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Acta Medica Alanya

Editorial

Editöryal

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What We Learned in the Short Term From the 2023 Kahramanmaraş Earthquakes in Terms of Orthopedic Trauma: A Brief Literature Review

2023 Kahramanmaraş Depremlerinden Ortopedik Travma Açıcından Kısa Dönemde Neler Öğrendik: Kısa Bir Literatür Derlemesi

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ABSTRACT

In this article, we have attempted to briefly review retrospective research articles related to Orthopedics and Traumatology in the context of the earthquakes centered in Kahramanmaraş to date.

Key Words: Kahramanmaras Earthquake, Musculoskeletal, İnjury, Orthopedics, Trauma, Fasciotomy, Crush Syndrome, Amputation, Compartment Syndrome, Epidemiology

ÖZET

Bu yazıda Kahramanmaraş merkezli depremler bağlamında bu güne kadar olan dönemde Ortopedi ve Travmatoloji konulu geriye dönük araştırma makalelerini kısaca gözden geçirmeye çalıştık.

Anahtar Kelimeler: Kahramanmaraş depremi, Kas-iskelet sistemi, Yaralanma, Ortopedi, Travma, Fasiyotomi, Ezilme sendromu; Amputasyon, Kompartman sendromu, Epidemiyoloji

The earthquakes in Kahramanmaraş/Pazarcık on February 6th, followed by those centered in Kahramanmaraş/Elbistan, have been deemed the most severe natural disasters in the history of the Republic of Turkey. Every major disaster deserves retrospective studies, enabling us to learn how to enhance emergency healthcare services after such events. Many injuries that allow for survival in an earthquake are orthopedic injuries. Consequently, orthopedic surgeons play a pivotal role in providing care for earthquake victims. Reporting the demographic characteristics and clinical outcomes of orthopedic injuries after an earthquake can be valuable for formulating policies and guidelines to prepare for, respond to, and improve future disaster management [1-2].

Following previous earthquakes in our country, patient demographics, clinical data, undesirable events related to organization, and coordination were reported. Nevertheless, after the Kahramanmaraş-centered earthquakes, it became evident that these lessons were underutilized, and necessary improvements were not effectively implemented [1]. The most substantial obstacle to improving healthcare interventions after disasters is the challenge of collecting accurate data. Nevertheless, data related to earthquakes should be collected, rigorously analyzed and published to take necessary measures for future earthquakes [1]. In this article, we have attempted to briefly review retrospective research articles related to Orthopedics and Traumatology in the context of the earthquakes centered in Kahramanmaraş to date.

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Some controversial issues have arisen in previous reports on earthquakes in our country and around the world, as well as in meetings held after the Kahramanmaraş earthquake. These include the timing of fasciotomy, when and how to decide on limb salvage or amputation for specific patients, and whether fractures in limbs without life-threatening injuries should be treated immediately or deferred/transferred in earthquake-affected areas. Other important issues include the timing of procedures such as fasciotomy, amputation, external and internal fixation, wound care, closure, antibiotic therapy, support therapies for crush syndrome (such as fluid and dialysis), approaches to children, the elderly, and young patients, and differences in the approach to upper and lower extremity injuries. These issues require further research reports.

For this editorial short review, articles related to earthquakes have been searched on PUBMED and Google Scholar databases using the keywords "Kahramanmaras earthquake, musculoskeletal, injury, orthopedics, trauma, fasciotomy, crush syndrome; Amputation, compartment syndrome, epidemiology" from the Kahramanmaraş earthquakes of February 6th until the short term. Research articles were reviewed, and, as far as we could investigate, a total of 12 research articles in the format of orthopedic trauma, authored by at least one Orthopedic Surgeon, were accessed. To the best of our evaluation, these studies covered topics related to epidemiology, demographics, organization, triage, crush syndrome, fasciotomy, amputation, wound care, and wound closure.

Regarding the issues mentioned above that are considered controversial and/or require more research reports, experiences related to certain topics have been shared in retrospective studies conducted after the earthquakes centered in Kahramanmaraş [3-14]. In research studies presenting epidemiological and demographic data after the Kahramanmaraş earthquakes, Asfuroğlu ZM and colleagues [3] reported significant changes in the patient admission procedures at orthopedic and traumatology clinics after the earthquake, with an increase in the number of international patients and trauma-related diagnoses. Gök M and colleagues [4] observed that the most intensive period of emergency room admissions occurred within the first 24 hours after the earthquake, and the peak period for surgical procedures was within 24-48 hours. They also noted that crush syndrome was the most common cause of death. Kulakoğlu B and colleagues [5] reported that the highest patient influx occurred within the first 24 hours after the earthquake, with isolated soft tissue injuries and fractures being the most frequent injuries related to high-energy trauma. Akkaya M and colleagues [6] reported that during the initial five days, the majority of patients were young adults. A total of 173 orthopedic surgeries were performed, including internal/external fixation, upper/lower extremity fasciotomy, amputation, and soft tissue debridement.

In studies related to organization and triage, Özdemir G and colleagues [7] reported that patient data were archived using applications like WhatsApp, and patient interventions were carried out in a team-based, multi-disciplinary approach coordinated by a consulting orthopedic surgeon. They emphasized that a triage system utilizing effective communication and organization strategies could be beneficial in future disasters. Özel M and colleagues [8] found that the Mangled Extremity Severity Score (MESS) might be useful in predicting amputations in patients transferred to centers following limb crushing injuries in major earthquakes. Regarding crush syndrome, fasciotomy, and amputation, Kundakci B and colleagues [9] stated that the timing of fasciotomy should not be delayed and emphasized that amputation was life-saving in cases of severe lower extremity injuries. Bingol O and colleagues [10] highlighted that the risk of amputation increased with the duration of rescue from debris and that delayed fasciotomies were associated with a higher amputation risk. Furthermore, Yalın M and colleagues [11] reported that in adults and pediatric patients with acute kidney injury due to crush syndrome, there was no increase in the risk of death after fasciotomy. They found that the number of fasciotomy incisions was associated with the development of sepsis and that the frequency of crush syndrome and mortality rates were relatively low in pediatric patients. Kilic E and colleagues [12] reported that the MESS score was a useful scoring system for determining the level of amputation after trauma but that hyperbaric oxygen therapy did not change the level of amputation in patients who underwent fasciotomy. In studies related to wound care and closure, Ulusoy S and colleagues [13] indicated that methods such as debridement creams containing collagenase, wound and skin antiseptics, negative pressure wound therapy, and hyperbaric oxygen therapy could provide satisfactory results in the short term for earthquake-related wound care and treatment. Kılıçarslan K and colleagues [14] reported that in patients with cruris fasciotomy due to acute compartment syndrome, vacuum-assisted closure required more grafts and increased treatment costs compared to the subcuticular polydioxanone method.

In conclusion, while the studies we have summarized made important contributions to our existing knowledge. However, when we examine articles related to earthquakes, both from our country and abroad, it is observed that information regarding patient records and long-term follow-up of patients is insufficient. we currently lack sufficient information on patient records and long-term follow-up data. for patients who have undergone limb-preserving treatments, amputations, or experienced

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significant morbidity in the aftermath of the Kahramanmaraş-centered earthquakes. Records regarding patients who develop kidney failure after limb-saving treatment and experience significant morbidity in the later stages or require organ transplantation are also insufficient [15]. Therefore, long-term research on earthquake-related injuries, both orthopedic and from other medical disciplines, is necessary.

Finally, the physical and psychological rehabilitation of earthquake survivors with orthopedic injuries is crucial, and collaboration with relevant experts is necessary [1]. Furthermore, research related to rehabilitation and prosthetic applications for earthquake-related amputations, as well as rehabilitation for earthquake-related limb injuries, spinal cord injuries, and peripheral nerve damage, is important. After this disaster, the review of the process has shown that we need to update our knowledge and share it with colleagues working on this subject in a scientific sense, considering that we are in an earthquake-prone region.

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Research Article

Araştırma

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Validity and Reliability of the "Visual Object and Space Perception Test" in Turkish

Visual Object and Space Perception" Testinin Türkçe Versiyon Geçerlik ve Güvenirliği

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ABSTRACT

Aim: Although many tests evaluating visual perceptual impairment have been developed in the clinic, it is seen that the tests within Turkish validity and reliability are insufficient. Visual Object and Space Perception (VOSP) Test, which evaluates visual and spatial perception separately, is distinguished from other visual perceptual tests as a sensitive battery. In our study, it is aimed to analyze Turkish cultural adaptation, validity, and reliability of VOSP Test, which evaluates visual-spatial functions in stroke survivors and healthy individuals.

Methods: Twenty-seven stroke patients with right hemisphere lesions and 54 healthy individuals were included in our study. Criterion validity method has been used to examine the validity. Internal consistency, Cronbach alpha and test-retest methods have been used for the reliability of the test.

Results: Eighty-one participants [45 (55.6%) female] have been included in our study. The mean age was 46.04±14.74 years. Turkish version of the VOSP test has been found to be sufficient and reliable according to the Cronbach Alpha Coefficient (0.923).

Conclusion: In the light of the findings of our study, it has been concluded that the Turkish version of the VOSP Test is a valid and reliable measurement tool and that this test can be benefited by scientific and clinical studies.

Key Words: Visual Perception, Space Perception, Stroke, Reliability, Validity

ÖZET

Amaç: Klinikte görsel algısal bozukluğu değerlendiren birçok test geliştirilmiş olmasına rağmen Türkçe geçerlilik ve güvenilirliği bulunan testlerin yetersiz kaldığı görülmektedir. Görsel ve uzaysal algıyı ayrı ayrı değerlendiren "Görsel Nesne ve Uzaysal Algı (Visual Object and Space Perception, VOSP)" hassas bir batarya olarak diğer görsel algısal testlerden ayrılmaktadır. Çalışmamızda inme geçiren bireyler ve sağlıklı bireylerde görsel-uzaysal işlevleri değerlendiren VOSP Testinin Türkçe kültürel adaptasyon, geçerlik ve güvenirlik analizinin yapılması amaçlanmıştır.

Yöntem: Çalışmamıza sağ hemisfer lezyonuna bağlı inme tanısı almış 27 hasta birey ve 54 sağlıklı birey dahil edildi. Geçerliliğin incelenmesinde kriter geçerliği yönteminden yararlanıldı. Testin güvenilirliği için; iç tutarlılık, Cronbach Alpha ve test-tekrar test yöntemlerine başvuruldu.

Bulgular: 81 katılımcı [45 (%55,6) kadın] dahil edildi. Yaş ortalaması 46,04±14,74 idi. VOSP testinin Türkçe versiyonu Cronbach Alfa katsayısına göre yeterli ve güvenilir bulundu (ICC=0,923).

Sonuç: Çalışmamızın bulguları ışığında, VOSP Testinin Türkçe versiyonunun geçerli ve güvenilir bir ölçme aracı olduğu, bilimsel ve klinik çalışmalarda bu ölçekten yarar sağlanabileceği sonucuna ulaşıldı.

Anahtar Kelimeler: Görme Algısı, Uzaysal Algı, İnme, Geçerlik, Güvenirlik

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Introduction

Visual spatial perception is a complex process involving the stimulus and the identification of its localization [1]. Two different components of this process can be mentioned as visual and spatial perception. Visual perception component is defined as the ability to recognize and distinguish visual stimuli and interpret these stimuli by combining them with previous experiences. As for the spatial perception component, it is defined as the ability to provide information about the object's position in space, their relationships among themselves, their relationships between their parts of the body, and their body's relationships with objects including the mental rotation of objects, the ability to imagery and, above all, the ability to visualize [2].

Visuospatial abilities are mandatory for functional tasks in everyday life and deficits of them frequently occur following a stroke [3]. It is stated that incidence of visuoperceptual deficits varies between 20-54% after stroke and it prevents their independence in daily life activities by effecting the motor recovery of patients [4,5]. In addition, it is among the cases that are reported for it to put the person's safety at risk in daily life by increasing the risk of falling [6]. In this context, it is very important to evaluate visuospatial perception with valid and reliable tests. Also, National Clinical Guidelines recommend that every patient with stroke who appears to have perceptual difficulties should assess with a standardized measurement [7].

It is seen in the literature that many different scales are used to assess visual perceptual disorders [8]. However, most of these scales do not evaluate visuospatial perception as a whole. They either assess only spatial perception, as in the Judgment of Line Orientation Test or only assess object detection, as in the *Benton Face Recognition Test*. In addition, most visual tests are intensively influenced by other cognitive skills such as the Clock test [9-12]. In this sense, Visual Object and Space Perception Battery (VOSP), which is different from many evaluations used in the literature, was developed by Warrington and James in 1991 to evaluate both visual and spatial perception separately. Schintu et al. was revealed that subtest of VOSP related to parietal, temporal and frontal areas in their study designed with voxel-based lesion symptom mapping approaches [13]. Furthermore, it was distinguished from other tests with its specific evaluation of visual and spatial skills by minimizing the need for motor, attention, recall and executive functions. One of the advantages of this test is that the object decision test included in the VOSP battery allows the evaluation of aphasic patients [14].

It has been stated that VOSP, developed specifically to reveal certain problems in individuals with right hemisphere damage, detects the presence of disorders in visuospatial skills, or is sensitive to changes in these skills, seen in various neurologic diseases such as Dementia with Lewy Bodies, and atypical Parkinsonian syndrome [15,16]. Although there are studies showing that this test is used in many populations, including the healthy elderly population, there is no information that it is reliable for use in the stroke patient population [17,18].

In our study, we aim to analyze the Turkish cultural adaptation, validity, and reliability of the VOSP Test, which evaluates visuospatial functions, and to introduce it into clinical use.

Methods

Design

This study has been conducted with the approval of İstanbul Medipol University Non-Interventional Clinical Research Ethics Committee on 6 August 2020 (no. E-10840098-772.02-34222/approval number 606). All individuals who agreed to participate in the study have been informed about the study and written consent has been obtained from all of them.

Participants

Patients who applied to İstanbul Medipol University, Faculty of Medicine, Department of Neurology and Necmettin Erbakan and Selçuk University Faculty of Medicine Department of Neurology were conducted. Patients who diagnosed with stroke due to right hemisphere lesion because of clinical and radiographic examinations by neurologist were included. Also, healthy individuals have been placed in our study.

The inclusion criterias for patient participation were (1) between the ages of 18-80, (2) able to communicate in Turkish, (3) diagnosed with stroke with a right hemisphere lesion, (4) a score above 24 on the Standardized Mini Mental State Examination (SMMSE), (5) at least primary school graduate, (6) individuals who signed the informed consent form.

The exclusion criterias of patients' participation were; (1) those with visual problems due to peripheral causes, (2) having a neurological disease other than stroke, (3) individuals with severe hearing impairment, (4) not being able to understand or speak the Turkish language.

The inclusion criterias of healthy controls were; (1) between the ages of 18-80, (2) able to communicate in Turkish, (3) at least primary school graduate, (3) a score above 24 on the SMMSE, (4) individuals without any neurological disease.

Measurements

Demographic Form

The demographic information form has been filled in verbally by the researcher. Information about the individuals' age, gender, education level, anamnesis, diagnosis have been included.

Visual Object and Space Perception Battery (VOSP)

Before the VOSP test is analyzed, a preliminary test of visual sensory efficiency (shape detection screening test) is performed to determine whether the patient has adequate visual and sensorial capacity to finish the VOSP subtests [14].

Shape Detection Screening Test*

Visual object and spatial perception can only be evaluated meaningfully in patients with sufficient visual sensory capacity. Normal acuity is necessary for the cognitive tasks of visual perception. But in addition to this, it is also necessary to distinguish between shapes. The VOSP Test is not suitable for patients with significant impairment in the shape detection screening test. Individuals who score 15 or less are considered to fail this test. There are two different cards. One of them is a practice card to explain the task at the beginning. Other one is stimulus cards (20 cards) to calculate the score. On the cards, there is the letter X, partially erased, on the irregular background patterns. The individual is asked to report whether the letter X is exists on the pattern. Some cards do not include X letter [14].

VOSP and Subtests

The VOSP Test was developed by Warrington and James in 1991 to assess visuospatial perception. It consists of 8 subtests, four of which are visual object perception (incomplete letters, silhouettes, object decision, progressive silhouettes) and four space perception (dot counting, position discrimination, number location, cube analysis). There is no order in the tests. The total number of correct points is recorded as the score. The test has no set administration time and is administered at an appropriate rate for the patient [14].

Test 1: Incomplete Letters

The test consists of 20 (70% deleted) stimulus cards and 2 (30% deleted) practice cards used to explain the test. The letters F and B are shown on the practical cards and the individuals are asked to name them. Patients with speech disorders are asked to identify the letters by drawing or signs. If the individual cannot name or identify the practice cards, the test is canceled. The total number of correct answers is recorded as a score (maximum score =20) [14].

Test 2: Silhouettes

The test consists of two sequences as 15 animal silhouettes and 15 object silhouettes. The individual is first shown the silhouette of an animal and is asked to name it by being explained that it is an animal drawing. For individuals with speech disorders, this task may involve identifying the animal through any means available. Then the individual is asked to name the objects or to describe each silhouette by using signs or by describing how the object is used. If 5 errors are made in both series, the test should be ended. The total number of silhouettes named or described is recorded as a score (maximum score=30) [14].

Test 3: Object Decision

The test consists of 20 cards. Each card contains three distractions and a real two-dimensional object. Distractions consist of object-like shapes, but they are purely imaginary. Individuals are asked to choose the real object. The total number of correct answers is recorded as a score (maximum score =20) [14].

Test 4: Progressive Silhouettes

The test consists of two series, a silhouette of a gun and a silhouette of a trumpet. In each series, there are 10 silhouettes created by rotationally changing the angle of view of the lateral axis from 90 degrees to 0 degrees. If the individual is unable to name the silhouette, they are encouraged to define it indirectly or by means of signs. The number of attempts now that each object can be identified is collected and recorded as a score. However, in this test, it means that the sooner individuals identify the object, the better the level is. The number of attempts required to identify each object is summed and recorded as the score (number of attempts =10+10) [14].

Test 5: Dot Counting

Test stimuli consist of black dot sequences on a white card. There are 10 stimulus cards in total in the test. The individual is asked how many dots there are on the card. If the first card is answered incorrectly, the individual is asked to point to each dot. If the individual has pointed incorrectly,

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(but not because of omissions), the dot counting test is ended. The total number of correct answers is recorded as a score (maximum score =10) [14].

Test 6: Position Discrimination

An identical or different matching task comparing the position of a point in a square with the position of a point in a second square provides information about the right hemisphere lesion. 20 stimulus cards are shown in this test. Each card consists of 2 squares placed horizontally. One of the squares has a black dot imprinted exactly in the center, and the other a black dot imprinted just off-center. Individuals are asked to show the dot in the center of the square. The reminder "Look at both squares before deciding." is made for individuals who constantly choose the right or left square. The total number of correct answers is recorded as a score (maximum score =20) [14].

Test 7: Number Location

Ten stimulus cards are shown in the test. In the upper square there are randomly placed numbers (from 1 to 9), and in the lower square there is a black dot corresponding to one of the numbers above. The task in this test is to find the number corresponding to the position of the dot. There are two practice cards used to explain the task. If a mistake is made on the first card, the individual is informed about the correct number before moving on to the second practice card. If the individual answers both practice cards incorrectly, the test is ended. The total number of correct answers is recorded as a score (maximum score =10) [14].

Test 8: Cube Analysis

There are two practice cards and 10 stimulus cards to explain the task in the test. The difficulty level of the test is increased by adding hidden cubes. The individual is asked to say how many cubes are on the cards. If they fail to calculate both practice cards, the test is ended. The total number of correct answers is recorded as a score (maximum score =10) [14].

Translation and Cultural Adaptation Steps

Permission to administer the VOSP Test has been obtained from its author, Elizabeth Warrington. The VOSP Test was translated from its original language English to Turkish by 1 expert physiotherapist and 2 experts' psychologists who are with sufficient knowledge in the field of cognitive rehabilitation. The translations were combined into a single form. Later, the scale was translated back into English by a linguist who is not a healthcare Professional with a good level of English. The new English scale was sent to the original author of the test for approval. To evaluate the scale for cultural adaptations, a pre-test study was conducted with 20 people and necessary corrections were made (Figure).

Sample Size

We have used univariable and multivariable linear regression models to estimate the association between the VOSP score and severity of illness, mean values of right hemisphere lesion with age, gender, and education levels as co-variables, to avoid over-adjustment due to the relatively small sample size. Coefficients with 95% confidence intervals (CIs) have been reported.

Data Analysis

IBM SPSS software (Version 22.0, IBM Corp., Armonk, NY) statistical package program has been used to analyze the data. Number-percentage ratios, mean, standard deviation, median, and minimum-maximum values have been used in the descriptive statistics of the data. Kolmogorov-Smirnov test was used to evalute the normal distrubiton of the variables. For the analysis of the obtained scores, Kruskal Wallis test has been used for more than two independent groups and Mann Whitney U test has been used for two independent groups. The criterion validity method was used to examine the validity. For the reliability and internal consistency of the test Cronbach Alpha and Test-Retest reliability methods have been used. The Cronbach-*a* coefficient is interpreted as highly reliable if it is $a > 0.80$. For test-retest reliability, Intraclass Correlation Coefficient (ICC) has been calculated by choosing a one-way random effects model.

Results

107 volunteers participated in our study, including 42 patients and 65 healthy individuals. 5 patients have been excluded due to the Covid-19 pandemic, 10 patients have been excluded for scoring less than 24 in SMMT, and 11 healthy individuals have been excluded due to transportation. 81 participants [45 (55.6%) female] have been joined. Mean age was 46.04±14.74 (min-max=21-78) years. There were statistically significant differences between the under 50 years old and over in the incomplete letters (p=0.018), silhouettes (p=0.001), progressive silhouettes (p=0.000), cube analysis (p=0.004), and VOSP total (p=0.000) tests. The results of the VOSP tests according to the age of par-

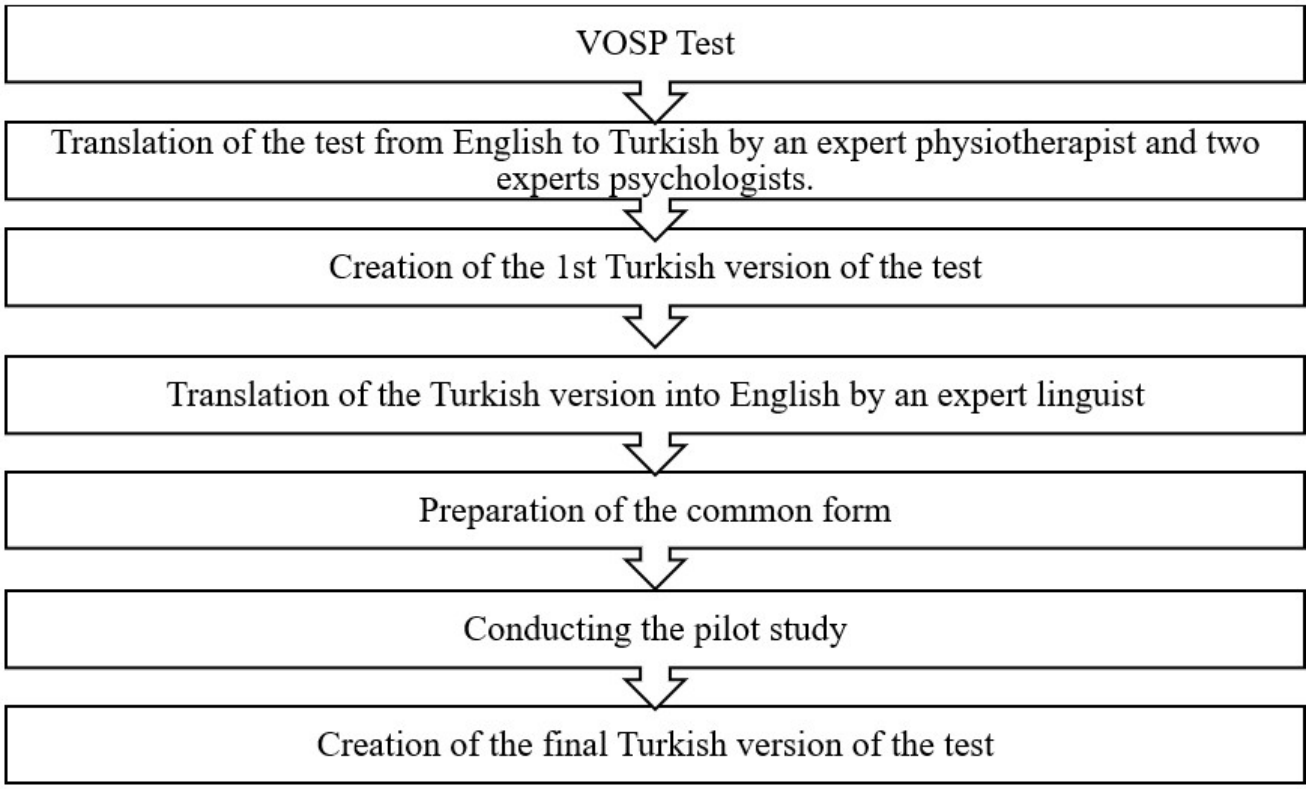

Figure 1. Translation Stages

VOSP Visual Object and Space Perception Test

ticipants were shown in Table 1. There was no statistically significant difference between the education levels of participants in dot counting (p=0.773), position discrimination (p=0.463), and cube analysis (p=0.179) tests. In accordance with the education level of participants the VOSP tests results were given in the Table 2.

Translation and Cultural Adaptation

Due to the feedback and suggestions of the committee and invited patients, changes have been made to the Turkish version of the questionnaire to better align the translated version with the original, to adapt the questionnaire to Turkish culture, and to ensure all items were easily understandable. The corkscrew card has been removed from the silhouettes test. For individuals who did not understand the letter X in the shape detection screening test, the word "cross" has been used instead of X. The word "cube" has been used instead of "solid brick" in the cube analysis test. After these changes, all participants in the pre-test said that the questionnaire was easy to answer, items were clear, they had no doubt and knew all the objects in the questionnaire. The consensus version of the questionnaire was not further modified during the study.

Reliability

Cronbach-*a* has been used to evaluate the homogeneity of the questions regarding the internal consistency within the test. The internal consistency of VOSP was excellent (Cronbach-*a*=0.923). The ICC value for the test-retest reliability was found between 0.881-0.951 at the confidence interval of 95%, which suggests a high level of test-retest reliability (Table 3).

Discussion

This study showed that the VOSP Test has been found a valid and reliable test in which we planned to investigate the Turkish validity and reliability of the VOSP test, which is used to evaluate visual and spatial perception disorders, in patients with stroke due to right hemisphere lesion and healthy individuals in our study.

The VOSP test, is a comprehensive assessment scale and used in various neurological diseases-to evaluate visual and spatial perception separately with different subtests [1,14,16,19]. Although VOSP is mostly studied in healthy individuals, also with Alzheimer's and mild cognitive impairment, there is no reliability study conducted on individuals with stroke [1,13,14].

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Table 1. The VOSP Results of Participants According to The Age

VOSP	<50 years (N=45)				>50 years (N=36)				p
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Incomplete letters	18.955	0.320	7.0	20.0	18.305	0.325	12.0	20.0	0.018*
Silhouettes	19.155	0.575	8.0	27.0	15.777	0.671	9.0	23.0	0.001**
Object decision	17.333	0.419	4.0	20.0	15.944	0.608	9.0	20.0	0.124
Progressive silhouettes	13.911	0.585	5.0	19.0	10.694	0.628	3.0	18.0	0.000**
Dot counting	9.911	0.042	9.0	10.0	10.000	0.000	10.0	10.0	0.068
Position discrimination	19.377	0.273	10.0	20.0	19.305	0.254	13.0	20.0	0.629
Number location	8.577	0.443	1.0	20.0	8.027	0.426	2.0	10.0	0.531
Cube analysis	9.488	0.163	4.0	10.0	8.833	0.259	3.0	10.0	0.004**
VOSP Total	116.755	1.804	82.0	133.0	107.583	2.070	77.0	127.0	0.000**

SD *Standard Deviation*, Min *Minimum*, Max *Maximum*, VOSP *Visual Object and Space Perception*, *p<0.05, **p<0.01

Table 2. The VOSP Results of Participants According to The Education Level

		N	Mean	SD	Min	Max	p
Incomplete letters	Primary	34	18.294	0.366	12.0	20.0	0.006**
	Secondary	14	17.714	0.879	7.0	20.0	
	College and more	33	19.454	0.123	18.0	20.0	
Silhouettes	Primary	34	16.676	0.732	8.0	26.0	0.000**
	Secondary	14	15.000	0.907	9.0	21.0	
	College and more	33	19.787	0.630	9.0	27.0	
Object decision	Primary	34	15.617	0.652	4.0	20.0	0.009**
	Secondary	14	16.500	0.635	13.0	20.0	
	College and more	33	17.939	0.445	9.0	20.0	
Progressive silhouettes	Primary	34	10.882	0.724	3.0	18.0	0.000**
	Secondary	14	10.571	0.953	6.0	18.0	
	College and more	33	14.939	0.523	8.0	19.0	
Dot counting	Primary	34	9.970	0.029	9.0	10.0	0.773
	Secondary	14	9.928	0.071	9.0	10.0	
	College and more	33	9.939	0.042	9.0	10.0	
Position discrimination	Primary	34	19.205	0.272	13.0	20.0	0.463
	Secondary	14	19.285	0.507	13.0	20.0	
	College and more	33	19.515	0.305	10.0	20.0	
Number location	Primary	34	7.382	0.511	1.0	10.0	0.050*
	Secondary	14	8.214	0.672	3.0	10.0	
	College and more	33	9.363	0.414	5.0	20.0	
Cube analysis	Primary	34	8.823	0.317	3.0	10.0	0.179
	Secondary	14	9.285	0.194	8.0	10.0	
	College and more	33	9.545	0.131	8.0	10.0	
VOSP Total	Primary	34	107.325	2.446	77.0	126.0	0.000**
	Secondary	14	107.243	2.529	92.0	121.0	
	College and more	33	120.152	1.453	96.0	133.0	

SD *Standard Deviation*, Min *Minimum*, Max *Maximum*, VOSP *Visual Object and Space Perception*, *p<0.05, **p<0.01

Table 3. The Test-retest Reliability of Turkish Version of VOSP

VOSP	Test scores	Re-test scores	ICC	%95 CI
	X±SD	X±SD		
Incomplete letters	18.46±2.61	18.67±2.08	0.751	0.612-0.840
Silhouettes	16.54±4.27	17.65±4.26	0.931	0.892-0.955
Object decision	15.95±3.47	16.71±3.26	0.917	0.871-0.947
Progressive silhouettes	6.84±3.13	12.48±4.17	0.655	0.463-0.778
Dot counting	9.84±0.49	9.95±0.22	0.733	0.460-0.816
Position discrimination	19.18±1.82	19.34±1.70	0.756	0.620-0.843
Number location	8.00±2.69	8.33±2.79	0.763	0.632-0.848
Cube analysis	8.99±1.87	9.19±1.35	0.752	0.614-0.840
VOSP total	104.18±14.79	112.68±13.01	0.923	0.881-0.951

X *Mean*, SD *Standard Deviation*, VOSP *Visual Object and Space Perception*, ICC *Intraclass Correlation Coefficient*

In the recent studies, it is stated that each hemisphere has complementary role in object recognizing but space perception mainly depends on integrity of the right hemisphere. The right hemisphere is dominant in object and space perception [13, 20]. However, the VOSP test was especially designed for the right hemisphere damage [14]. In the light of this information, stroke patients with right hemisphere damage were selected for our study.

The VOSP test is a good neuropsychometric test for detecting visual perceptual problems and even revealing the deterioration of existing problems [18,21]. Consistent with the literature, we found that VOSP total and subtest scores were lower in individuals with right hemisphere stroke, where visual perceptual problems are common, than individuals without any cognitive impairment [22].

The VOSP test has been reported to be a sensitive battery in measuring visuospatial functions [1,15,23]. When we scan the literature, in addition to internal consistency being seen in the different subtests of the test, it has been reported in various studies that the internal consistency of the silhouettes test is high in general [17,23,24]. It has been reported that the silhouettes test is more than 90% sensitive in detecting progress in individuals with mild cognitive impairment and Alzheimer's disease [23]. In our study, we have also found that there is high internal consistency in silhouettes (ICC: 0.931) and object decision (ICC: 0.917) tests and test total score (ICC: 0.923), which is in line with the literature.

In addition to the perception of depth and object size, there are age-related changes in visual acuity, accommodation, adaptation to darkness, color and peripheral vision, and visual processing speed decreases with age [25,26]. The decrease in performance with age in the visual perception part of the VOSP test is associated with this situation, and it is not clear whether normal aging affects space perception disproportionately compared to visual perception [27]. In our study, in which we examined our cases based on the age of 50, we found that individuals without any cognitive impairment over the age of 50 had lower scores than the total of cube analysis and the VOSP test in all visual tests, except for object decision, while those who had a stroke due to right hemisphere lesion had a lower score than only the silhouettes test. These results support the literature [14,17,18].

Studies on the effect of gender on VOSP have generally concluded that this factor has no effect on the test [16,18,21]. It is also seen that there is no significant consistency in studies indicating gender differences [24]. In our study, when the effect of the gender factor on the VOSP test was examined, it was seen that among all participants, women had higher scores in visual perceptual tests, and there was no gender difference in spatial perceptual tests, except for the position discrimination test. When the group who had a stroke due to right hemisphere damage was examined, it was found that there was no difference between the genders. When the effect of education on the scale was examined, individuals with high education level had better results in the total score of the test and visual perceptual tests, and in the patient group only in the progressive silhouettes test, individuals with higher education level had better results. As a matter of fact, it has been stated in other studies in the literature that education level is an important determining factor [18,24].

During cultural adaptation of the test, some modifications were made for better understanding and scoring. In studies on the analyzes of VOSP in the literature, it was reported that there were problems in both the object and animal naming parts of the silhouettes test, and that people could not name some cards in this section accurately. In this case, it is advised that the naming can be accepted

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as correct if it has almost the same meaning or synonyms with the objects on the cards (hare for rabbit card), or if the names of young animals or the diminutive suffix/adjective/noun expressions are used (lamb for sheep card etc.) and the subcategories of correct answers are given (winter shoes for shoe card etc.) [19]. Similar problems have been encountered in our study (such as calf or ox for the cow, battery car for the tractor, lizard for the crocodile, folding chair or coffee table for deckchair, hand broom for dustpan, adjustable wrench for spanner etc.), and it has been decided to score each option as correct after developers' approval. However, since the corkscrew card in the objects section of this test was named by only two people and many of the participants were religious and non-alcoholic individuals, it has been thought that sociocultural factors might be a factor, and this card has been removed from the test, and with developers' approval, this section has been evaluated out of 29 points instead of 30 points. A similar issue occurred with the trumpet card in the progressive silhouettes test. Out of the individuals who could not name the trumpet, the answers of the ones who correctly described the object on this card and called it clarinet or flute, which is one of the more familiar instruments in Turkish culture, or called it saxophone, has been accepted as correct with developers' approval.

The "Incomplete Letters" test, which consists of the English alphabet, makes the application of the test difficult in some countries. Due to the letter difference in the alphabet, the incomplete letters subtest was not used in the validity study on Chinese individuals, and the VOSP was adapted to consist of 7 subtests [24]. In the adaptation phase of the scale to Turkish in our study, no changes have been made in the test, due to the fact that there is no difference from the English alphabet except for the letters "W" and "X" and, since the answers given to these letters by individuals who do not speak English, "double V" or "Inverted M" instead of W, and "cross" instead of X have been approved by developers. For a similar reason, before moving on to the subtests of the VOSP Test, instead of the "X" used in the shape detection screening test, the word "cross" has been used for individuals who did not know this letter. In the cube analysis test, the "Solid Brick" question asked to the individuals has not been understood, so the word "Cube" has been used instead of this description.

Limitations

There are several limitations in this study that could be addressed in future research. First, individuals who had a stroke after the right hemisphere lesion without cognitive impairment were heterogeneous in terms of gender and education level. Secondly, it was mainly based on urban residents, lacking data from rural areas. The different sociocultural areas. Finally, wide age range in our population was another limitation of our study.

Conclusion

When visual perceptual problems are not apparent, they can be easily overlooked and remain undetected. It is important to test visual and perceptual skills separately to identify affected areas, determine rehabilitation protocols, and organize daily activities. In this context, the VOSP test, which assesses both visual and spatial skills, presents advantages over many other tests as it is easy to score and has established cut-off values. In our study, it was determined that the VOSP test is a valid and reliable assessment tool for evaluating visual-spatial impairments and can be used in the Turkish population.

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Research Article

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The Predictive and Prognostic Value of Skeletal Muscle Mass in Cancer Patients with Distant Metastases

Uzak Metastazlı Kanser Hastalarında İskelet Kası Kütlesinin Prediktif ve Prognostik Değeri

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ABSTRACT

Aim: Skeletal muscle loss is an indicator of cachexia and a strong prognostic factor for some types of cancer. After strict standardization, we aim to evaluate both the predictive and prognostic value of low muscle mass (LMM) in common cancer types for first-line chemotherapy.

Method: This retrospective single-center study was conducted in a regional hospital between 2015 and 2020. Patients diagnosed with distant metastatic cancer were screened and included in the study if they had abdominal computed tomography 45 days prior to first-line chemotherapy. The relationship between LMM and progression-free survival (PFS), overall survival (OS), and objective response rate (ORR) was evaluated.

Results: Initially, 289 patients with metastatic cancer were included. The median duration of follow-up was 17 months, with a mean age of 61.09±13.03 years (range 25 to 95), and 45.9% of patients were female. In total, 50.5% of patients had LMM, which was adjusted for gender. LMM was associated with worse OS and PFS in univariate analysis (HR:1.598;1.216-2.100; p=0.001 for OS and HR:1.583;1.216-2.059; p=0.001 for PFS), and this positive association was maintained after adjusted for diagnosis and age. Better ORRs were obtained in respiratory and gastrointestinal tract cancers, breast, prostate and gynecological cancer in non-LMM groups.

Conclusions: LMM has not only prognostic value but also predictive value for many types of cancer. Therefore, the assessment of muscle loss should be incorporated as part of the initial routine clinical evaluation.

Key Words: Prediction, Muscle Mass, Prognostic Value, Cancer, Muscle Index

ÖZET

Amaç: İskelet kası kaybı, kaşeksinin bir göstergesi ve bazı kanser türleri için güçlü bir prognostik faktördür. Bu çalışmada sıkı standardizasyon sonrası, birinci basamak kemoterapi için yaygın kanser türlerinde düşük kas kütlesinin (LMM) hem prognostik hem de prediktif değerini değerlendirmeyi amaçlıyoruz.

Yöntem: Bu retrospektif tek merkezli çalışma 2015-2020 yılları arasında bir bölge hastanesinde yapılmıştır. Uzak metastatik kanser tanısı alan hastalar tarandı ve birinci basamak kemoterapiden 45 gün önce abdomen bilgisayarlı tomografisi olan hastalar çalışmaya dahil edildi. LMM ile genel sağkalım (OS), progresyonsuz sağkalım (PFS) ve objektif yanıt oranı (ORR) arasındaki ilişki değerlendirildi.

Bulgular: Çalışmaya metastatik kanserli 289 hasta dahil edildi. Hastaların %45,9'u kadın olup medyan takip süresi 17 ay ve ortalama yaşı 61,09±13,03 yıldır (min-max: 25-95). Cinsiyete göre düzeltme sonrası, toplamda hastaların %50,5'inde LMM vardı. LMM, univariate analizde daha kötü OS ve PFS ile ilişkilendirildi (OS için HR:1,598;1,216-2,100;p=0,001 ve PFS için HR:1,583;1,216-2,059;p=0,001) ve bu pozitif ilişki tanı ve yaşa göre düzeltme yapıldığında da devam etti. LMM olmayan gruplarda solunum sistemi, gastrointestinal sistem, meme, prostat ve jinekolojik kanserlerde daha iyi ORR'ler elde edildi.

Sonuç: LMM sadece prognostik değere sahip değildir, aynı zamanda birçok kanser türü için prediktif değere de sahiptir. Bu nedenle kas kaybının değerlendirilmesi, ilk muayenede rutin klinik değerlendirmede yer almalıdır.

