBG and autologous HSCT with the diagnosis of refractory/ultra high-risk NBL between 2011 and 2021 were included in the study. Patient's data were obtained retrospectively using an electronic or manuscript medical record.

Results: Tandem autologous HSCT and MIBG treatment were applied to 11 patients with a median age of 40.5 months who were diagnosed with refractory/ultra high-risk neuroblastoma. In our study, the overall survival rate was 54.5% and event-free survival (EFS) was 39% at a median follow-up of 20.7 (7.8-105.6) months. Of the 6 surviving patients, 4 are followed in complete remission and 2 in partial remission.

Conclusion: Our study is the only study that includes a group of patients who received tandem high-dose MIBG and tandem autologous HSCT transplantation in refractory NBL patients without cytokine and immunotherapy. Tandem transplantation and MIBG therapy in ultra-high-risk NBL cases remain promising with acceptable toxicity. Adding immunotherapy to this treatment protocol and combining it with new treatment modalities such as anaplastic lymphoma kinase (ALK), which also takes into account genetic characteristics, may increase survival and event-free survival rates.

Keywords: Neuroblastoma, tandem stem cell transplantation, children, tandem MIBG

INTRODUCTION

The spectrum of neuroblastic tumors (this term includes neuroblastomas, ganglioneuroblastomas, and ganglioneuromas) is one of the most common lethal malign diseases that arise from primitive sympathetic ganglion cells (1). Neuroblastoma (NBL) is the most common extracranial malignancy overall in the first year of life as the cause of 15% mortality of childhood cancers and disease is characterized by a heterogeneous group of histopathologic appearance, biologic characteristics with onset usually before age 5 years old (2-4). Neuroblastomas constitute 13% of childhood cancers in Turkey (5). Remarkably divergent courses of tumors are typically associated with patient age, tumor stage, and histology, genetic and chromosomal abnormalities (6). Global surveys estimate the occurrence of NBL in the range of eight to ten per million children 0-14 years (2,3).

NBL remains distinct from other solid tumors. The disease has ranged from extremely aggressive metastatic disease to spontaneous regression forms, this is probably due to their unique histological and biological character (6). While the most adrenal gland involvement was observed (40%), up to 25% followed by abdominal, 15% thoracic, 10% other nervous sympathetic systems; location of the tumor determines the symptoms including abdominal mass or pain, weakness, constipation and bladder dysfunction, palpable non-tender subcutaneous nodules, anemia, and bone pain. Elevated levels of catecholamines

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may lead to significant morbidity and death but also "atypical" presentation such as horner syndrome, proptosis, periorbital ecchymoses, or opsoclonus myoclonus syndrome expected (1,2,6). Previous studies have shown that the most robust prognostic factors are age, stage, metastasis, histology, Stage of the tumor at the time of diagnosis, and amplification of the N-Myc oncogene (7). Markers associated with a poor prognosis include ferritin, lactate dehydrogenase (LDH), and neuron-specific enolase (NSE). However, these markers have become less important due to the discovery of N-Myc amplification, deletion of chromosome 1, and heterozygosity at 11q23 (1,7).

Standard care of high-risk NBL is defined as multi-modal treatment which includes induction chemotherapy, surgical tumor resection, immunotherapy, consolidative high-dose chemotherapy with autologous hematopoietic stem cell transplantation (HSCT), post-consolidation therapy to treat minimal residual disease including isotretinoin, post-transplant radiotherapy (8,9,10). Detection of GD2 and ALK expression in neuroblastoma cells brings up alternative treatment options (11). Survival is around less than %15 before these intense therapies but, it is still below 50% despite all these treatment regimens (9,10). The use of high-dose chemotherapy with autologous HSCT rescue and Iodine 131 (^{131}I) metaiodobenzylguanidine (MIBG) has still insufficient in survival however, patients with the high-risk disease requires treatment with multi-modal therapy (3). Especially, refractory and ultra high-risk patients (which including treatment response) have an extremely poor prognosis; despite gradual improvements chemotherapy, surgery, radiotherapy, and HSCT with %10-15 survival (8,12).

Studies over the past few years show that survival of NBL patients undergoing tandem transplantation is significantly better, by the time tandem HSCTs are getting the standard first-line therapy in The United States of America step by step (8,13,14). There are still institutes that apply myeloablative regimens consolidated with immunotherapy and isotretinoin as standard therapy (15). Thus, improvement of treatment strategies of stem cells is warranted.

In this study, the results of tandem high-dose MIBG and tandem autologous HSCT in patients who were diagnosed as refractory after first-line treatments and considered with ultra-high risk NBL in Erciyes University Medical Faculty Pediatric Bone Marrow Transplant Center were shared. In comparison to previously published bone marrow transplantation experience, our data is the only study with both tandem high-dose MIBG and tandem autologous HSCT transplantation for pediatric ultra high-risk neuroblastoma patients.

MATERIAL AND METHOD

Patients

Eleven patients who underwent tandem MIBG and autologous HSCT with the diagnosis of refractory/ultra high-risk neuroblastoma at Erciyes University Pediatrics Bone Marrow Transplant Center between January 2011 and January 2021 were included in the study. Patient's data were obtained retrospectively using an electronic or manuscript medical record.

The study was carried out with the permission of Erciyes University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 08.07.2020, Decision No: 2020/371). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Diagnosis, Risk Classification, Genetic Study

All patients were diagnosed with tissue biopsy. Molecular analyzes were performed on tissue samples taken at the time of diagnosis at Dokuz Eylül University Oncology Institute. Bone marrow aspiration and biopsy were used for staging the disease, MIBG, positron emission tomography/computed tomography (PET CT) and/or whole-body magnetic resonance imaging (MRI) were used as imaging modalities. In the evaluation of patients' response to treatment, biochemical parameters such as lactate dehydrogenase, ferritin, NSE; if there is bone marrow involvement at the time of diagnosis or if additional symptoms developed, bone marrow aspiration and biopsy; MIBG, PET CT and/or MRI were used as imaging modalities. The patients were staged according to the International Neuroblastoma Staging System (INSS) (16). The patients' response to treatment was evaluated using the international neuroblastoma response criteria (17).

Treatment Protocols

All patients received the Turkish Pediatrics Oncology Group Neuroblastoma 2009 (TPOG 2009) treatment protocol. Tumor resection was performed before and/or after the protocol according to the size, localization and invasion of the tumor. Interim evaluation was made after the 3rd or 4th cycle. Following the interim evaluation, hematopoietic stem cells were collected from the patient with the help of an apheresis device using a central venous catheter. During the stem cell collection stage, granulocyte colony-stimulating factor (GCSF) was administered at a dose of 10 mcg/kg to the patients. Enough cells for two transplants were collected and cryopreserved. 8-12 mCi/kg dose of MIBG was given to the patients who completed the induction treatment and underwent surgery.The first transplantation was performed with conditioning regimen consisting of busulfan and melphalan 3 weeks after the first MIBG.

MIBG was repeated at a dose of 8-12 mCi/kg after first autologous HSCT in patients. In the second autologous HSCT, a conditioning regimen containing melphalan, etoposide and carboplatin was used. Nine cycles of 13-cis retinoic acid were given in the post-transplant maintenance treatment (**Figure 1**).

Statistical Analysis

Categorical data were expressed as the number of patients and percentage and continuous variables were expressed as the mean±standard deviation or median (minimum-maximum). Nominal variables were expressed as number of cases and percentage (%). The Kaplan-Meier method was used to estimate survival probabilities.

RESULTS

The median age of 11 patients (8 boys, 3 girls) included in the study was 40.5 (range, 22.1-72.9) months. The clinical features of the patients are summarized in **Table 1**.

The median value of CD34 (+) cell count in the stem cell product given in the first transplantation was 7.5 (range, 4.55-17.1) $\times 10^6$ cells/kg. The median day of myeloid and platelet engraftment was detected as +12 (9-22) days and +18 (12-28) days, respectively. The mean time between two HSCT was 4.7±0.5 months. In the second HSCT, 5.7 (2.7-16.7) $\times 10^6$ cells/kg CD34(+) cells were given. The median day of myeloid and platelet engraftment was +11 (9-15) days and +19 (16-21) days, respectively.

Only one patient did not have bone metastases at the time of diagnosis (Patient 6). One patient died on the 45th day of the second transplantation due to venoocclusive disease and sepsis, and 4 patients died due to progressive disease. Transplant-related mortality was 4.5%. After the second HSCT, 3 patients (Patient 9, 10, and 11) with residual tumors were given radiotherapy for local control. Retinoic acid maintenance therapy was given to the surviving patients (Patient 2, 5, 8, 9, 10, and 11).

In our study, the overall survival rate (OS) was 54.5% and event-free survival (EFS) was 39% at a median follow-up of 20.7 (range, 7.8-105.6) months (**Figure 2**). Of the 6 surviving patients, 4 are followed in complete remission and 2 in partial remission (**Table 1**).

Table 1. Patient characteristics

Patient number	Sex	Diagnosis age (month)	Tumor type	Histology	Stage	N-Myc	Outcome
1	Boy	26.7	Ganglioneuroblastoma	Poorly Differentiated	4	Amplified	Exitus
2	Boy	24.4	Neuroblastoma	Intermixed	4	Amplified	Remission
3	Boy	24	Neuroblastoma	Undiferantiated	4	Not amplified	Exitus
4	Girl	35.9	Neuroblastoma	Undiferantiated	4	Not amplified	Exitus
5	Boy	45.6	Neuroblastoma	Undiferantiated	4	Not amplified	Remission
6	Boy	13.7	Neuroblastoma	Undiferantiated	4	Amplified	Exitus
7	Girl	88.2	Neuroblastoma	Undiferantiated	4	Not amplified	Exitus
8	Girl	31.7	Ganglioneuroblastoma	Poorly Differentiated	4	Not amplified	Remission
9	Boy	13.1	Neuroblastoma	Undiferantiated	4	Amplified	Remission
10	Boy	33.8	Neuroblastoma	Poorly Differentiated	4	Amplified	Partial Remission
11	Boy	48.8	Ganglioneuroblastoma	Poorly Differentiated	4	Not amplified	Partial Remission

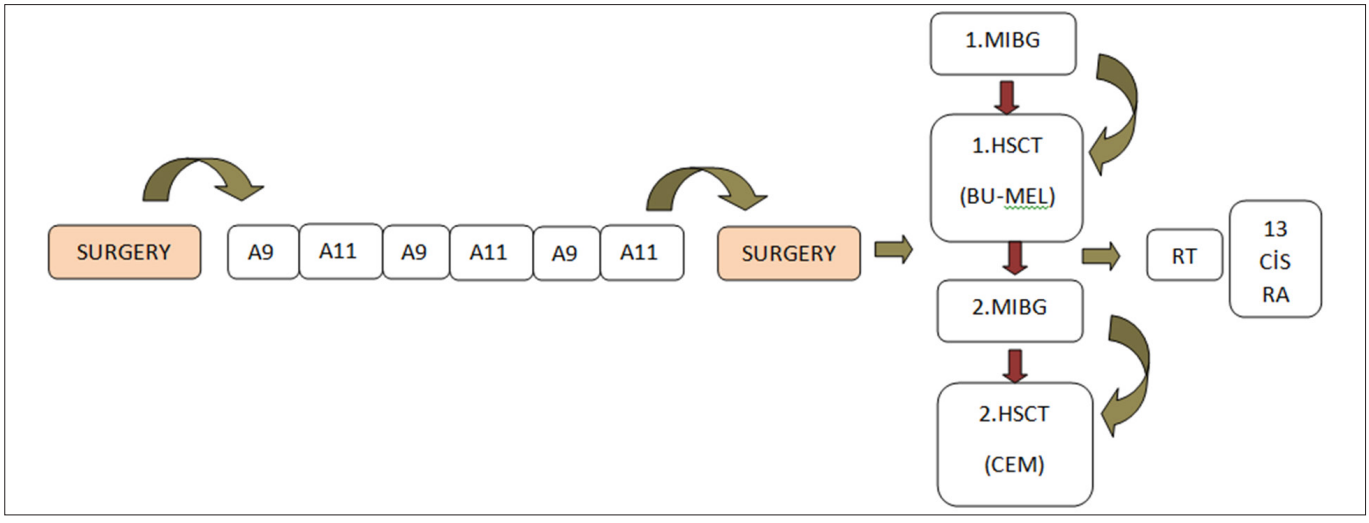

Figure 1. Treatment scheme

A9: Vincristine, dacarbazine, ifosfamide, adriamycin; A11: Cyclophosphamide, etoposide, cisplatin; Bu: Busulfan; Mel: Melphalan; CEM: Carboplatin, etoposide, melphalan; HSCT: Hematopoietic stem cell transplantation; MIBG: Iodine 131 (131I) metaiodobenzylguanidine ; RT: Radiotherapy; 13 CİS RA: 13-cis retinoic acid