Anahtar Kelimeler: Prediktif Değer, Kas Kütlesi, Prognostik Değer, Kanser, Kas İndeksi

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Introduction

Cachexia has been defined as "a complex metabolic syndrome associated with underlying illness and characterized by the loss of muscle with or without the loss of fat mass"[1]. Cancer cachexia, a multifactorial wasting syndrome, is associated with loss of weight, reduced food intake and elevated inflammatory response along with alterations in metabolism, and is thought to account for 20-30% of cancer related deaths [2, 3]. Recently, calculation of muscle mass mostly determined by computed tomography (CT) scanning, is considered the gold standard way to measure muscle parameters. The strong correlation between the CT-based measurement of cross sectional muscle area and total body muscle mass was previously demonstrated [4]. Skeletal muscle loss is an indicator of cachexia and appears to be a strong negative prognostic factor in aging, various chronic diseases, and also in some malignancies [5]. Most of the studies conducted were designed to assess the relationship between sarcopenia and toxicity, and increased toxicity with chemotherapy is shown in patients with muscle loss for many types of cancer [5]. Therefore, the use of muscle index for chemotherapy dose calculation can provide a more accurate method of toxicity management than body surface area, as well as provide the most appropriate effective dose of chemotherapy for sarcopenic patients.

Moreover, patients with muscle loss are particularly vulnerable to major physiologic stress, therefore, muscle loss is shown to be associated with postoperative outcomes in esophageal carcinoma, colorectal cancer, liver metastasectomy, hepatocellular carcinoma, pancreatic carcinoma, melanoma, bladder cancer and gynecological cancers following malignancy resection [6]. Awareness of the poor prognostic significance of muscle loss for patients undergoing surgical intervention can provide an estimate in terms of weighing clinical benefits versus potential complications. Although inconsistent results have been obtained, the prognostic role of muscle loss has also been investigated for many cancer types in metastatic disease in the last few years [5-14]. To assess only the prognostic and predictive value of muscle loss, it is necessary to study in a standardized patient data set in terms of treatment and clinicopathological prognostic features. We therefore performed a study to evaluate the predictive and prognostic values of skeletal muscle loss (SML) by calculation of muscle area with CT in standardized patients with respiratory tract cancer (RTC), gastrointestinal tract (GIT) cancer, bladder cancer, breast cancer, gynecological cancer, and prostate cancer who were treated with first line chemotherapy.

Materials and methods

Study design

This retrospective single-center study was performed at a regional hospital in Turkey. In this study, all performed procedures involving human participants complied with the ethical standards of the institutional research committee (ethics approval number: 2019/9-13). From June 2015 to December 2020, patients diagnosed with distant metastatic cancer were screened and included if the patients had an abdominal CT 45 days prior to first-line chemotherapy.

The primary study endpoint was to assess the relationship between overall survival (OS) and skeletal muscle index (SMI), and the secondary endpoint was to evaluate in terms of objective response rate (ORR) and progression-free survival (PFS) after first-line chemotherapy.

Treatments

None of the patients were treated with immunotherapy and targeted tyrosine kinase inhibitors or endocrine therapies. In the first line setting platinum doublet therapy was administered to all patients with lung cancer (platinum plus taxanes/pemetrexed/gemcitabine for non-small cell lung cancer [NSCLC] and platinum with etoposide for small-cell lung cancer [SCLC]). Patients with ALK fusion or EGFR mutation were excluded from the NSCLC group. Patients with mesothelioma who were unsuitable for surgical intervention were treated with platinum and pemetrexed with folbiol at the first line setting. Monoclonal antibody was added to backbone chemotherapy with fluoropyrimidine plus oxaliplatin or irinotecan according to K-RAS, BRAF and N-RAS mutation status and tumor sidedness in all patients with colon cancer. Gastric cancer patients were evaluated for her-2 staining and trastuzumab was added to backbone platinum plus fluoropyrimidine-based chemotherapy. Biliary tract cancer patients were treated with platinum plus gemcitabine for the first line. Patients with pancreatic cancer were treated with FOLFIRINOX in the case of better performance status, or otherwise with platinum and gemcitabine. Patients with bladder cancer treated with cisplatin with gemcitabine in the case of suitable performance status, or otherwise with carboplatin plus gemcitabine. Patients with breast cancer were evaluated pathologically in terms of estrogen, progesterone, her-2 stating, and ki-67 proliferation index for the first line optimal treatment option, therefore anthracycline plus cyclophosphamide was initiated in the case of the presence of visceral crisis or symptomatological disease, and trastu-

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zumab was added to backbone chemotherapy (platinum with taxanes) in patients with her-2 positive disease. Patients treated with endocrine therapy at the first line were excluded from the study in breast cancer group. Patients with high volume disease treated with docetaxel plus dexamethasone for the first line setting were included in this study. Endometrial and ovarian cancer patients treated with paclitaxel plus carboplatin were evaluated as gynecological cancers. Respiratory tract cancers (RTC) were divided into two groups; NSCLC and SCLC/mesothelioma, and gastrointestinal tract cancers (GIT) were also divided into two groups; colorectal cancer and non-colorectal GIT cancer. Patients who received at least one cycle of chemotherapy were included in the present study.

Assessed Parameters

Medical records were obtained for patient and tumor characteristics, metastatic sites, number of metastatic organs, presence of bone metastasis, body composition parameters (such as height), treatment names and data regarding clinical follow up to assess survival outcomes.

OS was defined as the date of diagnosis to the date of death or the end of follow-up, whichever occurred first. The date of diagnosis was assumed as the date of metastasis. PFS was defined as the date of the first cycle of first line chemotherapy to document disease progression or death, whichever occurred first. Switching to another regimen due to treatment intolerance or patient demand was not considered a progression and these patients were excluded. Response rates were evaluated as partial response, complete response, stable disease and progression according to Recist 1.1 criteria. In the case of complete or partial response, it was assumed that these patients showed an objective response (ORR).

Muscle mass was measured from 2-mm-thick CT images (64-slices, Toshiba Medical System, Otowara, Japan). To calculate muscle mass, the abdominal cross-sectional area of muscle at the level of the L3 vertebra (m.psoas, m.erector spinae, m.transversus abdominis, m.quadratus lumborum, m.rectus abdominis, m.obliquus internus, m.obliquus externus) was calculated by freehand ROI. Muscle mass was measured in cm^2 and corrected for height as m^2, resulting in a lumbar skeletal muscle index (SMI) in cm^2/m^2. Low muscle mass (LMM) was defined as an SMI of 42 cm^2/m^2 for men and 36.8 cm^2/m^2 for women according to the median value of the patients.

Statistical analyses

Quantitative variables were described as median with range and means with standard deviation [SD] while qualitative variables were presented as frequencies with proportions. Chi-square and/or Fischer-exact test for rates were used to detect significant differences between qualitative variables. OS and PFS were estimated by the Kaplan-Meier method and the Log Rank test was used to compare the effect of SMI and other parameters on survival. Then multivariate Cox proportional hazard models were used for PFS and OS. Associations between SMI and ORR were calculated by multivariate logistic regression analysis adjusting for age and diagnosis. The statistical software package SPSS 22.0 (SPSS, Chicago, IL, USA) were carried out for statistical analyses, and $P < .05$ indicated a statistically significant difference.

Results

Patient characteristics and LMM rates

Initially, 341 metastatic cancer patients with available CT scans who received first line chemotherapy were included. Among these, 52 patients who did not have CT imaging up to 45 days before starting chemotherapy were not included in the analysis due to the possible risk of tumor progression during this period. All patients were stage 4 and not amenable to surgery or other curative treatments. All patients' Eastern Cooperative Oncology Group (ECOG) performance scores were 0 or 1. The median duration of follow-up was 17 months (1-60) and 72.1% of patients were lost at follow-up. The mean age was 61.09 ± 13.03 years (range 25 to 95), and 9.7% of patients were over 75 years old. 45.9% of the patients were female, the remaining was male.

In total, 50.5% of the patients had low muscle mass, when the median cutoff value was chosen as SMI of 42 cm2 / m2 for men and 36.8 cm2 /m2 for women, which was adjusted for gender. The rate of LMM was 59.5%, 52.0%, 52.2%, 47.3%, 30.0%, 31.2%, and 41.2%, in patients with RTC, colorectal cancer, non-colorectal GIT cancer, prostate cancer, bladder cancer, breast cancer, gynecological cancer, respectively.

While LMM was more common in patients over 75 years of age, it was less common in patients with bladder and prostate cancer (Table 1).

Progression-free survival and overall survival according to LMM

In total, the median OS was 13 months (95%CI 9.53-16.5) in patients with LMM while it was 21 months (95%CI 17.2-24.8) in patients without LMM (p=0.001). Additionally, median PFS was 5 months (95%CI 3.46-6.53) in patients with LMM whereas it was 9 months (95%CI 6.41-11.58) in patients without LMM (p< 0.001). The median OS and PFS

Table 1. Descriptions and the parameters that can affect low muscle mass

	N (%)	Median SMI cm2 /m2	OR	95%CI	P value
Diagnosis					
RTC*	74 (25.6)	35 (17.6-68)	1 (ref)		
Colorectal cancer	50 (17.3)	37.8 (20.8-67)	0.74	0.36-1.51	0.41
Non-CR GIT	67 (23.2)	38 (20.5-56.7)	0.75	0.38-1.47	0.39
Breast cancer	55 (19.0)	37.5 (27-53.0)	0.61	0.30-1.23	0.17
Bladder cancer	10 (3.5)	44.2 (26.1-60)	0.29	0.07-8.66	0.09; 0.036***
Prostate cancer	16 (5.5)	45.9 (26.2-63)	0.31	0.08-1.02	0.047; 0.036***
Gynecological cancer	17 (5.9)	37.6 (32-53.0)	0.48	0.166-1.14	0.176
Number of metastatic organs					
1 to 4			-	-	0.89
Age					
≤75 vs >75			2.890	1.23-2.94	0.015
Bone metastasis					
Presence vs absence			0.87	0.46-1.67	0.68

*RTC: Respiratory tract cancer, **Non-colorectal gastrointestinal tract cancers, ***adjusted for age. OR: Odds ratio; CI: confidence interval.

were not statistically different according to the number of metastatic sites (p=0.297, p=0.440, respectively) and the presence of bone metastasis (p=0.223, p=0.134, respectively). In general, LMM was associated with worse OS and PFS in univariate analysis (HR: 1.583, 95%CI 1.216-2.059, p=0.001) for PFS and HR: 1.598, 95%CI 1.216-2.100; p=0.001 for OS) and this positive association was maintained after adjusted for diagnosis and age (HR: 1.563, 95%CI 1.191-2.051, p=0.001 for PFS and HR: 1.477, 95%CI 1.110-1966; p=0.008 for OS). The curve of multivariate Cox regression analysis of the effect of LMM on OS and PFS is shown in Figures 1 and 2.

The OS and PFS according to diagnosis and LMM

The median overall survival was 11 (8.4-13.6) months, 10.0 (4.9-15.1) months, 18 (12.0-23.9) months, 8 (4.99-11.0) months, 60 (Non reached [NR]-NR) months, 14 (2.98-19.8) months, 32 (21.2-42.8) months, 25 (19.2-30.8) months in patients with NSCLC, other RTC, colorectal cancer, non-colorectal GIT cancer, bladder cancer, breast cancer, gynecological cancer, and prostate cancer, respectively.

When we evaluated the effect of LMM on survival according to diagnosis; as was shown in Table 2 the negative association of LMM with OS was maintained after adjusting for age in patients with SCLC/mesothelioma and breast cancer on the other hand there was no association between LMM and OS for the other types of cancer.

LMM was associated with worse PFS in patients with SCLC/mesothelioma, breast cancer, and gynecological cancer, whereas the association was not observed for the other cancers (Table 2).

Overall response rates according to diagnosis

In total, higher ORRs were obtained in patients without LMM when compared with the patients with LMM after adjusting for age and diagnosis (HR: 3.410; 95%CI: 1.954-5.951, p<0.001). ORR was assessed for diagnosis, thus better ORRs were achieved in the group of NCSLC, SCLC/mesothelioma, colorectal cancer, non-colorectal GIT cancer, breast cancer, prostate cancer, and gynecological cancer, while statistically significance could not be reached except for NSCLC and gynecological cancer possibly due to the low number of patients (Table 3).

Discussion

In this study, an extremely high rate of 50.5% of the patients had low muscle mass, when the median cutoff value was chosen as SMI of 42 cm2 /m2 for men and 36.8 cm2 / m2 for women after adjusting for gender. In general, LMM was found to be significantly associated with worse OS and PFS in univariate analysis. Additionally, this positive association was maintained after adjusting for diagnosis and age (HR 1.563, 95%CI 1.191-2.051, p=0.001 for PFS and HR 1.477, 1.110-1966; p=0.008 for OS). Unlike other studies, we also studied the effect of LMM on ORR, and we demonstrated that LMM was associated with worse ORR independent of age and diagnosis (HR; 95%CI 3.410; 1.954-5.951, p= <0.001). Therefore, demonstrating the predictive value of muscle loss for treatment response with this patient data set, which we define with sharp lines in the patients' inclusion criteria, can provide guidance for the benefit of additional therapies in addition to conven-

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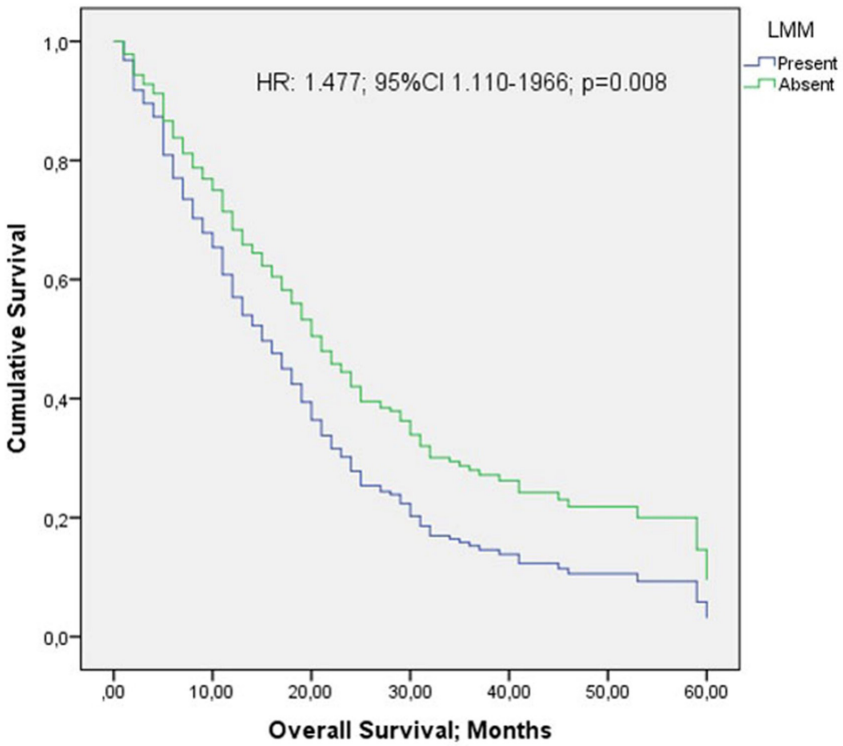

Figure 1. Overall survival curve according to LMM by Cox regression analysis adjusted for age and diagnosis. HR: Hazard ratio; CI: Confidence interval; LMM: Low muscle mass

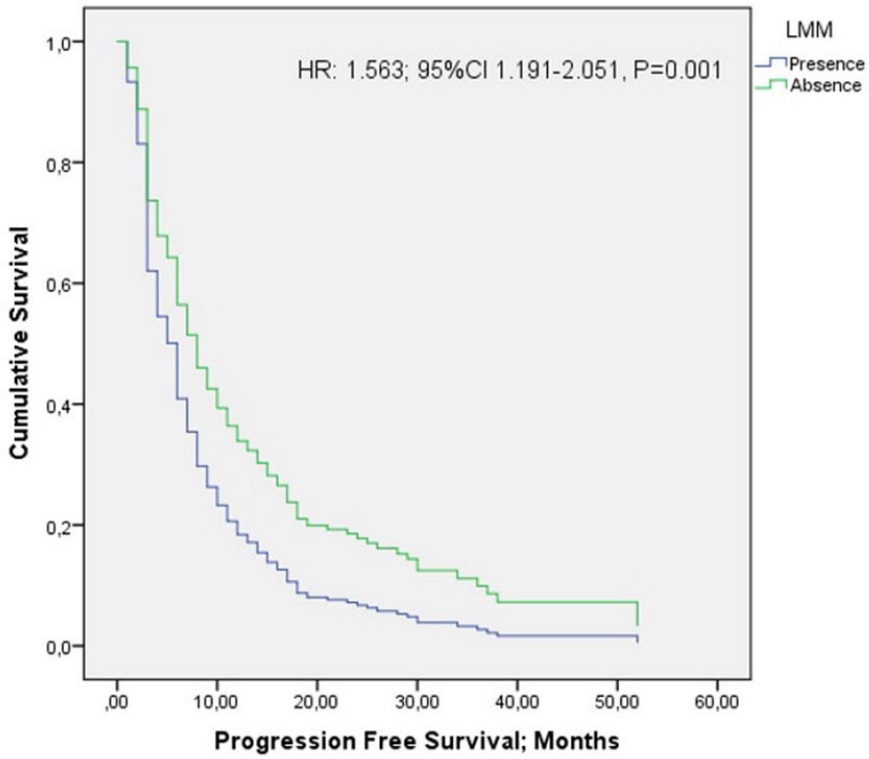

Figure 2. Progression-free survival curve according to LMM by Cox regression analysis adjusted for age and diagnosis. HR: Hazard ratio; CI: Confidence interval; LMM: Low muscle mass

Table 2. Progression-free survival and overall survival according to LMM by Cox proportional hazards models

	median OS, months		PFS		OS	
Diagnosis	**LMM**	**non-LMM**	**HR; 95%CI***	**P valus**	**HR; 95%CI***	**P value**
Respiratory tract						
NSCLC	10	12	1.591 (0.82-3.07)	0.22	1.534 (0.769-3.06)	0.225
SCLC and mesothelioma	5	16	*2.509 (1.00-5.28)*	*0.050*	*2.566 (1.05-6.27)*	*0.039*
GIS						
Colorectal cancer	16	19	0.761 (0.38-1.53)	0.44	0.997 (0.52-1.98)	0.99
Non-CR GIT cancer**	8	12	1.402 (0.83-2.36)	0.20	1.268 (0.75-2.15)	0.38
Breast cancer	32	NR	*3.493 (1.54-7.92)*	*0.003*	*2.647 (1.08-6.49)*	*0.033*
Bladder cancer	5	14	3.155 (0.29-34.84)	0.348	1.295 (0.15-11.2)	0.81
Prostate cancer	30	NR	1.514 (0.32-7.27)	0.611	1.609 (0.34-7.51)	0.54
Gynecological cancer	25	25	*5.480 (1.43-21.01)*	*0.013*	1.646 (0.39-6.98)	0.49

*Adjusted for age, **non-CR GIT: non-colorectal gastrointestinal tract; HR: Hazard ratio; CI: Confidence interval; PSF: Progression-free survival; OS: Overall survival; LMM: Low muscle mass; NSCLC: non-small cell lung cancer; SCLC: small-cell lung cancer, Non reached: NR.

Table 3. Overall response rates according to LMM by multivariate logistic regressions analysis

	N(%)		ORR %		OR; 95%CI	P value
	LMM	non-LMM	LMM	non-LMM		
NSCLC	30 (63.8)	17 (36.2)	18.0%	47.1%	2.120; 1054-4261	*0.043*
SCLC - mesothelioma	14 (51.9)	13 (48.1)	14.3%	46.2%	2.036; 0.999-4.148	0.070
CRC	26 (52.0)	24 (48.0)	57.7%	75.0%	1.545; 0.756-3.159	1.97
Non-CRT GIT cancer	35 (52.2)	32 (47.8)	18.2%	37.5%	1.567; 0.983-2.496	0.082
Breast cancer	26 (47.3)	29 (52.7)	56.5%	78.6 %	1.676; 0.848-3.315	0.091
Bladder cancer	3 (30.0)	7 (70.0)	50.0%	57.1%	1.067; 0.521-2.182	0.858
Prostate cancer	5 (31.2)	11 (68.8)	60.0%	81.8%	1.500; 0.534-4.214	0.35
Gynecological cancer	7 (41.2)	10 (58.8)	42.9%	100%	-	*0.015*

ORR: Overall response rate; CI: Confidence interval; LMM: Low muscle mass; NSCLC: non-small cell lung cancer; SCLC: small-cell lung cancer; CRT: Colorectal cancer; GIT: gastrointestinal tract

tional therapies, especially in patients with muscle loss who need a rapid treatment response.

Among parameters, muscle loss was less common in patients with bladder cancer and prostate cancer, than in other types of cancer in the study, which may be associated with early diagnosis due to the early onset of symptoms. While early diagnosis is generally possible for colon cancer, it can be thought that either a rapid disease course, delayed diagnosis or nutrition intake problems contribute to the achievement of high muscle loss rates in patients with metastatic process. On the other hand, considering the general life span of non-colorectal gastrointestinal system cancers and thoracic cancers with a median of less than 1 year, the high rates of muscle loss observed initially in aggressive cancers seem to be compatible with the natural course. Additionally, the high rates of muscle loss seen in breast cancer at the time of diagnosis were considered as normal, since patients who were not suitable for hormonal intervention and who were indicated for chemotherapy due to high disease burden were included in the study. When we evaluated other parameters, the presence of bone metastasis and metastatic organ involvement did not affect muscle loss levels. Statistically higher LMM was found in patients older than 75 years, so age was included in the multivariate analysis when evaluating the effect of LMM on survival for different types of cancer.

When the type of cancer was evaluated separately; LMM was a significant prognostic factor for OS for SCLC/ mesothelioma and breast cancer, whereas it did not reach a statistically significant level for the NSCLC, colon cancer, non-colorectal GIT cancers, bladder cancer, prostate cancer, and gynecological cancer, although numerical association was obtained (Table 2).

The association of OS and LMM has been studied before for advanced breast cancer patients [7, 8]. There was in-

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consistent result about the prognostic significance of muscle loss in patients with metastatic stage breast cancer. According to a meta-analysis of a total of six studies (5497 breast cancer patients), patients with muscle loss were shown to be associated with a significantly higher risk of mortality, compared to breast cancer patients without muscle loss. On the other hand, although the association was valid for the early stage of breast cancer, it was not for metastatic breast cancer [9]. Different from the previous studies, we only included patients who needed chemotherapy at the first line and were not eligible for endocrine treatments. In this regard, muscle loss appears to have prognostic significance for metastatic breast cancer patients with high tumor volume or symptomatic disease with a cut-off value of 36.8 cm^2/m^2. Better PFS and ORR were additionally achieved in patients without LMM in the breast cancer subgroup. Our study showed that screening breast cancer patients with high tumor burden for muscle loss is very important and additional interventions such as physical education, aerobic and resistance exercises, and nutritional supplements may probably prevent loss of muscle mass and reverse this poor prognosis thanks to this awareness [10,11].

Patients with RTC and GTC who are cachexic by the conventional criterion (involuntary weight loss) and by two additional criteria (muscle depletion and low muscle attenuation) presented with a poor prognosis, regardless of overall body weight according to a large scale study [12]. However, the evaluation of RTCs as a single title can produce inaccurate results. Therefore, in this study we divided RTCs into two groups of NSCLC and other respiratory tract cancer, including SCLC and mesothelioma. Additionally, standardization was provided in terms of treatment differences. Thus, worse OS and PFS were observed with LMM in patients with SCLC and mesothelioma, but not in the NSCLC group of RTCs. This can be explained by the fact that if cachexia develops at the beginning in cancers with aggressive prognoses such as SCLC and mesothelioma, although they present early symptoms compared to NSCLC, this may be a biomarker that indicates a more unfavorable prognosis (Table 3).

On the other hand, even though muscle loss had a numerically poor prognostic effect on survival, no statistically significant difference was found for PFS and OS in patients with colorectal and non-colorectal gastrointestinal tract cancer. When we evaluated the effect of muscle loss after adjusting for diagnosis and age, we reached a statistically significant conclusion that LMM is generally an independent prognostic factor for OS and PFS. Therefore, this result can be related to an insufficient number of patients was included in the GIT cancers in the present study. Higher ORR was achieved in patients without LMM, whereas it was more pronounced in patients with non-CR GIT cancers.

In our study, patients with distant metastatic bladder cancer were studied, and OS benefit was demonstrated in patients without LMM, consistent with the literature. Additionally we also showed PFS benefit in patients without LMM, even though it did not reach a statistically significant level, due to the possible low number of patients included in the study. On the other hand, the ORRs were similar between two groups and LMM did not predict the treatment responses with platinum plus gemcitabine.

In this study, we included the castration-sensitive prostate cancer patients with high volume disease who were treated with docetaxel at the first line. Previously it was shown that patients with muscle loss experienced higher toxicity and shorter survival while receiving docetaxel in patients with castration-resistant disease [14]. Additionally, the prognostic significance of muscle loss was shown in patients treated with androgen deprivation therapy at first line [15]. Unlike the previous studies, we demonstrated that LMM did not affect both PFS and OS in castration-sensitive prostate cancer patients treated with docetaxel in the first line. Therefore, predictive/prognostic significance of LMM should be evaluated in different clinical scenarios for prostate cancer.

In this study, we also examined patients with metastatic gynecological cancer for whom cytoreductive surgery is not suitable. Although muscle loss has a prognostic significance in the operable early stages of gynecological cancer, there were discordant results for the advanced stage of gynecological cancers [16, 17]. We found that although the prognostic effect of muscle loss on PFS and predictive value on ORR was observed, it did not translate into an overall survival contribution. Consistent with our study, metastatic gynecological cancers show a lower level of LMM when compared to RTC and GIT, and muscle loss has a lower prognostic significance compared to other cancers. In this regard, thanks to the higher ORR obtained, preoperative chemotherapy can be considered in borderline resectable disease without LMM. However, screening for muscle loss may not be considered a priority in the treatment plan for gynecological cancers for a long time.

This study has a number of limitations. Retrospective data analysis and insufficient number of patients are the main limitations of our study. This situation may have led to statistically negative results, although clinically significant results were obtained. However, we understand that the presence of LMM has not only prognostic value, but also predictive value in general. Our study will shed light on conducting prospective randomized studies comparing the response rate and survival time of patients with and without initial muscle loss by the studies with high statistical power and a number of patients for each type of cancer. When our findings are evaluated together with the results of previous studies, it may be considered that LMM may have a negative prog-

nostic effect, especially for SCLC / mesothelioma and breast cancer. Additionally, although statistically significant results were not obtained for the metastatic stage of non-colorectal GIT cancers and bladder cancers, clinically relevant survival differences were shown. Moreover, lower ORR was obtained in patients with LMM in general except for bladder cancer. Therefore, assessment of muscle loss should be incorporated as part of routine clinical evaluation especially for patients with metastatic SCLC/mesothelioma, breast cancer, non-colorectal GIT cancers, and bladder cancer to guide better additional treatment strategies. Additionally, one of the most important limitations of the study is that only muscle mass was evaluated, and muscle function could not be evaluated due to the retrospective design of our study. Although performing functional evaluation may provide a more optimal evaluation, even muscle mass evaluation alone seems to be a remarkable and predictive and prognostic biomarker that can guide the clinician according to our study.

Conclusions

In conclusion, LMM has not only prognostic value but also predictive value for many types of cancer. Therefore, the assessment of muscle loss should be incorporated as part of the first routine clinical evaluation.

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The Effect of Monthly Mean Global Horizontal Solar Radiation and Sunshine Duration on Vitamin D Levels in Young Women

Genç Kadınlarda Aylık Ortalama Küresel Yatay Güneş Radyasyonu ve Güneşlenme Süresinin Vitamin D Düzeyine Etkisi

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ABSTRACT

Aim: The most important reason for vitamin D deficiency is the lack of synthesis in the skin. The synthesis of vitamin D can be affected by many variables such as geographical region, race, season, monthly average daily sun exposure duration (MADSD), monthly average daily global horizontal solar radiation (MADGHSR). In this study, we aimed to investigate possible association between vitamin D levels and MADSD and MADGHSR in young women.

Methods: This is a retrospective study evaluating the levels of Vitamin D classified by age, seasons, months, MADGHSR and MADSD in women aged between 15-45 years admitted to a secondary state hospital.

Results: All of the women involved were at reproductive age and approximately 94.6% of them had vitamin D levels below 30 ng/mL. The median (minimum-maximum) vitamin D level was 16.1 (3.6-49.4) ng/mL in summer and 14.3 (3.2-49.8) ng/mL in winter (p=0.001). The rate of vitamin D deficiency (<20 ng/mL) was 68.1% in summer and 75.1% in winter (p<0.001). The median MADGHSR was 4.6 (3.4-5.7) hours in winter and 6.9 (5.2-7.2) hours in summer and the median MADSD was 3.3 (1.6-3.5) watt/m^2/day in winter and 15.2 (12.2-15.8) watt/m^2/day in summer. Vitamin D level was weakly correlated with age (r=0.082, p=0.002), MADSD (r=0.075, p=0.001) and MADGHSR (r=0.062, p=0.006).

Conclusion: We found that MADGHSR and MADSD had an effect on vitamin D synthesis in addition to factors related with personal and environmental situations. We suggest that routine optimal dose vitamin D replacement is necessary in geographies similar to the population in our study.

Key Words: Vitamin D Deficiency, Daily Sunshine Duration, Global Solar Radiation

ÖZET

Amaç: D vitamini eksikliğine neden olan en önemli neden ciltte sentez eksikliğidir. D vitamini sentezi yaşanılan coğrafi bölge, ırk, mevsim, maruz kalınan aylık ortalama günlük güneşlenme süresi (MADSD), aylık ortalama günlük küresel yatay güneş radyasyonu (MADGHSR), gibi pekçok değişkenden etkilenebilir. Bu çalışmada genç kadınlarda D vitamini düzeyi ile MADSD ve MADGHSR değerleri arasındaki ilişkinin araştırılmasını amaçladık.

Yöntem: Bu çalışma retrospektif olarak ikinci basamak bir devlet hastanesine başvuran 15-45 yaş arasındaki kadınlarda yapıldı ve D vitamini düzeyleri yaş, mevsimler, aylar, MADGHSR ve MADSD değerlerine göre sınıflandırıldı.

Bulgular: Çalışmaya dahil edilen tüm kadınlar üreme çağında olup yaklaşık %94,6'sının D vitamini düzeyi 30 ng/mL'nin altındaydı. Ortanca (minimum-maksimum) D vitamini düzeyi yazın 16,1 (3,6-49,4) ng/mL, kışın 14,3 (3,2-49,8) ng/mL idi (p=0.001). D vitamini eksikliği (<20 ng/mL) oranı yazın %68,1 ve kışın %75,1 olarak saptandı (p<0.001). Medyan MADGHSR kışın 4,6 (3,4-5,7) saat ve yazın 6,9 (5,2-7,2) saat ve medyan MADSD kışın 3,3 (1,6-3,5) watt/m^2/gün ve yazın 15,2 (12,2-15,8) watt/m^2/gün bulundu. D vitamini düzeyinin yaş (r=0.082, p=0.002), MADGHSR (r=0.062, p=0.006) ve MADSD (r=0.075, p=0.001) ile zayıf korelasyon gösterdiği saptandı.

Sonuç: Bireysel ve çevresel faktörlerin yanı sıra MADGHSR ve MADSD'nin vitamin D sentezi üzerinde etkili olduğunu bulduk. Çalışmamızdaki popülasyona benzeyen coğrafyalarda rutin optimal doz D vitamini takviyesinin gerekli olduğunu düşünüyoruz.

Anahtar Kelimeler: D Vitamini Eksikliği, Günlük Güneşlenme Süresi, Küresel Güneş Radyasyonu

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Introduction

Vitamin D deficiency is a leading health issue in most of the developed and developing countries due to numerous influential factors. Vitamin D is very important for bone mineralization and its deficiency has negative impacts on many bodily systems [1-4]. Moreover, the importance of vitamin D for the immune system has also been emphasized in studies after coronavirus disease 2019 (COVID-19) outbreak [5,6].

Vitamin D is a steroid hormone and dietary vitamins, D2 (ergosterol) and D3 (cholecalciferol), are transported to fat cells by chylomicrons. The vitamin D synthesis is completed in the skin, liver, and kidney, respectively and the most important synthesis site is the skin. The lack of vitamin D synthesis in the skin is the most crucial step causing vitamin D deficiency in the absence of liver and kidney failure. Vitamin D synthesis begins with the penetration of ultraviolet B (UVB) radiation into the skin, in the epidermis, at a wavelength of 290-315 nm and its absorption by 7-dehydrocholesterol (7-DHC). After taking the form of previtamin-D, it exits the cells and then enters the bloodstream. Then, 25-hydroxylation in the liver and 1 alpha-hydroxylation in the kidney occurs. Vitamin D metabolism is regulated by parathormone (PTH), serum calcium (Ca^{+2}), fibroblast growth factor 23 (FGF23), phosphorus (P), and other factors [7,8].

Increased body weight, insufficient dietary intake of vitamin D, use of sunscreen, and wearing clothes that cover the whole body reduce the effect of the sun and vitamin D synthesis. In addition, there are other factors that related with vitamin D synthesis, including race, skin colour (melanin content in the skin), geographic region, seasons, monthly mean daily global horizontal solar radiation (MADGHSR) and monthly mean daily sun exposure (MADSD) [9,10]. The MADSD and MADGHSR can be calculated using various meteorological and geographical data. MADSD can be estimated by analyzing the average daily hours of sunshine recorded at a specific location over a month. This data can be obtained from local meteorological stations or global databases. MADGHSR is calculated using models that account for various factors, such as the angle of incidence of solar radiation, atmospheric conditions, and the Earth's tilt and orbit. A widely used model for estimating solar radiation is the Angström-Prescott equation [11,12]

A vitamin D level of 30 ng/mL and above is essential not only for calcium metabolism but also for all other body functions that involve role of vitamin D leading to its transformation to previtamin D3, which is rapidly converted to vitamin D3. Season, latitude, time of day, skin pigmentation, aging, sunscreen use, and glass all influence the cutaneous production of vitamin D3. Once formed, vitamin D3 is metabolized in the liver to 25-hydroxyvitamin D3 and then in the kidney to its biologically active form, 1,25-dihydroxyvitamin D3. Vitamin D deficiency is an unrecognized epidemic among both children and adults in the United States. Vitamin D deficiency not only causes rickets among children but also precipitates and exacerbates osteoporosis among adults and causes the painful bone disease osteomalacia. Vitamin D deficiency has been associated with increased risks of deadly cancers, cardiovascular disease, multiple sclerosis, rheumatoid arthritis, and type 1 diabetes mellitus. Maintaining blood concentrations of 25-hydroxyvitamin D above 80 nmol/L (approximately 30 ng/mL(4). Although there are different threshold values determined around the world, vitamin D level is defined as insufficiency if it is below 30 ng/mL, deficiency if it is below 20 ng/mL and severe deficiency if it is below 12 ng/mL [13]. Studies have shown that 5.9-13% of the world population has severe vitamin D deficiency and 24-40% of them has vitamin D deficiency, although these rates vary across regions [14]. Additionally, in similar academic studies, it was reported that 41.4% of the general population in the United States of America (USA) had a vitamin D deficiency [15].

Vitamin D synthesis is affected by seasonal differences, latitude, and the time and angle of UVB rays reaching the earth. Additionally, environmental factors, clothing, ethnic group of the individual, and the geographical region of residence also have impacts on vitamin D synthesis [4,7,15,16].

The present study was conducted in the Eastern Black Sea Region in the north east of Turkey, which is located between 39-40°E longitude and 40-41°N latitude and at a mean altitude of 24 m above the sea level. In this region, typical Black Sea climate with moderate summers and mild winters is seen and all seasons are cloudy. Most of the women living in this region wear clothes that cover most of the body due to the weather conditions as well as their traditional lifestyles and religious preferences.

In this study, we aimed to investigate relationship between vitamin D levels and MADSD and MADGHSR values in female patients between the ages of 15-45 residing in the Akçaabat district in Trabzon province, which is located in the north-east of Turkey and receives relatively little sunlight. We also aimed to determine whether environmental factors or sunlight exposure were more effective on vitamin D synthesis.

Materials and Methods

This descriptive study retrospectively examined reproductive-aged women (15-45 years old), who presented to Akçaabat State Hospital, a secondary care hospital in

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Trabzon province, and had their vitamin D level status measured. Vitamin D levels classified according to age, seasons, months, MADGHSR and MADSD.

Measurements of serum 25-hydroxyvitamin D (25(OH)D) levels were performed using an enzyme chemiluminescence immunoassay method on an autoanalyzer (Beckman Coulter UniCel DXI 800 Immunoassay Systems, California, USA). These measurements were retrieved from the hospital data system and prescription data were obtained from the prescription drug monitoring program. Patients were excluded from the study that has inadequate medical records, kidney failure, liver failure, calcium metabolism disorders, malabsorption, intestinal diseases, and those who received vitamin D replacement within the last six months.

The MADSD and MADGHSR metrics are employed to evaluate solar energy potential and to compare different regions. MADSD is expressed in hours and represents the duration of sunlight available for solar energy production. MADGHSR is expressed in kWh/m^2/day or MJ/m^2/day and takes into account both direct and diffuse sunlight. These two parameters play a significant role in designing solar energy systems suited to a specific location [11,12].

This study was approved by Ethics Committee of Karadeniz Technical University (Number:2019-54).

Statistical Analysis

SPSS for Windows version 23.0 (Armonk, NY: IBM Corp.) was used for analyzing the data. The categorical variables were expressed by frequencies (n) and percentages (%) and the continuous variables were expressed as mean ± standard deviation (SD) and/or median (minimum-maximum). The normal distribution of data was analyzed by Kolmogorov-Smirnov test. Mann-Whitney *U* and Kruskal-Wallis tests were used for the comparison of independent continuous variables. Mann-Whitney *U* test was also used for post-hoc analysis of groups that showed a significant difference on the Kruskal-Wallis test. The categorical variables were compared by Chi-square test or Fisher's test, followed by post-hoc Bonferroni correction test. The correlation between continuous variables were evaluated using Spearman's Correlation test. A *p* value of <0.05 was considered as significant.

Results

A total of 1901 women were included and the mean age of the patients presented to hospital in summer was 31.1±7.9 years and in winter was 30.2±8.6 years. The median (min-max) vitamin D level was 16.1 (3.6-49.4) ng/mL in summer and 14.3 (3.2-49.8) ng/mL in winter (p=0.001). The median MADGHSR was 4.6 (3.4-5.7) hours in winter and 6.9 (5.2-7.2) hours in summer. The median MADSD was 3.3 (1.6-3.5) watt/m^2/day in winter and 15.2 (12.2-15.8) watt/m^2/day in summer. Moreover, MADSD values showed remarkable variation during the six months (Table 1).

The vitamin D level was significantly higher in summer than in winter (p<0.001). A significant difference was found during the six months with regard to vitamin D levels (p<0.001), which was due to the significantly higher vitamin D levels in July and August compared to the other months. Similarly, a significant difference was found between the three age groups in terms of vitamin D levels, as vitamin D levels in the 35-45 age group were significantly higher than in other age groups (p=0.008) (Table 2).

Table 1. Meteorological data and characteristics of study group

Season / Month	N	Vitamin D (ng/mL) Mean ±SD	Age (years) Mean ± SD	MADGHSR* (hours) Median (min-max)	MADSD**(Watt/m²/day) Median (min-max)
Winter	896	15.9 ± 7.5	30.2±8.6	4.6 (3.4-5.7)	3.3 (1.6-3.5)
November	287	16.5 ± 7.1	30.5±8.8	3.3	5.7
December	205	15.3 ± 8.2	28.6±8.5	1.6	3.4
January	404	15.7 ± 7.3	30.8±8.3	5.2	4.6
Summer	1005	17.3 ± 7.3	31.1±7.9	6.9 (5.2-7.2)	15.2 (12,2-15.8)
June	440	16.1 ± 7.5	30.9±7.6	7.2	15.8
July	428	18.2 ± 7.1	31.2±5.4	5.2	15.2
August	137	18.5 ± 6.9	31.5±8.7	6.9	12.2
All months	**1901**	16.6 ± 7.4	**30.6±8.3**	**12,2 (3.4-15.8)**	**5.5 (1.6-7.2)**

*MADGHSR: Monthly average daily global horizontal solar radiation. **MADSD: Monthly average daily sunshine duration. These data were obtained from the Regional Directorate of Meteorology as only monthly average.

Table 2. Comparison of vitamin D levels by season, months and age groups.