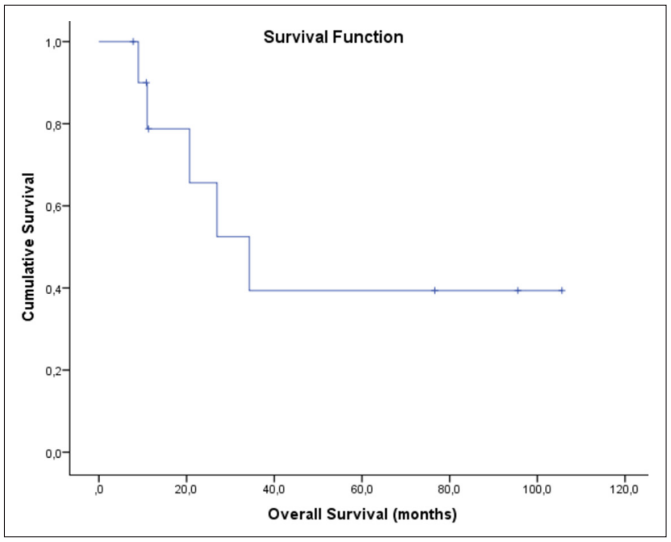

Figure 2. Overall survival of patients

DISCUSSION

Neuroblastoma is related to the disproportionate morbidity and mortality among the cancers of childhood and has been shown that high-dose chemotherapy with autologous stem cell rescue has improved the outcomes for children with high-risk neuroblastoma (8,18). In non-metastatic patients, chemotherapy, surgery, and/or radiotherapy are often cured. However, 40% of these patients are high-risk or ultra high-risk cases which is extremely difficult to provide cures for these patients. The current therapies including high-dose chemotherapy and HSCT are border on tolerability limits with insufficient effectiveness. Therefore, alternative protocols aiming at curative treatment are required.

The high-risk tumors include a heterogeneous group of patients with a wide range of outcome spectrum. Treatment of 5 phases with high-risk tumors includes; induction, surgery, metastatic remission, myeloablative chemotherapy with autologous HSCT, radiotherapy, and biological differentiation therapy (19). Main modalities have been standardized however, autologous HSCT approaches vary considerably. In the literature, autologous HSCT was performed with MIBG to be applied once to patients in the high-risk group, and the overall response rate was found to be 27% with the 31% 3-year EFS (20). In 2012, Qayed et al., showed that patients who were randomized to high dose chemotherapy and HSCT had a significantly improved at 3-year EFS compared with high dose chemotherapy and HSCT (4-year EFS 59.3±6.7% vs. 26.8±9.2%, p=0.01) (21). The Children's Oncology Group (COG) Phase 3 trial (NCT00567567) compared carboplatin-etoposide-melphalan and thiotepa based tandem HSCT. They found significantly increased 3 year EFS (3-year) scores (61.8% versus 48.8%; p=.0082) (14). Follow up results of this randomized controlled trial data in 2019 comparing tandem and single transplants show 85 of 176 tandem HSCT were in complete remission/very good partial response, and 121 of 176 were needed immunotherapy (8). Another immunologically based study compared post-transplant dinutuximab beta effects was also concluded that improved EFS (5-year) results with superior significant improvement in OS (5-year) (66.7% versus 11.4%; p=.0007) (22). Major tandem studies are summurized in **Table 2** (8,23-27). The experience of allogeneic HSCT in high-risk neuroblastoma patients is increasing over time (27,28).

In our study, the overall survival rate of ultra high-risk patients was 54.5% and EFS was 39% without cytokine and immunotherapies. In our patients with minimal toxicity, this tandem treatment modality provides encouraging results. Our results appear to be better than the previously high-risk studies eventhough immunotherapies were not used at all. MIBG is an adrenal imaging analogue marker initially developed at the University of Michigan fifty years ago; however, by the time with studies as therapy has directed on palliation for patients with refractory disease. The majority of authors focusing on patients with MIBG avid, non-responsive or progressive disease with safety profile, and also potential efficacy

Table 2. Tandem Hematopoietic Stem Cell Transplantation Data

Study	Patient	Pre-transplant remission status	Conditioning regimen	Additional treatment	OS	EFS
Park et al.	176	PR, CR, MR	Cy-TT/CEM	RT, 13 Cis RA, IT	74% (3-year)	61% (3-year)
Pasqualini et al.	26	PR, CR	TT/ Bu-Mel	RT, 13 Cis RA	69% (3-year)	37.3% (3-year)
Suh et al.	18	PR, CR, MR	CEM/Bu-Mel/TT-Cy	RT, 13 Cis RA, single MIBG, IT	83% (5-year)	64% (5-year)
Lee et al.	54	PR, CR, MR	CEC/TT-Mel	RT, 13 Cis RA, single MIBG, IT	72.4% (5-year)	58.3% (5-year)
Sung et al.	50	PR, CR, MR	CEC/TT-Mel-TBI/ TT-Mel	RT, 13 Cis RA, IT	77% (5-year)	71.4% (5-year)
George et al.	82	-	CEC/TBI-Mel	RT, 13 Cis RA	64% (5-year)	54%(5-year)-PFS
Our Data	11	PR, PD	Bu-Mel/CEM	RT, 13 Cis RA, tandem MIBG	54.5% (3-year)	39% (3-year)

CR: Complete response, PR: Partial response, MR: Mixed response, PD: Progressive disease, TT: Thiotepa, Bu: Busulfan, Mel: Melphalan, TBI: Total body irradiation, CEM: Carboplatin, etoposide, melphalan, CEC: Carboplatin, etoposide, cyclophosphamide, Cy: Cyclophosphamide, RT: Radiotherapy, 13 Cis RA: 13 Cis retinoic acid, MIBG: Iodine 131 (^{131}I) metaiodobenzylguanidine, IT: Immunotherapy, PFS: Progression free survival, OS: Overall survival, EFS: Event free survival

of this regimen, the combination of chemotherapy and stem cell rescue is being considered within COG for the refractory neuroblastoma patients (29). As a result, our study suggested that tandem MIBG and tandem autologous HSCT combination might indeed improve the EFS of the ultra high-risk refractory patients.

The limitations of the study were that it is retrospective design and the sample is relatively small. In addition, the patients were not given cytokine and immunotherapy/ anti-GD2 antibodies. Survivors should be evaluated for any toxicity.

CONCLUSION

This study is the only case series study with both tandem high-dose MIBG and tandem autologous HSCT transplantation in refractory NBL patients without cytokine and immunotherapy. Tandem transplantation and MIBG therapy in ultra high-risk NBL cases remain promising with acceptable toxicity. Our data show that a significant contribution may be added to the survival of ultra high-risk NBL patients based on this dual protocol. Adding immunotherapy to this treatment protocol and combining it with new treatment modalities such as anaplastic lymphoma kinase (ALK), which also takes into account genetic characteristics, may increase survival and EFS rates.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 08.07.2020, Decision No: 2020/371).

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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The relationship between preoperative anxiety level, ABO blood types and birth outcomes in cesarean sections

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ABSTRACT

Objective: There is a relationship between ABO blood groups, which are part of the genetic phenotype, and various psychiatric diseases. Our primer aim in this study is to examine the relationship between ABO blood groups and preoperative anxiety (POA) levels in patients undergoing elective cesarean section. The secondary aim is to determine the relationship between POA levels and birth outcomes in pregnant women.

Material and Method: A total of 132 patients with different ABO blood groups who were scheduled for elective cesarean section under spinal anesthesia between August 2020 and July 2021 at the Medical Faculty Hospital were included in this prospective observational study. State Anxiety Inventory scores (SAI) were used to assess preoperative anxiety in groups A, B, AB, and O. Birth outcomes (fetal birth weight, birth sex and APGAR scores at the 1st and 5th minutes) were recorded.

Results: There were 55 people (41.7%) in the A blood group, 43 people (32.6%) in the O blood group, 25 people (18.9%) in the B blood group, 9 people (6.8%) in the AB blood group. A total of 73/132 (55.3%) patients with SAI score >40 were identified. There was no significant difference in mean SAI scores in A, B, AB and O blood groups (p=0.531). Fetal birth weight was found to be lower in patients with high SAI scores (p=0.044).

Conclusion: There was no relationship between ABO blood groups and preoperative anxiety and birth outcomes in elective cesarean sections under spinal anesthesia, but low fetal birth weight was found in pregnant women with high anxiety. More studies with larger sample sizes in the future are needed to confirm the results of our study.

Keywords: ABO blood types, preoperative anxiety, birth outcomes, spinal anaesthesia, caesarean section

INTRODUCTION

Antenatal preoperative anxiety (POA) is a common problem that can cause negative physiological responses in pregnant women due to pain and fear of delivery. POA is more common in cesarean section, which is the most common obstetric surgery (1-4).

High-level anxiety symptoms before cesarean section cause an increase in catecholamine levels and thus may result in increased heart rate, blood pressure, and cardiac stimulation (1,2,5). However, many studies have shown that maternal stress causes many negative clinical outcomes such as low birth weight and preterm delivery (5-7). For these reasons, predetermining the factors that trigger POA by clinicians can reduce these negative outcomes.

While POA level varies according to age, gender, type of operation and negative operation experience, genetic factors also affect anxiety level (8). Multifactorial factors play a role in the etiology of many psychiatric diseases. These factors are generally genetic and environmental factors. Studies examining the genetics of psychiatric diseases have shown that gene-environment interaction is determinant, especially in anxiety disorders (9). Blood group types are genetically inherited from parents and ancestors (9,10). Observational studies investigating ABO blood types, preoperative anxiety and phenotypic factors are limited in the literature. To the best of our knowledge, a study examining ABO blood types and preoperative anxiety was found in the literature (11). In this study, POA levels of patients with AB blood group were found to be higher than those with other blood groups (11).

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When the literature is examined, it has been found that ABO blood groups are associated with many clinical conditions, including diabetes, various types of cancer, and cardiovascular diseases (12,13). Thus, it reveals that ABO blood group types may be associated with some diseases. For these reasons, we hypothesized in this study that there is a relationship between ABO blood types and preoperative anxiety levels in CS deliverys, which is a stressful surgical procedure.

The main aim of current study is to evaluate the relationship between POA levels according to ABO blood groups in patients undergoing elective cesarean section. The secondary aim is to determine the relationship between POA levels and birth outcomes in pregnant women.