Parameters	Vitamin D level (ng/mL)			*p*
	Median	Minimum	Maximum	
Season				
Summer	16.1	3.6	49.4	*<0.001*
Winter	14.3	3.2	49.8	
Month*				
June[a]	14.4	4.5	49.4	
July[b]	17.4	3.6	47.9	
August[b]	17.2	5.5	40.7	*<0.001*
November[a]	15.2	3.5	46.7	
December[a]	13.1	3.2	49.8	
January[a]	14.2	3.5	43.0	
Age (years)* (n=1473)				
15-24[a]	14.0	3.4	48.6	*0.008*
25-34[a]	14.1	3.2	49.4	
35-45[b]	15.6	3.5	49.8	
Total	15.2	3.2	49.8	

In the comparison of age groups according to different cutoff values, a significant difference was found only between women with severe and non-severe vitamin D deficiency (≤12 ng/mL and >12 ng/mL, respectively) (p<0.001). In addition, while severe vitamin D deficiency was found in 26.4% of women aged 35-45, it was found to be significantly more common in women aged 25-34 (34.4%) and women aged 15-24 (37.1%) (p =0.001) (Tables 3 and 4).

In terms of seasonal variation, there was a significant difference between the cutoff values of 12 and 20 ng/mL. Severe vitamin D deficiency (≤12 ng/mL) was detected in 25.4% in summer and 34.2% in winter (p<0.001) and vitamin D deficiency (<20 ng/mL) was 68.1% in summer and 75.1% in winter (p<0.001). At a cutoff value of 30 ng/mL, there was no significant difference between summer and winter (p=0.438) (Table 3 and 4). There was a weak correlation between vitamin D level and age (r=0.082, *p=0.002*), MADSD (r=0.075, *p=0.001*) and MADGHSR (r=0.062, *p=0.006*) (Table 5).

Table 3. Comparison of vitamin D levels in age and seasonal groups.

≤12		Vitamin D Level (ng/mL)				
		12 -20	20 - 30	>30	Total (n)	
Age groups (years)						
15-24	n (%)	163 (37.1)	168 (38.3)	90 (20.5)	18 (4.1)	439 (100)
25-34	n (%)	172 (34.4)	205 (41.0)	97 (19.4)	26 (5.2)	500 (100)
35-45	n (%)	141 (26.4)	238 (44.6)	120 (22.5)	35 (6.5)	534 (100)
Total	**N (%)**	**476 (32.3)**	**611 (41.5)**	**307 (20.8)**	**79 (5.4)**	**1473 (100)**
Season						
Summer	n (%)	255 (25.4)	429 (42.7)	260 (25.8)	61 (6.1)	1005 (100)
Winter	n (%)	306 (34.2)	367 (41.0)	176 (19.6)	47 (5.2)	896 (100)
Total	**N (%)**	**561 (29.5)**	**796 (41.9)**	**436 (22.9)**	**108 (5.7)**	**1901 (100)**

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Table 4. Comparison of vitamin D levels in age and seasonal groups according to different cut-off values

≤12		Vitamin D (ng/mL)			Vitamin D (ng/mL)			Vitamin D (ng/mL)			Total (n)
		>12	*p*	≤20	>20	*p*	≤30	>30	*p*		
Age groups (years)*											
15-24	n	163[a]	276[a]		331	108		421	18		439
	%	37.1	62.9		75.4	24.6		95.9	4.1		100
25-34	n	172[a]	328[a]	*0.001*	377	123	0.178	474	26	0.235	500
	%	34.4	65.6		75.4	24.6		94.8	5.2		100
35-45	n	141[b]	393[b]		379	155		499	35		534
	%	26.4	73.6		71.0	29.0		93.4	6.6		100
Total	**n**	**476**	**997**		**1087**	**386**		**1394**	**79**		**1473**
	%	**32.3**	**67.7**		**73.8**	**26.2**		**94.6**	**5.4**		**100**
Season											
Summer	n	255	750		684	321		944	61		1005
	%	25.4	74.6	*<0.001*	68.1	31.9	*0.001*	93.9	6.1	0.438	100
Winter	n	306	590		673	223		849	47		896
	%	34.2	65.8		75.1	24.9		94.8	5.2		100
Total	**n**	**561**	**1340**		**1357**	**544**		**1793**	**108**		**1901**
	%	**29.5**	**70.5**		**71.4**	**28.6**		**94.3**	**5.7**		**100**

Chi Square test
*a, b: There is no significant difference between variables with the same letter.

Table 5. Correlation of Vitamin D levels with age, MADGHSR and MADSD

	Age		MADGHSR*		MADSD**	
	r	***p***	**r**	***p***	**r**	***p***
Vitamin D level	0.082	0.002	0.062	0.006	0.075	0.001

Spearman's Correlations test
*MADGHSR: Monthly average daily global horizontal solar radiation, **MADSD: Monthly average daily sunshine duration

Discussion

The mean vitamin D level in this study cohort was 16.6±7.4 (range, 3.2-49.75) ng/ mL. It was also noted that in 94.4% of the participants the vitamin D level was ≤30 ng/mL whereas it was ≤20 ng/mL in 73.8% and ≤12 ng/mL in 32.3% of the participants. A study conducted in Turkey by Ogus et al. reported that 50% of the participants had a vitamin D level of ≤20 ng/mL [17]. In another study, Alagol et al. reported vitamin D deficiency in 66.6% of reproductive aged Turkish women [18]. A study from USA showed that 42% of female participants aged 15-49 years had a vitamin D level of ≤15 ng/mL at the end of winter [19]. Aydın et al. evaluated the vitamin D levels of athletes and reported that 21.6% of them had severe deficiency (<11 ng/mL), 42.7% had deficiency (11-20 ng/mL), and 19.5% had insufficiency (21-30 ng/mL) [20]. In our study, the prevalence of vitamin D deficiency and insufficiency was remarkably higher than those reported in the literature.

In our study, although the difference between the median vitamin D levels in summer and winter was low (1.8 ng/mL), it was still statistically significant (p=0.001). Some other studies, however, have reported more differences between summer and winter for vitamin D levels. Ogus et al. examined vitamin D levels in Ankara province, Turkey, which has a continental climate, and reported a mean vitamin D level of 17.23 ng/mL for winter and a mean level of 26.26 ng/mL for summer [17]. Carnevale et al. reported that a 25(OH)D level of <12 ng/ml was detected in 17.8% and 2.2% of all participants in winter and summer, while it was detected in 27.8% and 3.4% of female participants, respectively [21]. The study also noted that the mean vitamin D level in women was 15.2±5.68 ng/mL in winter and

30.7±8.01 ng/mL in summer. In our study, seasonal variation was lower than expected when compared to those reported in other studies.

A study conducted by Oren et al. in Israel at 30-33°N latitude reported mean vitamin D levels of 21.6±8 ng/mL and 23.9±8 ng/mL for winter and summer, respectively [22]. The authors also noted that the prevalence of vitamin D insufficiency (<30 ng/mL) was 82.9% and 82.6% and vitamin D deficiency (<15ng/mL) prevalence was 21.7% for summer and 22.9% for winter. There was no significant difference between the two seasons for vitamin D levels. In contrast, the rates of severe deficiency (12 ng/mL) in our study were relatively lower (25.4% in summer and 34.2% in winter).

In our study, there was a significant difference between the two seasons for median MADGHSR and MADSD values. Additionally, vitamin D level was weakly correlated with age, MADGHSR, and MADSD. This finding does not support other studies that reported a positive correlation between vitamin D levels and sun exposure and seasonal differences [4,16,17,23,25].

It is commonly known that most of the women living in Turkey wear clothes that cover most of their body due to the weather conditions as well as their traditional lifestyles and religious preferences. Additionally, according to the latest research, 58% of Turkish women wear headscarves and this rate can reach up to 70% depending on the region [26].

Alagol *et al.* evaluated the impact of clothing on vitamin D levels and showed that clothing preferences had a significant impact on vitamin D synthesis [18]. This phenomenon could be the reason as to why we could not detect the expected effect of seasonal differences on our patients' vitamin D levels. In addition, based on this finding, it can also be assumed that although sun exposure is highly important for vitamin D synthesis, individual and other environmental factors may be more effective [4,15,22,27].

The present study was conducted on relatively young women (age of 30.6±8.3 years) and it is a common fact that vitamin D level is also important in terms of public and pregnancy health in reproductive aged women. Meaningfully, studies have found that low vitamin D levels are related with preeclampsia, gestational diabetes mellitus, pregnancy-induced hypertension, congenital heart diseases, and miscarriage [27,28,29]. Similarly, we also found a high rate of vitamin D deficiency in young women, which is a public health problem in this respect.

Because of the retrospective nature of our study, other factors such as body mass index and nutrition that could affect vitamin D synthesis could not be evaluated.

Among the limitations of this study are its retrospective design, which precluded the analysis of potential factors influencing vitamin D synthesis, such as body mass index and dietary habits. The demographic diversity of the sample is limited, given the focus on young women. While the study examined the impact of specific factors such as clothing preferences and sun exposure on vitamin D levels, other potential environmental and individual factors were not sufficiently explored. Generalizing the findings to broader and diverse populations, considering geographic, cultural, and individual variations, is challenging. Furthermore, the inability to fully ascertain the expected seasonal variations in vitamin D levels underscores the need for more comprehensive studies in this field.

Conclusion

The findings of this study indicate that geographical region, MADGHSR and MADSD values, and individual and environmental factors have a direct effect on sun exposure. It was also revealed that the regional clothing habits of our population was an important factor for low vitamin D levels. We suggest that routine optimal dose vitamin D supplementation is necessary for women that reside in similar geographical regions and have similar clothing preferences to the present study.

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Research Article

Araştırma

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Postpartum Type 2 Diabetes Mellitus Frequency of Patients with Gestational Diabetes Mellitus

Gestasyonel Diabetes mellitus hastalarında Doğum Sonrası Tip 2 Diabetes Mellitus Sıklığı

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ABSTRACT

Aim: This study aims to examine the frequency of type 2 diabetes mellitus (DM) in the postpartum period and its relationship with other risk factors.

Materials and Methods: Patients who were diagnosed, followed up and treated for gestational diabetes in Malatya Turgut Özal University, Malatya Training and Research Hospital Endocrinology outpatient clinic and who underwent Oral Glucose Tolerance Test (OGTT) in the postpartum period were included.

Results: In our retrospective study, 157 patients were included. The mean age was 31.8±5.6 years. After being followed up with her follow-up and treatment throughout the pregnancy, OGTT administered with 75 grams of glucose was performed at the postpartum 8th week. Impaired glucose tolerance (IGT) was detected in 23 patients (14.64%), impaired fasting glucose (IFG) in 18 patients (11.46%), and type 2 DM in 17 patients (10.8%). When evaluated in terms of the presence of DM in the postpartum period, no difference was found regarding the history of gestational DM in the anamnesis (p=0.305) and the presence or absence of family history (p=0.095). In terms of the presence of DM, there was a significant difference between the patients receiving insulin therapy and those receiving diet therapy (p=0.001).

Conclusion: We can say that type 2 DM development in the postpartum period is associated with high maternal age and an increase in body mass index (BMI). Being pregnant with the ideal weight to be achieved by lifestyle changes may decrease the risk of type 2 DM and diabetes-related complications in the long term.

Key Words: Diabetes, Gestational, Obesity, Insulin

ÖZET

Amaç: Çalışmamızda postpartum dönemde tip 2 diabetes mellitus (DM) sıklığı ve risk faktörleri ile ilişkisinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Malatya Turgut Özal Üniversitesi, Malatya Eğitim ve Araştırma Hastanesi Endokrinoloji polikliniğinde gestasyonel diyabet tanısı alan, takip ve tedavisi edilen ve postpartum Oral Glikoz Tolerans Testi (OGTT) yapılan hastalar dahil edilmiştir.

Bulgular: Çalışmaya 157 hasta dahil edildi. Yaş ortalaması 31,8±5,6 idi. Gebelik boyunca takibi ve tedavisi ile izlendikten sonra postpartum 8. haftada 75 gramlık OGTT yapıldı. Yirmi üç hastada (%14,64) bozulmuş glikoz toleransı (BGT), 18 hastada (%11,46) bozulmuş açlık glikozu (BAG) ve 17 hastada (%10,8) tip 2 DM tespit edilmiş olup 99 hastamızın (%63,5) normal glikoz değerleri tespit edilmiştir. Postpartum dönemde DM varlığı açısından değerlendirildiğinde anamnezinde gestasyonel DM öyküsü (p=0,305) ve aile öyküsü varlığı ya da yokluğu açısından farklılık saptanmadı (p=0,095). DM varlığı açısından insülin tedavisi alan hastalar ile diyet tedavisi alan hastalar arasında ise anlamlı farklılık vardı (p=0,001).

Sonuç: Çalışmamızın sonucunda postpartum dönemde tip 2 DM gelişiminin anne yaşının yüksekliği ve vücut kitle indeksi (VKİ) artışının beraberliği ile ilişkili olduğunu söyleyebiliriz. Yaşam şekli değişikliği ile sağlanacak ideal kilo ile gebe kalmak tip 2 DM riskin ve uzun vadede diyabete bağlı komplikasyonlarda azalma gösterebilecektir.

Anahtar Kelimeler: Diyabet, Gebelik, Obezite, İnsülin

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Introduction

Gestational Diabetes Mellitus (GDM) is a glucose intolerance first noticed during pregnancy and it is seen in 7% of all pregnancies [1]. Some of the patients diagnosed during pregnancy are type 2 diabetes patients who have not been recognized before [2-4]. Among the most crucial known risk factors are advanced maternal age, family history, sedentary life and high body mass index (BMI) [5].

Physiological insulin resistance and concomitant hyperinsulinemia develop to provide adequate nutrition to the fetus and due to hypermetabolic events during pregnancy. Increased placental in pregnancy hormones such as lactogen hormone, progesterone, cortisol, growth hormone, prolactin and their insulin in the second-trimester disruption of receptor substrate-1 (IRS-1) activity, followed by insulin with increased adipose tissue and genetic predisposition in the 3rd trimester. Sensitivity disorder causes insulin resistance. To compensate for this resistance, the pancreas must secrete more insulin. In patients with underlying chronic insulin resistance, this compensation mechanism cannot occur and beta-cell dysfunction develops [6].

Gestational diabetes may cause preeclampsia, spontaneous abortions in the mother, polyhydramnios, macrosomia, birth trauma and increased mortality in the fetus. Especially in the first 10 weeks, high HbA1C causes microcephaly, anencephaly, congenital heart diseases, and pathologies, such as hypoglycemia, hyperbilirubinemia, and hypocalcemia in the neonatal period. In children, advanced obesity, childhood diabetes, motor development disorder, and attention deficit can be seen [7].

More than 10% of women who develop gestational DM are diagnosed with type 2 DM immediately after delivery, and more than 70% in ten-year follow-ups. This metabolic uncontrollability experienced during pregnancy adversely affects maternal and child health in the long term, and may recur in the next pregnancy for GDM, which improves after subsequent delivery [8]. Therefore, early diagnosis of gestational diabetes, initiation of effective treatment of patients with risk factors, and postpartum follow-up are extremely important in reducing maternal/child morbidity and mortality due to GDM .

In our study, the patients who were diagnosed with gestational diabetes by applying to our polyclinic and treated. We aimed to present the results of the glucose loading test performed at the postpartum 8th week together with the demographic characteristics and the relationship with the risk factors in patients who developed permanent type 2 DM.

Material and Method

Our study was designed retrospectively and patients between January 1, 2018 and April 1, 2023 were included. In the general follow-ups of pregnancy, according to the screening program of the American Diabetes Association, the patients who did not have DM history, as a routine diagnosis and screening test at 24-28 weeks, in the morning and after at least eight hours of fasting, a 75-gram oral glucose tolerance test (OGTT) was performed and glucose values were determined. If fasting glucose ≥ 92 mg/dl, an hour postprandial glucose ≥ 180 mg/dl, and 2-hour postprandial glucose ≥ 153 mg/dl is high, it was considered gestational diabetes and is referred to Malatya Turgut Özal University, Malatya Training and Research Hospital Endocrinology outpatient clinic applicant gestational diabetes pregnant patients diagnosed with diabetes mellitus were included. After these patients applied to the endocrinology outpatient clinic, their family history, history and pre-pregnancy weight were questioned regarding the presence of DM, and pre-pregnancy BMI was calculated. In addition, a physical examination of the patients during pregnancy was performed and height, weight and BMI measurements were evaluated. In addition to the OGTT results, HbA1c, insulin, lipid profile, urea, and creatinine biochemical tests were requested for patients diagnosed with GDM. GDM diet (25-30 kcal/kg) and exercise (4 days a week for half an hour) were recommended to each patient in the treatment. Insulin therapy was started in the control examination for patients who were not regulated by diet and exercise therapy.

Postpartum applications of patients whose pregnancy continues with close follow-up and treatment, along with recommendations, blood sugar follow-up and 75 grams oral glucose tolerance test were performed and if the fasting blood glucose is ≥126 mg/dl, impaired fasting glucose (IFG), If the postprandial blood glucose is between ≥140-199 mg/dl, impaired glucose tolerance (IGT), random single value or postprandial blood glucose ≥200 mgr/dl was divided into groups as type 2 DM. The rest were considered normal.

Exclusion criteria: Pregnant women under 18 years of age**,** diagnosed with type 1 diabetes or type 2 diabetes before pregnancy, and pregnant women with kidney or liver failure were excluded from this study.

Ethics Committee**:** This retrospective research was conducted in compliance with the Declaration of Helsinki's guiding principles and authorized by the Malatya Turgut Ozal University Clinic Ethics Committee (Approval no: 2023/27).

Statistical analysis: SPSS 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. All data were expressed as mean

± standard deviation or number (percent). Mann-Whitney U test was performed to compare the group with or without DM in terms of age, BMI, insulin dose, FPG, and HbA1c averages. The chi-square test was used to compare the presence or absence of DM with the groups of gestational DM history, family history, and treatment status. A p-value of <0.05 was considered significant.

Results

A total of 157 volunteer patients with no previous diagnosis (Diabetes, impaired fasting glucose, impaired glucose tolerance) were included in our study. The age range was 21- 44 years and the mean age was 31.8±5.6. The number of pregnancies was between one and six, with an average of 2.5±1.1.

There was a history of GDM in 64 (40.8%) patients who participated in this study, and a family history in 108 (68.2%) patients. Forty-nine (31.2%) patients had both a history of GDM and a family history.

When the anamnesis of the patients was questioned, 13 (8.3%) patients had birth complications in their previous pregnancies and four (2.5%) patients had fetal anomalies.

Diet and exercise therapy (69 patients; 43.9%) were recommended as non-pharmacological treatment for pregnant patients diagnosed with gestational diabetes. In case of insufficient blood sugar regulation, insulin therapy was added once (66 patients; 42.0%), twice (17 patients; 10.8%), and four times (5 patients; 3.2%).

After the pregnancy follow-up of the patients was followed at certain intervals, a 75-gram glucose test was performed in the 8th week for blood sugar follow-up during and after delivery. Patients with postprandial blood sugar or blood sugar ≥200 mg/dlt at any time were considered to have persistent type 2 DM. Impaired glucose tolerance (IGT) was detected in 23 patients (14.64%), impaired fasting glucose (IFG) in 18 patients (11.46%), type 2 DM was detected in 17 patients (10.8%), and 99 patients (63%, 5) normal glucose values were present.

The mean age, pre-pregnancy BMI, pregnancy BMI and postpartum BMI, mean insulin dose, and HbA1c mean of patients with postpartum DM were significantly higher than the mean of those without DM (Table 1).

When evaluated regarding the presence of DM in the postpartum period, no difference was found between patients with and without a history of gestational DM (p=0.305). There was no difference in terms of the presence or absence of family history (p=0.095). Of the patients with DM, 16 (94.1%) were receiving insulin therapy and one (5.9%) was receiving diet therapy. Regarding the presence of DM, there was a significant difference between the patients receiving insulin therapy and those receiving diet therapy (p=0.001) (Table 2).

Table 1. Comparison of age, BMI, insulin dose, FPG, HbA1c averages of pregnant women with and without DM

	DM available (n=17)	no DM (n=140)	P
Age (year) mean ±SD (n=157)	36.9±4.3	31.2±5.5	**0.001**
Pre-pregnancy BMI (kg/m^2) mean ±SD (n=157)	30.8±3.3	27.7±4.4	**0.006**
Pregnancy BMI (kg/m^2) mean ±SD (n=157)	33.9±3.8	30.3±4.5	**0.001**
Post-pregnancy BMI (kg/m^2) mean ±SD (n=157)	32.4±3.4	28.8±4.3	**0.001**
Mean insulin dose (Units) mean ±SD (n=89)	21.6±7.2	13.9±5.2	**0.001**
Pregnancy FPG (mg/dl) mean ±SD (n=157)	118.5±31.7	113.1±34.7	**0.216**
Postpartum FPG (mg/dl) mean ±SD (n=157)	94.9±15.7	94.6±21.6	**0.659**
Pregnancy of HbA1c (%) mean ±SD (n=157)	7.7±0.5	6.5±0.5	**0.001**
Postpartum HbA1c (%) mean±SD (n=157)	7.4±0.5	5.7±0.5	**0.001**

n: number of patients, SD: standard deviation; p-values were obtained by Mann-Whitney U test.

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Table 2. Comparison of pregnant women with and without DM in terms of gestational DM history, family history and treatment methods

	DM available n (%)	No DM n (%)	P
Has a history of gestational DM (n=64)	9 (52.9)	55 (39.3)	0.305
No history of gestational DM (n=93)	8 (47.1)	85 (60.7)	
There is a family history (n=107)	15 (88.2)	92 (65.7)	0.095
No family history (n=50)	2 (11.8)	48 (34.3)	
Diet therapy (n=69)	1 (5.9)	68 (48.6)	0.001
Insulin therapy (n=88)	16 (94.1)	72 (51.4)	

n: number of patients; p-values were obtained by chi-square test.

Discussion

Gestational diabetes mellitus (GDM) is the most common metabolic disorder of pregnancy. It is defined as glucose intolerance characterized by hyperglycemia, which has not been detected before and first appears during pregnancy [7,8]. The prevalence of GDM in our country varies between 1.9% and 27.9%, and it is shown in various sources that it is 7.7% on average. Less part of these cases are pregestational diabetes, and 90% of them are gestational diabetes diagnosed during pregnancy [9,10]. It causes an increase in the risk of type 2 DM that may develop after birth, together with the risk it carries during pregnancy and delivery [11].

In this study, the relationship between these risk factors and the development of type 2 DM in the postpartum period of gestational diabetes patients with increased maternal age, BMI, gestational DM in previous pregnancies, and a family history of DM were investigated.

According to our data, there was no significant difference in the family history of DM persistence in the postpartum period in the group without DM persistence (p= 0.095). However, postpartum DM persistence was significantly higher in patients using insulin (p=0.001) and patients with high BMI (p=0.001).

In the study of Özay et al., 260 pregnant patients were divided into two groups with BMI <25 and ≥25, and the risk of developing gestational diabetes in the patient group with BMI ≥25 was significant (p=0.001). In addition, it was found that the frequency of GDM increased significantly in patients with increased gravida and multiparity (p-values 0.006 and <0.001, respectively) [12].

However, there are fewer publications on pregnancy follow-up after delivery. There is a significant decrease in insulin requirement due to the sudden decrease in placental hormones with anti-insulin effect after delivery [13]. Patients with GDM who are not insulin dependent before pregnancy and are controlled by diet alone may not need insulin after delivery. The insulin dose for type 2 diabetic women is minimal within 1-3 days postpartum. For women with type 1 diabetes, small doses to be determined by blood glucose levels may be needed [14].

In the study of Lin et al. in 302 patients with GDM, 43% of women who had postpartum diabetes have detected the mellitus [15]. In our study, postpartum diabetes mellitus development was lower at 10.8%. We think this can be achieved with close follow-up, treatment and weight control of our patients.

Since the incidence of DM development in the postpartum period is higher than those without GDM, pregnant women who were followed up with the diagnosis of GDM should be screened for DM after delivery. There are many studies supporting this finding in the literature. In studies conducted on this subject, the incidence of DM development after GDM ranges from 6.06% to 34.6% [16,17].

In another study, 235 patients with GDM were metabolically evaluated at week 6, with Type 2 DM in 17 (13.4%), impaired glucose tolerance in 37 (29.1%) patients, and normal in 73 (57.5%) patients. [18]. In our study, after OGTT was performed at the postpartum 8th week of 157 volunteer GDM patients, 23 patients (14.64%) had impaired glucose tolerance (IGT), 18 patients (11.46%) had impaired fasting glucose (IFG), and 17 patients (10%,) type 2 DM was detected. Normal glucose values were determined in 99 patients (63.5%). It was noted that the presence of type 2 DM was proportional to increasing maternal age and BMI. Regarding the presence of DM, there was a significant difference between the patients receiving insulin therapy and those receiving diet therapy (p=0.001).

In the study conducted by Turker et al., in which 40 GDM patients were included, it was observed that postpartum Type 2 DM developed in seven patients (17.5%) in the follow-up of metabolic values in the postpartum period [19]. In our study, postpartum DM was observed in 17 patients (10.8%) in proportion to high BMI and increased maternal age. The number of those who needed insulin was higher, especially during pregnancy.

While the rate of developing type 2 DM within five years postpartum in mothers who gave birth varies from 5 to 50%, it was determined that the risk of developing type 2 DM in 15-25 years is 50-70% [20]. In a study conducted by Krishnaveni et al. on this subject, the frequency of diabetes was higher in the 5th year of postpartum screening of South Indian GDM patients compared to pregnant women without GDM (37% vs. 2%) [21]. In our study, the rate of type 2 DM was 10.8% in our OGTT results at the postpartum 8th week. Our data show consistency with previous study results.

Among the shortcomings are the retrospective nature of this study and the relatively low number of patients. The limitations of our study are that the number of patients is in a certain range since it is retrospective and single-center. Despite this, we think that patient follow-ups do not adversely affect the results due to the regularity and completeness of the data.

Conclusion

Pregnancy is a process that creates opportunities for diabetes screening and the determination of future risks. The coexistence of increasing maternal age and sedentary life, in other words, the increase in BMI will increase the risk of developing type 2 DM in the postpartum period in women with gestational diabetes. Close follow-up and treatment of women with GDM during the postpartum period should not only reduce the risk of developing diabetes but also prevent or delay micro and macrovascular complications.

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ORCID and Author contribution: ***L.K. (0000-0001-8283-4516)*** Concept and Design, Data Collection, Interpretation of Results, Literature Search, Critical Review. Final Approval

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Research Article

Araştırma

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Association of Hypophosphatemia with Morbidity and Mortality in Patients with COVID-19

COVID-19'lu Hastalarda Hipofosfateminin Morbidite ve Mortalitesi ile İlişkisi

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ABSTRACT

Background and Objective: In critical cases, electrolyte disturbances such as hypophosphatemia have been shown to be associated with mortality and in our study, we aimed to examine the relationship between hypophosphatemia, a symptom disorder with COVID-19, and death.

Material and Methodology: This study is a retrospective, cross-sectional investigation that encompasses patients diagnosed with COVID-19 and subsequently admitted for treatment at our hospital. Based on their serum phosphate levels, the patients were bifurcated into two distinct categories: those with serum phosphate levels below 2.5 mg/dl, classified as hypophosphatemic, and those with levels above this benchmark, categorized as non-hypophosphatemic. The comparisons drawn between these two cohorts were facilitated using a range of statistical methodologies, and the resulting findings were subsequently analyzed and interpreted within this framework.

Results: Compared to the hypophosphatemia group, the diagnoses of DM (p<0.001) and CKD (p=0.015) were statistically significantly higher in the group without hypophosphatemia. A statistically significant difference was found between phosphorus groups and mortality and length of stay (p<0.001). In addition, age and length of hospitalization were found to be statistically significantly higher in those who died compared to those who were alive (p<0.001; p=0.002).

Conclusion: Hypophosphatemia has been shown to be associated with mortality in patients with COVID-19, as in many studies and in our study, and it may be a biomarker in predicting severe disease.

Key Words: COVID-19, Phosphorus, Diabetes Mellitus, Mortality

ÖZET

Amaç: Kritik hastalıklarda hipofosfatemi gibi elektrolit bozukluklarının mortalite ile ilişkisi gösterilmiştir. Biz de çalışmamızda COVID-19'lu hastalarda bir elektrolit bozukluğu olan hipofosfateminin mortalite ile ilişkisini incelemeyi amaçladık.

Yöntemler: Bu retrospektif kesitsel çalışma, hastanemizde COVID-19 tanısı alıp, yatarak tedavi gören hastaları içermektedir. Hastalar, serum fosfor düzeylerine göre iki gruba ayrılmıştır: serum fosfor düzeyi 2.5 mg/dl'nin altında olanlar (hipofosfatemi) ve bu seviyenin üzerinde olanlar (hipofosfatemi olmayanlar). İki grup arasındaki karşılaştırmalar, çeşitli istatistiksel yöntemler kullanılarak yapılmış ve sonuçlar bu veriler ışığında değerlendirilmiştir.

Bulgular: Hipofosfatemi grubuyla karşılaştırıldığında, hipofosfatemi olmayan grupta DM (p<0,001) ve KBY (p=0,015) tanıları istatistiksel olarak anlamlı yüksekti. Fosfor grupları ile mortalite ve yatış süresi arasında da istatistiksel olarak anlamlı farklılık saptandı (p<0.001). Ayrıca yaş ve yatış süreleri sağ olanlara kıyasla exitus olanlarda istatistiksel olarak anlamlı yüksek bulundu (p<0.001; p=0.002).

Sonuç: Hipofosfatemi yapılan birçok çalışmada ve bizim çalışmamızda da olduğu gibi COVID-19'lu hastalarda mortalite ile ilişkisi gösterilmiştir ve şiddetli hastalığı öngörmede bir biyobelirteç olabilir.

Anahtar Kelimeler: COVİD-19, Fosfor, Diyabetis Mellitus, Mortalite

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Introduction

The COVID-19 epidemic, Coronavirus disease 2019 (COVID-19), which caused a respiratory pandemic for the first time in the world, has infected more than 139 million people today and caused the death of approximately 3 million people [1,2]. In many studies, it has been shown that COVID-19 causes diseases and complications in the cardiovascular, gastrointestinal (GI) and urogenital systems as well as the respiratory system [2]. COVID-19 virus infection can cause electrolyte disorder and fluid imbalance in the body by affecting the gastrointestinal and urogenital system, and this situation can be dangerous and fatal if not controlled [2]. Electrolyte and acid-base imbalances occupy an important place in many serious diseases, as well as in COVID-19 disease, which causes severe viral pneumonia together with acute respiratory distress syndrome (ARDS) from asymptomatic infection [2-4].

In a small number of studies, it has been shown that electrolyte disorders such as hyponatremia, hypokalemia, hypochloremia, and hypocalcemia are among common electrolyte disorders that increase mortality and morbidity in patients [1,4,5]. In acute critical illnesses, patients may become prone to serum phosphorus disorders [6]. Hypophosphatemia, especially observed in critically ill patients, is a common electrolyte disorder associated with numerous side effects [7]. The prevalence of hypophosphatemia reported in critical illnesses varies between 10% and 80% in different studies [6].

Especially hypophosphatemia, which is among the sepsis findings, is also associated with high morbidity and mortality in Covid-19 disease [6-7]. In Covid-19 infection, hypophosphatemia develops as a result of hypovolemia, tissue hypoxia, septic systemic inflammation (cytokine storm), heart failure, rhabdomyolysis, and immune complex deposition [8]. Hypophosphatemia is potentially life-threatening, as phosphate is one of the main intracellular anions required in numerous biological processes [8-9].

During the course of critical illnesses, electrolyte disturbances such as hypophosphatemia frequently occur and have been shown to significantly impact mortality rates [6, 7]. Interestingly, such disturbances are often overlooked in our clinical practice or considered secondary in diagnostic and treatment processes. However, recent research has indicated that hypophosphatemia could potentially have a substantial effect on a patient's overall health status and prognosis.

The COVID-19 pandemic has broadly reshaped the focus of medical research, with many investigators trying to understand the biological and physiological changes caused by the disease [1,4,5]. In this context, our study aims to examine the possible impact of hypophosphatemia, an electrolyte disturbance that COVID-19 may induce, on mortality rates.

The fundamental rationale for this study is to understand the potential influence of electrolyte disturbances on clinical progression and mortality rates in COVID-19 patients. Through this study, we aim to gain more insight into the effect of hypophosphatemia associated with COVID-19 on mortality and to apply this knowledge in our clinical practices. Our hypothesis is that hypophosphatemia in COVID-19 patients has a significant effect on mortality rates. If this hypothesis is confirmed, it could be suggested that strategies maintaining electrolyte balance could play a crucial role in the management of COVID-19.

Materials and Methods

Study Design and Patients

This study, consisting of 673 RT–PCR positive COVID-19 hospitalized patients is a retrospective cross-sectional study conducted at Bakırköy Dr. Sadi Konuk Training and Research Hospital.

Patients were tested for SARS-CoV-2 according to the epidemiological and clinical criteria specified in the National Guideline for the Diagnosis and Treatment Protocol for SARS CoV-2 Infection circulated by the Ministry of Health of the Republic of Turkey. Nasopharyngeal and oropharyngeal specimens were collected from patients once and samples were tested for SARS-CoV-2 using real-time RT-PCR at our hospital. Informed consent was obtained from each subject before the study. Bakirkoy Dr. Sadi Konuk Training and Research Hospital Medical Research Ethics Committee approved the study. Work was done to protect patient privacy and in accordance with the Declaration of Helsinki. (Ethical approval date:30/04/2020, Approval number: 2020-09-15). Necessary permissions were obtained from the hospital administration for the study.

Data were obtained from patient files and hospital registry system. Demographic characteristics of patients (age, gender), RT-PCR result (+/-), radiological findings, laboratory findings (leukocyte, platelet, lymphocyte, hemoglobin, hematocrit, neutrophil, eosinophil counts, and urea, creatinine, albumin, lactate dehydrogenase (LDH)), C-reactive protein (CRP), ferritin, procalcitonin, fibrinogen, prothrombin time (PT), activated partial thromboplastin time (APTT), D-Dimer, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine kinase (CK) , co-morbid diseases (diabetes mellitus, hypertension, ischemic heart disease, etc.), COVID-19 treatments, need for intensive care, length of stay were recorded, and complications and survival of the patients in the hospital were also evaluated.

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Statistical Analysis

SPSS 25.0 package program was used for data analysis in the study. Descriptive data on the socio-demographic information of the participants are given in the form of frequency tables (N and %). Data belonging to continuous variables are given as mean±SD.

When the data of the study were analysed in terms of normality assumptions, Kolmogorov-Smirnov values were determined as p>0.05. Independent t test, one of the parametric tests, was used to determine whether there was a significant difference between various variables and mortality and phosphorus groups. ROC analysis results of phosphorus values predicting mortality are given. Chi Square test or Fisher's Exact test was used to compare categorical variables. Finally, the results of Multivariate Logistic Regression on Mortality Presence of Various Clinical Factors are given. p<0.05 was considered statistically significant.

Results

A total of 673 COVID-19 patients were included in this study and were divided into two groups, hypophosphatemia and non-hypophosphatemia, according to their serum phosphorus level at presentation. Values of serum phosphorus level below 2.5 mg/dl were considered as hypophosphatemia. The mean age of the patients was 61.72±12.79 years.

Of the patients, while 64.2% (n=432) were male, 35.8% (n=241) were female (Table 1). The mean hospital stay at the time of admission was 16.98±11.01 days (Table 1).

Diabetes mellitus was the most common comorbidity with 66.1%(n=445) of the patients included in the study. (Table 1)

Compared to the hypophosphatemia group, DM (p<0.001) and CKD (p=0.015) were found to be statistically higher in the group without hypophosphatemia. (Table 2)

Table 1. Distributions of Descriptive Information Pertaining to Patients

		N	%
Gender	Female	241	35,8
	Male	432	64,2
Phosphorus	Low	362	53,8
	Normal-High	311	46,2
Mortality	Ex	178	26,4
	Discharge	495	73,6
		Mean±SD	**Median (min-max)**
Age		61,72±12,79	63,00 (18,00-97,00)
Hospitilazition		16,98±11,01	11,01 (2,00-104,00)
Phosphorus		2,47±0,78	0,78 (0,40-8,50)
		Absent N (%)	**Present N (%)**
Hypertension		308 (45,8)	365 (54,2)
Diabetes Mellitus		228 (33,9)	445 (66,1)
COPD-Asthma		607 (90,2)	66 (9,8)
CKD		631 (93,8)	42 (6,2)
CVD		516 (76,7)	157 (23,3)
CHF		626 (93,0)	47 (7,0)
Cancer		648 (96,3)	25 (3,7)
Chronic Liver Disease		670 (99,6)	3 (0,4)
Other		584 (86,8)	89 (13,2)

Table 2. Comparison of Various Variables According to Phosphorus Groups

	Phosphorus		
Gender, N(%)	**Normal-High N=311**	**Low N=362**	**p**
Female	119 (49,4)	122 (50,6)	0.218[a]
Male	192 (44,4)	240 (55,6)	
Age, Mean±SD	61,40±11,66	62,00±13,70	**0.545[b]**
Hospitilazition, Mean±SD	13,30±8,93	20,14±11,64	**<0.001[b]**
HT, N(%)			
Absent	134 (43,5)	174 (56,5)	0.196[a]
Present	177 (48,5)	188 (51,5)	
DM, N(%)			
Absent	63 (27,6)	165 (72,4)	**<0.001[a]**
Present	248 (55,7)	197 (44,3)	
COPD-Astım, N(%)			
Absent	281 (46,3)	326 (53,7)	0.897[a]
Present	30 (45,5)	36 (54,5)	
CKD, N(%)			
Absent	284 (45,0)	347 (55,0)	**0.015[a]**
Present	27 (64,3)	15 (35,7)	
CVD, N(%)			
Absent	229 (44,4)	287 (55,6)	0.084[a]
Present	82 (52,2)	75 (47,8)	
CHF, N(%)			
Absent	291 (46,5)	336 (53,6)	0.700[a]
Present	20 (43,5)	26 (56,5)	
Cancer, N(%)			
Absent	300 (46,3)	349 (53,8)	0.970[a]
Present	11 (45,8)	13 (54,2)	
Chronic Liver Disease, N(%)			
Absent	311 (46,4)	359 (53,6)	0.253[b]
Present	0 (0,0)	3 (100,0)	
Other, N(%)			
Absent	280 (47,9)	304 (52,1)	**0.021[a]**
Present	31 (34,8)	58 (65,2)	
SpO2	90,91±5,56	88,89±7,43	**<0.001**
HbA1c	8,72±2,33	8,71±2,15	0.971
HGB	12,7±1,9	13,02±1,92	**0.032**
HTC	38,17±5,09	38,73±5,21	0.160
WBC	8142,84±4406,23	8277,29±4577,1	0.699
Lymphocyte	1281,4±762,31	1106,75±902,95	**0.007**
Neutrophil	6343,06±4048,14	6634,59±4179,4	0.360
Eosinophil	48,44±106,21	27,42±82,48	**0.004**
PLT	232,05±97,41	204,32±95,59	**<0.001**
Glucose	224,01±114,24	198,33±97,58	**0.003**

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Table 2. Comparison of Various Variables According to Phosphorus Groups *(continued)*

AST	43,34±34,04	51,82±35,77	**0.002**
ALT	38,62±39,39	38,72±34,63	0.971
Urea	48,72±31,14	47,64±26,33	0.626
Creatinine	1,59±4,83	1,08±0,84	0.067
LDH	379,85±180,83	445,25±228	**<0.001**
Albumin	7,63±19,19	14,6±15,34	**<0.001**
Ferritin	381,34±455,57	323,34±654,02	0.204
Triglyceride	176,73±144,83	284,29±508,99	**<0.001**
CK	334,3±593,74	612,62±1045,74	**<0.001**
Procalcitonin	26,26±69	74,39±145,75	**<0.001**
CRP	84,6±77,45	56,42±84,63	**<0.001**
Fibrinogen	522,83±122,08	538,79±147,21	0.130
PTZ	14,54±5,82	15,19±7,42	0.226
APTT	38,03±26,47	36,24±9,58	0.233
D-Dimer	0,82±1,42	0,88±1,33	0.543

a=Chi Square test, b=Independent t test, p<0.05 is statistically significant

A statistically significant difference was found between phosphorus groups and mortality (p<0.001). While the mortality of the patients in the group without hypophosphatemia was 14.5%, it was found as 36.7% in the group with hypophosphatemia.

A statistically significant difference was found between the phosphorus level and mortality among the patients who were discharged (p<0.001). The mean phosphorus level was higher in the discharged group compared to the group with mortality (Table 3).