MATERIAL AND METHOD

Study Design

This prospective cross-sectional study was conducted at Yozgat Bozok University Faculty of Medicine Hospital (Yozgat, Turkey) between August 2020 and July 2021. The study was carried out with the permission of Yozgat Bozok University Clinical Research Ethics Committee (Date: 29.07.2020, Decision No: 2017-KAEK-189_2020.07.29_07). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Participants

A total of 132 pregnant women between the ages of 18-45 whose gestational age was 36-41 weeks, who gave written informed consents and who underwent elective cesarean section with medical indications under spinal anesthesia were included in the study. The numbers of the A, O, B and AB blood type groups were 55, 43, 88 and 9, respectively. Patients with a known psychiatric or neurological disease, drug users, patients who did not have the mental competence to understand the survey questions or were illiterate, patients scheduled to undergo an emergency cesarean operation, urgent patients were not included in the study.

Procedures

Preoperatif anxiety levels in patients who were referred to an anesthesia outpatient clinic due to elective cesarean section were evaluated using the State Anxiety Inventory (SAI). Maternal demographic and medical data (smoking status, educational level, employment status, anesthesia history) were recorded for prenatal evaluation by anesthetists. The gestational week was calculated based on the ultrasound imaging performed during the first trimester by the obstetrics department. Following delivery, fetal birth weight, birth sex and APGAR scores at the 1st and 5th minutes were recorded. The health status of the newborns was evaluated using the 10-point APGAR score, which evaluates five parameters (heart rate, respiratory effort, muscle tone, reflex irritability and color) with a score from 0 to 2. A total score of 7 or higher suggests that the newborn is in good health, while a total score of 4 to 6 means the newborn needs medical attention, and scores of 3 or lower indicate a critical condition (14).

Preoperative anxiety assessment

The SAI scale (20 items) determines how the individual feels at a certain time and under certain conditions. The SAI scale is scored between 20 and 80 points, where higher scores indicate a higher level of anxiety. The SAI is widely used to assess anxiety in pregnancy, and their validity and reliability have been demonstrated (1). In the evaluation of the scale score, 40 points was considered the threshold value, as is common in the literature (1), scores below 40 were classified as 'low to moderate' anxiety, and scores above 40 were classified as 'high' anxiety.

Statistical Analysis

Data were analyzed using SPSS v.20.0 (SPSS Inc., Chicago, IL, USA) software. Data were expressed as mean±standard deviation. The normality of distribution was checked using the Kolmogorov–Smirnov test and histograms. Normally distributed continuous variables are reported as mean ± standard deviation (SD), non-normally distributed variables as median [interquartile range], and categorical variables as counts (%). Differences in non-normally distributed variables between two independent groups were assessed using the non-parametric Mann–Whitney U-test. A one-way ANOVA, chi-square test, or Kruskal-Wallis pairwise comparisons were used to identify the difference among the A, B, AB, and O blood type groups in some variables. All comparative analyzes were 2-tailed, and a P-value of less than 0.05 was considered to be statistically significant.

RESULTS

Of the 132 female patients included in the study, 41.7% (n=55) A, 32.6% (n=43) O, 18.9% (n=25) B, and 6.8% (n=9) AB were patients with blood group. The mean age of blood groups A, B, AB and O was 28.8±6.0, 31.6±5.8, 31.5±7.3 and 29.3±5.9, respectively. Blood groups did not differ significantly according to sociodemographic (age, gravity, parity, gestational age, smoking status, education level, employment status, anesthesia history) and clinical characteristics. Fetal birth weight was similar between groups (p=0.456). There was no difference between ABO blood groups according to birth sex (p=0.230). The 1st min and 5th min APGAR scores were similar between the groups (0.747, 0.539, respectively) (**Table 1**).

Table 1. Demographic and clinical characteristics of patients according to ABO blood groups

Variables		A (n= 55)	B (n= 25)	AB (n= 9)	O (n= 43)	Total (n=132)	P value
Age (years)		28.8±6.0	31.6±5.8	31.5±7.3	29.3±5.9	29.7±6.0	0.198[a]
Gravity		2 [2 to 3]	3 [2 to 4]	3 [2 to 4]	3 [2 to 4]	3 [2 to 4]	0.296[b]
Parity		1 [1 to 2]	2 [1 to 2]	2 [1 to 2]	2 [1 to 3]	1 [1 to 2]	0.280[b]
Gestational age (weeks)		38 [38 to 39]	38 [38 to 39]	38 [38 to 39]	38 [38 to 39]	38 [38 to 39]	0.992[b]
APGAR score 1st min		8 [8 to 9]	8 [7 to 9]	8 [7 to 9]	9 [7 to 9]	8 [8 to 9]	0.747[b]
APGAR score 5th min		9 [9 to 10]	9 [8 to 10]	10 [9 to 10]	10 [9 to 10]	9 [9 to 10]	0.539[b]
Fetal birth weight (grams)		3175±454	3327±373	3283±432	3287±480	3248±447	0.456[a]
Birth sex	Male	36 (65.5)	11 (44)	4 (44.5)	22 (51.2)	73 (55.4)	0.230[*]
	Female	19 (34.5)	14 (56)	5 (55.5)	21 (48.8)	59 (44.6)	
Smoking status, n (%)	Yes	14 (25.5)	8 (0.32)	1 (11.2)	15 (34.9)	38 (28.7)	0.462[*]
	No	41 (74.5)	17 (0.68)	8 (88.8)	28 (65.1)	94 (71.3)	
Educational level	Primary school	20 (36.3)	8 (32)	3 (33.3)	18 (39.5)	49 (36.3)	0.906[#]
	Secondary school	26 (47.2)	10 (40)	4 (44.4)	17 (39.5)	57 (43.1)	
	University	9 (16.3)	7 (28)	2 (22.3)	8 (18.6)	26 (19.6)	
Employment status	Employed	21 (38.1)	10 (40)	3 (33.4)	14 (32.5)	48 (36.4)	0.913[*]
Anesthesia history	Yes	36 (65.5)	16 (64)	5 (55.5)	30 (69.7)	87 (66.0)	0.859[*]
	No	19 (34.5)	9 (36)	4 (44.5)	13 (30.3)	45 (34.0)	

Values are quoted as the mean ± SD and median [interquartile range] for continuous variables and percentage for categorical variable. [a]One-way ANOVA test and [b]Kruskal-Wallis test were used to identify the difference among the A, B, AB, and O blood type groups in some variables. *Chi-square Test; #Fisher's exact test. SAI, State Anxiety Inventory.

Table 2. Comparison of patients' SAI scores according to ABO blood groups

Variables	A (n= 55)	B (n= 25)	AB (n= 9)	O (n= 43)	Total (n=132)	P value
SAI score	42.1±10.9	45.4±11.9	43.4±11.0	41.5±10.7	42.6±11.0	0.531[a]
Patients with SAI score >40, n (%)	31 (56.4)	15 (60.0)	5 (55.6)	22 (51.2)	73 (55.3)	0.921[#]

Values are quoted as the mean ± SD for continuous variable and percentage for categorical variable. [a]One-way ANOVA test was used to identify the difference among the A, B, AB, and O blood type groups in SAI score. [#]Fisher's exact test, SAI: State Anxiety Inventory

SAI score was used to assess preoperative anxiety in patients with blood groups A, B, AB, and O. Then, the relationship of different blood groups with SAI scores was examined. The mean SAI score of the patients was 42.6±11.0. A total of 73/132 (55.3%) patients with SAI score >40 were identified. The number of patients with high preoperative anxiety did not differ according to ABO blood groups (p=0.921). There was no significant difference in mean SAI scores in A, B, AB and O blood groups (p=0.531) (**Table 2**). The lowest SAI score was found in O blood group (41.5±10.7) and the highest SAI score was found in B blood group (45.4±11.9).

Detailed information about the differences between pregnant women with and without high anxiety is presented in Table 3. There was no statistically significant difference between maternal age and gestational week and POA groups (p=0.367, p=0.436, respectively). Fetal birth weight was found to be lower in patients with high SAI scores (p=0.044) (**Table 3**). However, there was no significant correlation between SAI scores and APGAR scores (respectively, p=0.055, p=0.066) (**Table 3**).

Table 3. Characteristics of the mother and the newborn according to preoperative anxiety groups

Variables	Patients with SAI score <40	Patients with SAI score >40	P value
Maternal characteristics			
Age (years)	30 ± 6	29 ± 6	0.367[a]
Gestational age (weeks)	38 [38 to 39]	38 [38 to 39]	0.436[b]
Newborn characteristics			
Fetal birth weight (grams)	3327 ± 468	3187 ± 421	0.044[a]
APGAR score 1st	9 [8 to 9]	8 [7 to 9]	0.055[b]
APGAR score 5th	10 [9 to 10]	9 [9 to 10]	0.066[b]

Values are quoted as the mean ± SD and median [interquartile range] for continuous variables. [a]Independent sample t test, [b]Mann Whitney U test, SAI: State Anxiety Inventory.

DISCUSSION

Although many studies have investigated the relationship between various psychiatric diseases and blood groups, the relationship between POA and ABO blood groups has not been studied in pregnant women with high anxiety levels who will have a cesarean delivery. The primary aim of this prospective, observational study was that preoperative anxiety scores of pregnant women in cesarean deliveries were associated with ABO blood group types; The secondary aim was to test the hypothesis that POA scores affect birth outcomes. Several important findings were identified in the current study. Firstly; POA levels of pregnant women did not differ according to ABO blood group types. Secondly; Lower fetal birth weight was found in pregnant women with high POA levels. Finally; There was no significant correlation between POA levels and APGAR scores.

Many studies have shown that genetic factors such as blood group antigens are associated with the development and severity of some diseases (diabetes, cardiovascular diseases, endocrine diseases) (12,13,15). It has been determined that genes controlling blood group antigen

expression are associated with genes encoding dopamine beta-hydroxylase, catechol-O-methyl-transferase and argininosuccinate synthetase activities that regulate neuropsychiatric responses. It has even been claimed that genes encoding ABO blood group antigens control dopamine beta-hydroxylase activity (15,16). For these reasons, the potential role of the ABO blood group in psychiatric diseases (schizophrenia, bipolar and unipolar depression) and personality traits, which are thought to be predominantly inherited, has been investigated in various studies, but conflicting results have been found (17-19).

When the literature was examined, in a study examining the relationship between dental anxiety and blood group in children, the highest level of dental anxiety was found in those with AB blood group (20). Similarly, Feng Xu et al. (11), preoperative anxiety levels were found to be higher in people with AB blood type. In another study examining blood groups and stress levels, it was found that pathological and general stress levels were found in people with the lowest O blood group (21). Similarly, in the current study, the mean SAI score was found in the lowest O blood group (41.5±10.7) and the highest in B blood group (45.4±11.9). However, no significant relationship was found between the POA levels of pregnant women and ABO blood groups. The reason for these results may be related to the effect of many variables on POA, including surgical factors other than blood groups.

In the literature, there are studies reporting a relationship between maternal ABO blood groups and adverse birth outcomes (most commonly preeclampsia, venous thromboembolism, chorioamnionitis, and postpartum hemorrhage) (22,23). Premature birth and low birth weight are among these negative outcomes associated with blood type. In a recent study, it was reported that pregnant women with maternal B blood group had lower fetal birth weight, but there was no relationship between preeclampsia and gestational week and blood groups (22). In the same study, the authors reported that fetal growth may be adversely affected as a result of placental insufficiency due to the etiological role of blood groups in arterial thrombosis. However, in this study, the authors did not analyze other adverse pregnancy risk factors such as gestational diabetes mellitus, smoking, chorioamnionitis and venous thromboembolism (22). According to the results of our study, no significant difference was found between ABO blood groups and fetal birth weight, APGAR scores and gestational week. This issue, which are still controversial, need to be investigated with further studies.

Another important result in our study is that pregnant women with high POA levels have lower fetal birth weights and similar APGAR scores. POA, which is more common in cesarean deliveries, has been shown to cause many adverse birth outcomes such as premature birth, low birth weight, and low APGAR scores (5-7). It has been reported that the autonomic stress response triggered by maternal anxiety may cause negative fetal outcomes due to uterine artery vasoconstriction (7,24). On the other hand, although there are studies reporting that there is no relationship between maternal anxiety and APGAR scores (25), there are also studies reporting that maternal anxiety causes low APGAR scores (4,7). There is still no consensus on whether there is a true relationship between POA and adverse birth outcomes.