To differentiate the presence of mortality, the estimation of the Phosphorus parameter was statistically significant (p<0.001). The AUC in the ROC analysis designed to differentiate the phosphorus values from mortality was 0.703 (95%[CI], 0.654-0.751) (Figure 1). Phosphorus values at a cut-off value of ≤2.31 have a sensitivity of 66.3% and a specificity of 66.1% in the diagnosis of one-month mortality.

A statistically significant difference was found between the length of stay and the phosphorus groups (p<0.001). The length of stay was found to be longer in the hypophosphatemia group than in the other group. (Table 2)

In the hypophosphatemia group, compared to the non-hypophosphatemia group; lymphocyte (p=0.007), eosinophil (p=0.004), platelet (p<0.001) counts were found to be significantly lower, while AST (p=0.002), LDH (p<0.001), triglyceride (p<0.001), CK (p<0.001) levels were found to be significantly higher. Glucose (p=0.003) and CRP (p<0.001) measurements; It was found to be lower in the group with hypophosphatemia. (Table 2)

No statistically significant difference was found between mortality and gender (p=0.219). Patient age (p<0.001) and length of stay (p=0.002) showed a statistically significant difference between mortality and discharged groups. Age and length of hospital stay were higher in the group with mortality compared to the discharged group (Table 3).

At first admission, lymphocyte (p=0.004), neutrophil (p=0.013), eosinophil (p=0.001), PLT (p=0.003), AST (p=0.031), urea (p<0.001), creatinine (p=0.003) 0.036), LDH (p<0.001), albumin (p<0.001), CK (p<0.001), procalcitonin (p<0.001), CRP (p<0.001), fibrinogen (p=0.003) and D-Dimer (p =0.042) levels showed a statistically significant difference between mortality groups. Neutrophil, AST, urea, creatinine, LDH, CK, procalcitonin, CRP, fibrinogen and D-Dimer measurements were higher in the group with mortality compared to those who were discharged. Lymphocyte, eosinophil and PLT measurements were found to be higher in the discharged group compared to the mortality group. (Table 3)

As a result of univariate analysis; a statistically significant difference was found between the groups in terms of age, length of stay, CRF, lymphocyte, neutrophil, eosinophil, PLT, AST, urea, creatinine, LDH, CK, procalcitonin, CRP, fibrinogen, D-Dimer values according to mortality status (p< 0.05). These variables, which were found to be significant as a result of univariate analysis, were included in the Multivariate logistic regression model. According to the results of the multivariate logistic regression model, the increase in age (OR: 1.05% 95% CI: 1.03-1.08), the increase in urea values (OR: 1.01% 95% CI: 1.01-1.01), the increase in LDH values (OR: 1.00 95% CI: 1.00-1.01) 1.01), an increase

Table 3. Comparison of Various Variables According to Mortality Groups

	Mortality		
Gender, N(%)	**Discharged N=495**	**Ex N=178**	**p**
Female	184 (76,3)	57 (23,7)	0.219[a]
Male	311 (72,0)	121 (28,0)	
Age, Mean±SD	60,45±13,20	65,25±10,87	**<0.001[b]**
Hospitilazition, Mean±SD	16,21±11,21	19,12±10,16	**0.002[b]**
Phosphorus			
Normal-High	266 (85,5)	45 (14,5)	**<0.001[a]**
Low	229 (63,3)	133 (36,7)	
Phosphorus, Mean±SD	2,58±0,65	2,16±1,01	**<0.001[b]**
HGB	12,90±1,85	12,80±2,08	0.583
WBC	8053,54±4470,26	8664,61±4549,75	0.120
Lymphocyte	1244,3±857,07	1029,37±790,67	**0.004**
Neutrophil	6262,98±4022,93	7158,65±4317,97	**0.013**
Eosinophil	42,94±102,35	21,00±66,77	**0.001**
PLT	223,76±95,65	198,69±99,91	**0.003**
AST	46,14±33,76	52,77±38,64	**0.031**
ALT	39,52±38,71	36,32±31,2	0.321
Urea	44,2±24,11	59,08±36,43	**<0.001**
Creatinine	1,05±0,83	2,05±6,31	**0.036**
LDH	387,12±176,44	492,38±268,56	**<0.001**
Albumin	8,89±17,42	18,28±16,04	**<0.001**
Ferritin	365,04±588,67	310,33±501,02	0.297
CK	375,55±704,06	787,2±1190,52	**<0.001**
Procalcitonin	32,05±71,06	107,57±187,81	**<0.001**
CRP	77,82±78,79	46,44±88,32	**<0.001**
Fibrinogen	520,36±123,97	561,23±161,72	**0.003**
D-Dimer	0,78±1,34	1,04±1,43	**0.042**

a=Chi Square test, b=Independent t test, p<0.05 is statistically significant

in procalcitonin values (OR:1.01, 95% CI:1.01-1.02) is risky for the presence of mortality, an increase in phosphorus values (OR: 0.49, 95% CI: 0.35-0.70) for the presence of mortality. It has been determined that it reduces the risk.

Discussion

Although Hypophosphatemia is defined as a serum phosphorus level below 2.5 mg/dL in adults, distinct symptoms of hypophosphatemia rarely occur unless the serum phosphorus level is less than 2 mg/dL in different studies. In our study, we accepted values below 2.5 mg/dl as hypophosphatemia [10].

In present study; male patients constituted 64.2% of the total patients. This situation was considered to be because of the higher immunological response in male patients and the protective effect of female sex hormones. In other studies in the literature, it has been associated with the severe course of Covid 19 disease and the higher hospitalization rates in male patients than female patients. [11-12].

Malinowska et al. stated in their study that hyperphosphatemia may contribute to the development of severe COVID-19. However, in many other studies; it has been shown that hypophosphatemia is associated with increased mortality in patients with Covid 19, especially in critical diseases such as sepsis. In our study, similar to previous studies, mortality was significantly higher in patients with hypophosphatemia than in patients with low serum phosphorus levels [1,14,15].

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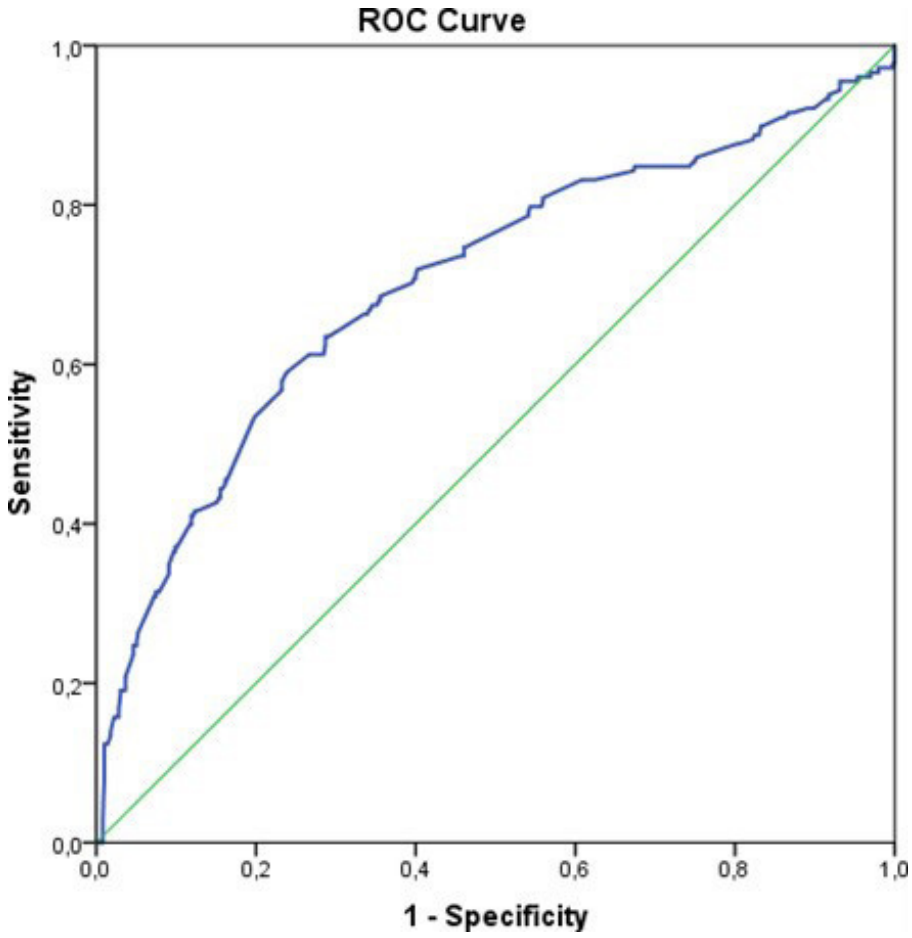

Figure 1. ROC Curve Related to Phosphate Levels According to Mortality A statistically significant correlation has been discerned between mortality rates and a threshold phosphate level of 2.26 (p=0.001; p<0.01). It could be deduced that instances with phosphate levels at or exceeding 2.26 demonstrate a mortality risk that is elevated by a factor of 4.363. For phosphate, the calculated odds ratio stands at 4.363 (95% Confidence Interval: 3.028-6.286).

It has been shown that some biomarkers may be associated with severe disease in patients with Covid 19 who have severe systemic disease [16-17]. In our study, similar to previous studies, neutrophil count, Ast, urea, Ldh, Ck, Crp, fibrinogen and D-Dimer measurements were found to be higher in the mortality group compared to the patients who were discharged. Lymphocyte, eosinophil and platelet count measurements were higher in the discharged group compared to the mortality group. The presence of biomarkers such as clinical and inflammatory markers in predicting severe disease is undoubtedly of great importance for the clinical management of the disease. In addition to these biomarkers, blood phosphorus level can be added, but more comprehensive studies are needed for this.

Three basic mechanisms are held responsible for the formation of hypophosphatemia. First, inadequate intake caused by malnutrition, malabsorption, etc. reasons; the second one is an excess of excretion in the form of loss from the gastrointestinal tract and kidneys [18-19]. The third is transition from the extracellular space to the intracellular space. This situation mostly occurs during the treatment of diabetic ketoacidosis, refeeding after prolonged fasting, and acute respiratory alkalosis [20-22]. In Covid 19 patients, especially in the severe course of the disease, hypophosphatemia may be observed due to the transition from extracellular to intracellular due to inadequate intake, malabsorption, gastrointestinal damage, renal damage and respiratory alkalosis [13].

In our study, diabetes mellitus was found to be the highest comorbid condition with 66%. The reason for this is -similar to the literature- the probability of severe disease and hospital admission in diabetic patients was higher than in non-diabetic patients [23]. However; it should not be ignored that in patients with diabetes mellitus, insulin therapy, which is administered differently from other patients, increases the transfer of extracellular phosphate into the cell, in other words, hypophosphatemia can be observed with transcellular shift [20].

As a result, hypophosphatemia is an electrolyte disorder that has been shown to be associated with mortality in

patients with Covid 19, as in many studies including ours, and it can be a biomarker in predicting severe disease. However, it is not yet known whether correction of hypophosphatemia reduces mortality. More comprehensive observational studies are needed to learn all of these. Our study has some limitations as it is a retrospective observational study and was conducted in a single center.

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Acta Medica Alanya

Research Article

Araştırma

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Apoptosis Induction Through Increased TRPV1 Activation by Synergic Effect of Melatonin and Doxorubicin in Human Osteosarcoma and Chondrosarcoma Cell Lines

İnsan Osteosarkoma ve Kondrosarkoma Hücre Hatlarında Melatonin ve Doksorubisinin Sinerjik Etkisi Yoluyla Artan TRPV1 Etkinliği Üzerinden Apopitoz Uyarımı

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ABSTRACT

Aim: We aimed to reveal the role of doxorubicin (Dox), melatonin (Mel) and transient receptor potential Vanilloid 1 (TRPV1) channels in bone and cartilage cancer cells during the treatment process. Human Bone Osteosarcoma (Saos-2/An1) and Human Chondrosarcoma (Hs 819.T) cell lines were used to prepare in-vitro experiment models.

Methods: Both cell lines were cultured at 37°C. We have separated each cell line into five groups as follows: Controls, Dox, Dox+Capsazepine (Cpz), Dox+Melatonin (Mel), and combined Dox+Mel+Cpz given group. Capsaicin and capsazepine were added to cell culture mediums to activate or inactivate the TRPV1 channels, respectively. Cytosolic calcium, apoptosis, intracellular reactive oxygen, mitochondrial depolarization, caspase-3 and caspase-9 levels were measured.

Results: Increased apoptotic activity was detected in doxorubicin given cell lines (Group II) when compared with the controls ($p<0.001$). There was also a significantly higher apoptotic level in Dox+Mel group (Group IV), when compared with only Dox given group ($p<0.001$). TRPV1 inhibition applied groups (Group III and V) have had lower apoptotic levels than other drug administered groups ($p<0.001$).

Conclusion: This study has indicated that apoptotic effects of Dox and Mel on both osteosarcoma and chondrosarcoma were strictly associated to TRPV1 channels, and that TRPV1 channels played an important role in whole mitochondria dependent pathways of apoptosis, which in turn may lead to increased intracellular Ca+2 levels and mitochondrial depolarization.

Key Words: Osteosarcoma, Chondrosarcoma, TRPV1, Doxorubicin, Melatonin

ÖZET

Amaç: Kemik ve kıkırdak kanseri hücrelerinde doksorubisin (Dox) ve melatonin (Mel) ile birlikte geçici reseptör potansiyeli olan Vanilloid 1 (TRPV1) kanallarının tedavi sürecindeki rolünü ortaya çıkarmayı amaçladık. İn-vitro deney modellerinin hazırlanmasında İnsan Kemik Osteosarkomu (Saos-2/An1) ve İnsan Kondrosarkomu (Hs 819.T) hücre hatları kullanıldı.

Gereç ve Yöntem: Her iki hücre hattı da 37°C'de kültürlendi. Her hücre hattını aşağıdaki gibi beş gruba ayırdık: Kontroller, Dox, Dox+Kapsazepin (Cpz), Dox+Mel ve kombine Dox+Mel+Cpz verilen grup. Kapsaisin ve kapsazepin, sırasıyla TRPV1 kanallarını etkinleştirmek veya inaktive etmek için hücre kültürü ortamlarına eklendi. Sitozolik kalsiyum, apoptoz, hücre içi reaktif oksijen, mitokondriyal depolarizasyon, kaspaz 3 ve kaspaz 9 seviyeleri ölçüldü.

Bulgular: Doksorubisin (Dox) verilen hücre hatlarında (Grup II) kontrollere göre artmış apoptotik aktivite saptandı ($p<0.001$). Ayrıca Dox+Mel grubunda (Grup IV), sadece Dox verilen grupla ($p<0.001$) karşılaştırıldığında anlamlı derecede daha yüksek bir apoptotik seviye vardı. TRPV1 inhibisyonu uygulanan gruplar (Grup III ve V) diğer ilaç uygulanan gruplara göre daha düşük apoptotik düzeylere sahiptir ($p<0.001$).

Sonuç: Bu çalışma, Dox ve Mel'in hem osteosarkom hem de kondrosarkom üzerindeki apoptotik etkilerinin TRPV1 kanalları ile kesin olarak ilişkili olduğunu ve TRPV1 kanallarının apoptozun tüm mitokondriye bağımlı yolaklarında önemli bir rol oynadığını ve bunun da hücre içi Ca+2 düzeylerinde artışa ve mitokondriyal depolarizasyona yol açabileceğini göstermiştir.

Anahtar Kelimeler: Osteosarkom, Kondrosarkom, TRPV1, Doksorubisin, Melatonin

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Introduction

Osteosarcoma and chondrosarcoma cases are the common malignant tumors of the musculoskeletal system [1-3]. Osteosarcoma originates from the bone cells and is generally detected in long bones. For this reason, bones of upper and lower extremities are frequently involved. Other bone or soft tissue involvements have also been reported [2,3]. Whereas, chondrosarcoma is a less commonly encountered condition rather than osteosarcoma in bones and surrounding soft tissues. Chondrosarcoma is more commonly seen in pelvic, femoral, acetabular or acromioclavicular regions in contrast to osteosarcoma. Besides, spinal involvement has also been reported [3]. Radiotherapy, surgery and chemotherapy are used in medical management of these tumors, although the chemotherapeutic approach is still controversial in chondrosarcoma cases [1].

Osteosarcoma is more sensitive to chemotherapy and radiotherapy, when compared to chondrosarcoma [2]. Data established from studies conducted in recent years suggest that favorable outcomes have been established from chemotherapy applications in various types of cancer, especially osteosarcoma and Ewing cancer [3]. However, chemotherapy resistance seen in chondrosarcoma cells restricts treatment strategies in this area. Doxorubicin is a very effective first-line treatment agent used in high-grade osteosarcoma cases. Although cisplatin is widely administered in combined treatments in osteosarcoma, doxorubicin has recently been preferred as a chemotherapy agent in chondrosarcoma cases which are resistant to cisplatin [4]. Melatonin is an antioxidant hormone which is released mainly from epiphysis gland as well as testes, retina, ovary, skin, and intestines. Melatonin plays a crucial role in regulation of various biological pathways such as reproduction and circadian rhythms in the human body. Protective effect of melatonin has been reported in healthy cells, and its pro-apoptotic effect is a well-known fact in cancer cells [5]. Transient receptor potential (TRP) channels are tetramers assembled from sub units with six membrane-spanning domains, are permeable to monovalent cations and calcium ions, and are comprised of mammalian large six subfamily of ion channel proteins including TRPA (ankyrin), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin) and TRPP (polycystin) and TRPV (vanilloid) [6]. There are various factors affecting TRP channels such as body temperature changes, blood pH level, injury healing, cations, cytokines and mechanical stress [7]. Moreover, reactive oxygen species (ROS), reactive nitrogen species and other electrophilic compounds are also important mediators for TRP channels [8]. It has been reported that TRPV1 channels play an active role in pathogenesis and treatment of diseases just like other TRP channels. Stimulation of TRPV1 channels induces apoptosis in cancer cell populations and prevents their development in various tissues such as osteosarcomas, gliomas, colon and pancreas [9]. However, we have not come across any research article about the relationship between chondrosarcoma and TRPV1 channels in the literature. In this study, we aimed to investigate the possible effect of combined doxorubicin and melatonin use in osteosarcoma and chondrosarcoma cell cultures, and the role of TRPV1 channels in mitochondria-dependent intracellular apoptotic pathway mechanism.

Materials And Methods

Cell Preparation and Culture

Human Chondrosarcoma (Hs 819.T) cell line was purchased from ATCC. Human Bone Osteosarcoma and (Saos-2/An1) cell lines were obtained from Ankara ŞAP (Culture Collection of Animal Cells, Foot and Mouth Disease) Institute, Ankara, Turkey. The protocols were designed according to the standard process for cell cultures. Cells were cultured with 1% penicillin / streptomycin and 10% fetal bovine serum containing Dulbecco's modified Eagle's medium (DMEM). The distribution of cells was as follows: 1×10^6 cells in each of 6-8 flasks (sterile filter cap, 5 ml, 25 cm^2). Saos-2 and Hs 819.T cells were incubated at 37°C at 5% CO_2 in a humidified incubator. Cell lines were then incubated with chemical compounds when 75–85% confluence level was reached. Flasks that did not grow during standard time were excluded from the study. Additionally, triple repetitions were made during the analysis, and in-group agreement was observed in established data.

Reagents and Stains

DMEM, Trypsin–EDTA, Fetal Bovine Serum (FBS) and penicillin-streptomycin (Pen-Strep) and Dihydrorhodamine-123 (DHR 123), Dimethyl sulfoxide (DMSo) were obtained from Sigma Aldrich (St. Louis, MO), and dye was purchased from a commercially available company (Fura-2 (AM) calcium fluorescent, Calbiochem, Darmstadt, Germany). Likewise, commercially available kits were utilized for mitochondrial stain (5,50, 6,60-tetrachloro-1,10,3,30-tetraethyl benzimi dazolyl carbocyanine iodide (JC-1), probenecid (Santa Cruz, Dallas, Texas, USA), Pluronic® F-127 (Biovision, San Francisco, USA)). Caspase substrates (Caspase 3 (AC-DE-VD-AMC) and Caspase 9 (AC-LEHD-AMC)) were purchased from Enzo (Lausen, Switzerland). Biocolor APOPercentage assay (Belfast, Northern Ireland) was used for obtaining the releasing buffer.

Study Groups

A total of five groups were adjusted for study design as follows:

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Group I (Control): A control group was designed with empty cell culture for observing normal development in routine conditions.

Group II (Dox): In Dox groups, 2 µM doxorubicin was applied to Saos-2 and Hs 819.T cells for 24 hrs [10].

Group III (Dox+Cpz): In Dox+Cpz groups, 2 µM doxorubicin was applied to Saos-2 and Hs 819.T cells for 24 hrs; and then TRPV1 channel antagonist Capsazepine (Cpz, 0.1 mM, 30 min) was applied.

Group IV (Dox+Mel): In Dox+Mel groups, 2 µM doxorubicin and 0.1 mM Melatonin was applied to Saos-2 and Hs 819.T cells for 24 hrs [10,11].

Group V (Dox+Mel+Cpz): In Dox+Mel+Cpz groups, 2 µM doxorubicin and 0.1 mM Melatonin was applied to Saos-2 and Hs 819.T cells for 24 hrs; and then TRPV1 channel antagonist Capsazepine (Cpz, 0.1 mM, 30 min) was utilized.

During calcium signaling analysis (Fura-2/AM), Saos-2 and Hs 819.T cells were stimulated on 20th cycles with 0.1 mM Cap (TRPV1 channel agonist) in the existence of 1.2 mM Calcium and calcium free buffer in extracellular environment. Cells were treated with Cap (0.1 mM, 10 min) for activation of TRPV1 channel prior to intracellular ROS, mitochondrial depolarization, caspase-3 and caspase-9 measurements and analyses.

Measurement of Intracellular Free Calcium Concentration

Free intracellular calcium ion level was determined using Fura-2 fluorescent stain. Saos-2 and Hs 819.T cells were treated with doxorubicin and melatonin as indicated in the group descriptions (no chemicals were applied to the control group) and were treated separately for two times in calcium and non-calcium solutions. Cellular response to agonists (Cap, 0.1 mM) which added with the automated injector during the analysis was detected by evaluating fluorescence emission intensity at 510 nm in individual wells using a plate reader equipped with an automated injection system (Synergy TM H1, Biotek, USA) at alternating excitation wavelengths of 340 and 380 nm every 3 s for 50 acquisition cycles (cycle:3 s; gain:120). $[Ca^{+2}]i$ in cells was expressed as the average emission at 510 nm in individual wells in response to excitation at 340 nm (Ca^{+2} bound) / 380 nm (Ca^{+2} Free Fura 2 AM) normalized to initial fluorescence emission obtained during the first twenty cycles. The $[Ca^{+2}]i$ evaluation and other process such as staining was applied according to the methods described by Martinez et al. [12,13].

Determination of Apoptosis Level

Apoptosis (programmed cell death) is a highly regulated mechanism involved in cell death, and it is essential for development and cellular homeostasis. It is clear that excessive increase in Ca^{2+} concentration by disrupting the intracellular Ca^{2+} balance may affect apoptosis. Apoptosis analysis was performed using the APOPercentageTM commercial assay kit. Apoptosis levels were carried out regarding the producer instruction [13,14]. APOPercentage dye binding to phosphatidylserine lipids has reflected red color in apoptotic cells. Detection of red stained apoptotic cells was achieved using spectrophotometry (multiplate reader) at 550 nm (SynergyTM H1, Biotek, USA).

Measurement of Reactive Oxygen Species (ROS) and Mitochondrial Depolarization Levels

Oxidative stress caused by reactive oxygen species (ROS) can induce activation of some TRP ion channels, induce rapid depolarization of inner mitochondrial membrane potential, and subsequent impairment of oxidative phosphorylation. Damaged mitochondria produce more ROS and the intracellular mitochondria-dependent apoptosis pathway becomes more effective. Saos-2 and Hs 819.T cell lines (10^6 cells/ml per group) were incubated with DHR123 fluorescent oxidant dye [13]. An automatic microplate reader (Synergy™ H1, Biotek, USA) was used for determination of the Rh123 fluorescence intensities. Emission wavelengths of the analysis was 543 nm and excitation wavelengths of the analysis was 488 nm. The data as fold change over the level before treatment was presented. Quantification of the mitochondrial membrane depolarization was performed by measuring the fluorescence intensity of the cationic dye of JC-1 a. Wavelength of 485 nm (green) for excitation, wavelength of 535 nm for emission, and the red signal emitted at 540 and 590 nm wavelengths were measured using SynergyTM H1, (Biotek, USA) [13,14]. Data were shown as fold change over the level before treatment as emission ratios (590/535).

Measurement of Caspase-3 and Caspase-9 Enzyme Activities

Caspase-3 and caspase-9 enzyme activity levels have increased following the increment in mitochondrial depolarization level. Caspase-9 mediates the intracellular apoptotic process, while caspase 3 is the ultimate apoptotic inducer. Caspase-3 and caspase-9 activities were evaluated by using reported literature [13]. Caspase-3 (AC DEVD-AMC) and caspase-9 (AC-LEHD-AMC) substrates cleavages were performed with automatic Synergy™ H1 microplate reader (Biotek, USA) with 360 nm and 460 nm wavelengths. The values were shown as fold change over the level before treatment (experimental / control).

Statistical Analysis

Whole statistical analyses were performed using GraphPad Prism v-7.04 for Windows (San Diego California, USA). Significant variances were evaluated using one-way ANOVA and parametric values were shown as means ± standard deviation (SD). P values less than 0.05 were taken as statistically significant.

Results

There was a significant difference between Dox and Dox+Mel groups in Human Bone Osteosarcoma (Saos-2/An1) and Human Chondrosarcoma cell cultures (Hs 819.T). Dox and Dox+Mel treatments increased the intracellular calcium levels via stimulation of TRPV1 channels in osteosarcoma and chondrosarcoma cells ($p<0.001$). In Dox+Mel treated osteosarcoma and chondrosarcoma cell lines, intracellular calcium levels of TRPV1 significantly increased when compared with Dox applied groups ($p<0.001$). Ca^{2+} levels prominently decreased after administration of TRPV1 channel blocker capsazepine (Cpz) in Dox+Cpz and Dox+Mel+Cpz groups, when compared with doxorubicin (Saos-2: $p<0.001$, Hs 819.T: $p<0.05$) and Dox+Mel groups (Saos-2 and Hs 819.T: $p<0.001$) (Figure 1). In evaluation of the programmed cell death and intracellular ROS levels, we have observed a remarkable increment in apoptosis ($p<0.001$). Also, Dox+Mel treatment significantly increased the intracellular ROS and apoptosis levels by activation of TRPV1 channels compared to the doxorubicin groups ($p<0.001$). However, programmed cell death (apoptosis) and intracellular ROS levels prominently decreased in Saos-2 and Hs 819.T with use of the TRPV1 channel blocker Cpz in Dox+Cpz and Dox+Mel+Cpz groups, when compared with only Dox ($p<0.001$) and Dox+Mel groups ($p<0.001$) for both analyses (Figures 2 and 3). In Dox and Dox+Mel administered groups, mitochondrial membrane potential, caspase-3 and caspase-9 levels have significantly increased via activation of TRPV1 channels, when compared to the control group ($p<0.001$). We have also found that Dox+Mel treatment increased the mitochondrial depolarization, caspase-3 and caspase-9 levels when compared to only Dox given group ($p<0.001$). However in Saos-2 and Hs 819.T cells, pro-

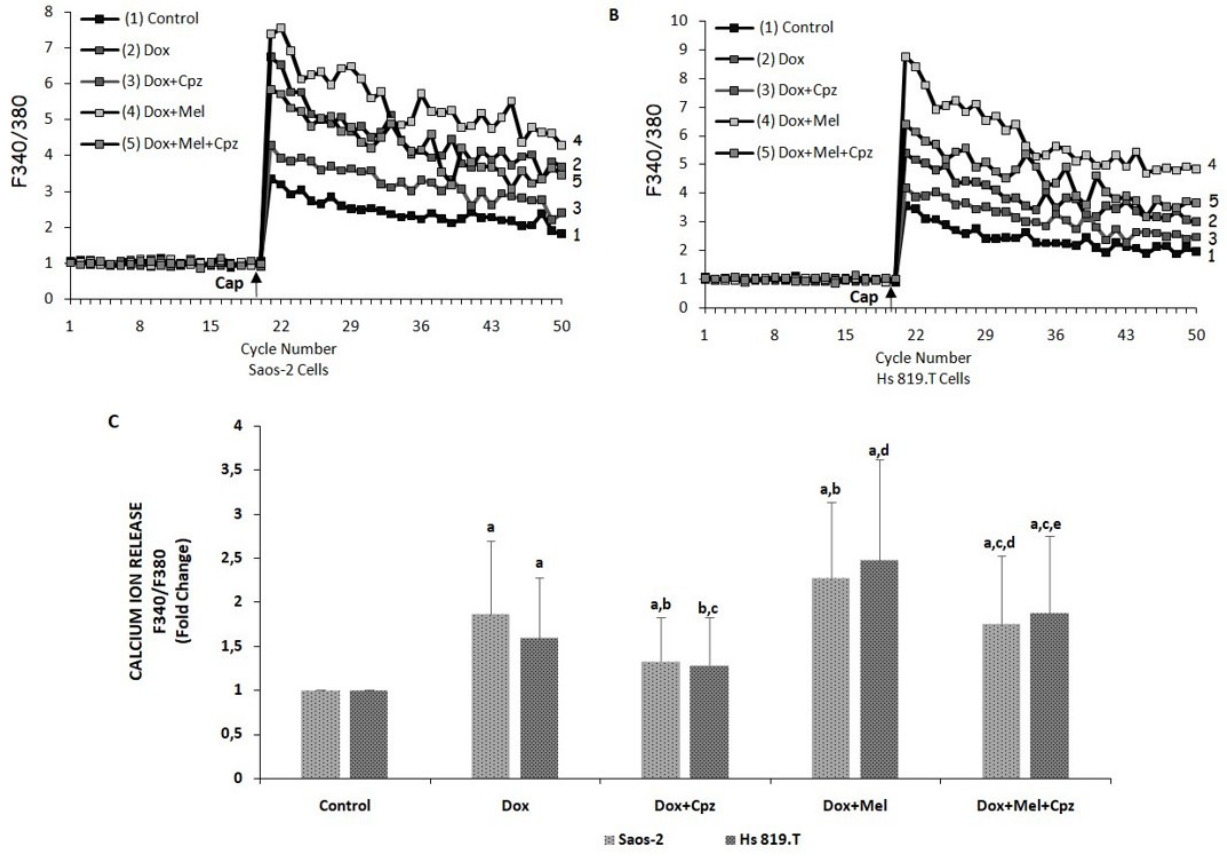

Figure 1. In vitro effect of doxorubicin (2 µM, 24 hrs) and Mel (0,1 mM, 24 hrs) on the free intracellular calcium increase ([Ca2+]i) through TRPV1 channels in Saos-2 (A) and Hs 819.T (B) cells and cellular calcium ion release (C). (n=3 and mean ± SD). The cells are stimulated by capsaicin (Cap and 0.1 mM on 20th cycle) but they were inhibited by capsazepine (Cpz and 0.1 mM for 30 min). Saos-2: ap<0.001 vs control, bp<0.001 and cp<0.05 vs Dox, dp<0.001 vs Dox+Mel. Hs 819.T: ap<0.001 and bp<0.05 vs control, cp<0.05 and dp<0.001 vs Dox, ep<0.001 vs Dox+Mel.

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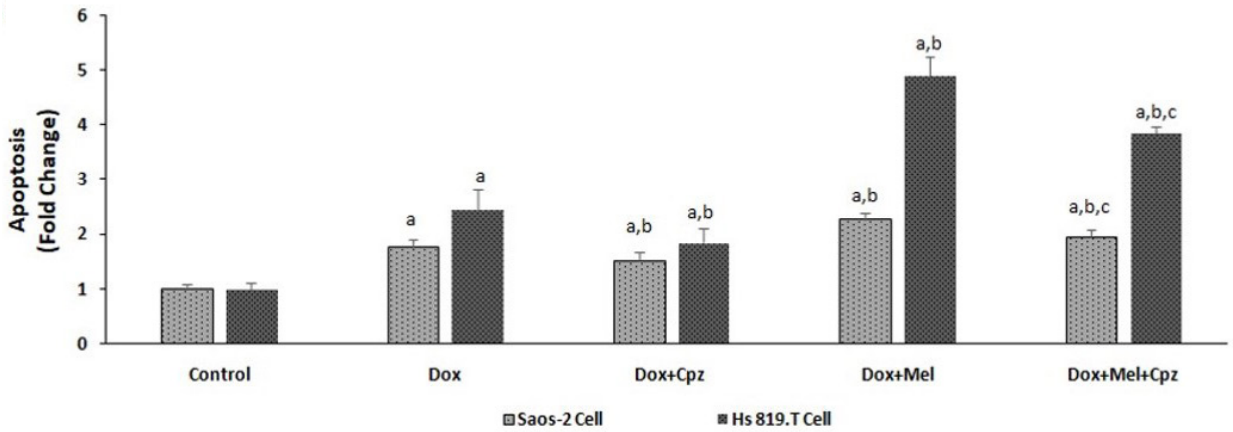

Figure 2. The effect of doxorubicin (2 µM, 24 hrs) and Mel (0,1 mM, 24 hrs) on apoptosis levels in the Saos-2 and Hs 819.T cells (mean ± SD and n=10). ap<0.001 vs control, bp<0.001 vs Dox, Cp<0.001 vs Dox+Mel.

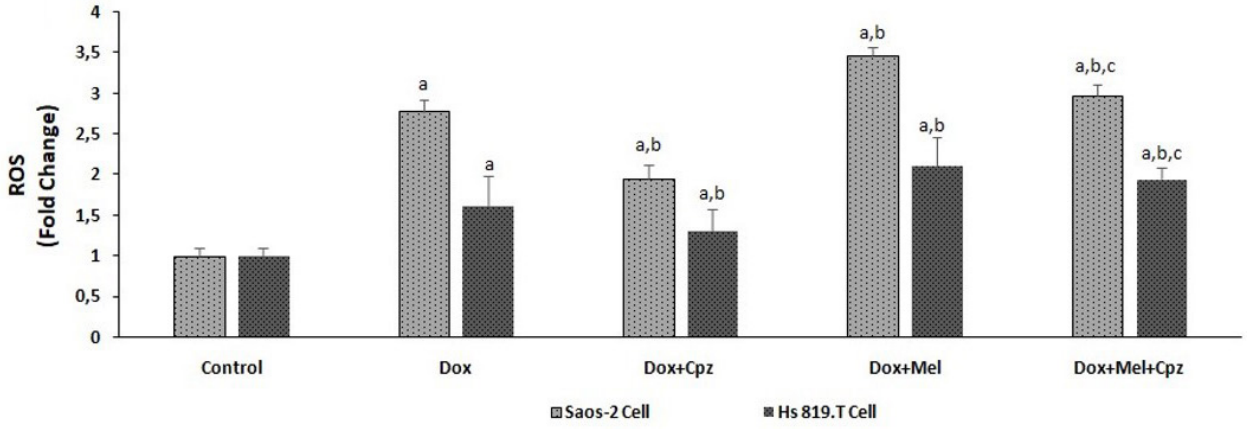

Figure 3. The effect of doxorubicin (2 µM, 24 hrs) and Mel (0,1 mM, 24 hrs) on Reactive Oxygene Species levels in Saos-2 and Hs 819.T cells (mean ± SD and n=10). ap<0.001 vs control, bp<0.001 vs Dox, Cp<0.001 vs Dox+Mel.

grammed depolarization, caspase-3 and caspase-9 levels prominently decreased with use of TRPV1 channel blocker capsazepine (Cpz) in Dox+Cpz and Dox+Mel+Cpz groups, when compared with only Dox ($p<0.05$ and $p<0.001$) and Dox+Mel given groups ($p<0.05$ and $p<0.001$, respectively) (Figures 4, 5 and 6).

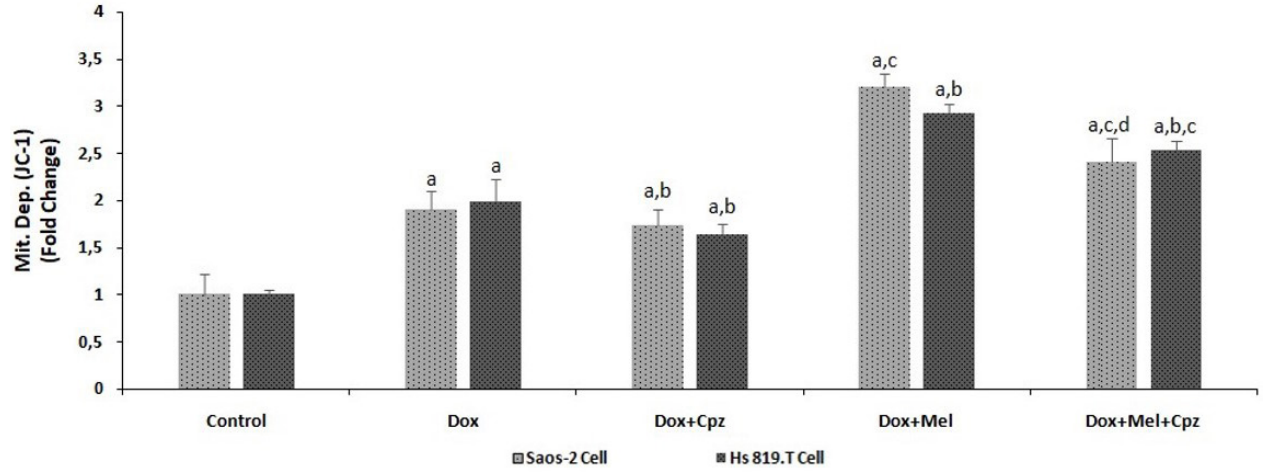

Figure 4. The effect of doxorubicin (2 µM, 24 hrs) and Mel (0,1 mM, 24 hrs) on Mitochondrial Depolarization levels in Saos-2 and Hs 819.T cells. (mean ± SD and n=10). Saos-2: ap<0.001 vs control, bp<0.05 and cp<0.001 vs Dox, dp<0.001 vs Dox+Mel. Hs 819.T: ap<0.001 vs control, bp<0.001 vs Dox, Cp<0.001 vs Dox+Mel.

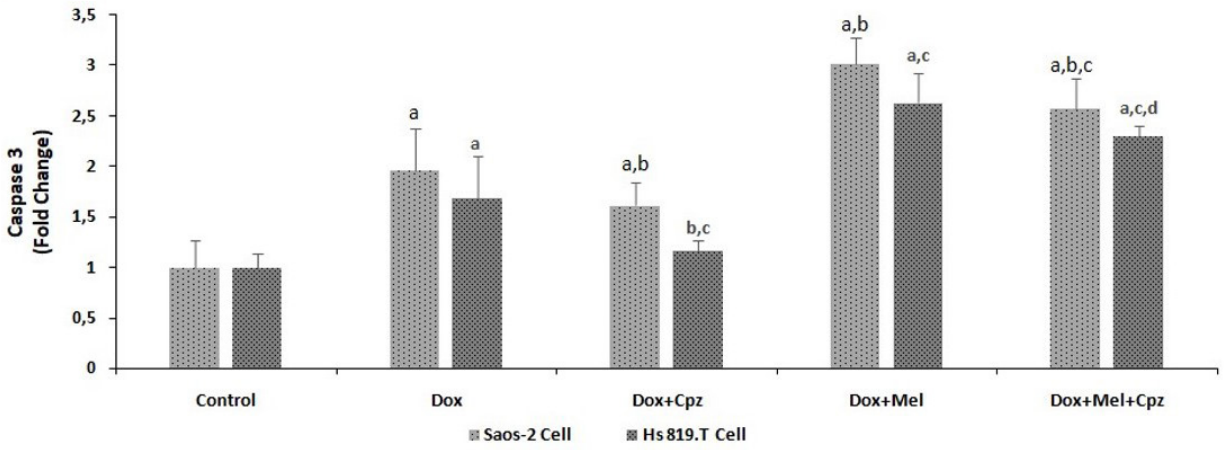

Figure 5. The effect of doxorubicin (2 μM, 24 hrs) and Mel (0,1 mM, 24 hrs) on Caspase 3 levels in Saos-2 and Hs 819.T cells. (mean ± SD and n=10). Saos-2: ap<0.001 vs control, bp<0.001 vs Dox, Cp<0.001 vs Dox+Mel. Hs 819.T: ap<0.001 and bp<0.05 vs control, cp<0.001 vs Dox, dp<0.001 vs Dox+Mel.