This study has some strengths and limitations. One of its strengths is that, to the best of our knowledge, it is the first original study in the literature to investigate the relationship between AB0 blood groups and preoperative anxiety in pregnant women. Other strengths are that the study is prospective and blood groups are obtained from data of the Hospital Blood Bank, not verbally. The limitations are; rare blood groups require more patients due to the small number of patients, and Rh sub blood groups are not included in the study.

CONCLUSION

We planned this study with the hypothesis that there may be a relationship between ABO blood groups, which are known to be associated with many psychiatric disorders and various personality types, and preoperative anxiety. As a result, it was determined that there was no relationship between ABO blood groups and preoperative anxiety and birth outcomes in cesarean section, but low fetal birth weight was found in pregnant women with high anxiety. For this reason, the identification of pregnant women with high preoperative anxiety levels and removing anxiety may contribute to the prevention of negative birth results to the fetus such as low birth weight. However, we think that our results are interesting and further studies are needed in different races and geographical regions to clarify these results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Yozgat Bozok University Clinical Researchs Ethics Committee (Date: 29.07.2020, Decision No: 2017-KAEK-189_2020.07.29_07).

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

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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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Is there a relationship between the liver SUVmax values in FDG-PET/CT imaging and non-alcoholic fatty liver disease score?

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ABSTRACT

Aim: Non-alcoholic fatty liver disease is one of the most common causes of liver disease worldwide with an estimated prevalence of 20%–30% in adult population. Following the widespread utilization of PET in the evaluation of malignant diseases, F-18 FDG have also been reported to be used in non-malignant processes. The aim of this study is to elucidate whether the FDG SUVmax values determined by PET/CT in different adipose tissue samples and the liver change according to NAFLD score. During our desktop research we did not find any published article therefore, it is the first study in this field.

Materials and Method: A total of 230 patients who applied to Dicle University Faculty of Medicine, Department of Nuclear Medicine between March and April 2020 and who have been conducted FDG PET/CT for diagnosis, staging, restaging and evaluation of response to treatment were included in the study. Patients were divided into three groups according to their NAFLD score as patients with fibrosis score <-1,455 (the group in which severe fibrosis was excluded) as group-1, and those with NAFLD score between -1.455-0.676 (inter-mediate score) as group-2. and patients with a NAFLD score >0.676 (severe fibrosis group) group-3.

Results: Liver SUVmax levels were found to be significantly higher in group-3 than group-1. No significant difference was observed between group-2 and group-3. SUVmax levels measured from supracalvicular region, posterior scapular region and mesentery region were not different from each other in all three groups. Glucose-corrected liver SUVglu levels were found to be significantly lower in group-1 than group-3 (p=0.001). In terms of liver SUVglu levels, group-1 and group-2 and group-2 and group-3 did not differ statistically from each other. Supracalvicular SUVglu, posterior scapular SUVglu and mesenteric SUVglu groups were not different from each other.

Conclusions: The most important result of this study could be elaborated with increased FDG uptake in NAFLD. Liver FDG uptake increases as the severity of NAFLD increases as demonstrated by the NAFLD score.

Keywords: SUVmax, FDG-PET/CT, nonalcoholic steatohepatitis score

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of liver disease worldwide. NAFLD is a broad terminology that includes simple fatty liver spectrum of chronic liver disease that progresses to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and end-stage liver (1-4). The excessive accumulation of triglycerides and cholesterol in the liver is classified as alcoholic fatty liver disease or non–alcohol-related fatty liver disease (NAFLD). NAFLD is the most common chronic liver condition in the developed countries, with an estimated prevalence of 20%–30% in adult population (5,6).

PET/CT technology is a non-invasive imaging method commonly utilized as it provides important metabolic and functional data and high spatial resolution. Fluorine (F)-18, a radiopharmaceutical glucose analogue is a labeled Fluoro-2-deoxy-D-glucose (FDG) compound is mostly used in routine practice (7). The sensitivity and

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specificity of FDG PET/CT is quite high in metastatic liver lesions and other possible metastatic foci due to the whole body scanning advantage (8). Lymphocytes involved in infectious and inflammatory events, such as neutrophils and macrophages increased intracellular hexokinase and surface glucose transporter present a similar pattern to malignant cells and show affinity for F-18 FDG (7,8) .

Adenosine triphosphate located inside the inflammatory cells phosphorylates FDG and increases concentration cytoplasmic conversion of FDG to FDG 6-phosphate. However, as FDG-6-phosphate is not the convenient substrate for glucose-6-isomerase enzyme it cannot enter into metabolic reactions and accumulates inside the cells. The accumulation of FDG due to increased glucose transport activity during inflammatory processes on glucose metabolism and the amount of FDG accumulated derives information about the pathological areas showing impaired glucose metabolism in three dimensional display (9,10).

SUV is a semiquantitative measurement which corresponds to measured activity normalized for body weight/surface area and injected dose (11, 12). The formula for calculation of SUV is region of interest (ROI) activity (mCi/mL) x body weight (g) / injected dose (mCi). SUV is a proportional value without units, rather than being an absolute value in characterization of lesions, so for the quantification of tumour FDG uptake there was always a need for a site in the body which is presumed to have normal FDG uptake (13,14). If the 18F-FDG uptake in the target lesion is greater than in the liver in terms of SUV, the hypermetabolic focus would be considered abnormal (15).

Study Hypothesis

The aim of this study is to elucidate whether the FDG SUVmax values determined by PET/CT in different adipose tissue samples of the liver and body change according to NAFLD score.

Fatty liver has been elaborated with different methods in previous studies. In this research we preferred to proceed by calculating the NAFLD score, considering it as a practical method. During our desktop research we did not find any published article therefore, it is the first study in this field.

MATERIAL AND METHOD

The study was carried out with the permission of Dicle University Medical Faculty Non-interventional Studies Ethics Committee (Date: 2021, Decision No: 358). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 230 patients who applied to Dicle University Faculty of Medicine, Department of Nuclear Medicine between March and April 2020 and who have been conducted FDG PET/CT for diagnosis, staging, restaging and evaluation of response to treatment were included in the study. Because the study was designed retrospectively, no written Informed consent form was obtained.

NAFLD fibrosis score is a non-invasive, simple and easy-to-apply scoring system, which is obtained using 6 variable parameters (age, presence of impaired fasting glucose (IFG) or diabetes, body mass index (BMI), AST/ALT ratio, platelet count, and albumin) and allows to accurately determine the presence, absence and severity of fibrosis(16). The files of the patients were examined (blood tests in the last month were examined), and the patients with ALT, AST, albumin and platelet levels in their files were included in the study. The height and weight of the patients were measured and their BMI (body mass index) was calculated. Patients were divided into three groups according to their NAFLD (Non-alcoholic fatty liver disease) score. According to the score defined by Angulo P et al. (16), patients with fibrosis score <-1,455 (the group in which severe fibrosis was excluded) as group-1, and those with NAFLD score between -1.455-0.676 (inter-mediate score) as group-2. and patients with a NAFLD score >0.676 (severe fibrosis group) were classified as group-3.

In group-1, a total of 28 of patients had diabetes or IGT and 66 did not. In group-2, 37 had diabetes or IGT, while 50 did not. In group-3, 41 of the patients had diabetes or IGT, 8 did not.

SUVmax value was calculated by drawing 1 cm diameter ROI (region of interest) from the periphery of the right lobe of the liver. In addition, SUVmax values were measured from the fatty planes in the right supraclavicular region, from the subcutaneous tissue of the posterior part of the left scapula, and from the mesenteric area of the abdomen. All three groups were compared and was investigated whether there was a difference between the SUV values obtained from the supraclavicular region, especially the liver, and the mesentery of the infracalvicular region. In addition, patients' glucose-adjusted SUV values were SUVmax×blood glucose level/100 were calculated via SUVglu in order to compare 3 groups (17).

Method and Device Information

For the FDG PET/CT imaging, patients were required to fast for at least 6 h and have a blood glucose level of 140 mg/dL. FDG at a dose of 0.1 mCI/kg was injected intravenously into the patients. After the injection, the patients were kept in a special lead-coated room for 1 h

for the medication to spread through the whole body, and a CT scan of the whole body (from vertex to knees) was performed. Subsequently, whole-body emission scanning was performed with PET. A 2016 model Siemens Horizon brand PET/CT device with 3D-TOF was used for imaging. The slice thickness of the device was 3 mm, and the images were created according to PET iterative and by the CT bp-LOR reconstruction processing method. The low-dose CT device used for anatomical detail and attenuation correction was adjusted to 80 mA and 120 kV (Siemens Healthcare, GmbH Henkestrasse 127, 91052 Erlangen, Germany).

Anthropometric Evaluations

According to the height and body weight data obtained from the file records of the patients, the BMI of the patients was calculated with the formula weight(kg) / height(m)2.

Inclusion Criteria

Patients who had blood tests in the last month and whose bio-distribution of F-18 FDG in the whole body was within normal limits.

Exclusion Criteria

Patients with liver cancer or liver metastasis were not included to the study as these cancer types may affect liver SUV. Patients with alcoholic stetohepatitis, viral hepatitis, pregnancy, autoimmune hepatitis, drug-induced toxic hepatitis, cirrhosis, insulin and pioglitazone using individuals have been excluded from the study. Imaging was performed in patients who received chemotherapy after waiting at least two weeks from the last chemotherapy

Statistical Analysis

IBM SPSS 21.0 for Windows statistical package program was used for the statistical evaluation of the data. Measurable variables were presented as mean ± standard deviation, and categorical variables as numbers and percentages (%). The chi-square ($\chi 2$) test was used for comparing categorical variables. Oneway Anova and Post-Hoc Bonferroni were used for comparing measurable variables. Pearson correlation test was used to show correlation between NAFLD score and liverSUVmax, liverSUVglu age and glucose levels. A P value ≤0.05 indicated a statistically significant difference.

RESULTS

The mean ages of all three groups were statistically different from each other. The mean age of group-1 was statistically significantly lower than group-2 and group-3 (p<0.001). The mean age was not different between group-2 and group-3. There was no difference between the groups in terms of gender. Group-1 mean body weight was significantly lower than group-2 (p=0.03). There was no difference in body weight between group-2 and group-3.

There was no difference between the three groups in terms of height and BMI. Fasting blood glucose was found to be different from each other when all three groups were compared (p=0.001). Fasting blood glucose level was found to be significantly lower in group-1 than group-2 (p<0.0001).

The proportion of patients with impaired glucose tolerance and diabetes was found to be significantly higher in group-3 and group-2 than in group-1. Gender distribution, age, anthropometric parameters, glucose AST , ALT, albumin and platelet levels of the groups have been presented in **Table 1**.

Tablo 1. Sex distribution, age, anthropometric parameters, glucose AST ,ALT Albumin and platelet levels of the groups

	Group 1 (n=94)	Group 2 (n=87)	Group 3 (n=49)	P value
Age (years)±SD	48.01±13.92	59.5±13.3	62.7±13.4	0.0001
Gender (female/male)	55/39	40/47	29/20	>0.05
BMI (kg/m2)	25.0±4.8	26.7±4.7	25.9± 5.5	>0.05
Glucose (mg/dl)	97.94±23.44	106.6±21.16	115.78±31.09	0.001
AST (IU/L)	24.46±23,33	27.5±18.5	41.4±47.7	0.004
ALT (IU/L)	23.11±22.87	22.2 ±23.4	21.3±23.9	>0.05
Platelets (cell/ml)	331±109	239±74	170±95	0.0001
Albumin (g/dl)	3.89±0.56	3.76±0.63	2.99±0.75	0.0001

AST levels were significantly different from each other in the three groups (p=0.004). While the AST level of group-1 was found to be significantly lower than group-3 (p=0.003), the AST level of group-2 was found to be significantly lower than group-3 (p=0.02). ALT levels did not differ between groups.

All three groups were different from each other in terms of platelet levels. Group-1 had lower platelet levels than group-2, while group-2 had a significantly lower platelet level than group-3 (p=0.0001).

The albumin levels in the three groups were significantly different from each other (p=0.0001). While the albumin level of group-1 was found to be significantly lower than group-3 (p=0.0001), the AST level of group-2 was found to be significantly lower than group-3 (p=0.0001).

Liver SUVmax levels were found to be significantly higher in group-3 than group-1. No significant

difference was observed between group-2 and group-3. SUVmax levels measured from supracalvicular region, posterior scapular region and mesentery region were not different from each other in all three groups. NAFLD score showed a positive correlation with the liver SUVmax (r=0.254, p=0.02) liverSUVglu(r=0.284, p=0.01), age (r=0.482, p=0.001) and (r=0.225, p=0.03) glucose levels. (**Table 2**, **Figure 1**).

Glucose-corrected liver SUVglu levels were found to be significantly lower in group-1 than group-3 (p=0.001). In terms of liver SUVglu levels, group-1 and group-2 and group-2 and group-3 did not differ statistically from each other. Supracalvicular SUVglu, posterior scapular SUVglu and mesenteric SUVglu groups were not different from each other (**Table 2**, **Figure 2**).

Table 2. NAFLD score of the groups, SUV measurements from the liver supraclavicular supscapular and mesenteric region, and SUVglu measurements corrected for glucose level

	Group 1 (n=94)	Group 2 (n=87)	Group 3 (n=49)	P value
NAFLD score	-2.80±1.18	-0.447±0.643	2.223±1.279	0.0001
Liver SUVmax	3.39±0.74	3.66±0.73	3.68±0.77	0.02
Supraclavicular region SUVmax	0.57±0.12	0.53±0.12	0.52±0.10	>0.05
Posterior scapular SUVmax	0.55± 0.12	0.53±0.16	0.55±0.15	>0.05
Mesenteric SUV	0.65±0.36	0.60± 0.12	0.61± 0.15	>0.05
Liver SUVglu	3.79±1.76	4.50±1.85	5.22 ± 2.79	0.001
Supraclavicular region SUVglu	0.63±0.26	0.65±0.29	0.72±0.34	>0.05
Supscapular SUVglu	0.61±0.30	0.64±0.28	0.75 ±0.34	>0.05
Mesenteric SUVglu	0.25±0.18	0.72±0.30	0.86 ±0.45	>0.05

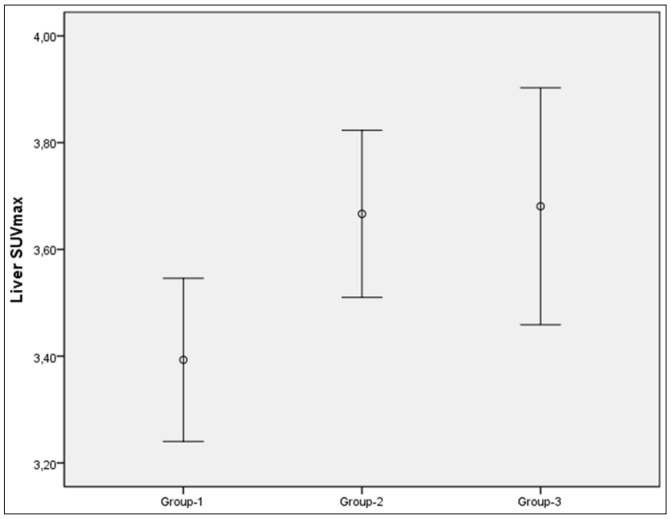

Figure 1. Liver SUVmax values of all three groups. The liver SUVmax value of group-1 was found to be significantly lower than group-3. Group-1 and group-2 Liver SUVmax values were not different. Liver SUVmax values of group-2 were not lower than group-3.

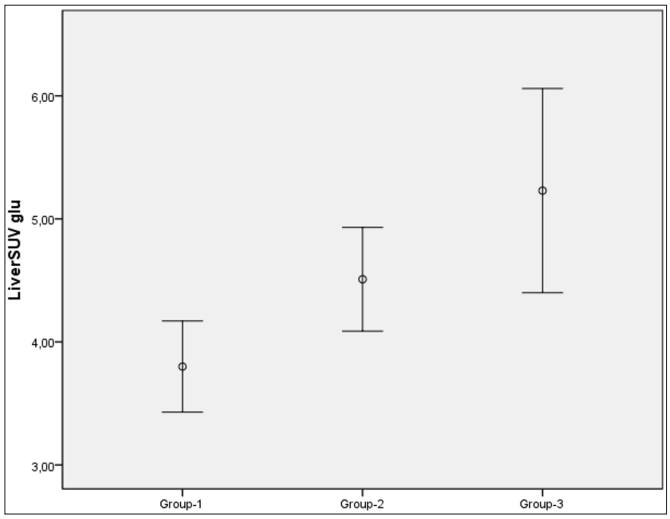

Figure 2. Liver SUVglu values of all three groups. The liver SUVglu value of group-1 was found to be significantly lower than group-3. Group-1 and group-2 Liver SUVglu values were not different. Liver SUVglu values of group-2 were not lower than group-3.

DISCUSSION

In our study, patients with a high NAFLD score have increased liver FDG Uptake. We did not find a similar study in our literature review. The articles in the literature on this subject are limited to FDG uptake measurements in patients with steatohepatitis, and fibrosis has been evaluated with either an invasive method such as liver biopsy or liver MRI, which is very expensive or invasive for the patient (18,19). In our study, this easy and reproducible non-invasive model was used and its relationship with increased liver FDG uptake was emphasized.

The majority of patients with NAFLD will have fatty liver and liver inflammation resulting in altered hepatic FDG kinetics(20, 21). Fatty liver disease, which means accumulation of fat in the form of triglycerides and cholesterol in the liver cells, might induce some sort of inflammation and FDG uptake may be increased as a result of irreversible FDG accumulation in inflammatory cells, suggesting that FDG PET could be developed as a potential imaging approach to NASH (22). The reason for increased FDG uptake in fatty liver can be explained by increased Kupffer cell activity and FDG trapping (23).

In this study we found that liver SUV levels were found to be significantly higher in group-3 than group-1. No significant difference was observed between group-2 and group-3. SUV levels measured from supracalvicular region, posterior scapular region and mesentery region were not different from each other in all three groups. Glucose-corrected liver SUVglu levels were found to be significantly lower in group-1 than group-3. In terms of liver SUVglu levels, group-1 and group-2 and group-2 and group-3 did not differ statistically from each other. Supracalvicular SUVglu, posterior scapular SUVglu

and mesenteric SUVglu groups were not different from each other. The most important phrase that one could derive from this data could be elaborated as, FDG uptake increased in NASH and FDG SUVmax has been detected statistically significantly higher in the group with higher NAFLD scores compared to the group with lower NAFLD scores. FDG uptake may be increased as a result of irreversible FDG accumulation in inflammatory cells, suggesting that FDG PET could be developed as a potential imaging approach to NASH (24, 25).

In this study fasting blood glucose was found to be different from each other when all three groups were compared. Fasting blood glucose level was found to be significantly lower in group-1 than group-2. Blood glucose levels can affect liver FDG SUVmax and therefore FDG SUVmax may have been different in the group with high NAFLD scores. FDG SUVglu levels, in which FDG uptake is calculated according to the patient's blood glucose level, can provide a correction opportunity according to glucose levels (26). In our study, FDG SUVglu levels were found to be significantly higher in the group with high NAFLD score. Based on these findings, the reason for the increase in FDG uptake seems to be due to a different cause than the patient's blood glucose levels.

The AST level of group-1 was found to be significantly lower than group-3, the AST level of group-2 was found to be significantly lower than group-3. ALT levels did not differ between groups. All three groups were different from each other in terms of platelet levels. Group-1 had lower platelet levels than group-2, while group-2 had a significantly lower platelet level than group-3. The albumin levels of group-1 was found to be significantly lower than group-3. Ozulker et al. (27) found a statistically significant difference between the body weight, serum ALT levels, DM, and glucose levels of the patients with fatty liver and the control group. Patients with fatty liver disease have higher AST and ALT levels .

In some cases, NAFLD score may clarify the reason of increase in FDG uptake. Liver biopsy is performed to identify fibrosis and in addition to this the fibrosis score of patients can be predicted with lower FDG doses (28). In this case the calculation of the NAFLD score can reveal whether the FDG increase is due to the increase in the NAFLD score. The liver FDG SUV values are used as reference SUV values and if the NAFLD score is high in the patient, then the FDG SUVmax values will increase, thus causing false judgements because the reference SUV levels change. Currently liver biopsy is the gold standard in NASH to show inflammation, fibrosis and the severity of the disease. However, this procedure is invasive and has difficulties in routine implementation. In this study we have shown that a relationship can be established between FDG uptake and the severity of the disease (29).

Limitations of the Study

The main limitation of this study could be elaborated by its study population as all the enrolled individuals were cancer patients. One of the limitations of our study is that it had a retrospective nature and the mean age was different between the groups.

CONCLUSION

The most important result of this study could be elaborated with increased FDG uptake in NASH. Liver FDG uptake increases as the severity of NASH increases as demonstrated by the NAFLD score. An increase in NAFLD score causes the formation of the inflammatory pathway leading to fibrosis. Future prospective studies with larger number of patients where inflammatory markers are to be measured may enlighten this situation better.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dicle University Medical Faculty Non-interventional Studies Ethics Committee (Date: 2021, Decision No: 358).

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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Evaluation of studies on molecular biology and genetics related to COVID-19 with data mining

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ABSTRACT

Aim: The aim of this study was to examine the most common studies about molecular biology and genetics related to COVID-19. In addition, the aim was also to determine the subject focus of studies about COVID-19 during the pandemic with data mining.

Material and Method: Review and research articles, book chapters, conference abstracts, case reports and mini reviews published between March 2020 and July 2021 were included in this study. We retrieved only articles from the genetics discipline. The MeSH heading "genetics [GENET]" was used including the specific fields in the MeSH hierarchy of cytogenetics, genomics, human genetics, immunogenetics, molecular biology, pharmacogenetics, phenomics, radiation genetics, toxicogenetics, gene ontology, microbial genetics, behavioral and population genetics.

Results: A total of 6234 research articles were evaluated in our study. Of the 85966 terms, 5833 met the threshold from title and abstract extraction. We showed that betacoronavirus, viral pneumonia, viral RNA, spike glycoprotein, coronavirus, middle-aged and animals were the most repetitive terms. Clinical laboratory techniques, polymerase chain reaction and reverse transcriptase polymerase techniques were the main focus for the detection of COVID-19. We found that molecular-based COVID-19 studies were most frequently published by the Journal of Medical Virology, Viruses, and PLoS One. We found that the institutes where molecular-based studies investigating COVID-19 were conducted are in the United States (USA), China and England. The USA and China were in the first rank for countries that conducted the most frequent molecular-based COVID-19 studies, and Turkey was in 19th place in terms of published molecular COVID-19 studies.

Conclusion: It is important to identify the issues and mechanisms most frequently investigated in molecular-based studies related to COVID-19. Scientific approaches founded on evidence-based data may be beneficial to find the curative treatment for COVID-19 infection and to effectively prevent this infection.

Keywords: COVID-19, genetics, molecular biology, data mining

INTRODUCTION

With the worldwide COVID-19 pandemic, humanity is facing a global health threat. There are more than one hundred million infected individuals affected by COVID-19 due to the rapid spread of the virus, resulting in the death of more than four million people. (1).