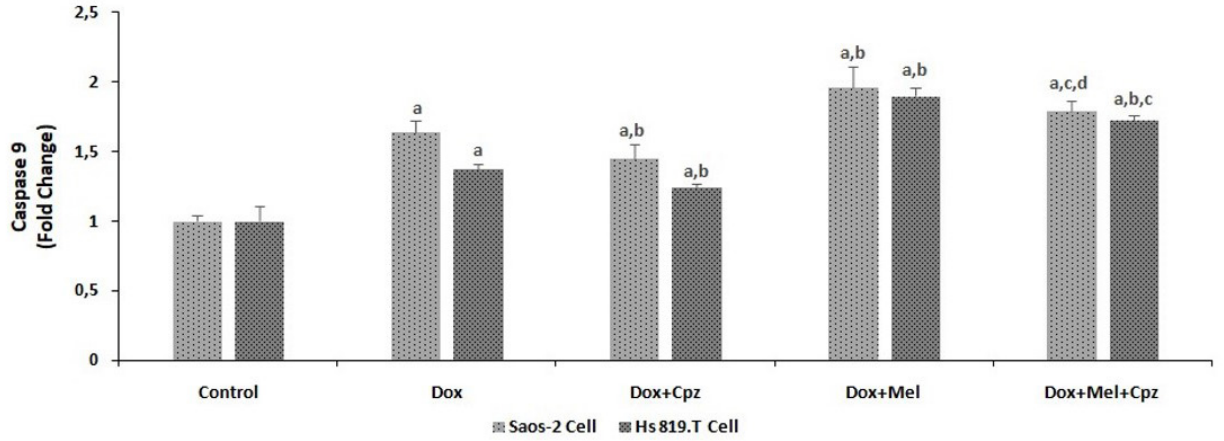

Figure 6. The effect of doxorubicin (2 μM, 24 hrs) and Mel (0,1 mM, 24 hrs) on Caspase 3 levels in Saos-2 and Hs 819.T cells. (mean ± SD and n=10). Saos-2: ap<0.001 vs control, bp<0.001 and cp<0.05 vs Dox, dp<0.001 vs Dox+Mel. Hs 819.T: ap<0.001 vs control, bp<0.001 vs Dox, Cp<0.001 vs Dox+Mel.

Discussion

Osteosarcoma and chondrosarcoma are rarely seen cancer types worldwide, and are among the leading causes of mortality. There are numerous chemotherapy protocols widely used during the preoperative and postoperative periods in osteosarcoma treatment [14]. Chemotherapy agents such as cisplatin and doxorubicin are frequently preferred in both types of cancer. Although no resistance to cisplatin is observed in osteosarcoma, it has been shown in the literature that chondrosarcoma is resistant to cisplatin [15]. TRP channels have six different subtypes in mammals; TRPA, TRPC, TRPM, TRPML, TRPP and TRPV. The selectivity of TRP channels has a wide spectrum, ranging from nonselective cation channels to highly selective Ca^{2+} channels [16]. Increased intracellular oxidative stress level due to the blocking or hyperactivity of intracellular mechanisms of chemotherapeutics used in cancer treatment may activate the channels which are sensitive to oxidative stress. These channels can play an important role in different processes such as intracellular Ca^{2+} metabolism, phagocytosis, cell motility, homeostasis and inflammation [17]. While there are many articles in the literature about the expression and roles of TRP channels in osteosarcoma cells, there is no article on the presence or function of these channels in chondrosarcoma so far. TRPV1 channels have been reported to have effect on invasion, proliferation, differentiation, and vascularization of cancer cells [18]. It has been indicated that TRP channels altered intracellular calcium concentrations and they had influence on regulation of Ca^{2+} release in numerous cell organelles. Intracellular Ca^{2+} concentration is variable and has been reported to increase in cases such as increased proliferation, apoptosis and abnormal differentiation, which are

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important markers of cancer invasion. doxorubicin is an agent which is used therapeutically in many types of cancer including osteosarcoma and chondrosarcoma. It kills cancer cells by blocking the DNA duplication and increasing ROS levels. Increased intracellular ROS levels trigger oxidative stress, which in turn result in irreversible changes in components such as intracellular lipids, proteins and nucleic acids [19,20]. Increase in intracellular Ca^{2+} levels leads to an increase in the amount of ROS, mitochondrial membrane depolarization, and increase in activation of caspase-3 and caspase-9 [18,20]. In our study, only Dox and Dox+Mel were administered to osteosarcoma (Saos-2) and chondrosarcoma (Hs 819.T) cell lines. Afterwards, the effect of these treatments on TRPV1 channels on intracellular mitochondria dependent cell death were investigated. A specific stimulator (Capsaicin) and inhibitor (Capsazepine) for TRPV1 channels were administered, and the intracellular Ca^{2+} level, intracellular ROS, mitochondrial depolarization, caspase-3 and caspase-9 values, as well as the degree of apoptosis were measured by examining the intermediate stages of apoptosis of osteosarcoma and chondrosarcoma cells. Established results were compared to the control group.

We have found that Dox and Dox+Mel treatments have resulted in TRPV1 channel stimulation, and that there was a significant increase in intracellular calcium ion levels, mitochondrial depolarization levels and intracellular ROS levels, which are mediated by TRPV1 channels in Dox and Dox+Mel groups. Oxidative stress-induced apoptosis levels were significantly increased compared to the control group, and melatonin triggered the mitochondria dependent cell apoptosis in both cancer cell lines. However, intracellular calcium, ROS, mitochondrial depolarization and caspase-3 / -9 levels were significantly decreased in Dox+Cpz and Dox+Mel+Cpz, when compared to Dox and Dox+Mel groups. Moreover, these effects of Dox and Dox+Mel on both cancer types achieved through TRPV1 channels and mitochondria dependent cell death processes were higher in Dox+Mel combination groups, when compared to Dox groups in both cancer cells. According to our literature review, there are very limited studies in the literature on the effect of chemotherapeutic agents on TRPV1 channels in osteosarcoma, but no study has conducted to examine the effects of chemotherapeutic agents on these channels in chondrosarcomas to date [18]. Melatonin is an antioxidant hormone released from the pineal gland and some other organs. In addition to its cardioprotective effect, melatonin is also used clinically as a pro-apoptotic agent in cancer treatment. It is well-known in the literature that melatonin is effectively used together with antineoplastic agents in treatment of bone, breast and colon cancer types. Melatonin is used together with chemotherapeutic agents with known efficacy such as cisplatin and doxorubicin [21]. In our study, both synergistic and comparative effects of melatonin and doxorubicin in osteosarcoma and chondrosarcoma were investigated. It has been observed that 0.1 mM of melatonin and 2 µM of doxorubicin combination increased apoptosis in cancer cells, when compared with the controls. Niu et al. have reported that melatonin increased the pro-apoptotic activity of doxorubicin in osteosarcoma cells by obtaining results similar to what we have established in our study [21]. Although there are limited studies conducted on the use of doxorubicin in chondrosarcoma, no reference has been found regarding the combined use of melatonin and doxorubicin. Kumari et al. have reported that the pro-apoptotic activities of doxorubicin increased in cancer cells depending on the dose at variable levels [21,22]. Intracellular cytosolic calcium ion level is fixed at 80-100 nanomolar concentrations through various ion channels. Excessive increase in intracellular calcium leads to increase in intracellular reactive oxygen species production. Besides, it also triggers mitochondrial depolarization and increases the level of caspase-3 and caspase-9. Increased caspase levels lead to increment in intracellular mitochondria-dependent apoptotic pathways and apoptosis [23]. Hsu et al have reported that five microgram/ml doxorubicin led to IC_{50} effect on human osteosarcoma cells, and it triggered apoptosis by increasing intracellular reactive oxygen species production, mitochondrial depolarization, caspase-9 and caspase-3 levels at given dose levels lower than five micrograms [24]. These results were similar to the 2 µM for 24 hours dose we used in our study for the same purpose. Administration of chemotherapeutic agents gives rise to increment in intracellular reactive oxygen species production and causes activation of TRPV1 channels which are sensitive to oxidative stress. Melatonin treatment given in combination with various chemotherapeutic agents may cause more activation in TRPV1 channels. However, we have not encountered any related study in the literature conducted to investigate the TRPV1 channel-mediated efficacy of doxorubicin alone or in combination with melatonin in osteosarcoma and chondrosarcoma cell lines. Koşar et al. have reported in a study in 2016 that combined doxorubicin and melatonin treatment administered to MCF7 breast cancer cells induced apoptosis by increasing intracellular calcium concentration, reactive oxygen species production, mitochondrial depolarization, caspase-3 and caspase-9 levels [25]. In our study, we have observed that intracellular calcium concentration, reactive oxygen species production, mitochondrial depolarization, caspase-3 and caspase-9 levels were similarly increased and apoptosis was induced after the combined use of doxorubicin and melatonin in osteosarcoma and chondrosarcoma cell lines.

Conclusion

As a result, we have concluded that apoptosis levels of osteosarcoma and chondrosarcoma cells increased more in both cell lines with the use of melatonin in addition to doxorubicin, and that the apoptotic effects of doxorubicin and melatonin were mediated by the indirect activation of TRPV1 channels. Future and more comprehensive studies are warranted to be conducted in the future to fulfill the aim to uncover the in vivo and in vitro effect of these and other agents which have efficacy on TRPV channels and calcium metabolism.

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Research Article

Araştırma

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Prognostic Significance of PNI, SIRI and LIPI in Non Small-Cell Lung Cancer

Küçük Hücreli Dışı Akciğer Kanserinde PBİ, SİYİ ve AİPİ'nin Prognostik Önemi

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ABSTRACT

Aim: Non-small cell lung cancer (NSCLC) is one of the 3 most common and deadly cancers. The aim of the current study is to investigate whether Prognostic Nutritional Index /PNI), Systemic Immune-Inflammation Index (SIRI), Lung Immune Prognostic Index (LIPI) has a prognostic significance in patients with metastatic NSLCL.

Methods: Patients diagnosed with pathologically confirmed metastatic NSCLC in 5 different hospitals in Turkey between 2016-2022 were included in our study and analyzed retrospectively. overall survival (OS) and progression-free survival (PFS) were recorded.

Results: The median PFS was 5.50 months, while the median OS was 16.03 months. Median OS was 14.86 months for the PNI-Low group and 17.2 months for the PNI-High group (p: <0.121). The median OS of the PNI-Low group was shorter than the PNI-High group, but there was no statistically significant difference between the groups. Median OS was 19.86 months for the SIRI-Low group and 14.23 months for the SIRI-High group (p: <0.112). Median OS was 17.76, 15.13, 13.73 months for the LIPI-Low, LIPI-intermediate group and LIPI-high group, there was no statistically significant difference between the groups (p: <0.391).

Conclusion: In conclusion, PNI and SIRI may be significant in a prospective study in a specific patient group to be performed with a larger number of patients to predict the prognosis of patients with metastatic NSCLC.

Key Words: Prognostic Nutritional Index, Systemic Immune-Inflammation Index, Lung Immune Prognostic Index, Non Small Cell Lung Cancer

ÖZET

Amaç: Küçük hücreli dışı akciğer kanseri (KHDAK) en sık görülen ve en çok ölüme sebep olan 3 kanserden birisidir. Mevcut çalışmanın amacı, metastatik KHDAK'lı hastalarda Prognostik Beslenme İndeksi (PBI), sistemik inflamatuvar Yanıt İndeksi (SİYİ), Akciğer İmmün Prognostik İndeksi (AİPİ)'nin prognostik bir öneminin olup olmadığını araştırmaktır.

Yöntem: Çalışmamıza 2016-2022 yılları arasında Türkiye'de 5 farklı hastanede patolojik doğrulanmış metastatik KHDAK tanısı almış hastalar dahil edilmiş ve retrospektif olarak incelenmiştir. Bazı hemogram parametreleri ve Idh, albumin gibi biokimyasal parametreler, ayrıca genel sağkalım (GSK) ve progresyonsuz sağkalım (PSK) kaydedilmiştir.

Bulgular: Çalışmamıza 297 hasta dahil edilmiştir. Median PSK 5,5 ay iken median GSK 16,03 ay idi. Median GSK PBİ-düşük grup için 14,86 ay, PBİ-yüksek grup için ise 17,2 ay ay idi (p: <0.121). PBİ-düşük grubunun medyan GSK'sı, PBİ-yüksek gruptan daha kısaydı, ancak gruplar arasında istatistiksel olarak anlamlı bir fark yoktu. Median GSK SİYİ-düşük grup için 19,86 ay iken, SİYİ-yüksek grup için ise 14,23 ay idi(p: <0.112). SİRİ-düşük grubunun medyan GSK'sı, SİYİ-yüksek gruptan daha uzundu, ancak gruplar arasında istatistiksel olarak anlamlı bir fark yoktu. Medyan GSK, AİPİ-Düşük grup için 17,76 (%95 GA 16,51 -19,02) ay, AİPİ- orta grup için 15,13 ay, AİPİ-yüksek grup için 13,73 ay (%95 GA 8,05 -19,41) bulundu. AİPİ -Düşük AİPİ -orta ve AİPİ -Yüksek grupları arasında istatistiksel olarak anlamlı bir fark yoktu (p: <0,391).

Sonuç: Sonuç olarak PNI ve SIRI daha çok hasta sayısıyla yapılacak spesifik bir hasta grubunda prospektif bir çalışmada KHDAK hastaların prognozunu tahmin etmek için anlamlı çıkabilir.

Anahtar Kelimeler: Prognostik Beslenme İndeksi, Sistemik İmmün İnflamasyon İndeksi, Akciğer İmmün Prognostik İndeksi, Küçük Hücreli Dışı Akciğer Kanseri

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Introduction

Non-small cell lung cancer (NSCLC) is one of the 3 most common cancers and the leading cause of death. They constitute approximately 85% of newly emerging lung cancers [1]. If we classify NSCLC according to histology, adenocarcinoma (AC) is in the first place with an incidence of 32%, while squamous cell cancer (SCC) is around 29% [2]. Despite new developments in chemotherapy and surgical techniques, NSCLC survival was around 5-10%. With the introduction of immunotherapy, this rate reached around 20-40% [3,4]. However, due to the possibility of developing fast-progression and hyper progression in patients using immunotherapy, the fact that recent studies are needed has appeared [5].

Access to genetic biomarkers in developing and underdeveloped countries is financially difficult. Therefore, there is a need for useful indices that can be used in daily clinical practice.

Malnutrition plays a significant role in the prognosis of cancer in many ways, in the immune system. Substances released from the tumor have a key role in cancer prognosis because they increase systemic inflammation through cytokines. At the same time, systemic inflammatory responses such as infection and trauma increase angiogenesis and play a role in tumor development [6].

Previously, indices created by hematological parameters emerged as prognostic markers in some cancers [7,8]. The prognostic nutritional index (PNI) is calculated based on the number of lymphocytes and albumin. A low PNI appears to have a poor prognostic value in small cell lung cancer (SCLC) and metastatic laryngeal cancer [9,10]. The Systemic Inflammatory Response Index (SIRI) is a comprehensive marker of inflammation calculated from monocyte, neutrophil, and lymphocyte counts. Studies in patients with SCLC have shown that elevated SIRI has poor prognostic value [11]. High SIRI was also found to be a poor prognostic factor in a study of stage 3 NSCLC patients undergoing definitive chemotherapy (CRT) [12]. The combination of LDH, which indicates neutrophil, leukocyte, and proliferation levels, resulted in a poor prognosis for patients with locally advanced NSCLC, leading to shorter overall survival (OS) and progression-free survival (PFS). However, there is a lack of research regarding the importance of PNI, SIRI, and LIPI as prognostic indicators in patients suffering from metastatic NSLCL.

In our current study, we aimed to investigate whether PNI, SIRI, LIPI have prognostic significance in patients with metastatic NSCLC.

Material And Methods

Our study included patients who underwent surgery between 2016 and 2022. was diagnosed with pathologically confirmed metastatic NSCLC in 5 different hospitals in Turkey. We retrospectively reviewed patient records and hospital databases and recorded patient demographic and hemogram parameters such as platelet, neutrophil, monocyte and lymphocyte count and biochemical parameters such as LDH and albumin. We also recorded demographic data, metastatic sites, and tumor mutational status. Survival outcomes were also recorded. Patients with additional malignancy, known autoimmune disease, steroid use, and active infection were excluded from the study.

PNI was determined by adding together 10 times the serum albumin value (in g/dl) and 0.005 times the peripheral lymphocyte count (per mm3). The formula for calculating SIRI is (neutrophil multiplied by lymphocyte) divided by monocyte. The dNLR was calculated prior to determining the LIPI. The value of dNLR was obtained by dividing the number of neutrophils by the difference between the total number of leukocytes and the number of neutrophils. If dNLR is less than or equal to 3 and LDH is less than or equal to the upper limit of normal, then it belongs to the low group of LIPI. If dNLR is greater than 3 or LDH is greater than the ULN, then it falls into the intermediate group of LIPI. If both dNLR and LDH are greater than the ULN, then it is categorized in the high group of LIPI.

Statistical Analysis

The statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA). Categorical variables are presented as n and %, while continuous variables are expressed as mean±SD in the descriptive statistics.

The study's data was assessed for normality assumptions, and comparisons between continuous variables and groups were made using the parametric tests of ANOVA and independent t-tests after analyzing the Kolmogorov-Smirnov values. To compare categorical variables, researchers employed either the chi square test or Fisher's Exact test. Patients in the high LIPI group have a dNLR score of 3 or less and LDH levels below the upper limit of normal. In the intermediate LIPI group, patients have a dNLR score greater than 3 or LDH levels above the upper limit of normal. The SIRI-high group consists of patients with both a dNLR score greater than 3 and LDH levels above the upper limit of normal.

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The Kaplan Meier method was used to compare survival time and disease-free survival according to several variables. Finally, multivariate Cox regression results based on risk of death and disease-free survival are presented. $p<0.05$ was considered statistically significant.

The research was approved by the ethics committee of Diyarbakir Gazi Yaşargil Training and Research Hospital in compliance with the Declaration of Helsinki and Good Clinical Practices. The study was carried out following these regulations and guidelines. (Approval Date-No:November 25th, 2022 - 2022/232).

Results

There were 297 patients in our study. The median mean age was 63 (53, 73). 245 (82.5%) of our patients were male. ECOG performance scale of 247 (83%) of our patients was 0 or 1. Of our patients, 174 (58.6%) were AC and 123 (41.4%) were scc. The metastasis site of 153 (51.5%) of our patients was bone, while 106 (35.7%) of them were liver. As a metastatic 1st line chemotherapy option, 100 (33.7%) patients received carboplatin + paclitaxel, 64 (21.5%) patients received a platinum regimen + gemcitabine, and 56 (18.9%) patients received a platinum regimen + pemetrexed combination.

ROC analysis results of various variables predicting death were calculated. While this value was 483.3 for PNI, it was found to be 1.8 for SIRI. There was no statistically significant difference between the group with a PNI score < 483.3 (PNI-Low) and the group with a PNI score ≥ 483.3 (PINI-High). However, a statistically significant difference was found between liver metastasis and PNI groups (p=0.014). General characteristics and demographic information of patients grouped according to PNI and SIRI are shown in Table I in detail. No statistically significant difference was found between the group with a SIRI score <1.8 (SIRI-Low) and the

Table 1. Comparison of various sociodemographic and clinical variables by SIRI group.

Variables		Total	SIRI		p	PNI		p
			LOW <1,8	HIGH ≥1,8		LOW <483,3	HIGH ≥483.3	
Age, Mean±SD		63,51±10,07				64,10±9,88	62,94±10,26	0.321
Gender, n (%)	Male	245 (82,5)	83 (78,3)	162 (84,8)	0.157	122 (83,6)	123 (81,5)	0.663[a]
	Female	52 (17,5)	23 (21,7)	29 (15,2)		24 (16,4)	28 (18,5)	
ECOG, n (%)	0	61 (20,5)	26 (24,5)	35 (18,3)	0.559	32 (21,9)	29 (19,2)	0.655
	1	186 (62,6)	65 (61,3)	121 (63,4)		91 (62,3)	95 (62,9)	
	2	44 (14,8)	13 (12,3)	31 (16,2)		19 (13)	25 (16,6)	
	3-4	6 (2,1)	2 (1,9)	4 (2,1)		4 (2,7)	2 (1,3)	
Histopathological Subtype, n (%)	Adenocarcinoma	174 (58,6)	61 (57,5)	113 (59,2)	0.787	90 (61,6)	84 (55,6)	0.293[a]
	SCC	123 (41,4)	45 (42,5)	78 (40,8)		56 (38,4)	67 (44,4)	
Smoking, n (%)	Yes	172 (57,9)	17 (16)	32 (16,8)	0.966	23 (15,8)	26 (17,2)	0.501
	No	49 (16,5)	61 (57,5)	111 (58,1)		83 (56,8)	89 (58,9)	
	Left	76 (25,6)	28 (26,4)	48 (25,1)		40 (27,4)	36 (23,8)	
COPD, n (%)	No	223 (75,1)	81 (76,4)	142 (74,3)	0.693	112 (76,7)	111 (73,5)	0.524[a]
	Yes	74 (24,9)	25 (23,6)	49 (25,7)		34 (23,3)	40 (26,5)	
Diabetes mellitus, n (%)	No	246 (82,8)	85 (80,2)	161 (84,3)	0.369	120 (82,2)	126 (83,4)	0.775[a]
	Yes	51 (17,2)	21 (19,8)	30 (15,7)		26 (17,8)	25 (16,6)	
Chronic kidney disease, n (%)	No	272 (91,6)	97 (91,5)	175 (91,6)	0.973	137 (93,8)	135 (89,4)	0.169[a]
	Yes	25 (8,4)	9 (8,5)	16 (8,4)		9 (6,2)	16 (10,6)	

PNI (Perioperative Nutritional Index), SIRI (Systemic Immune-Inflammation Index), SCC: squamous cell carcinoma, COPD: chronic obstructive pulmonary disease

Table 1. Comparison of various sociodemographic and clinical variables by SIRI group. *(continued)*

Variables		Total	SIRI LOW <1,8	SIRI HIGH ≥1,8	p	PNI LOW <483,3	PNI HIGH ≥483.3	p
Liver metastasis, n (%)	No	191 (64,3)	70 (66)	121 (63,4)	0.643	104 (71,2)	87 (57,6)	**0.014[a]**
	Yes	106 (35,7)	36 (34)	70 (36,6)		42 (28,8)	64 (42,4)	
Bone metastasis, n (%)	No	144 (48,5)	50 (47,2)	94 (49,2)	0.736	69 (47,3)	75 (49,7)	0.678[a]
	Yes	153 (51,5)	56 (52,8)	97 (50,8)		77 (52,7)	76 (50,3)	
Brain metastasis, n (%)	No	221 (74,7)	86 (81,1)	135 (71,1)	0.056	102 (70,3)	119 (78,8)	0.094[a]
	Yes	75 (25,3)	20 (18,9)	55 (28,9)		43 (29,7)	32 (21,2)	
Type of First-Line Chemotherapy, n (%)	Platinum Gemcitabine	64 (21,5)						
	Carboplatin-paclitaxel	100 (33,7)						
	Platinum-pemetrexed	56 (18,9)						
	Platinum-etoposide	3 (1,0)						
	Cisplatin-paclitaxel	8 (2,7)						
	Cisplatin-doxorubicin	15 (5,1)						
	Cisplatin-vinorelbine	5 (1,7)						
	Chemotherapy plus Immunotherapy	5 (1,7)						
	Immunotherapy	7 (2,4)						
	Single-Agent Chemotherapy	7 (2,4)						
	Target-specific	27 (9,1)						

group with a SIRI score ≥1.8 (SIRI-High). There was no statistically significant difference between LIPI-Low group, LIPI-intermediate group, and LIPI-high group (p>0.05). General characteristics and demographic information of patients grouped according to LIPI are shown in Table II in detail.

In the overall population, median PFS was 5.50 (95% CI 4.95-6.04) months and median OS was 16.03 (95% CI 14.13-17.93) months. Median PFS was 5.96 (95% CI 5.26-6.67) months in the low PNI group and 6.03 (95% CI 4.99-7.01) months in the high PNI group. The median PFS of the low PNI group was lower than that of the high PNI group, but there was no statistically significant difference between the groups (p < 0.108). Median PFS was 5.16 (95% CI 4.28-6.04) months in the SIRI-Low group and 4.80 (95%

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Table 2. Comparison of various sociodemographic and clinical variables by LIPI groups

Variables		Total	LIPI			p
			dNLR negative and LDH normal	One of the two is Positive	Both Positive	
Age, Mean±SD		63,51±10,07	62,95±9,63	64,14±10,01	63,20±11,16	0.636[c]
Gender, n (%)	Male	245 (82,5)	95 (84,1)	105 (81,4)	45 (81,8)	0.852[a]
	Female	52 (17,5)	18 (15,9)	24 (18,6)	10 (18,2)	
ECOG, n (%)	0	61 (20,5)	27 (23,9)	28 (21,7)	6 (10,9)	0.180[b]
	1	186 (62,6)	71 (62,8)	80 (62)	35 (63,6)	
	2	44 (14,8)	12 (10,6)	20 (15,5)	12 (21,8)	
	3-4	6 (2,1)	3 (2,7)	1 (0,8)	2 (3,6)	
Histopathological Subtype, n (%)	Adenocarcinoma	174 (58,6)	74 (65,5)	66 (51,2)	34 (61,8)	0.068[a]
	SCC	123 (41,4)	39 (34,5)	63 (48,8)	21 (38,2)	
Smoking, n (%)	Yes	172 (57,9)	23 (20,4)	14 (10,9)	12 (21,8)	0.132[a]
	No	49 (16,5)	67 (59,3)	76 (58,9)	29 (52,7)	
	Left	76 (25,6)	23 (20,4)	39 (30,2)	14 (25,5)	
COPD, n (%)	No	223 (75,1)	87 (77)	95 (73,6)	41 (74,5)	0.831[a]
	Yes	74 (24,9)	26 (23)	34 (26,4)	14 (25,5)	
Diabetes mellitus, n (%)	No	246 (82,8)	98 (86,7)	103 (79,8)	45 (81,8)	0.358[a]
	Yes	51 (17,2)	15 (13,3)	26 (20,2)	10 (18,2)	
Chronic kidney disease, n (%)	No	272 (91,6)	105 (92,9)	117 (90,7)	50 (90,9)	0.808[a]
	Yes	25 (8,4)	8 (7,1)	12 (9,3)	5 (9,1)	
Liver metastasis, n (%)	No	191 (64,3)	73 (64,6)	85 (65,9)	33 (60)	0.745[a]
	Yes	106 (35,7)	40 (35,4)	44 (34,1)	22 (40)	
Bone metastasis, n (%)	No	144 (48,5)	55 (48,7)	66 (51,2)	23 (41,8)	0.509[a]
	Yes	153 (51,5)	58 (51,3)	63 (48,8)	32 (58,2)	
Brain metastasis, n (%)	No	221 (74,7)	92 (81,4)	90 (70,3)	39 (70,9)	0.110[a]
	Yes	75 (25,3)	21 (18,6)	38 (29,7)	16 (29,1)	
	No-Not Viewed	245 (82,5)	93 (82,3)	108 (83,7)		
Type of First-Line Chemotherapy, n (%)	Platinum Gemcitabine	64 (21,5)				
	Carboplatin-paclitaxel	100 (33,7)				
	Platinum-pemetrexed	56 (18,9)				
	Platinum-etoposide	3 (1,0)				
	Cisplatin-paclitaxel	8 (2,7)				
	Cisplatin-doxorubicin	15 (5,1)				
	Cisplatin-vinorelbine	5 (1,7)				
	Chemotherapy plus Immunotherapy	5 (1,7)				
	Immunotherapy	7 (2,4)				
	Single-Agent Chemotherapy	7 (2,4)				
	Target-specific	27 (9,1)	62,95±9,63	64,14±10,01	63,20±11,16	

LIPI (Lung Immune Prognostic Index), dNLR: derived neutrophil lymphocyte ratio, SCC: squamous cell carcinoma, COPD: chronic obstructive pulmonary disease

CI 4.18-5.41) months in the SIRI-Low High group. The median PFS of the SIRI-Low group was longer than that of the SIRI-High group, but there was no statistically significant difference between the groups (p < 0.422). Median PFS was 5.3 (95% CI 4.39–6.20) months for the low LIPI group and 5.46 (95% CI 3.52–7.41) months for the intermediate LIPI group. For the high LIPI group, it was 5.36 (95% CI 4.64 -6.08). There was no statistically significant difference between LIPI-Low, LIPI-Intermediate and LIPI-High groups (p < 0.362). The shrinkage of the tumor in one of our patients with high PNI and low SIRI was supportive on a case-by-case basis. (Figure – 1)

If we look at the whole population, the median OS was 16.03 (95%CI: 14.13-17.93) months. The median OS was 14.86 (95% CI: 11.7 -18.03) months for the PNI-Low group and 17.2 (95% CI: 14.75-19.64) months for the PNI-High group. The median OS of the PNI-Low group was shorter than the PNI-High group, but there was no statistically significant difference between the groups (p: <0.121). Median OS was 19.86 (95% CI:16.31 -23.42) months for the SIRI-Low group and 14.23 (95% CI: 11.98-16.48) months for the SIRI-High group. The median OS of the SIRI-Low group was longer than the SIRI-High group, but there was no statistically significant difference between the groups (p: <0.112) (Figure 1). The median OS was 17.76 (95% CI 16.51 -19.02) months for the LIPI-Low group and 15.13 (95% CI: 12.15-18.11) months for the LIPI-intermediate group. For the LIPI-high group, it was found to be 13.73 (95% CI 8.05 -19.41). There was no statistically significant difference between LIPI-Low LIPI-intermediate and LIPI-High groups (p: <0.391).

Median PFS was 5.86 (95% CI: 5.34-6.38) months in those without brain metastases, and 4.70 (95% CI: 4.01-5.38) months in those with brain metastases. It was shorter in those with brain metastases than in those without PFS, and it was statistically significant (p=0.040). In patients without brain metastases, 2-year disease-free survival was 4.4%, while 5-year disease-free survival was 2.2%. In patients with brain metastases, 2-year disease-free survival was 0%.

Median OS was statistically significant according to histological subtype groups (p=0.001). The median OS was 8.06 (95%CI: 14.76-21.37) months in AC, and the median OS was 13.60 (95%CI: 10.55-16.64) months in SCC. The OS in AC is statistically significantly longer than SCC. While 2-year survival was 35.2% in AC patients, 5-year survival was 16.7%, and 2-year survival in SCC was 27.1%.

We conducted a Multivariate Cox regression analysis in order to identify factors that independently predict PFS (progression-free survival). In the group of individuals who did not have brain metastases, the duration of progression-free survival was observed to be longer, although the difference was not found to be statistically significant, in comparison to the group with brain metastases [HR (95% CI) = 1.29 (0.98-1.71), p: 0.68].

Multivariate Cox regression analysis was performed to find independent prognostic factors to determine OS). The OS was found to be longer in AC than in SCC [HR (95% CI) = 1529 (1.18-2.14), p: 0.002].

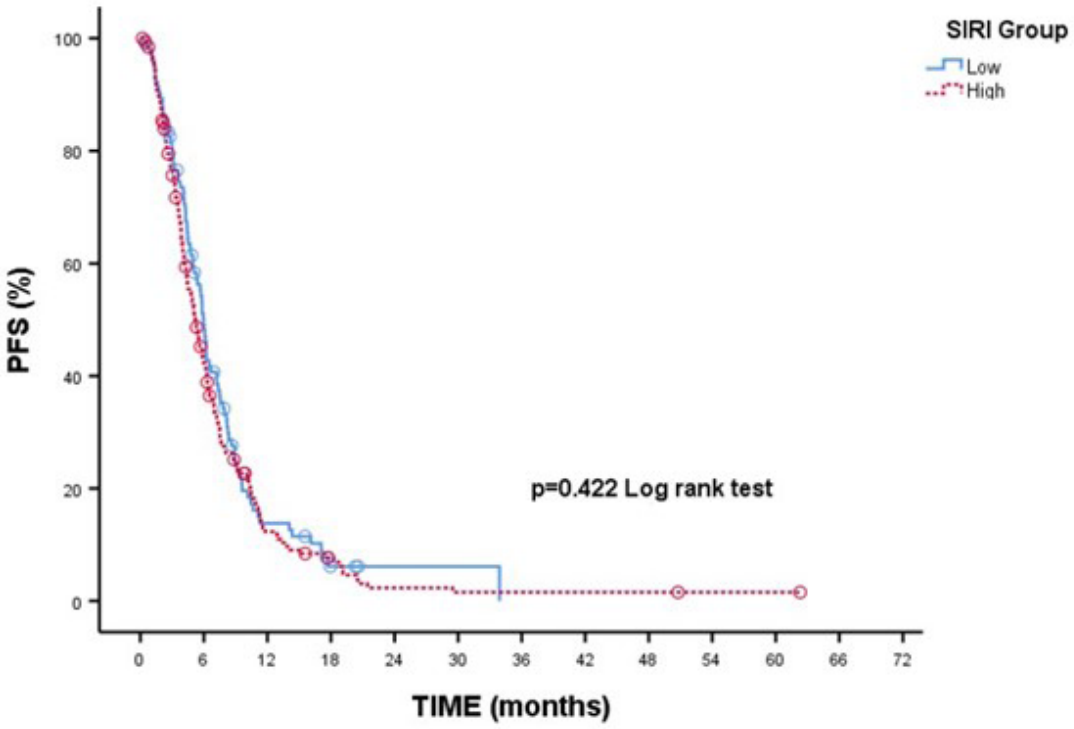

Figure 1. Progression Free Survival analysis functions by SIRI groups

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Table 3. Univariate analysis of PFS and OS

	mOS (95%CI)	p-value	mPFS (95% CI)	p-value
General	**16,03 (14,13-17,93)**		5,50 (4,95-6,04)	
Gender		**0.988**		0.093
Male	**16,00 (13,75-18,25)**		5,40 (4,72-6,07)	
Woman	**17,70 (14,48-20,91)**		5,90 (5,06-6,73)	
Histological subtype		0.001		0.315
AC	**18,06 (14,76-21,37)**		5,63 (4,94-6,32)	
SCC	**13,60 (10,55-16,46)**		5,40 (4,26-6,53)	
Liver Metastasis		**0.700**		0.018
No	**16,00 (13,67-18,32)**		5,06 (4,25-5,87)	
Yes	**16,03 (13,36-18,70)**		6,20 (5,48-6,91)	
Lung Metastasis		**0.734**		0.484
No	**16,06 (15,77-19,35)**		5,93 (4,98-6,88)	
Yes	**15,76 (13,53-18,00)**		5,30 (4,61-5,98)	
Bone Metastasis		**0.070**		0.333
No	**17,53 (13,88-21,17)**		5,80 (4,80-6,79)	
Yes	**14,60 (13,53-18,00)**		5,30 (4,59-6,01)	
Brain Metastasis		**0.540**		0.040
No	**16,06 (14,32-17,80)**		5,86 (5,34-6,38)	
Yes	**14,60 (9,84-19,35)**		4,70 (4,01-5,38)	
PDL		**0.167**		0.907
No-Not Viewed	**15,76 (13,62-17,91)**		5,63 (5,06-6,20)	
Positive	**18,06 (12,02-24,11)**		4,40 (2,94-5,85)	
SIRI		**0.112**		0.422
Low	**19,86 (16,31-23,42)**		**5,16 (4,28-6,04)**	
High	**14,23 (11,98-16,48)**		**4,80 (4,18-5,41)**	
PNI		**0.121**		0.108
Low	**14,86 (11,70-18,03)**		**5,96 (5,26-6,67)**	
High	**17,20 (14,75-19,64)**		**6,03 (4,99-7,01)**	
LIPI		**0.391**		0.362
dNLR negative and LDH normal	**17,76 (16,51-19,02)**		**5,30 (4,39-6,20)**	
One of the two is Positive	**15,13 (12,15-18,11)**		**5,46 (3,52-7,41)**	
Both Positive	**13,73 (8,05-19,41)**		**5,36 (4,64-6,08)**	
Type of First-Line Chemotherapy		**0.211**		0.787
Platinum Taxane	14,86 (11,75-17,97)		4,76 (3,80-5,73)	
Platinum-peme-trexed	18,10 (13,07-23,12)		5,36 (4,64-6,08)	

PFS: Progression-free survival, OS: Overall Survival, CI: Confidence interval, PS: performance status, LIPI (Lung Immune Prognostic Index), dNLR: derived neutrophil lymphocyte ratio, SCC: squamous cell carcinoma, COPD: chronic obstructive pulmonary disease, PNI (Perioperative Nutritional Index), SIRI (Systemic Immune-Inflammation Index)

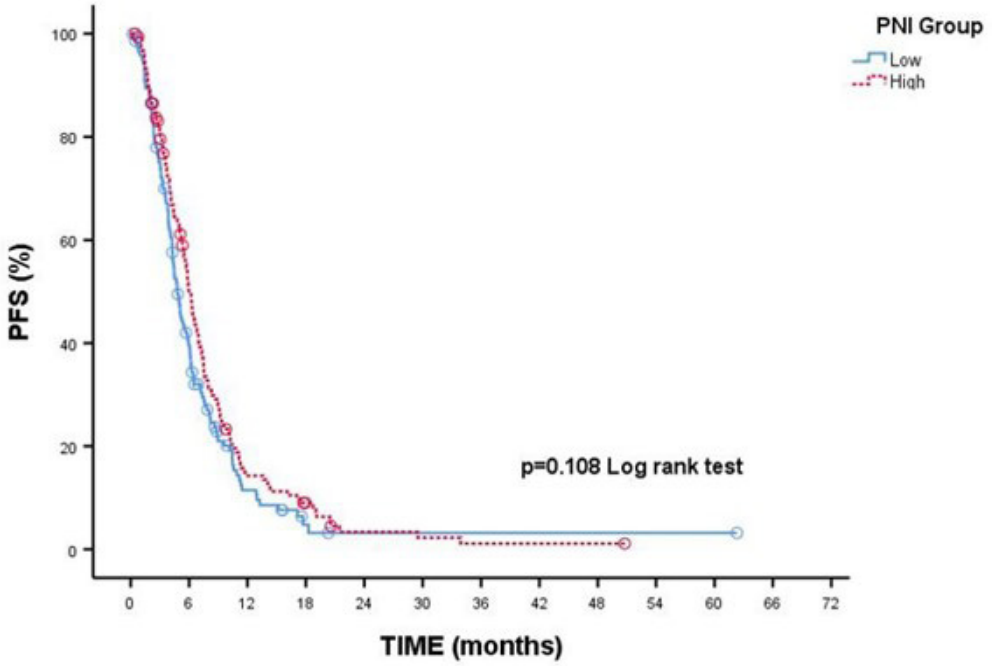

Figure 2. Progression Free Survival analysis functions by PNI groups

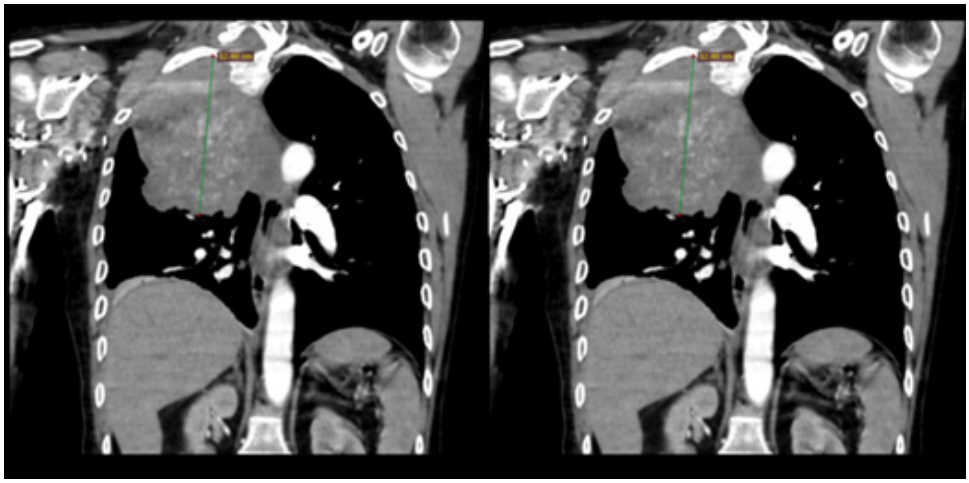

Figure 3. Coronal section computed tomography of the tumor shrinkage in a case with high PNI and low SIRI

Discussion

Many studies have been conducted to predict the prognosis of patients with metastatic NSCLC and prognostic factors have been identified. Unfortunately, no marker to be used in daily clinical practice has been found. Albumin is a negative acute phase reactant synthesized in the liver. Hypoalbuminemia can be caused by malnutrition, hyper catabolism caused by cancer cells, and increased inflammation due to cytokine release, which plays a role in the survival of cancer patients [14]. Lymphocytes contribute to prolonged survival by inhibiting apoptosis by secreting tumor necrosis factor alpha and interferon gamma, preventing tumor migration and invasion [15]. An increase in the number of neutrophils plays a role in tumoral pathogenesis by increasing the C-X-C motif chemokine ligand 8, nuclear factor kappa-B, Transforming growth factor-beta-β1 (TGF-β1) and vascular endothelial growth factor (VEGF) [16]. Meanwhile, the number of circulating monocytes is known to contribute to the tumor microenvironment by differentiation into macrophages and secreting proteases that degrade the extracellular matrix, leading to poor prognosis in various tumors [17]. We decided to conduct this study evaluating PNI, SIRI and LIPI, which we think will play a prognostic role in metastatic NSCLC.