According to the World Health Organization (WHO), approximately 80% of COVID-19 patients are asymptomatic, approximately 20% of them progress with respiratory tract symptoms, and 5% of these patients need respiratory support. Although the respiratory system is primarily affected including severe pneumonia, COVID-19 involvement in the heart, kidney, nervous system, liver and gastrointestinal system was also reported. Although different races, genders and age groups have equal susceptibility to the virus, i the disease has higher prevalence in people over the age of 60 years (2,3). Individuals with comorbidities such as cardiovascular diseases, hypertension, diabetes, asthma, chronic liver and chronic kidney disease have higher mortality rate (4,5).

Molecular and genetic mechanisms underlie all these COVID-19-related clinical manifestations and conditions that affect the course of the disease (6). Unfortunately, the molecular, biological and genetic mechanisms of the SARS-CoV-2 virus, which we recently encountered, are still not clearly known. Studies and articles dealing with molecular

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and genetic mechanisms, as well as clinical presentations of COVID-19, are being published in increasing numbers. (7, 8). However, the areas of focus in molecular biology and genetics-based COVID-19 studies, which have a very common study subject, that are most researched and which topics involve the most frequently asked questions are unknown. Determining which subjects and areas these studies focus on and which mechanisms they focus on, and taking scientific steps by combining the findings like puzzle pieces is the most powerful and rational course of action to eliminate the COVID-19 infection.

In this current data mining study, the aim was to determine the most common research topics in molecular biology and genetics studies related to COVID-19. Moreover, the aim was also to determine the subject focus of studies about COVID-19 during the pandemic.

MATERIAL AND METHOD

This current study is a computer based data-mining study. There is no need to obtain ethical committee approval. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this research, the study sample consisted of 6234 articles published between March 2020 and July 2021. We addressed all publications regardless of their number of co-authors. Review and research articles, book chapters, conference abstracts, case reports and mini reviews were included in the sample. We retrieved only articles from molecular studies in genetics discipline. The MeSH heading "Genetics [GENET]" was used, including the specific fields in the MeSH hierarchy of cytogenetics, genomics, human genetics, immunogenetics, molecular biology, pharmacogenetics, phenomics, radiation genetics, toxicogenetics, gene ontology, microbial genetics, behavioral and population genetics.

We choose the binary counting method to indicate the number of documents in which a term occurs at least once. For bibliographic mapping, the terms were extracted from MeSH headings, title and abstract fields. For title and abstract text analysis, the minimum number of occurrences of a term was set to 5 and relevance scores were calculated. Of the 85966 terms, 5833 met the threshold from title and abstract extraction. Then, the most relevant terms were selected based on the scores. The terms with low relevance scores were filtered out manually in order to focus on more informative terms. The calculation of relevance scores was performed according to Van Eck and Waltman (9).

Co-occurrence analysis was also performed for MeSH keywords. In the analysis, the relatedness of the items was determined based on the number of documents in which the items occur together. Fractional counting was used to determine the weight of a link. The minimum number of occurrences of a keyword was set to 1, so that all of the MeSH keywords (N=5240) met the threshold for co-occurrence analysis.

RESULTS

As mentioned above, 6234 published articles were included in this data mining study. In **Figure 1** (network diagram of MeSH key terms), betacoronavirus, viral pneumonia, viral RNA, spike glycoprotein, coronavirus, middle-aged and animals are the most repetitive terms and the studies about these terms have the highest link strength. It is noteworthy that almost all studies about COVID-19 are related to each other and the studies concentrate around betacoronavirus and RNA. In addition, studies in the field of COVID-19 genetics are shaped around the keywords shown in **Figure 2**. Clinical laboratory techniques, polymerase chain reaction (PCR) and reverse transcriptase polymerase techniques (RT-PCR) are the main focus for the detection of COVID-19.

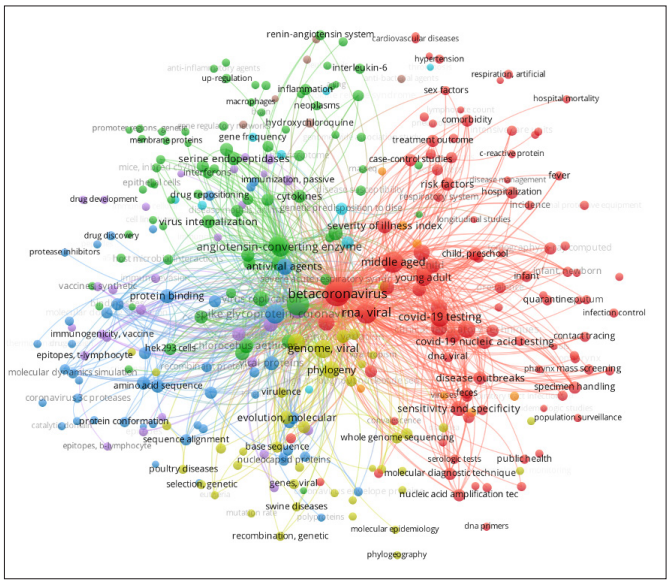

Figure 1. Network diagram of MeSH keywords

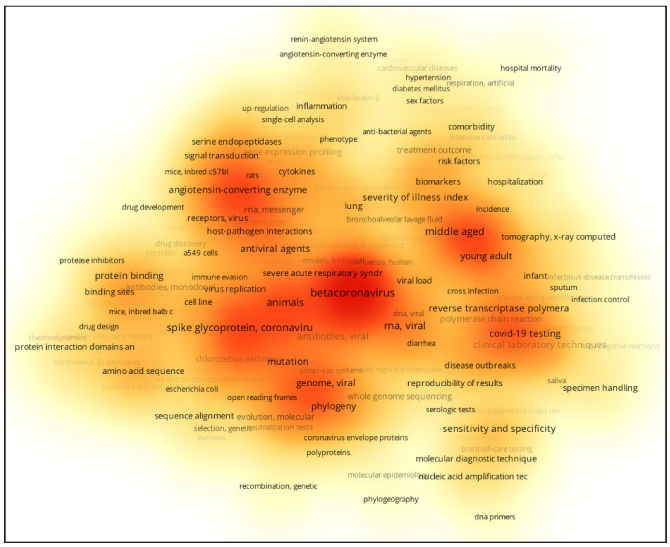

Figure 2. Occurence of MeSH data

Clusters of research fields in the MeSH data are shown **Table 1**. According to **Table 1**, all items are clustered by their links, occurrences and strengths. The subjects in each cluster were studied jointly. Viral pneumonia, betacoronavirus, viral RNA, middle aged and antibodies were in the first cluster. The topics in the first cluster were studied for middle-aged people. The most repeated item in the cluster was betacoronavirus which had the highest link strength, indicating the total strength of the co-authorship with other researchers. Cluster 1 also contained the most studied topics. In cluster 2, animal studies with angiotensin-converting enzyme 2 and peptidyl-dipeptidase-a have the highest links and occurrence. In cluster 3, spike glycoprotein, coronavirus, antiviral agents, protein binding, and viral proteins are included. Phylogeny, mutation, evolution, molecular, genetic variation, and chiroptera terms are included in cluster 4. In cluster 5, COVID-19 vaccines, antibodies, neutralizing, viral vaccines, antigens, viral, antibodies and monoclonal terms are included.

In **Figure 3**, the clustering results for the terms obtained from the titles and abstracts of the studies are given. Accordingly, the studies are gathered around 4 different clusters. The first cluster was carried out in relation to the subjects of receptor binding domain, rbd, phylogenetic analysis, bat, epitobe, viral entry, spike glycoprotein and pedv. In the second cluster, the topics are angiotensin, mouse, tmprss2, IFN, kidney, TNF, ACE2 expression and macrophage.

The studies that make up the 3rd cluster focus on COVID-19 detection, assay, PCR, qPCR and nasopharyngeal swab (**Table 2**).

The most repeated terms in the studies are COVID-19 detection, nasopharyngeal swab and PCR. The distribution of published articles by countries, university departments and number of documents is given in **Table 3**. Accordingly, USA, China and UK are the countries with the most published studies. The list of journals with the highest number of publications is given in **Table 4**. Journal of

Table 1. Clusters of research fields (from MeSH data)

Subject	Cluster	Links	Total Link Strength	Occurence
betacoronavirus	1	312	17695	2290
viral pneumonia	1	313	17186	2253
viral RNA	1	305	8382	1257
middle aged	1	292	8709	1163
reverse transcriptase polymerase chain reaction	1	243	3636	501
viral antibodies	1	272	4371	484
80 and over aged	1	254	3710	431
animals	2	308	10640	1488
angiotensin-converting enzyme 2	2	283	6833	827
peptidyl-dipeptidase-a	2	266	4177	455
virus replication	2	256	2691	342
host-pathogen interactions	2	275	2693	329
lung	2	266	2669	319
chlorocebus aethiops	2	240	2547	264
vero cells	2	237	2471	254
virus receptors	2	234	2277	232
serine endopeptidases	2	204	1792	230
cell line	2	233	1862	223
cytokines	2	240	1593	221
spike glycoprotein, coronavirus	3	298	7829	921
antiviral agents	3	289	3861	503
protein binding	3	224	2786	298
viral proteins	3	243	2056	276
amino acid sequence	3	207	2043	212
phylogeny	4	263	4003	558
mutation	4	277	3712	551
molecular evolution	4	209	1811	243
genetic variation	4	228	1557	216
chiroptera	4	181	1494	171
covid-19 vaccines	5	266	3483	460
antibodies, neutralizing	5	220	2587	273
viral vaccines	5	224	1962	221
viral antigens	5	188	907	99
monoclonal antibodies	5	151	908	90

Table 2. Extracted Items from the titles and abstracts

Label	Cluster	Links	Total Link Strength	Occurence
detection	3	1873	9739	713
assay	3	1642	6417	436
RT PCR	3	1568	5769	382
specimen	3	1175	3178	202
nasopharyngeal swab	3	1035	2969	189
binding	1	1204	2750	237
receptor binding domain	1	1000	2671	231
angiotensin	2	1006	2366	209
rbd (receptor-binding domain)	1	921	2341	191
reaction	3	925	2276	132
phylogenetic analysis	1	938	2162	200
mouse	2	926	2058	172
tmprss2	2	889	2035	182
RT qPCR	3	771	1917	126
s protein	1	881	1880	157
inf (interferon)	2	890	1712	122
bat	1	739	1706	146
woman	4	773	1639	109
adult	4	799	1544	105
epitope	1	642	1430	131
isothermal amplification	3	427	1398	72
reagent	3	537	1330	90
n gene	3	588	1306	81
reverse transcription polymerase chain reaction	3	675	1271	90
kidney	2	660	1247	92

Table 3. Distribution of molecular based COVID-19 studies by country and institution

Institution	Number of Documents	Country
Department of Microbiology, Icahn School of Medicine At Mount Sinai	22	USA
University of Chinese Academy of Sciences	15	China
Departmentof Zoology, University of Oxford	11	UK
Ihu-Méditerranée Infection	11	France
Chan Zuckerberg Biohub	10	USA
Department of Immunology, School of Medicine, Tehran Universityof Medical Sciences	10	Iran
Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern	10	Switzerland
Institute of Evolutionary Biology, University of Edinburgh	10	UK
Laboratório De Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz	9	Brazil
University of Chinese Academy of Sciences	9	China
Africa Health Research Institute, Durban, South Africa	8	South Africa
Broad Institute of Mit and Harvard	8	USA
Department of Biochemistry and Molecular Biology, University of Texas Medical Branch	8	USA

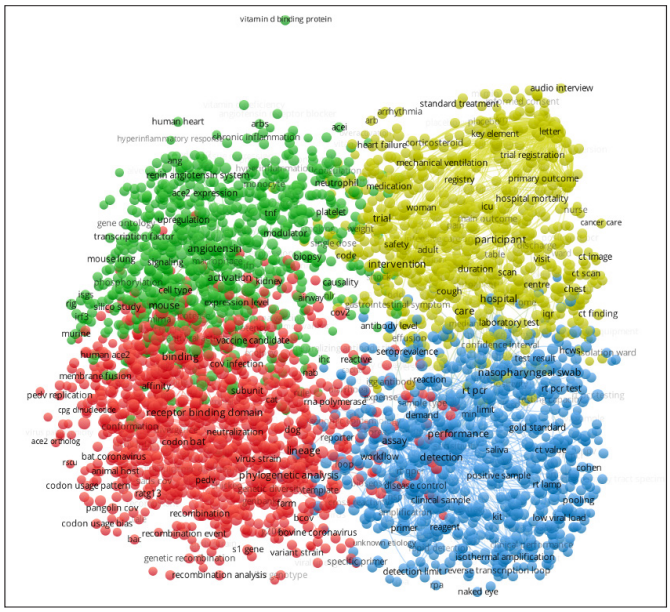

Figure 3. Clusters from title and abstract **extraction**

Medical Virology, Viruses, PLoS One, Scientific Reports, and Nature were found to be the journals with the most publications in 2020, 2021 and in total. Lastly, the list of journals which published molecular-based COVID-19 studies by year is found in **Table 5.**

Table 4. List of journals which published molecular based COVID-19 studies by years

Journal Name	2020	2021	Total
Journal of Medical Virology	95	104	199
Viruses	83	94	177
PLoS One	98	71	169
Scientific Report	70	80	150
Nature	61	56	117
Nature Communications	50	50	100
Frontiers in Immunology	52	32	84
International Journal of Molecular Sciences	52	23	75
Emerging Microbes & Infection	58	17	75
International Journal of Infectious Diseases	41	34	75
Infection, Genetics and Evolution	46	23	69
Science	38	31	69
Cell	36	26	62
Proceedings of the National Academy of Sciences	29	29	58
Journal of Clinical Virology	48	8	56
Journal of Virology	33	14	47
Signal Transduction and Targeted Therapy	32	19	51
Journal of Clinical Microbiology	41	6	47
BMC Infectious Diseases	30	17	47
Medical Hypotheses	34	12	46
Virus Research	29	14	43
Eurosurveillance	32	10	42
Emerging Infectious Diseases	30	12	42
Clinical Microbiology and Infection	25	15	40
Journal of Virological Methods	13	25	38
Archives of Virology	24	12	36
PLoS Pathogens	10	25	35
The New England Journal of Medicine	19	15	34
Nature Medicine	25	9	34
JAMA	17	17	34
The journal of Infectious Diseases	23	11	34
Biochem Biophys Res Commun	8	24	32
Genes (Basel)	19	11	30
Cell Host Microbe	15	15	30
Frontiers in Cellular and Infection Microbiology	20	9	29
British Medical Journal	4	23	27

Table 5. Distribution of molecular based COVID-19 studies by countries

#	Country	Study number
1	USA	2816
2	China	2267
3	Italy	637
4	Germany	353
5	France	342
6	United Kingdom	263
7	India	210
8	Canada	116
9	Japan	113
10	Spain	107
11	Korea	98
12	Switzerland	92
13	Netherlands	86
14	Australia	88
15	Saudi Arabia	72
16	Israel	57
17	Brazil	53
18	Denmark	43
19	Turkey	37
20	South Africa	37
21	Vietnam	37
22	Poland	31
23	Sweden	30
24	Pakistan	30
25	Russia	22
26	Finland	16

Note: In studies with multiple authors, if the countries in which the institutions are located are not the same, the study is equally distributed to the countries of the authors.

DISCUSSION

COVID-19 is a dangerous infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), for which we have no curative treatment (10). COVID-19 first emerged in late December 2019 in the Chinese province of Wuhan and soon spread globally around the world (11). It led to the declaration of the COVID-19 pandemic by the WHO. As of August 2021, it has caused approximately 200 million confirmed cases and approximately 4 million deaths (1). Due to the current number of cases, death rates and worldwide prevalence, it has become the pandemic of the century and is a major problem affecting humanity worldwide. In the last 1.5 years, scientific studies in almost every discipline around the world have focused on COVID-19. In this process, many studies were carried out about the molecular structure, biology and molecular mechanisms of COVID-19 (12). It is critical to elucidate the molecular structure and mechanism of action of COVID-19 to find a curative treatment and prevent the disease. To the best of our knowledge this current study is one of the most comprehensive studies in the literature.

In line with the data obtained from our study, we determined that "betacoronaviruses" are mostly investigated in molecular studies. In their study on betacoronavirus, Letko et al. (13) confirmed that lineage B betacoronaviruses can enter human cells through an unknown receptor and that human ACE2 is the receptor for SARS-CoV-2. Li et al. (14) suggested that betacoronaviruses may have a much more complex recombination mechanism than our current knowledge.

In molecular studies about COVID-19, "viral pneumonia" is the second most common topic. Tianyu et al. (15) showed that Xuebijing agent inhibits COVID-19 and reduces lung involvement by acting on the AKT1

pathway, a serine-threonine protein kinase protein that is effective in the inflammatory response. In another study, it was shown that when the damage-associated molecular pattern (DAMPs) from the coronavirus is combined with other risk factors such as air pollution, smoking or advanced age, the disease progresses more seriously and causes fatal coronavirus pneumonia (16).

Regarding viral RNAs, which is the third most common molecular study subject, Zhang et al. (17) showed that CoV nonstructural protein 14 (nsp14) functions as (guanine-N7)-methyltransferase (N7-MTase) involved in RNA cap formation. They suggested that it would be an ideal method for designing live attenuated vaccines for coronavirus by eliminating the viral RNA N7-MTase activity. In the study by Jesus et al. (18), they suggested that antisense RNA-mediated gene editing would increase the success of treatment and provide a cost-effective approach to treat COVID-19.

Another leading research topic is the reverse transcriptase polymerase chain reaction method used in the diagnosis of COVID-19. A common mutation in the spike protein of SARS-CoV-2, called D614G (A23403G), is known to occur (19). Al-Jaf et al. (20) reported that the qRT-PCR method is a suitable diagnostic method for the detection of this mutation because it is fast, effective and cost-effective. In a meta-analysis study by Sopp et al. (21) with COVID-19 data, they reported that SARS-CoV-2 RNA tested by qRT-PCR was rarely found in conjunctival samples. In a review article describing the production and distribution of mRNA vaccines in the COVID-19 process, production scales of SARS-CoV-2 RNA vaccines and mRNA vaccine production against new agents were mentioned. In this review, the topicality of the mRNA vaccine was emphasized (22).

While the United States and China are in the top ranking for countries that conducted molecular-based COVID-19 studies, Italy, Germany and France among European countries follow this ranking. Among the reasons for this ranking are the first detection of the virus in China, the population and the budget allocated to research. Our country of Turkey, on the other hand, ranks 19th in terms of published molecular COVID-19 studies, and an increasing number of comprehensive studies were accepted for publication in reputable journals. When molecular-based studies examining COVID-19 are evaluated on an institute basis, the USA, China and England share the top three places. The reason for this may be that there are sufficient devices and equipment on an institutional basis, experienced researchers and sufficient research budgets.

CONCLUSION

It is an obvious fact that clinical and molecular studies conducted during the COVID-19 pandemic will continue after the pandemic. However, it is important to determine in the subjects and areas where the molecular-based studies about COVID-19 are clustered and which mechanisms were investigated. Taking scientific steps according to the evidence-based data obtained will be the most beneficial and rational approach to find curative treatment for COVID-19 infection and to effectively prevent this infection.

ETHICAL DECLARATIONS

Ethics Committee Approval: This current study is a computer based data-mining study. There is no need to obtain ethical committee approval.

Informed Consent: Because of the study design 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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Case Report

HEALTH SCIENCES
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Interference of high dose intravenous vitamin C with blood glucose testing in a patient with COVID-19 infection

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ABSTRACT

Flash glucose monitoring system (FGMs) is an option for patients to monitor their glucose however, the presence of interfering substances can result in false blood glucose readings. The COVID-19 (SARS-2-Cov) pandemic has resulted in substantial damage to the public and currently, no effective treatment is available for this deadly disease. Intravenous vitamin C (VC) has been shown to attenuate the cytokine storm in COVID-19 infection. Studies suggest that vitamin C supplementation reduces blood glucose in diabetic patients. On the other hand, VC can affect glucose readings obtained by devices in varying degrees. Here, we present a diabetic patient diagnosed with COVID-19 infection, who had false increased blood glucose readings on FGM after VC treatment. A 45-year-old woman with T2DM was diagnosed with a COVID-19 infection. Intravenous VC at a dose of 2500mg five times was added to the standard treatment protocol due to COVID-19 pneumonia. Her flash glucose levels were consistently elevated during intravenous VC infusion and returned to her normal average values after cessation of infusion. Insulin was not given at the time of fluctuations in blood glucose readings because both fingerstick and venous blood glucose measurements at the time of VC infusion were not consistent with the FGM readings. False increase in blood glucose readings due to interfering substances should be kept in mind since correction with insulin or antidiabetics may lead to a potential for dangerous life-threatening hypoglycemic events.

Keywords: Flash glucose monitoring system, COVID-19, interference, high dose vitamin C

INTRODUCTION

Continuous glucose monitoring (CGM) is a new method that depends on interstitial glucose measurement via a subcutaneous sensor. CGM has been shown to reduce risks of hypoglycemia and hyperglycemia, glycemic variability, and improve HbA1c and patient's quality of life (1). Flash Glucose Monitoring system (FGMs), a subset of CGM, is designed as an alternative to traditional blood glucose monitoring, which allows patients to get individual blood sugar readings on demand. However, understanding the limitations of flash glucose monitoring (Abbott Freestyle Libre) due to the presence of interfering substances and the clinical status of the patient can be significant, and need to be considered when interpreting the glucose values provided. Dehydration, using high dose vitamin C (VC) or salicylic acid may lead to false readings with the system (2). The COVID-19 (SARS-2-Cov) pandemic, first reported in Wuhan, China, spreads to many countries, has resulted in substantial damage to the public. Currently, no effective treatment is available for this deadly disease. Coronaviruses increase oxidative stress that promotes cellular malfunction and ultimately results in organ failure. This process finally leads to serious cellular injury, organ failure, and death (3,4). The administration of anti-oxidizing agents along with proven conventional supportive therapies is believed to have an important role in controlling these medical situations. VC shows its antioxidant properties by the elimination of harmful reactive oxygen species, thus defending the cells from oxidative damage (5,6). It is known that high-dose VC provides a certain protection against viral infection. Intravenous VC may also attenuate the cytokine storm in the COVID-19 infection besides its antiviral properties (7). High-dose vitamin C is an example of a substance that has proven to alter blood glucose measurements on glucose devices. Here, we will discuss a type 2 diabetic patient diagnosed with COVID-19 infection, who had false increased blood glucose readings on FGM after VC treatment.

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CASE REPORT

A 45-year-old woman with T2DM was diagnosed with a COVID-19 infection. She was on metformin/sitagliptin and empagliflozin treatment and her last HbA1c and C-peptide were 8.2% and 1 ng/mL, respectively. She was on FGMs to improve her glycemic control. After diagnosed with a COVID-19 infection, she was admitted to the hospital since she had pulmonary infiltrations. Empagliflozin treatment was discontinued as recommended by the guidelines. She has started standard hydroxychloroquine (800 mg loading dose, 400 mg/day for 4 days), azithromycin (500 mg loading dose, 250 mg/day for 4 days), and oseltamivir (75 mg/day for 5 days) treatment and later inhaler treatment and, intravenous VC at a dose of 2500 mg for five times was added to the treatment protocol due to COVID-19 pneumonia. Following this, her flash glucose levels (Freestyle Libre) were consistently elevated with intravenous VC and returned to her normal average values after cessation of infusion (**Figure 1-2**). Both fingerstick and venous blood glucose measurements at the time of VC infusion were not consistent with the FGM readings. Dextrose or some other dextrose-containing fluid was not administered during hospital admission. Her diabetes medication dose was not increased and insulin was not given at the time of fluctuations in blood glucose readings. Her glucose levels returned to her normal average levels after discontinuation of VC treatment. She did not need an intensive care unit for COVID-19 infection. She was discharged on the 6th day of admission after recovery of COVID-19 infection.