In the study, we observed 297 patients overall, with 245 of them being men, accounting for 82.5% of the total. Out

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of the total number of patients, 174 (58.6%) were categorized as AC, while 123 (41.4%) were classified as scc. When considering the entire population, the middle point of progression-free survival (PFS) is 5.50 months with a range of 4.95-6.04 months, whereas the middle point of overall survival (OS) is 16.03 months with a range of 14.13-17.93 months. The overall traits and average lifespan of the patients included in our research align with what is stated in the existing literature[18]. While the median PFS was 5.96 months for the PNI-Low group, it was 6.03 months for the PNI-High group. The median PFS of the PNI-Low group was shorter than the PNI-High group, but not statistically significant (p: <0.108). While the median OS was 14.86 months for the PNI-Low group, it was 17.2 months for the PNI-High group. The median OS of the PNI-Low group was shorter than the PNI-High group, but there was no statistically significant difference between the groups (p: <0.121). In a meta-analysis of 4922 patients with metastatic NSCLC, lower PNI was found with shorter OS (HR: 1.59, 95% CI: 1.28–1.96, P = 0.001) and PFS (HR = 1.52, %CI = 1.26–1.83, P = 0.002) [19]. In a study of gastrointestinal cancers recruiting 3414 patients preoperatively, low PNI was associated with low OS (HR = 1.80, CI = 1.26–1.83, P = 0.002) [20]. In a study of 319 patients with metastatic and locally limited pharyngeal cancer Stage 1-4, a low PNI score was associated with poor OS [9].

In our study, the median PFS was 5.16 (95% CI: 4.28 -6.04) months for the SIRI-Low group and 4.80 (95% CI: 4.18-5.41) months for the SIRI-High group. The median PFS of the SIRI-Low group was longer than the SIRI-High group, but not statistically significant (p: <0.422). Median OS was 19.86 (95% CI:16.31 -23.42) months for the SIRI-Low group and 14.23 (95% CI: 11.98-16.48) months for the SIRI-High group. But it was not statistically significant (p: <0.112). In a meta-analysis of 38 studies with a predominance of gastrointestinal cancers, breast cancer, and head and neck cancer involving 10,734 patients, high SIRI was associated with poor OS (HR = 2.04, 95% CI = 1.82–2.29, P < .001). and PFS (HR = 2.08, 95% CI = 1.84–2.34, P < .001). At the same time, SIRI elevation was found to be correlated with low tumor size, lymph node involvement, and TNM (Tumor, Node, Metastasis) stage [21]. In a study of 176 patients with stage 3 NSCLC undergoing definitive chemoradiotherapy, low SIRI was found to be correlated with good prognosis (HR=1.868 CI:1.016–3.436) (p:0.018) [10]. In a study of 390 patients with locally limited NSCLC, preoperative high SIRI was associated with lower OS and PFS [22].

Median PFS was 5.3 (95% CI 4.39 -6.20) months for the LIPI-Low group and 5.46 (95% CI: 3.52-7.41) months for the LIPI-intermediate group. For the LIPI-high group, it was found to be 5.36 (95% CI 4.64 -6.08). There was no statistically significant difference between the groups (p: <0.362). Median OS was 17.76 (95% CI 16.51 -19.02) months for the LIPI-Low group and 15.13 (95% CI: 12.15-18.11) months for the LIPI-intermediate group. For the LIPI-high group, it was found to be 13.73 (95% CI 8.05 -19.41). There was no statistically significant difference between the groups (p: <0.391). A higher LIPI score was associated with longer OS in a meta-analysis of 12 studies of 4883 patients, the majority of whom were patients with NSCLC receiving immunotherapy. A higher LIPI score was associated with longer OS in a meta-analysis of 191 patients, the majority of whom were patients with malignant melanoma receiving immunotherapy [23].

In our study, there were 221 (74.7%) patients with brain metastases. PFS was found to be 5.86 (95% CI: 5.34-6.38) months in the group without brain metastases, and 4.70 (95% CI: 4.01-5.38) months in the group with brain metastases (p=0.040). Although it did not reach statistical significance in multivariate analysis, it was found to be longer [HR (95% CI) = 1.29 (0.98-1.71) (p:0.68)]. Our study is compatible with the literature in terms of the poor prognosis of brain metastases [24].

OS was found to be longer in AC compared to scc [HR (95% CI) = 1.59 (1.18-2.14) (p:0.002). Our study is compatible with the literature. [25,26].

The small number of patients is one of the limitations of our study because it is retrospective. Our study found a cut-off value of 483.31 for PNI and 2.34 for SIRI using ROC analysis, but a more accurate value can be calculated using methods with higher specificity and sensitivity, but this optimal value is not known. Prospective, well-designed studies with large numbers of patients are needed.

In conclusion, PNI and SIRI are inexpensive, practical, literature contributing markers and can be used in routine clinical practice to predict the prognosis of patients with metastatic NSCLC, if confirmed by prospective studies. Although not reaching statistical significance, low PNI and high SIRI were independent predictors of poor prognosis in patients with metastatic NSCLC.

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Research Article

Araştırma

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Assessment of Preanalytical Errors by Six Sigma Method and the Pareto Principle Analysis

Preanalitik Hataların Altı Sigma Metodu ve Pareto Prensibi Analizi ile Değerlendirilmesi

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ABSTRACT

Aim: In this study, we aimed to evaluate the preanalytical errors over a five year period using the Six Sigma methodology and Pareto Principle in the clinical biochemistry laboratory.

Methods: Five-year sample rejection data between January 2015 and December 2019 in the clinical biochemistry laboratory were analyzed and classified according to the reasons for rejection. Six Sigma levels for the total and each preanalytical error were calculated with Westgard online formula. Preanalytical errors were evaluated according to their frequencies ranks and percentages with Pareto principle.

Results: The overall rate of five-year total critical preanalytical errors was 1.91% and the sigma level was 3.6. According to Pareto's chart, the three most common errors among the five-year preanalytical rejections were clotted sample (42.49%, sigma value:4), insufficient sample (23.53%, sigma value:4.2), and wrong container (8.01%, sigma value:4.5).

Conclusion: Six Sigma is a quality management methodology used to evaluate laboratory performance processes according to universal quality criteria. Calculated sigma values of preanalytical errors in our laboratory were within the acceptable range. However, planned regulatory activities for frequently observed preanalytical errors should be a laboratory management strategy to reduce these error rates and improve our laboratory performance.

Key Words: Six Sigma; Quality Control; Preanalytical Errors

ÖZET

Amaç: Bu çalışmada, klinik biyokimya laboratuvarımızda altı sigma metodolojisi ve Pareto prensibi kullanılarak beş yıllık süreçteki preanalitik hataların değerlendirilmesi amaçlanmıştır.

Yöntem: Klinik biyokimya laboratuvarında Ocak 2015 ve Aralık 2019 tarihleri arasında gerçekleşen beş yıllık numune red verileri analiz edildi ve reddetme nedenlerine göre sınıflandırıldı. Toplam ve her bir preanalitik hata için gerçekleşen red verilerinin altı sigma düzeyleri, Westgard online formülü kullanılarak hesaplandı. Preanalitik hatalar, Pareto prensibi kullanılarak sıklık sıraları ve yüzdelerine göre değerlendirildi.

Bulgular: Beş yıllık toplam kritik preanalitik hataların genel oranı %1,91 ve sigma düzeyi 3,6 idi. Beş yıllık veriler Pareto grafiğine göre değerlendirildiğinde en sık karşılaşılan preanalitik hatalar pıhtılaşmış numune (%42,49, sigma değeri: 4), yetersiz numune (%23,53, sigma değeri: 4,2) ve yanlış numune kabı (%8,01, sigma değeri: 4,5) olarak belirlendi.

Sonuç: Altı Sigma, laboratuvar performans süreçlerini evrensel kalite kriterlerine göre değerlendirmek amacıyla kullanılan bir kalite yönetim metodolojisidir. Laboratuvarımızdaki preanalitik hataların hesaplanan sigma değerleri kabul edilebilir aralıktaydı. Ancak sık gözlenen preanalitik hatalara yönelik planlanan düzenleyici faaliyetler, bu hata oranlarının azaltılması ve laboratuvar performansımızın geliştirilmesi için bir laboratuvar yönetim stratejisi olmalıdır.

Anahtar Kelimeler: Altı Sigma; Kalite Kontrol; Preanalitik Hatalar

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Introduction

Clinical laboratories assume a critical role in patient safety. Therefore, they must enforce quality management and provide quality reports to produce more accurate and reproducible test results. The performance processes in laboratories consist of preanalytical, analytical, and post-analytical phases. The preanalytical phase is the period from the planning of which tests will be requested to the starting of the laboratory analysis [1]. Although all phases of the laboratory process are important for quality management, the majority of laboratory errors (46-68%) occur in the preanalytical phase [2]. The preanalytical phase consists of test requests, patient identification, sample collection, labeling, transportation, pipetting, and centrifugation. In case of any negligence in these steps, preanalytical errors may occur. The fact that many of these multidisciplinary preanalytical variables are difficult to control by the laboratory is a primary reason for the high prevalence of preanalytical errors [3].

Six Sigma is a quality management method that integrates accurate and precision evaluation, error identification, and process improvement. Six Sigma is a component of the continuous improvement approach used primarily in the manufacturing world and then used in hospital quality management since 1999 [4]. In the clinical laboratory, process performance should be evaluated according to accepted quality criteria, and all errors should be determined and controlled. Six Sigma is one of the quality management methods that can be used to evaluate the laboratory performance processes within universal criteria. The universal application steps of Six Sigma are "Defining, Measuring, Analysing, Improving, Controlling" which is called the DMAIC cycle [5]. Sigma values are defined as "defects per million opportunities (DPMO)" and the Six Sigma scale ranges from 0 to 6. A sigma value of 6 corresponds to 3.4 DPMO and a sigma value of 1 corresponds to 691462 DPMO (Table 1). As the sigma values increase, the error rates decrease and the reliability of the process increases. In an evaluation of laboratory processes, the lower limit sigma value is accepted as 4 to reduce systematic errors and ensure adequate performance [6]. Expressing laboratory quality data with sigma values makes the organization of corrective and remedial initiatives practical by providing a more accurate and easy assessment of the quality level.

There are different analysis methods to evaluate the variables and errors that cause low sigma levels. The Pareto Principle is one of the methods that list errors in order of frequency to analyze the causes of problems and compare them with each other [7]. The Pareto Principle, also known as the 80/20 rule, states that roughly 80% of the effects come from 20% of the causes [8]. In other words, a small number of factors or issues often account for the majority of problems or outcomes. In the quality management process, using the Pareto Principle ensures that efforts and resources are prioritized to tackle the critical issues that have the most significant impact. It allows us to focus on addressing the vital few causes that are responsible for most of the problems, rather than wasting resources on less significant factors.

Table 1. Process sigma levels according to defects per million opportunities

Sigma Level	DPMO*
1	691462
2	308538
3	66807
4	6210
5	233
6	3.4

*DPMO: defects per million opportunities

In this study, we aimed to evaluate the preanalytical errors over a five year period using the Six Sigma methodology and Pareto Principle in the clinical biochemistry laboratory.

Material and Methods

Materials

This observational study was conducted between January 2015 and December 2019 in the clinical biochemistry laboratory of the Adıyaman University Research and Education Hospital which is 400 bedded Tertiary Care Hospital.

Methods

The central laboratory is comprised of two departments (biochemistry and microbiology) and serves inpatients, outpatients, and emergency departments. All blood samples are collected in vacutainer by nurses/clinical staff, and transported to the central laboratory mostly by pneumatic system. Urine samples are transported to the central laboratory by the patients in sterile urine containers. Laboratory technicians observe the samples and requisition forms for any pre-analytical errors. If any error is observed, the sample is rejected and the reason for rejection is entered into the laboratory information system (LIS).

Preanalytical errors and rejection criteria of samples are as follows: missing test request, wrong test request, double test request, mislabeled samples, improper transport, absent sample, empty container, wrong container, inappropriate sample type, insufficient sample, inappropriate volume, haemolysed, clotted, lipemic.

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Statistical Analysis

We analyzed the five-year sample rejection data in LIS of the biochemical laboratory and classified them according to the reasons for rejection. The annual percentages of the rejected samples and their distribution according to the reasons were calculated in Microsoft Excel 2007 software program according to the formulas below.

Total critical errors frequency= (number of the total critical errors/number of the total requests) x 100

Preanalytical errors rates according to the reasons= (number of the preanalytical errors according to the reasons/ number of the total critical errors) x 100

DPMO and Six Sigma levels for the total and each preanalytical error were calculated with Westgard online formula (www.westgard.com/six-sigma-calculators).

Preanalytical errors were evaluated according to their ffrequencies ranks and percentages with the Pareto Principle applied in Microsoft Excel 2007 software program.

Results

The annual distributions of the total critical errors and each preanalytical error in the biochemistry laboratory between January 2015 and December 2019 were shown in Table 2. According to these data, the number of total requests for five years (hemogram, coagulation, cardiac markers, biochemistry, hormones, tumor markers, urine analysis, and urine drug levels) is 2809366, and the number of the total critical errors for five years is 53686. There was a permanent increase in the total number of samples between 2015 and 2019. While the total rejection percentage was 3.05 in 2015, the following years' range was be-

Table 2. Distribution of preanalytical errors for five years

Preanalytical error	2015		2016		2017		2018		2019		TOTAL	
	N	%	N	%	N	%	N	%	N	%	N	%
Missing test request	35	0.31	6	0.07	12	0.13	10	0.08	12	0.10	75	0.14
Wrong test request	43	0.38	315	3.47	311	3.34	469	3.79	314	2.71	1452	2.70
Double test request	795	7.00	855	9.43	577	6.20	814	6.58	1037	8.96	4078	7.60
Mislabeled samples	593	5.22	224	2.47	214	2.30	237	1.92	293	2.53	1561	2.91
Improper Transport	28	0.25	144	1.59	100	1.07	99	0.80	47	0.41	418	0.78
Absent Sample	48	0.42	9	0.10	28	0.30	18	0.15	10	0.09	113	0.21
Empty container	2371	20.88	113	1.25	127	1.36	75	0.61	104	0.90	2790	5.20
Wrong container	571	5.03	778	8.58	868	9.33	995	8.04	1087	9.39	4299	8.01
Inappropriate sample type	559	4.92	268	2.96	233	2.50	171	1.38	287	2.48	1518	2.83
Insufficient sample	2592	22.82	2185	24.10	2204	23.68	2704	21.86	2945	25.45	12630	23.53
Inappropriate volume	3	0.03	53	0.58	11	0.12	10	0.08	13	0.11	90	0.17
Haemolysed	701	6.17	341	3.76	207	2.22	210	1.70	246	2.13	1705	3.18
Clotted	3007	26.47	3754	41.41	4390	47.17	6526	52.75	5136	44.38	22813	42.49
Lipemic	12	0.11	20	0.22	25	0.27	34	0.27	41	0.35	132	0.25
Total critical errors	11358	3.05	9065	1.90	9307	1.56	12372	1.83	11572	1.68	53686	1.91
Total requests	372185		476638		595772		676478		688293		2809366	

tween 1.56 and 1.90; and the five-year total rejection rate was 1.91 percent.

The DPMO and sigma values calculated for each year of the distribution of rejected samples due to preanalytical errors were given in Table 3. The sigma level of total critical errors for five years was 3.6. Sigma levels for each of the preanalytical errors ranged from 3.9 to 5.9.

Pareto's chart, which ranked the distribution of preanalytical errors according to their frequencies and cumulative percentages, was given in Figure 1. According to the chart, among the five-year preanalytical rejections, the three most common errors were clotted sample (42.49%, sigma value: 4), insufficient sample (23.53%, sigma value: 4.2) and wrong container (8.01%, sigma value: 4.5).

Discussion

In this study, the total number of samples rejected in our laboratory over five years and their distribution according to the reasons for preanalytical rejection were examined. The sigma values of the total and each of the preanalytical errors were calculated separately and their distribution according to their frequency ranks and percentages were evaluated by Pareto's analysis.

According to our results, the percentage of total preanalytical errors for five years is 1.91%; and its sigma value is 3.6. In addition, the annual sigma values calculated separately for each of the preanalytical errors were 4 or over 4. When the distribution of total critical errors by years was evaluated, it was seen that the lowest sigma value was experienced in

Table 3. DPMO and Six Sigma values for preanalytical quality indicators

Preanalytical error	2015		2016		2017		2018		2019		TOTAL	
	DPMO	Sigma	DPMO	Sigma	DPMO	Sigma	DPMO	Sigma	DPMO	Sigma	DPMO	Sigma
Missing test request	94	5.3	13	5.7	20	5.7	15	5.7	17	5.7	27	5.6
Wrong test request	116	5.2	661	4.8	522	4.8	693	4.7	456	4.9	517	4.8
Double test request	2136	4.4	1794	4.5	968	4.6	1203	4.6	1507	4.5	1452	4.5
Mislabeled samples	1593	4.5	470	4.9	359	4.9	350	4.9	426	4.9	556	4.8
Improper Transport	75	5.3	302	5	168	5.1	146	5.2	68	5.4	149	5.2
Absent Sample	129	5.2	19	5.7	47	5.5	27	5.6	15	5.7	40	5.5
Empty container	6370	4	237	5	213	5.1	111	5.2	151	5.2	993	4.6
Wrong container	1534	4.5	1632	4.5	1457	4.5	1471	4.5	1579	4.5	1530	4.5
Inappropriate sample type	1502	4.5	562	4.8	391	4.9	253	5	417	4.9	540	4.8
Insufficient sample	6964	4	4584	4.2	3699	4.2	3997	4.2	4279	4.2	4496	4.2
Inappropriate volume	8	5.9	111	5.2	18	5.7	15	5.7	19	5.7	32	5.5
Haemolysed	1883	4.4	715	4.7	347	4.9	310	5	357	4.9	607	4.8
Clotted	8079	4	7876	4	7369	4	9647	3.9	7462	4	8120	4
Lipemic	32	5.5	42	5.5	42	5.5	34	5.4	60	5.4	47	5.5
Total critical errors	30517	3.4	19019	3.6	15569	3.7	18289	3.6	16813	3.7	19110	3.6

*DPMO: defects per million opportunities

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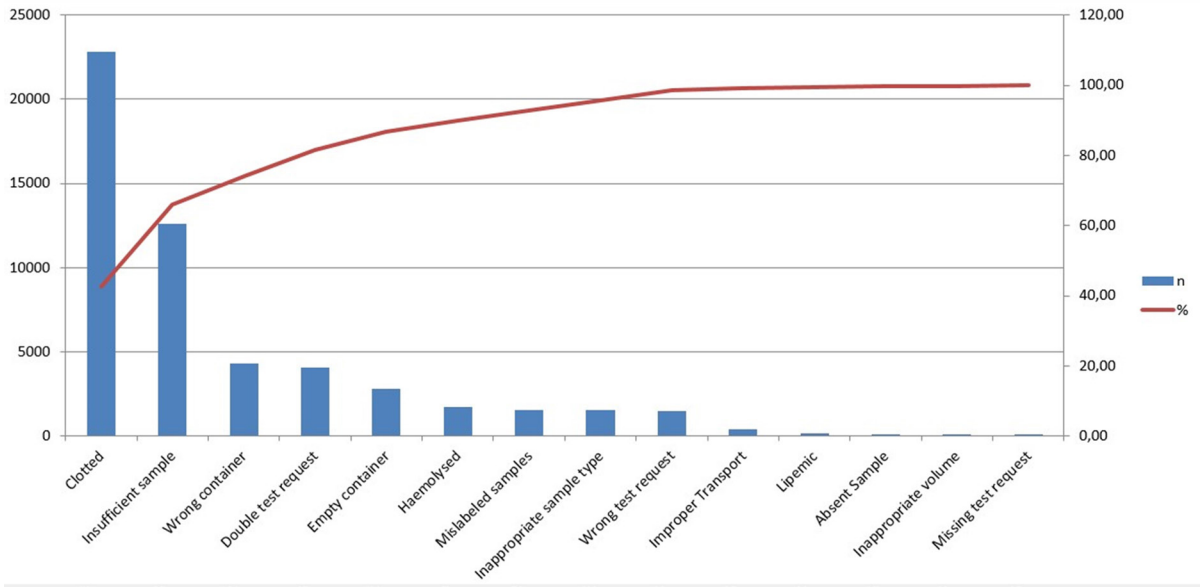

Figure 1. The Pareto's chart of preanalytical errors for five years (2015-2019)

2015, when the total number of requests was the least. The reason for this is thought to be due to the problems experienced from the hospital's moving to the new building, and installation process in 2015. Although the sigma value for the total critical errors was below 4; when 2015 and 2019 were compared, the number of total requests increased 1.85 times (total requests in 2015: n=372185; total requests in 2019: n=688293) and there was a minimal improvement in sigma values over the years (2015 sigma: 3.4, 2019 sigma: 3.7) (Figure 2). When the preanalytical errors were evaluated according to their causes, it was seen that the two most common errors for five years were clotted samples (42.49%, sigma: 4) and insufficient samples (23.53%, sigma: 4.2), respectively.

In the study of Ercan Ş. [9]. using the Six Sigma method to evaluate the reasons for preanalytical rejection for one-year period, the overall rate of critical preanalytical errors was 0.328%, with a Six Sigma value of 4.25. And although the most common cause of the error was clotted sample (32.7%; sigma: 4.375) similar to the present study, differently the error rate was lower and the sigma level was within the acceptable range. In the study of Mukhopadhyay et al. [10] using the Six Sigma method to evaluate the reasons for preanalytical rejection for two months, the overall rate of critical preanalytical errors was 2.11%, with a Six Sigma value of 3.6. These values were found close to the current study, but the duration of this study was shorter.

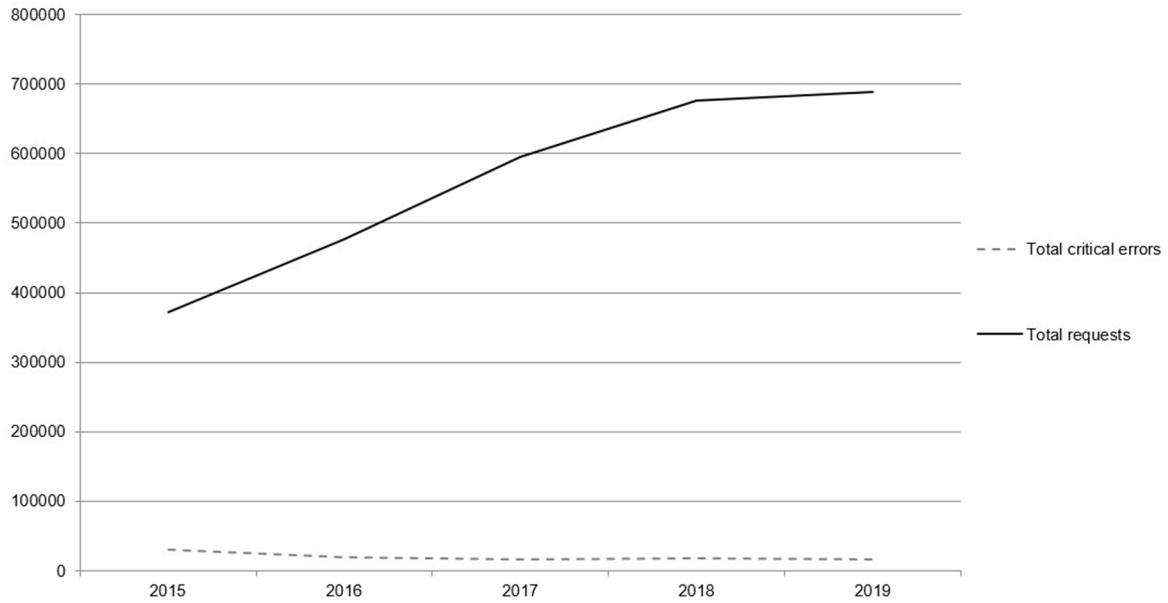

Figure 2. The alteration of total critical error and the number of total requests by years

Lay et al. [11] and Guimaraes et al. [12] evaluated the preanalytical errors and found that the overall rate of critical preanalytical errors was 2.7% and 0.57%, respectively, and the most common reasons for rejection were clotted and insufficient samples (percentages of clotted samples were 55.8% and 43.8%; insufficient samples were 29.3% and 24%, respectively). The six sigma method was not used in these two studies, and when compared with the present study according to the total critical error frequency rates, it was observed that the error rate observed in the present study (1.91%) was between those in these two studies. In addition, the most common causes of errors were found to be similar to the present study. There are many other studies in the literature that evaluated preanalytical phase performance and found that clotted and insufficient samples are common causes of rejection [13, 14, 15, 16, 17, 18]. While the total critical preanalytical error rate in the current study was similar to some studies in the literature, it was higher than most of them. In the current study, while the annual sigma values calculated separately for each of the preanalytical errors were 4 or over 4, the total error sigma level was below 4. Therefore, with this study, it was revealed that our laboratory should take regulatory and preventive actions to reduce the total error sigma rate and increase the sigma level to an acceptable level.

Pareto's chart showed that the two most common reasons for preanalytic rejection were caused by errors during phlebotomy. Clotted sample, which is the most common reason for rejection, may be caused by the excessive ratio of blood to anticoagulant in the tube and not mixing blood with anticoagulant sufficiently. This error may have been caused by the fact that the blood sample was not collected at the proper level specified on the tube during phlebotomy and then was not mixed properly. Clotted samples are not suitable for analysis as they cause inaccurate and incomplete laboratory results and cause clogging of analyzer probes. The second most common reason for preanalytical rejection in our laboratory was insufficient samples. Blood collection is accurately standardized with vacuum tubes with defined blood levels. However, in units such as neonatal, intensive care, and oncology, adequate blood can not be collected due to the incompatibility of the vascular structure of the patients. In addition, insufficient samples may be encountered due to the lack of knowledge and experience of the phlebotomists and nursing staff performing the phlebotomy procedure. An insufficient sample is not suitable for analysis because the amount of blood required for the analyzer cannot be provided.

To prevent all these errors caused mainly by the phlebotomy process and to improve the total quality management; Periodic training has been planned for technicians, nursing staff, interns, and doctors on phlebotomy, sample collection, and transportation. And also routine controls of responsible personnel were tightened during the period from blood sampling to entering the laboratory. After all development activities, the "Control" step will be carried out as the last step of the DMAIC cycle in the coming years.

In the present study, "Defining, Measuring, Analysing" steps of Six Sigma were performed to evaluate the preanalytical errors in the clinical biochemical laboratory over five years, and as the " Improving " step of Six Sigma, solutions suggestions for the most common errors were discussed according to Pareto principle. In the context of laboratory quality management, applying Pareto principle enables the identification of the most frequent or severe errors, leading to a better allocation of resources for corrective and preventive actions.

In conclusion, the Six Sigma method and the Pareto principle are effective and practical statistical approaches to solving problems, and continuous improvement should be a laboratory management strategy to make the processes more efficient and more effective.

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ORCID and Author contribution: ***S.B.O. (0000-0002-3427-9893); F.H. (0000-0002-6979-9469).*** All authors contributed to the study conception/design, material preparation, data collection and analysis. All authors read and approved the final manuscript.

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Acta Medica Alanya

Research Article

Araştırma

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Lack of Association Between *FNDC5* Gene Polymorphisms, Serum Irisin Levels and Allergic Rhinitis

FNDC5 Geni Polimorfizmleri, Serum İrisin Düzeyleri ve Alerjik Rinit Arasındaki İlişkinin Yokluğu

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ABSTRACT

Aim: Allergic rhinitis (AR) is an inflammatory nasal mucosa disease caused by type 1 immunoglobulin E-mediated reactions to allergen exposure. Irisin is a hormone released by skeletal muscles in response to exercise. There are studies that demonstrate the relationship of irisin with inflammation. We aimed to investigate the potential association between irisin coding fibronectin type III domain 5 *FNDC5* gene polymorphisms, serum irisin levels, and AR.

Methods: A case-control study was designed, involving 100 AR patients and 100 healthy controls. Genotyping of rs726344 and rs1746661 SNPs within the *FNDC5* gene was performed using PCR-RFLP method. Serum irisin levels were measured using ELISA.

Results: Genotyping of rs726344 SNP in patients revealed 90% GG and 10% GA genotypes, while in controls, it was 94% GG and 6% GA. The AA genotype was not detected in any case. For rs1746661 SNP, patients had 57% GG, 39% GT, and 4% TT genotypes, while controls had 58% GG, 36% GT, and 6% TT genotypes. No significant difference was found in rs726344 and rs1746661 SNPs between the patients and the control group. Serum irisin levels were 406.3±56.09 ng/ml in patients and 354.3±46.06 ng/ml in controls with no significant difference.

Conclusion: This is the first study aiming to investigate the relationship between the irisin protein, its encoding gene, and AR. No significant association was identified between *FNDC5* gene polymorphisms, serum irisin levels, and allergic rhinitis. While these findings suggest a limited role of these factors in AR, further studies are needed for more comprehensive understanding of the irisin-AR relationship.

Key Words: Allergic Rhinitis, Irisin, *FNDC5*, SNP, Genotyping

ÖZET

Amaç: Alerjik rinit (AR), alerjen maruziyetine bağlı olarak tip 1 immünoglobulin E-aracılı reaksiyonlarla oluşan bir enflamatuar burun mukozası hastalığıdır. İrisin, egzersize yanıt olarak iskelet kasları tarafından salınan bir hormondur. İrisin ile inflamasyon ilişkisini gösteren çalışmalar bulunmaktadır. Bu çalışmada, irisin kodlayan fibronektin tip III domain 5 *FNDC5* gen polimorfizmleri, serum irisin seviyeleri ve AR arasındaki potansiyel ilişkiyi araştırmayı amaçladık.

Yöntem: Çalışma, 100 AR hastası ve 100 sağlıklı kontrol bireyin yer aldığı bir vaka-kontrol çalışması şeklinde dizayn edilmiştir. *FNDC5* geninde bulunan rs726344 ve rs1746661 SNP'lerin genotipleme analizleri PCR-RFLP yöntemiyle gerçekleştirilmiştir. Serum irisin seviyeleri ELISA yöntemi ile ölçülmüştür.

Bulgular: Hasta grubunda rs726344 SNP genotipleme sonuçlarına göre, %90 GG ve %10 GA genotipleri saptanırken, kontrol grubunda sırasıyla %94 GG ve %6 GA genotipleri belirlendi. AA genotipi hiçbir olguda saptanmadı. rs1746661 SNP için ise hastalarda %57 GG, %39 GT ve %4 TT genotipleri, kontrollerde ise sırasıyla %58 GG, %36 GT ve %6 TT genotipleri saptandı. Hastalar ile kontroller arasında rs726344 ve rs1746661 SNP'lerinde genotipik farklılık saptanmadı. Serum irisin seviyesi, hastalarda 406.3±56.09 ng/ml iken, kontrollerde 354.3±46.06 ng/ml olarak bulundu ve aralarında anlamlı farklılık görülmedi.

Sonuç: Bu çalışma, irisin proteininin, kodlayıcı geni ve AR arasındaki ilişkiyi araştırmayı amaçlayan ilk çalışmadır. *FNDC5* gen polimorfizmleri, serum irisin seviyeleri ve AR arasında anlamlı bir ilişki saptanmamıştır. Bu bulgular, bu faktörlerin AR gelişiminde sınırlı bir rolünü göstermekle birlikte, irisin-AR ilişkisinin anlaşılması için ileri çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: Alerjik Rinit, Irisin, *FNDC5*, SNP, Genotipleme

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Introduction

In recent years, a continuous increase has been observed in the incidence of allergic rhinitis (AR) (OMIM #607154), a condition which is characterized by symptoms such as nasal congestion, sneezing attacks, and nasal discharge. For this reason, AR is emerging as an important health concern and challenge in modern medicine [1]. AR does not only affect an individual's social life but also results in substantial job loss and significant healthcare costs. The pathogenesis of AR involves nasal mucosal inflammation mediated by a type 1 immunoglobulin E (IgE)-triggered response upon allergen exposure [2]. The prevalence of AR is on the rise, with a global impact affecting approximately 10% to 25% of the population [3]. According to a study conducted in Turkey encompassing 9017 individuals from seven regions, the prevalence of AR was found to be 36.7% [3]. Genetic factors play a pivotal role in the etiology of AR. Although numerous single nucleotide polymorphisms (SNPs) potentially associated with AR have been identified, primarily in genes encoding cytokines and receptors, only a minority of these have demonstrated consistent validity in genetic studies, underscoring the complexity of the genetic landscape [4-7]. The genes contributing to susceptibility to AR have not been characterized to the extent of those associated with asthma and its correlated phenotypes [4].

Irisin, a myokine released from skeletal muscles during regular exercise, emerges as a key player in protecting individuals from metabolic disorders. Functioning as an autocrine, paracrine, and endocrine hormone, irisin stands out as a thermogenic protein capable of converting white adipose tissue into energy-expending brown adipose tissue. Its close associations lie with diseases such as type 2 diabetes, obesity, metabolic syndrome, and osteoporosis [8].

Furthermore, irisin is known to possess anti-inflammatory effects and has been shown to reduce systemic inflammation through various studies [9]. In a knockout mouse model of the fibronectin type III domain 5 (*Fndc5*) gene, which encodes irisin, metabolic parameters deteriorated alongside increased levels of pro-inflammatory cytokines (IL-6 and TNF-alpha) in the serum [8]. Conversely, diminished serum irisin levels have been observed in individuals with acute or chronic inflammation [10]. As a relatively recently discovered hormone, irisin is believed to play a role in development of the AR condition, particularly stemming from its association with systemic inflammation. Our research aims to investigate the relationship between genotypes of the rs726344 and rs1746661 SNPs within the *FNDC5* gene, serum irisin protein levels, and the development and clinical manifestations of AR in both AR patients and healthy control individuals.

Material and Methods

Study Group

The curent control-case study included a patient group of 100 adult individuals aged 18-65 years diagnosed with allergic rhinitis (AR) who presented to the Ear, Nose, and Throat (ENT) Diseases outpatient clinic of Alanya Alaaddin Keykubat University Alanya Education and Research Hospital, and a control group of 100 healthy adult individuals aged 18-65 years. Informed consent forms, signed by all participants, were obtained for the study.

The patient group consisted of Turkish subjects with isolated AR diagnosis and no chronic illnesses. Patients with a history of malignant disease, pregnancy, steroid use, obesity, diabetes, and signs of metabolic syndrome were excluded from the study. Total Immunoglobulin E (IgE) levels, duration of diagnosis, AR type (intermittent, persistent), and disease severity (mild, moderate, severe) were determined for the patients. Elevated serum total IgE levels were identified using a cutoff point of 150 IU/mL [11]. Patients were categorized into two groups based on total IgE levels: those with levels ≤ 150 IU/ml and those with levels > 150 IU/ml. The control group comprised healthy individuals with no previous diagnosis of AR.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis

Genomic DNA isolations were performed using Roche High Pure PCR Template Preparation Kit Version 20 (Roche Diagnostics GmbH, Mannheim, Germany) from peripheral blood samples collected in K3 EDTA tubes of both patients and healthy individuals. Following isolation, DNA concentrations and purities were determined by measuring absorbances at wavelengths of 260 nm and 280 nm using Biotek Synergy H1 Multimode Reader Take 3 (BioTek Instruments, Inc.). The gDNA samples were stored at -20°C.

The identification of two intronic SNPs, rs726344 (G>A) and rs1746661 (G>T), within the *FNDC5* gene in both patient and control subjects was carried out using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Initially, relevant gene regions were amplified using Polymerase Chain Reaction (PCR) with the gDNA of patients and controls. PCR mixture was prepared as described below: 0.4 µmol/L of each primer, 2.5 mmol/L $MgCl_2$, 0.8 mmol/L dNTP mix, 0.05 unit/L Taq DNA polymerase (Thermo Scientific), 0.01 pmol/ml gDNA template, and 1x Taq Buffer were added into PCR grade H_2O adjust total volume to 25 µl. The oligonucleotide primer sequences and PCR conditions for amplification were as follows: For the amplification of the SNP region with ID number rs726344, forward primer: 5'-CAGTGACTTCCCCTGAGCTT-3', reverse primer: 5'-CG-

ACAGTTCTGGGAAACAGA-3'; for the amplification of the SNP with ID number rs1746661, forward primer: 5'-TGGAGAGAGTTATGTAGGGGACA-3', reverse primer: 5'-CTTCCGCAGGCTTTATTCTG-3'. The PCR conditions for both regions were set as follows: initial denaturation at 95°C for 4 minutes, denaturation at 95°C for 45 seconds, annealing at 62°C for 30 seconds, extension at 72°C for 45 seconds, with 40 cycles, and a final extension at 72°C for 7 minutes.

Hpy188I restriction enzyme (New England Biolabs Ltd, UK) was used for genotyping the rs726344 SNP region, and HhaI restriction enzyme (New England Biolabs Ltd, UK) was employed for rs1746661 SNP. Reaction mixtures were prepared following the manufacturer's instructions and incubated overnight at 37°C. After digestion, the cleavage products were resolved on 3.0% (w/v) agarose gel and gels were run at 110 Volt then imaged using G:BOX Chemi XRQ gel documentation system (Syngene, Cambridge, UK). For rs726344, samples were identified as homozygous wild type if three PCR bands were observed at 132 base pair (bp), 92 bp and, 23 bp, homozygous variant genotype, if two PCR bands were observed at 224 bp and 23 bp, heterozygous variant genotype, if four PCR bands were observed at 224 bp, 132 bp, 92 bp, and 23 bp. For the rs1746661, samples were identified as homozygous wild type if a single but actually two PCR bands were present at 125 bp and 123 bp (seem like a single band due to their very close lengths), homozygous variant genotype, if a single PCR band was present at 248 bp, heterozygous variant genotype, if two PCR bands were observed at 248 bp and 125 bp (with 123 bp).

ELISA

The irisin protein levels of serum samples from 100 patients and 100 controls were determined using the enzyme-linked immunosorbent assay (ELISA) method. Blood samples of 5 mL were collected from both patient and control groups into gel tubes and allowed to clot at room temperature. After clotting, the samples were subjected to centrifugation at 2000 rpm for 10 minutes using a refrigerated centrifuge. Following centrifugation, serum samples were divided into portions in 1 mL Eppendorf tubes and stored at -20°C until the day of analysis. On the analysis day, serum samples belonging to both patient and control groups were thawed by taking them out from the deep freezer and allowing them to thaw at room temperature. The levels of serum irisin for both the patient and control groups were determined using the Human Protein Irisin (IS) kit (AFG Bioscience, China) according to the manufacturer's instructions.

Statistical Analysis

The frequencies of categorical variables between patients and controls were compared using the Chi-square test (Fisher's exact test), while continuous variables were compared using Student's t-test or Mann-Whitney test (for non-parametric variables). Shapiro-Wilk test was performed for assessing normality. Allelic and genotypic findings between controls and AR patients for the SNPs were compared using 2x2 contingency tables and two-sided Fisher exact test. The strength of the association was estimated using odds ratio (OR) and 95% confidence intervals (CI). For the comparison of genetic findings and irisin protein levels, the Kruskal-Wallis test was employed. The assessment of Hardy-Weinberg equilibrium was performed using an online calculation tool (https://www.cog-genomics.org/software/stats), while other statistical calculations were carried out using GraphPad Prism 7 software.

Results

Demographic and Clinical Findings

The mean age of the patient group was found to be 38.53 ± 13.01 years (Male n: 40, 40%, Female n: 60, 60%). In the control group, the mean age was 31.17 ± 11.9 years, and the gender distribution was consistent with that of the patient group. The duration of AR diagnosis in patients ranged from 18 to 72 months, with a mean of 29.36 months. Among the patients, 83 had intermittent AR, and 17 were diagnosed with persistent AR. Based on classification, 69 patients had mild AR, 21 had moderate AR, and 10 were classified as severe AR. The patients' total IgE levels were determined to be 169 ± 16.77 IU/ml (mean ± standard error of mean) with a range of 8 to 902.

Genetic Findings

The genotyping analysis of rs726344 SNP revealed that among the participants, the GG genotype was identified in 90 patients (90%) and 94 controls (94%), while the GA genotype was observed in 10 patients (10%) and 6 controls (6%). The AA genotype was not detected among both patients and controls. Regarding the genotyping of rs1746661 SNP, the results showed that among the participants, the GG genotype was determined in 57 patients (57%) and 58 controls (58%), the GT genotype was found in 39 patients (39%) and 36 controls (36%), and the TT genotype was present in 4 patients (4%) and 6 controls (6%) (Table 1). The genotypic findings of the representative cases for rs726344 and rs1746661 are shown in Figures 1 and 2, respectively.

According to the statistical analysis results, no significant difference in genotypic distribution was observed between the patient and control groups for the rs726344 and rs1746661 polymorphisms (Table 1).

According to the Hardy-Weinberg equilibrium analysis results, no significant deviation from Hardy-Weinberg equilibrium was observed for both SNPs (p=0.5781)

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Table 1. *FNDC5* SNP genotypes and allele frequencies in patient and control groups

SNP		n (%) Patients with AR	n (%) Control	OR** (95% CI)	p*
rs726344	*Genotype*	n= 100	n= 100		
	GG	90 (90%)	94 (94%)	Ref.	Ref
	GA	10 (10%)	6 (6%)	0.5745 (0.2006 to 1.518)	0.4353
	AA	0	0	-	-
	Allele	n=200	n=200		
	G	190 (95%)	194 (97%)	Ref.	Ref.
	A	10 (5%)	6 (3%)	0.5876 (0.2122 to 1.599)	0.5876
rs1746661	*Genotype*	n= 100	n= 100		
	GG	57 (57%)	58 (58%)	Ref.	Ref.
	GT	39 (39%)	36 (36%)	0.9072 (0.5088 to 1.609)	0.7682
	TT	4 (4%)	6 (6%)	1.474 (0.3935 to 4.812)	0.7443
	GT+TT	43 (43%)	42 (42%)	0.9599 (0.5395 to 1.705)	>0.9999
	Allele	n=200	n=200		
	G	153 (76.5%)	152 (76%)	Ref.	Ref.
	T	47 (23.5%)	48 (24%)	1.028 (0.6408 to 1.617)	>0.9999

*Fisher chi-square analysis.
**OR, Odds ratio.
CI, confidence interval; AR, allergic rhinitis; Ref, reference.
Genotype comparisons between patient and control groups were conducted using 2x2 contingency tables, with the format GG vs GA for rs726344, GG vs GT, GG vs TT, and GG vs GT+TT for rs1746661. Allele comparisons were performed using 2x2 contingency tables, with the format G vs A for rs726344 and G vs T for rs1746661.

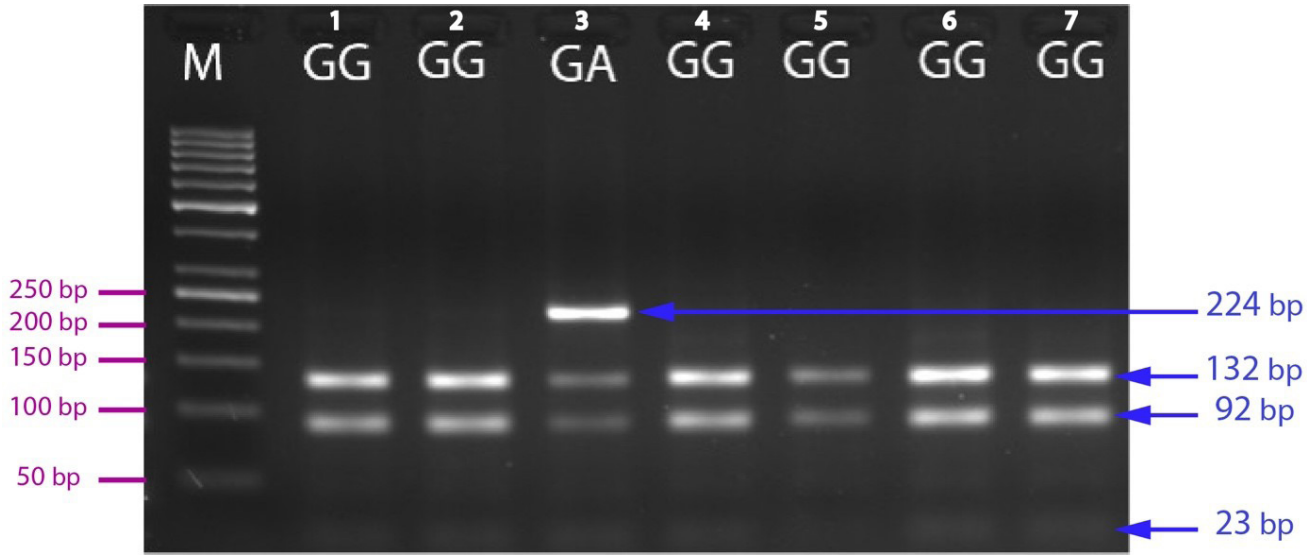

Figure 1. Gel electrophoresis of PCR-RFLP products for representative blood samples for the *FNDC5* gene variant rs726344 (G>A): M, 50 bp DNA molecular weight marker (Thermo Scientific), lanes 1, 2, 4, 5, 6 and 7, GG homozygous wild type; lane 3, GA heterozygous mutant.

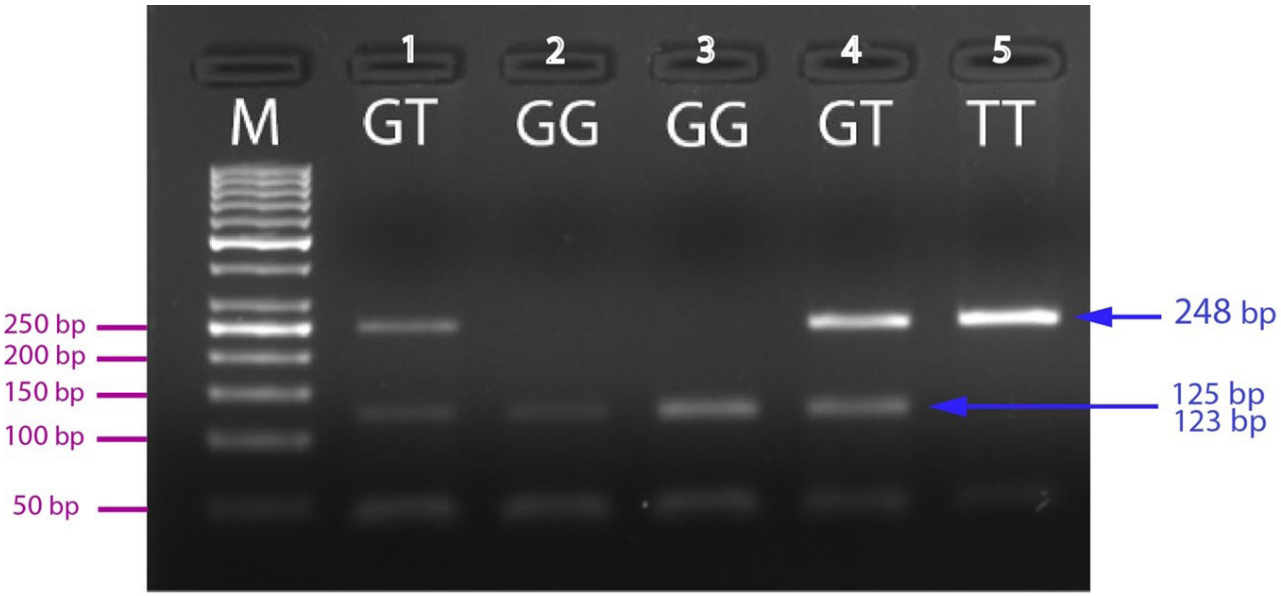

Figure 2. Gel electrophoresis of PCR-RFLP products for representative blood samples for the *FNDC5* gene variant rs1746661 (G>T): M, 50 bp DNA molecular weight marker (Thermo Scientific); lanes 1 and 4, GT heterozygous mutant; lanes 2 and 3, GG homozygous wild type; lane 5, homozygous mutant.

When genotype findings were compared according to the clinical and biochemical data (type of AR, disease severity and IgE levels) of the patients, no statistically significant difference was found for rs726344 polymorphism (Table 2) and rs1746661 polymorphism (Table 3).

Serum Irisin Levels

Serum irisin level in the patient group was 406.3±56.09 ng/ml and 354.3±46.06 ng/ml in the control group. There was no statistically significant difference between the serum irisin levels of the patient and control groups (p=0.757) (Figure 3). Additionally, no statistical difference was found between serum irisin levels of patients and controls with different genotypes for each polymorphism (p>0.05).

Discussion

AR, triggered by IgE-mediated reactions to inhaled allergens, stands as one of the most prevalent chronic disorders globally. In recent years, the incidence of AR has shown a dramatic rise, notably in Europe and Asia, thus elevating it to a concern of global proportions. AR is often accompanied by conditions such as asthma and conjunctivitis. Risk factors encompass inhaled allergens, occupational allergens, and genetic factors. While the contribution of genetic factors to the development of the disease is recognized, AR is noted for its genetic heterogeneity [12]. Given interpopulation variances, further genetic studies are required. The etiopathogenesis of AR remains incompletely elucidated.

Irisin, secreted by muscle tissue, is a hormone formed through cleavage of the membrane protein FNDC5. It is regarded as a mediator of the metabolic improvements induced by exercise. Irisin emerges as a prospective therapeutic target for metabolic and non-metabolic disorders [13].

We aimed to investigate the potential association between *FNDC5* gene polymorphisms, serum irisin levels, and AR. No statistically significant difference was found between serum irisin levels of the patient and control groups. Based on the genotyping of the rs726344 and rs1746661 SNPs within the *FNDC5* gene, no genotypic differences were found to be significant between the patient and control groups. AA genotype of rs726344 was not found in our cohort. It could be due to ethnic differences or the relatively small size of our cohort. Up to date, the associations of these variations have been explored in relation to conditions such as preterm birth, myocardial infarction, obesity, and type 2 diabetes [14-17]. However, their relation to a disease characterized by allergy has not been assessed. Given its close metabolic ties, particularly with metabolic diseases, it appears that irisin, as indicated by our current data, does not contribute to AR pathogenesis.

Over 40% of patients diagnosed with AR exhibit concomitant asthma, whereas individuals afflicted with asthma demonstrate a notably elevated prevalence of concomitant AR [18-19]. Therefore, evaluating asthma studies would be beneficial. Though no study has yet explored the relationship between AR and irisin, data regarding iri-

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Table 2. Comparison of clinical findings in patients with different rs726344 polymorphism genotypes

		GG (n)	GA (n)	OR** (95% CI)	p*
AR Type	**Intermittent**	73	10	0.000 (0.000 to 1.684)	0.2042
	Persistent	17	0		
Severity of AR	**Mild**	62	7	0.9490 (0.2522 to 3.894)	>0.9999
	Moderate + Severe	28	3		
IgE Level (UI/ml)	**≥151**	32	3	1.287 (0.3166 to 4.818)	>0.9999
	≤150	58	7		

*Fisher chi-square analysis.
**OR, Odds ratio; CI, confidence interval; AR, allergic rhinitis.
Genotype comparisons between groups were calculated using a 2x2 probability table as GG vs GA.

Table 3. Comparison of clinical findings in patients with different rs1746661polymorphism genotypes

		GG (n)	GT+TT (n)	OR** (95% CI)	p*
AR Type	**Intermittent**	50	33	2.165 (0.7872 to 5.679)	0.1829
	Persistent	7	10		
Severity of AR	**Mild**	37	32	0.6359 (0.2680 to 1.504)	0.3842
	Moderate + Severe	20	11		
IgE Level (UI/ml)	**≥151**	22	17	0.9613 (0.4462 to 2.110)	>0.9999
	≤150	35	26		

*Fisher chi-square analysis.
**OR, Odds ratio; CI, confidence interval; AR, allergic rhinitis.
Genotype comparisons between groups were calculated using a 2x2 probability table as GG vs GT+TT

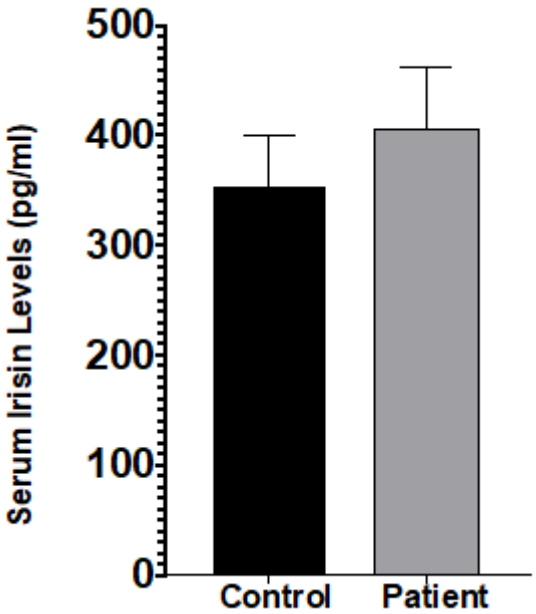

Figure 3. Serum irisin levels in patient and control groups

sin's role in asthma development are intriguing. In a study by Bulut et al. in 2019, immunomodulatory cytokine and irisin concentrations were evaluated in allergic asthmatic patients receiving omalizumab treatment, along with assessing their potential effects on cell surface markers. It was found that irisin and IL-1β concentrations were significantly higher in the serum of severe persistent asthma patients compared to the control group [20]. Asthma, a common chronic airway inflammatory disease, is characterized by a Th2-mediated immune response and excessive expression of inflammatory factors. There are few reports on the relationship between irisin and asthmatic lung injury. In 2022, Sun et al. performed a study wherein irisin was shown to improve overall condition and reduce lung tissue damage in an ovalbumin (OVA)-induced asthma mouse model. Additionally, irisin significantly suppressed inflammation factors in bronchoalveolar lavage fluid and serum, alongside considerably reducing OVA-induced PI3K/AKT phosphorylation. These findings suggest that irisin is an effective active molecule for asthma treatment and suppresses PI3K/AKT pathway phosphorylation in asthma progression and inflammatory factors [21]. Anxiety disorders and depressive states can accompany asthma. Szilasi et al. examined serum irisin levels in asthmatic patients with stress factors. They reported a decrease in irisin levels as stress levels increased [22]. The anti-inflammatory effects of irisin have also been demonstrated in-vitro. A statistically significant reduction in TLR4 protein levels was determined in irisin-treated cells [23].

It is known that physical exercise increases the secretion of the irisin [8]. The relationship between physical exercise-induced irisin secretion and AR remains complex, with varying results from different studies and no clear consensus on its impact on AR symptoms and IgE secretion. Aldred et al. found in 2010 that high-intensity exercise increased IgE secretion in AR patients [24]. Conversely, Tongtako et al. reported that acute and moderate-intensity exercise reduced AR symptoms [25]. The combination of exercise training alone and exercise training coupled with vitamin C supplementation has also been found to reduce rhinitis symptoms in AR patients [25]. These findings are interesting but further studies need to be done to clarify this topic.

Limitations

A limitation of our study is the relatively small size of the study group, which may have impacted the statistical power of our findings. The limited sample size might restrict the generalizability of our results to larger populations. Additionally, our study focused only on specific SNPs. While these SNPs were selected based on their potential relevance to our research question, our study did not encompass the entire sequencing of the *FNDC5* gene. Therefore, it is possible that other DNA variants within the *FNDC5* gene, which were not examined in this study, could play a role in the observed associations or outcomes related to AR. Further investigations involving a larger sample size, as well as a comprehensive analysis of the entire *FNDC5* gene, could provide a more comprehensive understanding of the genetic and molecular mechanisms underlying the relationship between irisin and AR.

Conclusion

Our study is the first to investigate the potential association between *FNDC5* gene polymorphisms, serum irisin levels, and AR. We found no significant association between the studied SNPs, serum irisin levels, and AR, suggesting that these specific genetic variations and irisin concentrations may not play a substantial role in the development of the condition. The findings of current study are preliminary data, the relationship between the irisin pathway and AR development needs to be clarified with further studies. The identification of alternative biological pathways related to AR and allergic diseases is crucial for both a comprehensive understanding of the etiopathogenesis of the disease and developing novel treatment protocols.

Conflict of Interest: The authors declare no conflict of interest related to this article.

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Ethics Committee Approval: The study was approved by the Alanya Alaaddin Keykubat University Clinical Research Ethics Committee on 24/03/2021 with decision number 06/09.

ORCID and Author contribution: ***D.D.E. (0000-0002-5887-3141):*** Concept and Design, Data collection, Literature search. Analysis and Interpretation, Manuscript Writing, Critical Review Final approval. ***H.G. (0000-0001-8653-0544):*** Data collection, Analysis and Interpretation, Manuscript Writing, Final approval.

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Acta Medica Alanya

Research Article

Araştırma

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Clinical Detection of Presence and Absence of Palmaris Longus Tendon in Somali Population

Somali Popülasyonunda Palmaris Longus Tendonunun Varlığı ve Yokluğunun Klinik Tespiti

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ABSTRACT

Aim: The aim of this study was to investigate whether the frequency of palmaris longus (PL) absence in Somali population differed from other populations in Africa and the rest of the world and to evaluate its association with sex and side of the limb involved.

Methods: Totally 1,000 participants (713 males and 287 females; age range: 7 to 80 years) were tested for the absence of PL tendon using Schaffer's test. In those with a negative Schaffer's test result, Thompson's test, Mishra's test I, Mishra's test II, and Pushpakumar's "two-finger sign" method were used to confirm its absence.

Results: Absence of PL tendon was found in 95 (9.5%) participants. The PL muscle was absent bilaterally in 42 (4.4%) and unilaterally in 53 (5.5%) participants. Unilateral absence of PL was higher in the right hand (3.0%) ($p>0.05$). Agenesis of the right side in males was significantly correlated with the left side in males ($rs=0.556$, $p<0.01$). Right agenesis in females was significantly correlated with its left side ($rs=0.625$, $p<0.01$). The incidence of right and left agenesis in females was more strongly correlated than that in males. Age was significantly correlated with left agenesis ($rs=0.154$, $p<0.01$) and right agenesis ($rs=0.145$, $p<0.05$) in females.

Conclusion: The prevalence of PL agenesis in the Somali population is lower than the reported incidence of 15%, but more significantly higher than some of the other African populations. These findings may be helpful, particularly for the surgeons in the region, for orthopedic and reconstructive surgeries using PL grafting.

Key Words: Palmaris longus, Tendon grafts, Variation, Somalia

ÖZET

Amaç: Bu çalışmada Somali popülasyonunda palmaris longus (PL) yokluğunun sıklığının Afrika ve dünyanın diğer ülkelerindeki popülasyonlardan farklı olup olmadığı araştırıldı ve bu durumun cinsiyet ve tutulan ekstremite tarafı ile olan ilişkisi incelendi.

Yöntem: Toplam 1000 katılımcı (713 erkek ve 287 kadın; yaş aralığı: 7-80 yıl) Schaffer testi ile PL tendon yokluğu açısından değerlendirildi. Schaffer test sonucu negatif olanlara tanıyı doğrulamak amacıyla Thompson testi, Mishra testi I, Mishra testi II ve Pushpakumar "iki parmak işareti" yöntemi yapıldı.

Bulgular: Katılımcıların 95'inde (%9.5) PL tendonu bulunmuyordu. Katılımcıların 42'sinde (%4.4) iki taraflı ve 53'ünde (%5.5) tek taraflı olarak PL kası yoktu. Tek taraflı PL yokluğu sağ elde daha yüksek oranda izlendi (%3.0) ($p>0.05$). Erkeklerde sağ taraflı agenezi, erkeklerde sol taraflı agenezi ile anlamlı düzeyde ilişkili bulundu ($rs=0.556$, $p<0.01$). Kadınlarda sağ taraflı agenezi, sol taraflı agenezi ile anlamlı düzeyde ilişkili bulundu ($rs=0.625$, $p<0.01$). Kadınlarda sağ ve sol taraflı agenezi insidansı, erkeklerinkine kıyasla daha yüksek düzeyde ilişkili idi. Yaş, kadınlarda sol taraflı agenezi ($rs=0.154$, $p<0.01$) ve sağ taraflı agenezi ($rs=0.145$, $p<0.05$) ile anlamlı düzeyde ilişkili bulundu.

Sonuç: Somali popülasyonunda PL agenezi prevalansı bildirilen %15'lik insidanstan daha düşük olup, diğer Afrika popülasyonlarının bazılarından anlamlı düzeyde daha yüksektir. Bu bulgular, özellikle bölgedeki cerrahlar için PL greftleme ile yapılan ortopedi ve rekonstrüktif ameliyatları açısından yararlı olabilir.

Anahtar Kelimeler: Palmaris longus, Tendon greftleri, Varyasyon, Somali

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Introduction

Palmaris longus (PL) is a superficial muscle of the forearm, mainly tendinous except for a small portion of its length, which is composed of a muscle belly [1]. It originates from the medial epicondyle of the humerus and, as a long and thin tendon, it lies medial to the flexor carpi radialis [1,2]. Its long tendon passes above the flexor retinaculum and attaches to the connective tissue of the palmar aponeurosis [1,2]. Its primary function is the augmentation of the palmar fascia that serves as an accessory flexor of the wrist and thumb abductor [3]. Due to the proximity to the surface, as a long muscle and clear tendon, PL is simple to identify and easily accessible [4]. Of note, PL is considered a non-essential muscle, as its absence does not severely affect the functions of the wrist and hands [1]. The PL tendon is one of the most commonly used donors for autologous tendon graft or tendon transfer [5]. Therefore, identifying the presence of a PL tendon is critical for reconstructive hand surgeries to minimize potential complications. To date, several physical examination maneuvers have been described to detect the PL tendon [5].

The PL tendon is considered to be congenitally absent in approximately 15% of the population [6]. However, the reported incidence of agenesis varies extensively by ethnicity, from 0.6% in Korea [7] to 64% in Turkey [8]. The PL has been studied considerably using various examination methods, and the prevalence of its absence varies significantly across different populations, 0.6 to 3.2% in Koreans, 1.02% in Ugandans, 3 to 4.2% in Japanese, 3.3 to 17.2% in Brazilians, 4.5 to 22.5% in African Americans, 4.5 to 55.2% in Egyptians, 3.8 to 5.6% in Chinese, 4.4 to 32.2% in Hungarians, 5.8 to 15.2% in Canadians, 6.7 to 25% in Nigerians, 6.1 to 11.7% in Malaysians, 11.6% in Czechs, 13.2 to 33.7% in Iranians, 12.8 to 18.3% in Iraqis, 11.7% in Ethiopians, 24% in North American Caucasians, 16.8% in UK, 16.7 to 27.9% in Saudis and Bahraini, and 14.5 to 64% in Turkish population [9,10]. In addition to the congenital absence of the PL, morphological variations have been identified. The tendon may be duplicated, reversed, bifid, or have an accessory muscle belly or divided to form an ulnar accessory slip distally [11].

In this present study, we aimed to determine the frequency of the absence of the PL in the Somali population and to compare it with relevant populations in Africa and the rest of the world. The second objective was to evaluate the association of PL agenesis with sex and the laterality of the limb.

Materials and Methods

Study Design and Study Population

This study was designed as a single-center, cross-sectional study. A total of 1,000 participants including 713 males and 287 females with an age range of 7 to 80 years were included. The participants consisted of patients visiting the Orthopedics and Traumatology Department of our hospital, hospital personnel from different areas, and soldiers from the TURKSOM military compound. Those a history of injury, surgery, scars on the wrist area, or any physical disabilities of their upper limbs were excluded from the study. All participants were informed about the nature of the study and a written informed consent was obtained on a voluntary basis. The study protocol was approved by the institutional Ethics Committee (No: 10909, Date: 07/04/2022). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Clinical Tests

All participants underwent physical examination of both wrists by an orthopedics specialist and a fifth-year (PGY5) orthopedics senior resident physician. The presence of PL tendons was examined using five assessment methods. The participants were initially asked to perform Schaeffer's test [12]; opposition of the thumb to the little finger and then flexed the wrist to assess the PL tendon (Figure 1A). If the tendon was unable to be visualized due to the inability to maneuver the technique, the additional tests of Thompson's [13] (Figure 1B), Mishra's test I and II [14] (Figure 1C and Figure 1D), and Pushpakumar's "two-finger sign" [15] (Figure 1E) were performed to confirm the absence of the tendon. If the participant had a positive result for any of the five tests, the presence of PL was considered. To regard to have an absence of a PL, the participant must have a negative test for all of the five tests (Figure 2). The Thompson's test involves flexion of the fingers to form a fist and flexion of the wrist with the thumb as opposed and flexed over the fingers. In the Mishra's test I, the participants were asked to abduct the thumb against resistance with the wrist slightly flexed. The Mishra's test II involves the abduction of the thumb against resistance and wrist flexion partially. In the Pushpakumar's "two-finger sign" method, the volunteers were made to extend the index and middle fingers fully with the wrist and other fingers flexed, followed by opposition and flexion of the thumb.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in number and frequency. The Chi-square test was used to identify the differences between the categorical variables. Since the data was non-normally distributed, the Spearman's rank order test was used for correlation analyses to assess the association of PL absence in both sexes unilaterally and bilaterally. A p value of <0.05 was considered statistically significant.

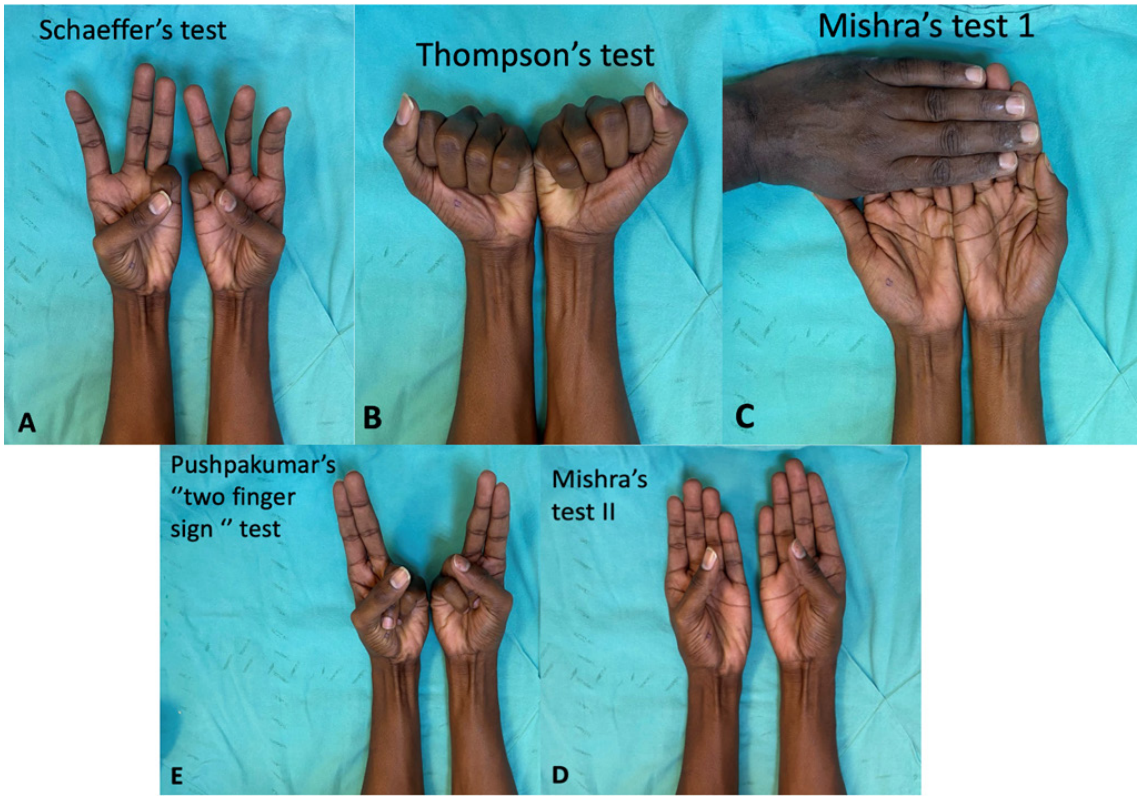

Figure 1. Presence of the palmaris longus tendon. A. Schaeffer's test; B. Thompson's test; C. Mishra's test I; D. Mishra's test II; E. Pushpakumar's "two-finger sign" test.

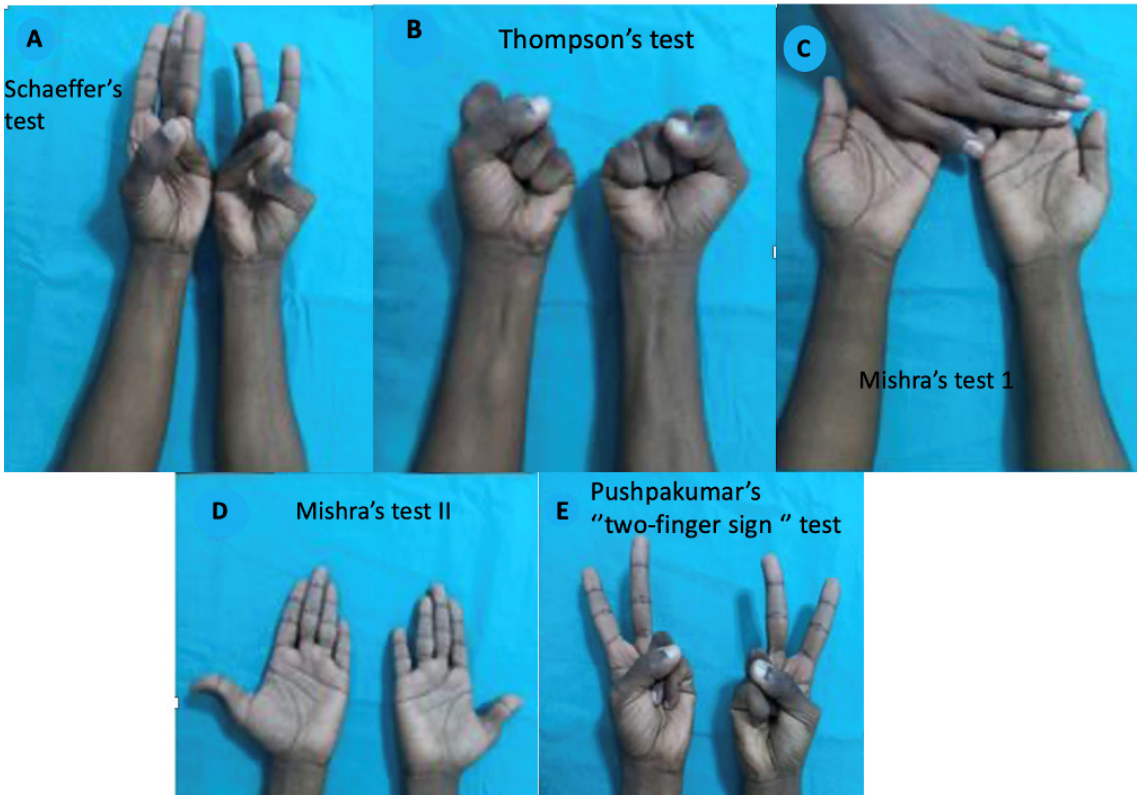

Figure 2. Absence of the palmaris longus tendon. A. Schaeffer's test; B. Thompson's test; C. Mishra's test I; D. Mishra's test II; E. Pushpakumar's "two-finger sign" test.

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Results

The overall PL absence was found in 95 (9.5%) participants (n=71 for males and n=24 for females). The PL muscle was absent bilaterally in 42 (4.4%) and unilaterally in 53 (5.5%) participants. There was no significant difference in the bilateral absence between the male (4.5%) and female (4.4%) participants (p>0.05). In addition, there was no significant difference in the unilateral absence between the male (6%) and female (4.4%) participants (p>0.05). Although the rate of unilateral absence of the PL was higher in the right hand (3.0%) than in the left hand (2.7%), it did not reach statistical significance (p>0.05). The rate PL absence in the right and left hands was higher in males than females, while there was no significant difference between two sexes (p>0.05, Table 1).

The Spearman's rank order tests revealed that the agenesis of the right side in males was significantly correlated with the left side in males (r_s=0.556, p<0.01). Similarly, right agenesis in females was significantly correlated with its left side (r_s=0.625, p<0.01). The incidence of right and left agenesis in females was more strongly correlated than that in males. Age was significantly correlated with left agenesis (r_s=0.154, p<0.01) and right agenesis (r_s=0.145, p<0.05) in females.

Discussion

In the present study, we attempted to investigate the prevalence of the absence of PL muscle in the Somali population. Our study results showed that the overall prevalence of PL absence was 9.5%. This ratio is comparable to the low prevalence of PL agenesis reported in African populations, Ghana (3.1%) [16], East Africa (4.4%) [17], Yoruba ethnic population in Nigeria (6.7%) [18], African Antiguan population (12.8%) [19], and the lowest number was reported in Zimbabwe (1.5%) [20]. On the other hand, a slightly higher prevalence of PL absence was identified in Ethiopian (15.3%) [21] and South African (19.6%) [22] populations. The lower rate of the absence of PL may be explained by the higher prevalence of manual labor in African populations. It is reasonable that manual labor anticipates more dynamic wrist motion and increasing instances in which the PL is brought into action. Manual labor also requires tensed palmar fascia for a strong grip, which may lead less occurrence of agenesis of the PL muscle [17,19]. This low frequency of absence of the PL in the Somali population supports the racial differences previously noted. This finding may be helpful for the surgeons working in this field regarding the probability of identifying the PL tendon for tendon grafts or various reconstructive procedures.

Although not statistically significant, our study in Somali population showed that the overall prevalence of bilateral and unilateral agenesis is more common in males than females. The prevalence of unilateral agenesis was higher than bilateral agenesis, and right agenesis occurred more commonly. These findings are consistent with studies in Uganda [17], Nigeria [18], and South Africa [22]. On the other hand, studies conducted in Turkey [9,10], African Antiguan [19], Zimbabwe [20], and South India [23] have shown that bilateral agenesis is more common in males than the females. These reports also emphasize that the left side agenesis was more significant than the right-side agenesis.

The main limitation to this study is the utilization of clinical tests to detect the presence of the PL tendon. This examination depends on the examiner compared to cadaveric

Table 1. Distribution of palmaris longus agenesis and its lateralization by sex

Sex	PLP n (%)	PLA n (%)	PLP n (%)	ULA n (%)	PLP n (%)	BLA n (%)	PLP n (%)	RULA n (%)	PLP n (%)	LULA n (%)
Female (n=287)	263 91.6%	24 8.4%	263 95.6%	12 4.4%	263 95.6%	12 4.4%	263 97.4%	7 2.6%	263 98.1%	5 1.9%
Male (n=713)	642 90.0%	71 10.0%	642 94.0%	41 6.0%	642 95.5%	30 4.5%	642 96.8%	21 3.2%	640 97.0%	20 3.0%
Total (n=1000)	905 90.5%	95 9.5%	905 94.5%	53 5.5%	905 95.6%	42 4.4%	905 97.0%	28 3.0%	903 97.3%	25 2.7%
Pearson's Chi-Square	0.606		1.008		0.005		0.218		0.986	
P value	0.436		0.315		0.946		0.641		0.321	

PLP: Palmaris longus present, PLA: Palmaris longus agenesis
ULA: Unilateral agenesis, BLA: Bilateral agenesis
RULA: Right unilateral agenesis, LULA: Left unilateral agenesis

Table 2. Correlation analysis of palmaris longus absence with age and sex

	Age	Female Right PL	Female Left PL	Male Right PL	Male Left PL
Age	1				
Female Right PL	0.145*	1			
Female Left PL	0.154**	0.625**	1		
Male Right PL	-0.007	-	-	1	
Male Left PL	-0.003	-	-	0.556**	1

*. Correlation significant at the 0.05 level (2-tailed).
**. Correlation significant at the 0.01 level (2-tailed).

studies where the presence of the PL can be determined by visualizing the actual muscle. However, the main strengths of this study include the use of various clinical tests to identify the presence of the PL, which increases the possibility that all the tendons crossing the wrist to be detected. The large sample size of this study is also another strength.

Conclusion

In conclusion, the prevalence of PL agenesis in the Somali population is lower than the reported incidence of 15%, but more significantly higher than some of the other African populations. These findings may be helpful, particularly for the surgeons in the region, for orthopedic and reconstructive surgeries using PL grafting.

Conflict of Interest: The authors declare that they have no potential conflict of interest regarding the investigation, authorship, and/or publication of this article.

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Research Article

Araştırma

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Relationship Between Fatigue, Cognitive Functions, Depression, and Disability in Multiple Sclerosis Patients

Multipl Sklerozlu Hastalarda; Yorgunluk, Kognitif Fonksiyonlar, Depresyon ve Özürlülük İlişkisi

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ABSTRACT

Aim: Multiple Sclerosis (MS) is a chronic, progressive, and neurodegenerative disorder of the central nervous system. It frequently leads to symptoms such as disability, cognitive impairment, fatigue, and depression. This study aims to examine the relationship between fatigue, cognitive functions, depression, and disability among individuals with MS.

Methods: Seventy-four MS patients were evaluated by using the Cognitive Failure Questionnaire (CFQ) battery. The Expanded Disability Status Scale (EDSS) was used in determining the level of disability, whereas the Fatigue Severity Scale (FSS) and the Beck Depression Inventory (BDI) were utilized in measuring the levels of fatigue and depression, respectively.

Results: Moderate positive correlations were found between EDSS and BDI (r1=0.342; p<0.001), between EDSS and FSS (r1=0.392; p<0.001), between CFQ and BDI (r1=0.451; p<0.001), between CFQ and FSS (r1=0.425; p<0.001), and between FSS and BDI (r1=0.424; p<0.001).

Conclusion: The results achieved in this study indicate that fatigue and depression increase as disability increases among Multiple Sclerosis patients and cognitive impairment is associated with both depression and fatigue.

Key Words: Multiple Sclerosis, Depression, Fatigue, Cognitive Failure

ÖZET

Amaç: Multipl skleroz (MS); kronik, ilerleyici ve nörodejeneratif bir merkezi sinir sistemi bozukluğudur. Multipl Skleroz; genellikle özürlülük, bilişsel yetersizlik, yorgunluk ve depresyon semptomlarına neden olur. Bu çalışmada amaç Multipl skleroz hastalarında bilişsel işlev, yorgunluk ve depresyonun özürlülük ile ilişkisini değerlendirmektir.

Yöntem: 74 MS hastası, Bilişsel Başarısızlık Anketi (CFQ) bataryası kullanılarak değerlendirildi. Engellilik düzeyini belirlemede Genişletilmiş Özürlülük Durumu Ölçeği (EDSS) kullanılırken, yorgunluk ve depresyon düzeylerini ölçmek için sırasıyla Yorgunluk Şiddeti Ölçeği (FSS) ve Beck Depresyon Envanteri (BDI) kullanıldı.

Bulgular: EDSS ile BDI arasında (r1=0.342; p<0.001), EDSS ile FSS arasında (r1=0.392; p<0.001), CFQ ile BDI arasında (r1=0.451; p<0.001), CFQ ile FSS arasında (r1=0.425; p<0.001) ve FSS ile BDI arasında (r1=0.424; p<0.001) orta düzeyde pozitif korelasyonlar bulundu.

Sonuç: Bu çalışmada elde edilen sonuçlar, Multipl Skleroz hastaları arasında engellilik arttıkça yorgunluk ve depresyonun arttığını, bilişsel bozulmanın ise hem depresyon hem de yorgunlukla ilişkili olduğunu göstermektedir.