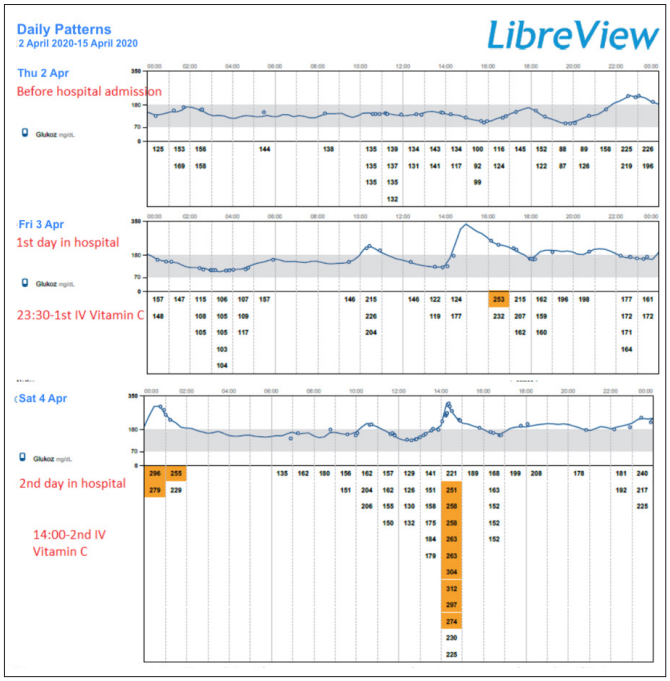

Figure 1. Flash glucose levels before hospital admission and 1st and 2nd days of hospital stay

DISCUSSION

Currently, no suitable vaccine or specific antivirals are available for COVID-19. Acute respiratory distress syndrome (ARDS) is considered as the virtual reason for mortality (7). Coronaviruses may lead to significant lung damage and death from ARDS via the activation of pulmonary capillary endothelium, infiltration by neutrophils, and enhanced oxidative stress as a result of "cytokine storm" (3,4). Enhanced oxidative stress is an important trigger in lung damage leading to acute lung failure with remarkably increased morbidity and mortality (8).

FGMs (Abbott Freestyle Libre) mechanically measures and constantly reads the glucose concentration in the interstitial fluid glucose collected by an inserted sensor filament just beneath the skin and record changes in an individual's glucose levels up to 14 days; this process reduces the necessity for countless and painful finger pricks currently used for glucose monitoring. FGMs are delicate and tolerable, which efficiently improve glycemic control (2). FGM systems can be exposed to heterograde interference from various drugs and nondrug compounds, such as acetaminophen, salicylic acid, and VC (9,10). It is known that vasopressor drugs and severe acute illness may also interfere with glucose readings, however, the patient was not admitted to the intensive care unit.

VC shows its antioxidant properties by the elimination of harmful reactive oxygen species, thus defending the cells from oxidative damage. VC at high concentrations (1,000–5,000 mmol/L) is administrated by intravenous access. According to a randomized cross-over study, supplementation with vitamin C tablets two times a day (2×500 mg/d, quite higher than the daily recommended dose) decreased postprandial hyperglycemia in diabetics

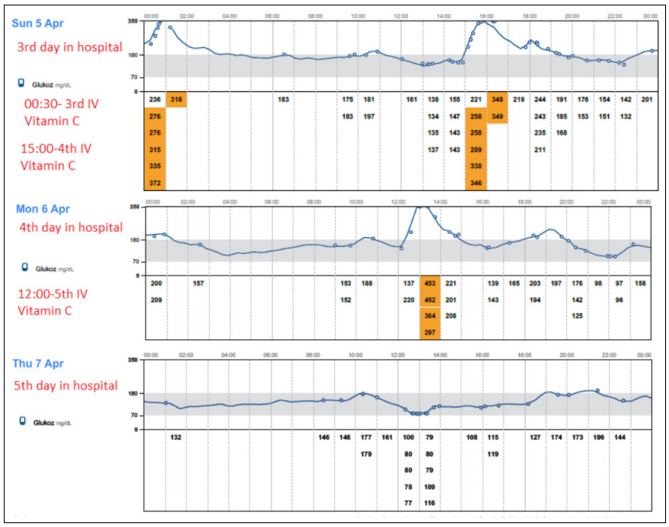

Figure 2. Flash glucose levels at the 3rd, 4th, and 5th days of hospital admission

(11). A meta-analysis showed vitamin C supplementation has beneficial effects on glycaemic control in diabetic patients (12). On the other hand, VC can variably affect glucose devices. The oxidization of ascorbic acid at the electrode surface, leading to more electrons and current production, which leads to a false increase in blood glucose reading (5). The antiviral activities of VC were known for decades and high-dose intravenous VC treatment was shown to be effective in patients with sepsis, ill with severe influenza, receiving mechanical ventilation (7). In addition to direct antiviral properties, intravenous VC may also attenuate the cytokine storm in the COVID-19 infection. Pneumonia caused by COVID-19 may lead to severe lung injury and prevent sufficient pulmonary oxygen enter that results in ARDS, with increased morbidity and mortality rate. Oxidative stress-induced by COVID-19 leads to cellular dysfunction and finally results in organ failure. ARDS is considered the main trigger of COVID-19's induced lung failure. An increase in oxidative stress by the production of free radicals and cytokines eventually causes critical cellular damage, organ dysfunction, and death. Anti-oxidants use with proven supportive therapies is considered to play a significant role in disease control. Intravenous VC and other antioxidant agents can be used for ARDS. Additionally, high dose intravenous VC is well-tolerable and effective (6).

In most cases of mild COVID-19 infection, antidiabetic medications should be followed as usual, except for sodium-glucose co-transporter-2 (SGLT2) inhibitors, which can lead to an increase in the risk of dehydration and diabetic ketoacidosis, necessitating careful renal function monitoring. Since there is a risk for lactic acidosis with metformin, discontinuation is suggested during hospitalization in case of moderate or severe disease (13). Our patient had mild disease and only empagliflozin treatment was discontinued.

Because no vaccine or specific antiviral medicine is available presently for this mortal disease, high-dose VC is safe and may be an option to mitigate COVID-19 associated pulmonary involvement. Correction of false increase in blood glucose readings as a result of high-dose VC may create a potential for dangerous life-threatening hypoglycemic events. Clinicians and patients need to be aware of the susceptibility of the blood glucose monitoring system to interfering substances.

ETHICAL DECLARATIONS

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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ARTICLE INDEX

ISSUE 2021/1

ISSUE 2021/2

ISSUE 2021/2

ISSUE 2021/3

ISSUE 2021/4

ISSUE 2021/4

ISSUE 2021/5

ISSUE 2021/5

ISSUE 2021/6

SUBJECT INDEX

D

E

F

G

S

T

U

V

W

X

Y

AUTHOR INDEX

D

E

G

H

I-İ

Z

REVIEWER LIST

A
ACAR Aydın
AKAY Neşet
AKDİ Ahmet
AKINCI ÖZYÜREK Berna
AKMANSU Müge
AKYOL Alparslan
ALANYA TOSUN Şebnem
ALPASLAN Mete
ARSLAN Serdar
ASİRVATHAM Raju
ATILGAN Hasan İkbal
ATILGAN Kadir Gökhan
ATİK Aziz
AYDIN Bahattin Kerem
AYDIN Gülçin
AYDOĞAN Tolga

B
BAÇÇIOĞLU Ayşe
BASTURK A
BAYRAK Muharrem
BEKTAŞ AKPINAR Nilay
BERBER İlhami
BERBER Ufuk
BEYAZIT Fatma
BEYAZIT Yavuz
BEYSEL Selvihan
BOLAT Esef
BOZKURT YILMAZ Hatice Eylül

C
CAN Güray
CANBEYLİ İbrahim Deniz
CESUR Salih

Ç
ÇETİNKAYA Mehmet
ÇADIRCI Kenan
ÇAPRAZ Aylin
ÇAPRAZ Mustafa
ÇARLIOĞLU Ayşe
ÇELİK Etem
ÇITIRIK Mehmet
ÇİFCİ Aydın
ÇOŞKUN Buğra
ÇULLU Neşat

D
DEMİR Mehmet Emin
DEMİRCİ Taner
DERİCİ Mehmet Kürşat
DOĞAN Emrah
DOĞAN Ruhi
DÜĞEROĞLU Harun

E
ERDOĞAN Elif
ERGÜN İhsan
EROĞLU Oğuz
ERSÖZ ALAN Burcu

G
GENÇOAMANOĞLU Gülenay
GÖKMEN KARASU Ayşe Filiz
GÖKMEN Süleyman
GÜL Serdar
GÜLTEKİN Yıldırım
GÜZEY ARAS Yeşim

H
HACIBEKİROĞLU Tuba

I
ISSIN Ahmet

K
KABALCI Mehmet
KAÇMAZ Birgül
KANDEMİR Hüseyin
KARADENİZ Muhammed
KARAHACI OĞLU Eray
KARAHAN İrfan
KARALEZLİ Ayşegül
KARATAY Eylem
KARLI Pervin
KAYA Ahmet
KAYA Mustafa
KESER ŞAHİN Havva Hande
KILIÇ Mustafa Kemal
KIRICI Pınar
KIZILARSLANOĞLU Muhammet Cemal
KİZİLGUL Muhammed
KOCA Gökhan
KOÇAK Erdem
KOÇAK FİDAN Nurdan
KORKMAZ Hakan
KORKMAZ Serdal
KORKMAZ Şükrü Alperen
KÖLÜKÇÜ Engin
KÖSE Hatice
KUTLUER Gülşen
KÜLTÜR Turgut

M
MİNİKSAR Ökkeş Hakan
MORTAŞ Tülay

Ö
ÖCAL Ruhsen
ÖĞDEN Mustafa
ÖTEN Erol
ÖZÇİFTÇİ Serhat
ÖZDEMİR Adnan
ÖZDEMİR Coşkun
ÖZEN Mehmet
ÖZKAN Derya
ÖZKUK Kağan
ÖZSOY Metin

P
PAÇ Mustafa
PEHLİVANLI Faruk
PEKER Kevser
PEKER Nurullah
POLAT Reyhan

S
SARI Oktay
SARI Tuğba
SEL Görker
SELVİ Özgür
Sema ÖZKAN Zehra
SİG Ali Korhan
SUNGUR Nezih

Ş
ŞAHAN Mehmet Hamdi
ŞAHİN Ercan
ŞAHİN Mustafa
ŞAHİN Orçun
ŞENCAN Ziya
ŞENER Emine
ŞENGÜL AYÇİÇEK Gözde
ŞİMŞİR Coşkun

T
TANOĞLU Alpaslan
TARHAN Gülnur
TAŞDELEN YEŞİLDAL Fatma
TEKELİ Arzu Esen
TEMOÇİN Fatih
TOĞRUL Cihan
TOPCU Ramazan
Toptaş Mehmet
TUTKUN Engin

U
ULUSOY M. Gürhan
UTKU Özlem Gül

V
VERGİLİ Özge

Y
YALÇIN Selim
YALIM Zafer
YENİÇERİ Murat
YERAL İlkin
YILDIZ Kadri
YILMAZ Yücel
YILMAZSOY Yunus
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