Anahtar Kelimeler: Multipl Skleroz, Depresyon, Yorgunluk, Bilişsel Yetersizlik

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Introduction

Multiple sclerosis (MS) is a chronic, progressive, autoimmune, neurodegenerative disorder affecting the central nervous system, characterized by axonal loss and demyelination in its pathogenesis [1]. The prevalence of MS in Turkey varies by region and was reported to have an average prevalence of 47.9/100,000 [2]. MS leads to brain atrophy and the development of widespread lesions or plaques in the brain and spinal cord [3]. It can cause disability in different domains, such as motor, sensory, visual, balance-coordination, and cognitive impairments in the central nervous system [4]. The prevalence of cognitive decline among individuals with MS ranges between 43% and 73%, the lifetime diagnosis rate of major depression was reported to be approx. 50%, and the prevalence of fatigue, which is closely associated with depression, was reported to be between 50% and 90% [5–7]. This study aims to assess the relationship between cognitive function, fatigue, depression, and disability among MS patients.

Materials and Methods

This study included 74 patients aged between 18 and 55 and diagnosed with MS by the 2017 McDonald criteria, who presented to the Neurology Clinic of Afyonkarahisar Health Sciences University Faculty of Medicine Hospital between 10 December 2022 and 01 February 2023. Patients, who had additional neurological diseases that could affect clinical evaluation and/or neurological and/or psychiatric conditions that could impact cognitive functions, those using antipsychotic medication, individuals with a history of severe head trauma, those with mental retardation or learning difficulties, pregnant or lactating women, alcohol or substance abusers, individuals with MS relapse, and/or those who had received corticosteroid treatment in the four weeks before assessment were excluded from the study. Patients' age, gender, education level, socioeconomic status, disease duration, medication usage, MS disease type, medical history, and neurological examination results were recorded, and their Expanded Disability Status Scale (EDSS) scores were determined by assessing the functional systems. The present study was carried out after obtaining written consent of the patients, supervised by medical students from the 3rd year of medical school. The cognitive impairment scale, fatigue severity scale, and Beck Depression Inventory were used for assessment.

Scales

Cognitive Failures Questionnaire (CFQ): CFQ is a self-report questionnaire that measures perception, memory, and motor function failures. It consists of 25 items, and participants rate the items on a five-point scale ranging between "never" and "always." The response options are: (0) Never, (1) Very rarely, (2) Occasionally, (3) Fairly often, and (4) Very often. CFQ scores can range between 0 and 100; higher scores indicate a greater tendency towards cognitive failures. CFQ's validity and reliability have been established [8–12].

Expanded Disability Status Scale (EDSS): EDSS is a scale utilized in assessing neurological examination findings among MS patients. Various subfunctions are evaluated to determine disability. The scale ranges between 0 (no disability) and 10 (death due to MS), with 20 levels in total [13].

Fatigue Severity Scale (FSS): FSS is a fatigue assessment scale, validity and reliability of which were tested in the Turkish language [14]. In this 9-item self-administered scale, each item is scored between 1 and 7 points. A cutoff value of 4 or higher indicates pathological fatigue [15].

Beck Depression Inventory (BDI): BDI is used for depression screening among MS patients. It includes 21 items rated on a scale of 0 to 3, assessing the degree of self-reported depression. A total score of 17 points or higher indicates depression (with a maximum score of 63) [16].

Patients were divided into two subgroups based on their EDSS scores: those with EDSS scores of 4 and above, and those with EDSS scores below 4. The patients were also divided into groups by disease duration: 5 years and above, and below 5 years. Statistical analyses were conducted within these subgroups.

Statistical Analysis

The data analysis was conducted with IBM Corporation's SPSS 24.0 statistical software package. Continuous variables are expressed using means, medians, and standard deviations, whereas categorical variables are expressed with numbers and percentages. The normal distribution of data was assessed using the Kolmogorov-Smirnov test. In cases of non-normal distribution of data, the Mann-Whitney U test was employed to examine differences between independent groups. The Kruskal-Wallis test was preferred for evaluating differences among three or more groups. Relationships between continuous variables were assessed using Spearman correlation analysis, considering the non-normal distribution of data [17]. A significance level of $p<0.05$ was considered for all statistical analyses.

Results

A total of 74 patients, comprising 54 (73%) females and 20 (27%) males, diagnosed with MS were included in this study. According to the subtypes of MS, 54 (73%) patients were classified as relapsing-remitting MS (RRMS), 17 (23%) as primary progressive MS (PPMS), and 3 (4.1%) as sec-

ondary progressive MS (SPMS). The mean age of the participants was 38.32±11.47 years. Examining the marital status, it was determined that 58 (78.4%) patients were married, and 16 (21.6%) were single. Regarding education, 4 (5.4%) patients were uneducated, 35 (47.3%) had completed primary education, 22 (29.7%) had completed high school, 10 (13.5%) had a university degree, and 3 (4.1%) had a master's degree.

The mean disease duration was 7.55±5.91 years, whereas the mean Expanded Disability Status Scale (EDSS) score was 2.83±1.54. The mean score on the Cognitive Failures Questionnaire (CFQ) was found to be 41.76±22.22, the mean Fatigue Severity Scale (FSS) score to be 4.45±1.93, and the mean Beck Depression Inventory (BDI) score to be 19.62±11.33. Symptoms of depression were present in 43 (58.1%) patients, and fatigue symptoms were present in 49 (66.2%) patients. Among the patients, 54 (73%) had an EDSS score of 4 or lower, whereas 20 (27%) had an EDSS score of 4 or higher. Considering disease duration, 31 (41.9%) patients had a disease duration of less than 5 years, and 43 (58.1%) had a disease duration of 5 years or more. Table 1 presents the statistical analysis of demographic and clinical data, along with mean, median, standard deviation, and minimum-maximum values. The statistical analysis and frequencies of demographic and clinical data are presented in Table 2.

There was no statistically significant difference between education level and CFQ, BDI, and FSS scores (p=0.644, p=0.937, p=0.727, respectively). Similarly, there was no statistically significant difference in marital status by CFQ, BDI, and FSS scores (p=0.259, p=0.524, p=0.507, respectively).

In Spearman correlation analysis, there was a strong positive correlation between age and disease duration (r1=0.597; p<0.001), in addition to the moderate positive correlations between EDSS and disease duration (r1=0.253; p<0.05), EDSS and BDI (r1=0.342; p<0.001), between EDSS and fatigue (r1=0.392; p<0.001), between CFQ and BDI (r1=0.451; p<0.001), between CFQ and fatigue (r1=0.425; p<0.001), and between fatigue and BDI (r1=0.424; p<0.001). Table 3 illustrates the Spearman correlation analysis of variables (Table 3).

Examining group analyses, significant differences were found in age, disease duration, BDI, and FSS scores by EDSS classification (p=0.02, p<0.001, p=0.027, p<0.001, respectively). Patients with EDSS scores of 4 and above exhibited significantly higher age, disease duration, BDI, and FSS scores. No statistically significant difference was found between CFQ and EDSS (p=0.105).

Using the disease duration for group analysis, it was determined that there were statistically significant differences in terms of age and EDSS (p<0.001, p=0.010, respectively). Patients with a disease duration of 5 years or more had significantly higher age and EDSS scores. However, there was no statistically significant difference between CFQ, BDI, FSS, and disease duration (p=0.200, p=0.645, p=0.089, respectively).

Discussion

In this study, a positive correlation was observed between disability and depression, fatigue, and disease duration in patients with MS. Similarly, a positive correlation was determined between cognitive functions and depression, as well as fatigue. In addition, a positive correlation was found between depression and fatigue. Among individuals with an Expanded Disability Status Scale (EDSS) score of 4 or higher, age, disease duration, depression, and fatigue were significantly elevated. Those with a disease duration of 5 years or more had significantly higher age and EDSS scores.

Multiple Sclerosis is the most prevalent neurological disease causing disability among young adults, and fatigue is one of its hallmark symptoms [18,19]. The pathophysiological mechanisms underlying fatigue include structural damage to white matter (WM) and gray matter (GM), inflammatory processes (both within and outside the central nervous system), incongruent connections due to distributed lesions or inflammation, and a heightened perception of internal states due to dishomeostatic condi-

Table 1. Statistical Analysis of Patients' Demographic and Clinical Data

(n=74)	Mean	Median	Std. Deviation	Minimum	Maximum	Test statistic*	p**
Age	38.32	37	11.47	18	70	0.976	0.172
Pain Duration	7.55	6	5.91	1	25	0.907	**<0.001**
EDSS	2.83	2.50	1.54	1	6.50	0.894	**<0.001**
CFQ	41.76	40.50	22.22	3	99	0.981	0.339
BDI	19.62	19	11.33	0	48	0.967	0.053
FSS	4.45	4.44	1.93	1	7	0.926	**<0.001**

*The Kolmogorov-Smirnov test was used for the analysis of normal distributions. ** p<0.05 was considered statistically significant.

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Table 2. Frequencies of Demographic and Clinical Data of Patients

		Frequency(n)	(%)	Test statistic *	p**
Gender	Female	54	(73)	0.555	**<0.001**
	Male	20	(27)		
Education level	Uneducated	4	(5.4)	0.855	0.855
	Primary education	35	(47.3)		
	High school	22	(29.7)		
	University degree	10	(13.5)		
	Master's degree	3	(4.1)		
Marital status	Married	58	(78.4)	0.507	**<0.001**
	Single	16	(21.6)		
MS clinical types	RRMS	54	(73)	0.593	**<0.001**
	PPMS	17	(23)		
	SPMS	3	(4.1)		
Disease duration classification	5 years or less	31	(41.9)	0.627	**<0.001**
	More than 5 years	43	(58.1)		
EDSS classification	Below 4	54	(73)	0.555	**<0.001**
	4 and above	20	(27)		
Fatigue	Absent	25	(33.8)	0.597	**<0.001**
	Present	49	(66.2)		
Depression	Absent	31	(41.9)	0.627	**<0.001**
	Present	43	(58.1)		

*The Kolmogorov-Smirnov test was used for the analysis of normal distributions. ** p<0.05 was considered statistically significant.

Table 3. Spearman correlation analysis between variables (n=74)

		Age	Year	EDSS	CFQ	BDI	FSS
Age	r	1					
Year	r	**0.597****	1				
EDSS	r	0.103	**0.253***	1			
CFQ	r	-0.145	0.039	0.186	1		
BDI	r	-0.052	-0.035	**0.342****	**0.451****	1	
FSS	r	0.150	0.213	**0.492****	**0.425****	**0.424****	1

** p < 0.001, *p < 0.05 values indicate the result of the Spearman correlation. CFQ: Cognitive Failures Questionnaire, EDSS: Expanded Disability Status Scale, BDI: Beck Depression Inventory, FSS: Fatigue Severity Scale

tions [18]. The prevalence of fatigue tends to increase with disease progression, eventually becoming a concern for approximately 80% of individuals with MS regardless of the MS phenotype. Fatigue is typically rated as one of the top two most inhibiting symptoms by individuals with MS; however, its severity fluctuates over days and between

days, posing challenges for clinical management [19]. The present study also demonstrated that the majority of patients exhibited fatigue symptoms as described in the literature. Furthermore, it was also revealed that fatigue tends to increase with increasing levels of disability.

When evaluating cognitive functions, despite clinical heterogeneity, cognitive decline has been consistently reported in patients with MS, regardless of the disease's processes. Deficits are most commonly observed in attention, processing speed, working memory, verbal fluency, and executive function. Moreover, individuals with MS tend to exhibit reduced performance in tasks related to social cognition, which includes interpersonal skills such as social perception, empathy, and theory of mind [20]. The underlying pathophysiology of cognitive impairment includes lesions in strategic cerebral white matter (WM) regions, microstructural WM damage, gray matter (GM) lesions, deep GM atrophy, and abnormal cerebral activation patterns [21]. Broadbent's Cognitive Failures Questionnaire (CFQ) is a tool widely used for evaluating cognitive function. Its validity and reliability were tested in Turkey, suggesting its utility in assessing non-demented cognitive functions [12]. In contrast to some previous studies, no significant relationship was found between increasing disability and cognitive function deterioration in the present study. However, most studies suggest that cognitive functions are associated with EDSS scores in MS patients. In general, a decline in cognitive function and an increase in disability levels are observed in parallel with the progression of the disease in MS patients [22]. Nevertheless, it's crucial to remember that there can be substantial variations among individuals; some might experience cognitive function decline, whereas others might experience minimal decline or none at all. The treatments used for MS can be useful in slowing down disease progression or alleviating symptoms. Different treatments might have diverse effects on cognitive functions and disability levels. Individuals with MS can develop various strategies to compensate for cognitive weaknesses, potentially resulting in fewer errors in daily life and a reduction in disability levels. The specific characteristics of the patients in the present study sample can influence the results. For instance, patients with milder symptoms or higher cognitive reserves might have been selected. Moreover, the limited number of patients might have contributed to an inconclusive correlation between cognitive functions and disability. Psychosocial factors such as stress, depression, and anxiety can influence both cognitive functions and disability levels. The worsening of depression and fatigue with increasing EDSS scores might indirectly relate to cognitive function impairment.

Regarding the relationship between MS and depression, it's evident that depression is a common finding among MS patients. Depressive disorders are experienced by up to 50% of patients, and major depression is a significant comorbidity of MS. Various dysfunctions, such as neuroinflammation, peripheral inflammation, gut dysbiosis, chronic oxidative and nitrosative stress, and neuroendocrine and mitochondrial abnormalities, are considered to contribute to the comorbidity between MS and major depression [23]. The present study, like others, also found a high prevalence of major depression among MS patients.

Fatigue, depression, and cognitive function impairment are closely interrelated clinical aspects among patients with MS. In a previous study, increased fatigue was found to not be associated with initial fatigue, cognitive impairment, disease variables, or disability levels. However, it was found to be related with higher anxiety, lower self-efficacy, and gender [19]. In this study, on the other hand, fatigue and depression were found to be associated with increasing disability scores, indicating a connection between fatigue and depression symptoms. Moreover, a similar relationship was identified between the increase in fatigue and depression and the increase in cognitive impairment. Similarly, another study demonstrated that psychological, demographic, and disease-related factors all contribute to inadequate performance in MS. For instance, depressive symptoms and anxiety were associated with inadequate performance in MS. More importantly, rates of inadequate performance were found to be higher in clinical populations characterized by fatigue and pain [24]. In another study, cognitive processing speed was not fully evaluated due to fatigue and depression, and it was determined that depression and fatigue contribute to cognitive processing speed and cognitive impairment [25].

Limitations of this study: The limitations of this study include not evaluating correlations and relationships between various cognitive tests used for cognitive function assessment, the limited number of patients due to the short study duration, and a lack of classification of patient treatments to assess their impact on cognitive functions.

Conclusion

As disability increases in MS, it likely contributes to higher levels of depression and fatigue. Additionally, depression and fatigue are correlated with each other, and these two factors probably negatively impact cognitive functions.

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Research Article

Araştırma

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Determination of Bone Developments of Rat Anterior and Posterior Extremity Bones in Prenatal and Postnatal Period by Double Staining Method

Sıçan Ön ve Arka Ekstremite Kemiklerinin Prenatal ve Postnatal Dönemdeki Kemik Gelişimlerinin İkili Boyama Yöntemi ile Belirlenmesi

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ABSTRACT

Aim: In our study, we aimed to determine the morphological development of the bones of the anterior and posterior extremity by staining the rat fetus and offspring skeletons with the double staining method.

Methods: In the current study, seven groups three prenatal (16th, 18th, and 20th days) and four postnatal (0th, 3th, 7th and 12th days) were formed from the foetuses and offsprings obtained from 13 pregnant rats. Then, it was stained with double staining method. Anterior and posterior extremity images of the fetuses and offsprings were examined under a stereo microscope, and ossification findings were determined. Total bone and ossification lengths as well as ossification areas were measured using the ImageJ software.

Results: The first cartilage destruction in fetuses occurred on the 16th day of pregnancy in the clavicle, scapula, humerus, radius and ulna; It was seen in the femur, tibia and fibula on the 18th day of pregnancy. The first ossification centres were in the clavicle, scapula and humerus on the 18th day of pregnancy; It was seen in the radius, ulna, femur, tibia, fibula and 2-5 metatarsal bones on the 20th day of pregnancy. The secondary ossification centre was seen on the 0th day (birthday) in the scapula and humerus, on the 7th day after birth in the ulna and radius, and on the 12th day after birth in the femur and tibia. In the study, while the ossification rate in 20-day-old foetuses was 48.9% in the humerus, 53.2% in the radius, 55.7% in the ulna, 33.6% in the femur, 43.2% in the tibia, 44.3% in the fibula, it was determined that it reached 69.7% in the humerus, 78.4% in the radius, 73.3% in the ulna, 63.5% in the femur, 75.5% in the tibia, and 69.2% in the fibula on the 12th day after birth.

Conclusion: In this study, we revealed the morphological changes of the anterior and posterior extremity bones of fetuses and offsprings in the normal developmental course. We think that these results will shed light on the studies to be conducted on the detection of skeletal anomalies in teratological studies and contribute to a more comprehensive evaluation of the findings to be obtained from the studies to be conducted.

Key Words: Rat, Double Staining, Ossification, Bone Development, Image J, Toxicology

ÖZET

Amaç: Çalışmamızda sıçan fetus ve yenidoğan iskeletinin ikili boyama yöntemi ile boyanarak, ön ve arka ekstremiteye ait kemiklerin morfolojik gelişimlerinin tespitini amaçladık.

Yöntem: Bu çalışmada 13 adet gebe sıçandan elde edilen fetüs ve yavrulardan 3'ü prenatal (16.,18. ve 20. gün) ve 4'ü postnatal (0, 3.,7. ve 12.gün) olmak üzere 7 grup oluşturuldu. Daha sonra ikili boyama yöntemiyle boyandı. Fetüs ve yavruların ön ve arka ekstremite görüntüleri stereo mikroskop altında incelenerek ossifikasyon bulguları belirlendi. ImageJ yazılımı kullanılarak toplam kemik ve ossifikasyon uzunlukları ile ossifikasyon alanları ölçüldü.

Bulgular: Fetüslerdeki ilk kıkırdak yıkımı clavicula, scapula, humerus, radius ve ulna'da gebeliğin 16. gününde; femur, tibia ve fibula'da ise gebeliğin 18. gününde görüldü. İlk kemikleşme merkezi clavicula, scapula ve humerusta gebeliğin 18. gününde; radius, ulna, femur, tibia, fibula ve 2-5 metatarsal kemiklerde gebeliğin 20. gününde görüldü. İkincil kemikleşme merkezi scapula ve humerusta 0. günde (doğum günü), ulna ve radiusta doğumdan sonra 7. günde, femur ve tibiada doğumdan sonra 12. günde görüldü. Çalışmada 20 günlük fetuslerde kemikleşme oranı humerus'ta %48.9, radius'ta %53.2, ulna'da %55.7, femur'da %33.6, tibia'da %43.2, fibula'da %44.3'ken, doğumdan sonraki 12. günde humerus'ta %69.7, radius'ta %78.4, ulna'da %73.3, femur'da %63.5, tibia'da %75.5, fibula'da %69.2'e ulaştığı tespit edildi.

Sonuç: Bu çalışmada, fetüslere ve yenidoğan yavrulara ait ön ve arka ekstremite kemiklerinin normal gelişim seyrindeki morfolojik değişimlerini ortaya koyduk. Bu sonuçların teratolojik çalışmalarda iskelet anomalilerinin (malformasyon, varyasyon ve diğer anomaliler) tespitine yönelik yapılacak çalışmalara ışık tutacağını ve yapılacak çalışmalardan elde edilecek bulguların daha kapsamlı değerlendirilmesine katkı sunacağını düşünmekteyiz.

Anahtar Kelimeler: Sıçan, İkili Boyama, Kemikleşme, Kemik Gelişimi, Image J, Toksikoloji

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Introduction

Skeletal evaluations are one of the standard evaluations of developmental and toxicology studies [1]. Today, different techniques are used to evaluate the skeletal system [1,2]. One of these techniques is double skeleton staining. Double skeletal staining provides simultaneous staining of both bone and cartilage areas of the skeleton. This method of Inouye has now become a safe method for skeletal staining of developmental and toxicology studies [3].

Many parameters such as fetal or offspring weight, headstern length, bone lengths, the number of ossification centers, where and in which time period the ossification centers ossify and how long they are, and whether there is a delay in the development of bones, are examined in double skeleton staining studies for skeletal evaluation. In the light of the data obtained from these parameters, evaluations are made about the development of the fetal or offspring skeleton [3-7].

In developmental toxicity studies using the double skeletal staining method, the pregnancy is terminated shortly before birth (often on the 20th day of pregnancy) and skeletal examinations are performed on the fetuses to examine the effect of the toxic substance given to pregnant animals on the fetus [8,9].

However, these evaluations on fetuses collected close to birth do not allow an explanation for the outcome of the prenatal induced change in the postnatal period. In particular, it does not provide information about when the changes detected on fetuses collected by cesarean section in fetal skeletal evaluations begin, how they develop, how the process continues and the reversibility of these changes in the postnatal period [10].

Considering that the development of the skeletal system begins in fetal life with intramembranous and endochondral ossification and continues after birth, it is necessary to know well the normal development of the skeleton in both prenatal and postnatal periods. For this reason, in our study, we aimed to reveal the normal morphological development of the bones of the anterior and posterior extremities during the rat skeletal development, both in the prenatal and postnatal periods, by staining the fetuses and offspring obtained from healthy pregnant rats with the double skeleton staining method.

We think that this study will shed light on developmental and toxicology studies covering prenatal and postnatal periods and contribute to a more comprehensive evaluation of the findings to be obtained from these studies in the light of the findings we have presented.

Materials and Methods

This study was carried out in the laboratories of Erciyes University Experimental Research Application and Research Center (DEKAM). The entire procedure related to the study was designed according to the principles of the Erciyes University Local Animal Ethics Committee (Date:10.08.2011 No:11/86) and was supported by the Erciyes University Scientific Research Projects Unit (No: TSY-11-3723).

Analysis of data; The data obtained from the research were analyzed using the SPSS 24.00 package program. Descriptive statistics (arithmetic mean, standard deviation, percentage) were used to analyze the data.

Selection and Mating of Experimental Animals

In this study, a total of 70 fetuses and offsprings obtained from 13 adult female Wistar-albino rats weighing between 170 and 270 grams were equally seperated to seven groups as follows: Three prenatal (16th, 18th, and 20th days of pregnancy), and four postnatal (0th, 3th, 7th and 12th days).

One female and one male rat were taken into the cages at 17 pm to obtain the fetuses and offsprings. Vaginal smears were taken from the female rats at 08.00 the next morning and examined under a microscope. The female rats that had a sperm positive vaginal smear were accepted to be on day 0 of pregnancy. The rats were fed standard on a 12 hours of light-12 hours of darkness in DEKAM (Experimental Studies Research and Implementation Center).

Pregnant rats were anesthetized with 75 mg/kg ketamine and 10 mg/kg xylazine on the 16th, 18th and 20th days of pregnancy, and their fetuses were explanted by opening the anterior abdominal wall under anesthesia.

Those who were damaged during the explantation procedure of the fetuses were not included in the study. A study group for the 16th, 18th and 20th days was formed, with at least 10 fetuses in each group. The remaining pregnant rats were fed until birth. After the rats gave birth, offspring were sacrificed under anesthesia with 75 mg/kg ketamine and 10 mg/kg xylazine on the 0th, 3th, 7th and 12th days of birth, and four separate groups were formed with at least 10 offspring in each group.

Preparation Of The Rat Fetuses And Offsprings For Staining

Height and weight parameters were measured for whole fetuses and offsprings. After the sacrifization and removal process, fetal and offspring tissues were kept in 95% eth-

yl alcohol for seven days, and then they were fixed. Following this procedure, they kept in pure aceton for three days to clear their oil. Then, their skins were peeled and their internal organs and eyes were removed. The staining process of the skeletons of rat fetuses and offspring was carried out within the framework of the protocol in Table 1 [10,11].

Right and left anterior and posterior extremity bones of fetus and offspring rats, whose skeletal system became fully visible after the transparency phase, which lasted for approximately 16 days, were examined under a stereomicroscope and their photographs were taken. The obtained images were opened in ImageJ program and total bone length, ossification length and ossification areas were calculated. For long-term storage of finished fetuses and offspring, they were kept in pure glycerin in separate containers.

Calculation of the ratio of bone and cartilage areas: The obtained photo images were opened in the ImageJ program [12]. Then, the boundaries of the structures were determined with the help of mouse to obtain the superficial areas of the images by selecting the polygon selections in the ImageJ program. Bone and cartilage surface areas were measured by performing Ctrl-M (measure) on the keyboard. Bone and cartilage surface areas were recorded in pixels. As a result, how much area the bone and cartilage occupy in the extremity was calculated as a percentage. The following formula was used for this.

$$V_v(\text{cartilage, bone}) = \frac{\sum Pcartilage}{\sum Pbone} \times 100$$

Similarly, in the measurement of the lengths of the bones and ossification centers, the calibration was made on the set scale in the ImageJ program, and then the length measurements were made using a straight line [12].

Results

Effects on growth parameters:

Before starting the staining process in all groups, the heights and weights of the fetuses and offspring were determined. The findings of the height (Head-Stern lengths) and weight measurements obtained from all groups are shown in Table 2.

Effects on cartilage destruction:

In our study, the first cartilage destruction was in the anterior extremity bones the clavicula, scapula, humerus, radius, and ulna were seen on day 16, and in the posterior extremity bones, on the femur, tibia, and fibula, on day 18 (Figure 1).

Effects on the general growth morphology of the skeleton:

In the general morphological examination of the whole skeleton after staining, it was observed that the first ossification took place in the mandible, maxilla, scapula,

Table 1. Double skeleton staining protocol

Technical Stages	Solutions	Time
Fixation	70% ethyl alcohol	4-7 days
Degreasing	Pure acetone	1-3 days
Double Staining	preparing the double staining solution 1st solution: 300mg Alcian Blue + 100ml 70% ethyl alcohol 2nd solution: 100mg Alizarin Red S + 100ml 95% ethyl alcohol 3th solution: 1st solution + 2nd Solution + 100ml Glacial acetic acid 4th solution: Prepared by adding 1700 ml of 70% ethyl alcohol to the first three solutions	7 days 38-40 °C incubated
Transparency	1) 1% KOH 2) 1% KOH (80 ml) +%20'lik glycerin (20 ml) 3) 1% KOH (50 ml) +%50'lik glycerin (50 ml) 4) 1% KOH (20 ml) +%80'lik glycerin (80 ml)	1-3 days 5-7 days 5-7 days 5-7 days
Safekeeping	100% pure glycerine	

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Table 2. Height (Head-Stern length) and weight (gr) measurements of fetuses and offspring groups

		PRENATAL GROUP			POSTNATAL GROUP			
	No	16-day old fetus	18-day old fetus	20-day old fetus	0-day-old offspring	3-day-old offspring	7-day-old offspring	12-day-old offspring
HEIGHT (mm)	1	11	17.4	31.1	41	45	57	61.2
	2	10.3	19.1	29.5	41.5	47.5	46.6	63.8
	3	13.3	20.1	32.9	43.0	48.3	52.2	61
	4	11.1	19.2	31.1	41.1	48.5	49.5	61
	5	11.2	19.5	30	41.5	46.1	48	73.0
	6	10.6	18.8	31.8	43	46.1	54.2	62.1
	7	10.4	18.4	30.5	40	43.5	52.2	53.2
	8	10.6	19	31.4	43.4	51	52.3	69.7
	9	11	17.9	31.7	40	44.2	43.2	56.5
	10	11	18	30.5	43.4	49.2	52.2	50
	11	-	18.7	32.4	39.8	45	51.5	54.2
	12	-	18	29.8	-	-	39.2	64.5
	13	-	19.2	33.7	-	-	-	-
$\bar{X}$±SD		**11.05±0.84**	**18.71±0.75**	**31.26±1.24**	**41.6±1.39**	**46.76±2.32**	**49.84±4.92**	**60.85±6.66**
WEIGHT (g)	1	0.24	1.98	3.5	5.11	7.46	10.67	14.53
	2	0.22	1.85	3.25	5.6	7.22	8.72	18.22
	3	0.24	2	3.07	4.94	6.11	10.84	11.4
	4	0.22	1.96	3.83	5	5.13	7.36	11.08
	5	0.23	2.4	3.39	4.82	6.6	7.56	15.59
	6	0.23	1.98	2.87	5.35	6.55	5.93	12.56
	7	0.24	1.74	3.36	5.59	7.36	10.08	20.34
	8	0.23	1.9	3.2	5.44	8	7.13	17.17
	9	0.22	2.1	3.03	5.21	7.88	10.37	10.68
	10	0.21	1.97	3.19	5.16	7.53	6.86	9.13
	11	-	1.98	3.2	5.59	7.29	10.65	18.31
	12	-	1.86	2.8	-	-	11.12	13.72
	13	-	1.80	2.9	-	-	-	-
$\bar{X}$±SD		**0.22±0.10**	**1.96±0.16**	**3.19±0.28**	**5.25±0.27**	**7±0.84**	**8.94±1.87**	**14.39±3.56**

clavicle and humerus bodies and extramitas vertebralis of the 1-8 ribs of the 18-day-old fetuses. It was observed that cartilage destruction continued in the bodies of other long bones and these regions had a spongy appearance (Figure 1).

Effects on the anterior extremity long bones:

The time of appearance of the first ossification centers of the anterior extremity skeleton bones is shown in Table 3, and the appearance of the first ossification centers of the anterior extremity skeleton is shown in Figure 2.

Effects on the posterior extremity long bones:

The first ossification center in the posterior extremity; femur, tibia, fibula, and 2-5 metatarsal bones at day 20 of gestation, 0-day-old offspring in 2-5 phalanges, and 3-day-old offspring in calcaneus, talus, 1st metatarsal and 1st phalanx (Figure 3).

Secondary ossification center in the anterior extremity was first seen in the scapula and humerus in 0-day-old offspring, while it was seen in the radius and ulna at the proximal end on the 7th day after birth and at the distal end on the 12th day after birth, and in the metetarcarpal bones on the 12th day after birth. Secondary ossification center in the posterior extremity was seen at the distal end of the femur, both the distal and proximal end of the tibia in the 12-dayold offspring (Figure 4).

In our study, bone length and ossification rate increased with age in all groups. Bone length, ossification length and ossification rates of anterior extremity and posterior extremity bones in prenatal and postnatal groups are given in Table 4.

Table 3. The time of first ossification center of the bones of the anterior extremity

	Bone Name	Prenatal period	Postnatal period
Anterior extremity	Clavicula	18th day before birth	-
	Scapula	18th day before birth	-
	Humerus	18th day before birth	-
	Radius	20th day before birth	-
	Ulna	20th day before birth	-
	Ossa carpi		12th day after birth
	Ossa metacarpi (2-4. Metacarpal bone)	20th day before birth	-
	Ossa metacarpi (5th metacarpal bone)		0 days old(birthday)
	Ossa metacarpi (1th metacarpal bone)	-	12th day after birth
	Phalanges (Both distal and proximal phalanges of the 2nd-4th finger)	20th day before birth	-
	Phalanges (Distal and proximal phalanx of the 5th finger)		0 days old(birthday)
	Phalanges (In the middle phalanx of the 2nd-5th finger and the proximal phalanx of the 1st finger)	-	3th day after birth
	Phalanges (distal phalanx of the 1st finger)	-	3th day after birth

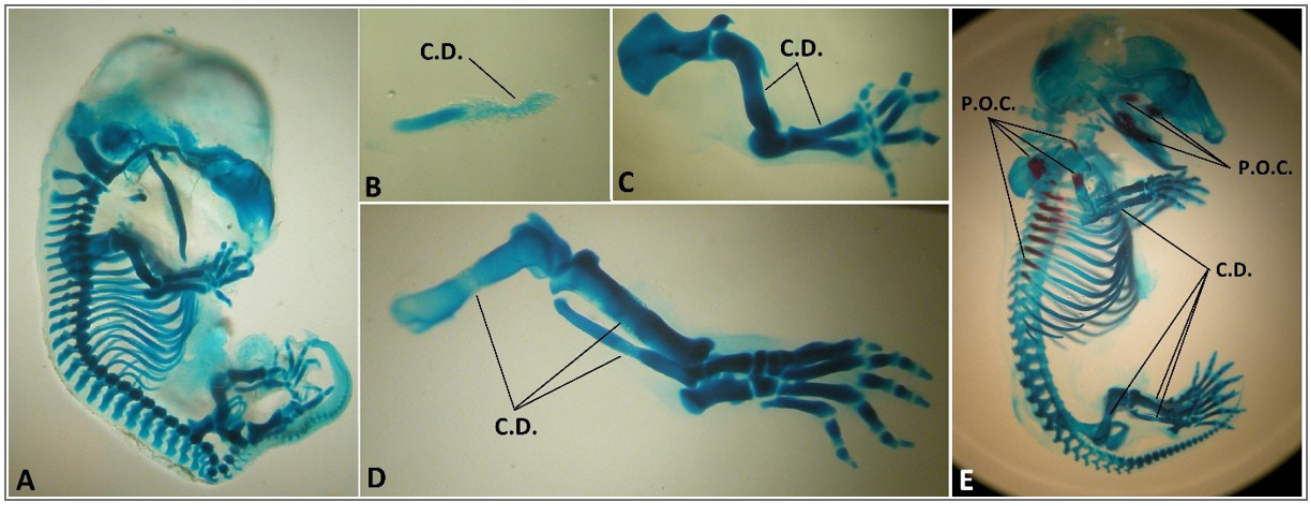

Figure 1. A: General view of the entire skeleton of the 16-day-old fetus. B: Clavicle (16-day-old fetus) C: Scapula, Humerus, Radius and Ulna (16 days old fetus) D: Posterior extremity (16-day-old fetus) E: General view of the entire skeleton of the 18-day-old fetus.
*C.D: Cartilage destruction, P.O.C: Primer ossification center

Discussion

In developmental toxicity studies, the degree of ossification is an indicator of skeletal maturity [11]. In the skeletal evaluations made using the double staining technique; parameters such as fetal or offspring weight, head-stern length, bone lengths, the number of ossification centers, where and when these centers appear, how long they are, whether there is a delay in bone development are examined [4,9,13-15]. When we scan the literature in the light of these parameters, we see that studies are generally carried out on 20-day-old fetuses and offspring. However, it is seen that the studies revealing the stages of skeletal development in the prenatal and postnatal groups are limited and the data in the findings of the control groups of the experimental studies are mostly shared.

Fetal weight and head-stern length are indicators of fetal skeletal development and are frequently evaluated in toxicology studies. In many studies, it has been reported that there is a decrease in weight and head-stern length in fetuses and offspring of pregnant mothers who have

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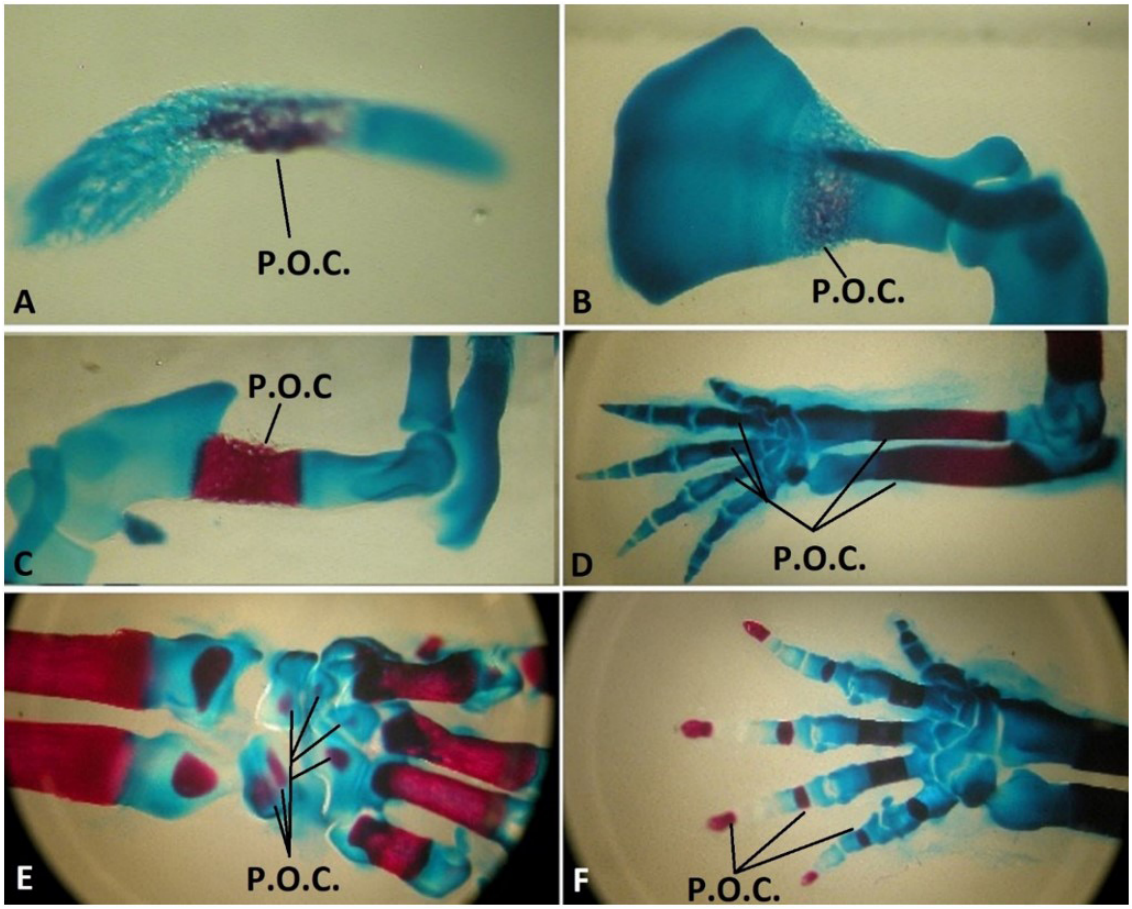

Figure 2. The appearance of primary ossification centers in the bones of the anterior extremity A: Clavicle (18-day-old fetus) B: Scapula (18-day-old fetus) C: Humerus (18-day-old fetus) D: Radius, ulna and ossa metacarpi (20-day-old fetus) E: Ossa carpi (12-day-old offspring) F: Ossa digitorum phalanges (0-day-old offspring).
P.O.C: Primer ossification center

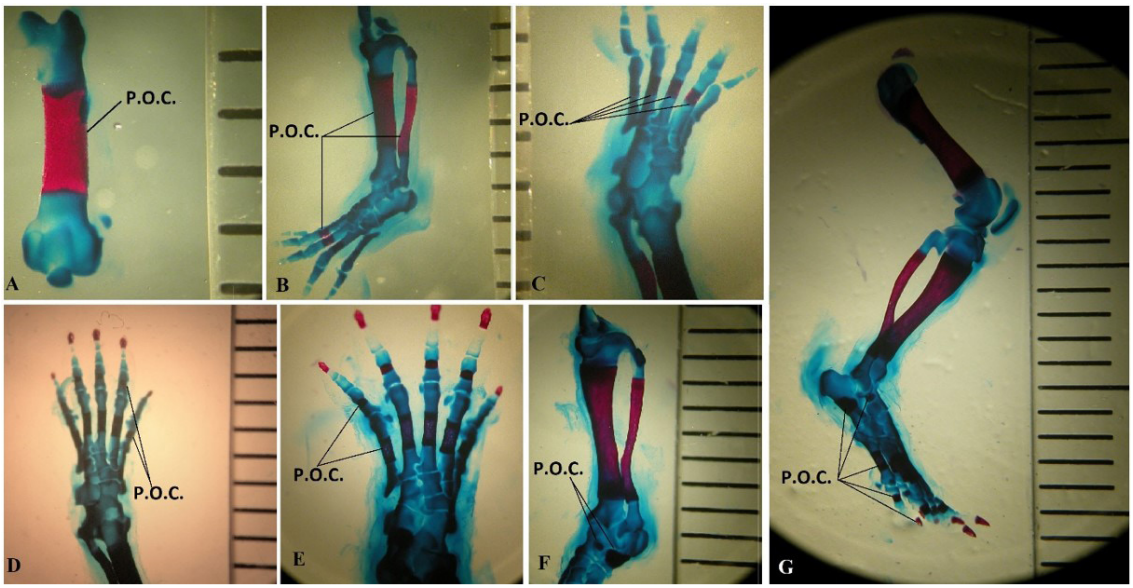

Figure 3. The appearance of the first ossification centers of the posterior extremity. A: Femur (20-day-old fetus) B: Tibia, fibula and metatarsal bones (20-day-old fetus). C: 2-5 metatarsal bones (0-day-old fetus) D-E: Ossa metatarsi and ossa digitorum phalanges (0-day-old offspring) F: Calcaneus and talus (3-day-old offspring) G: General view of the posterior extremity of the 3-day old offspring
P.O.C: Primer ossification center.

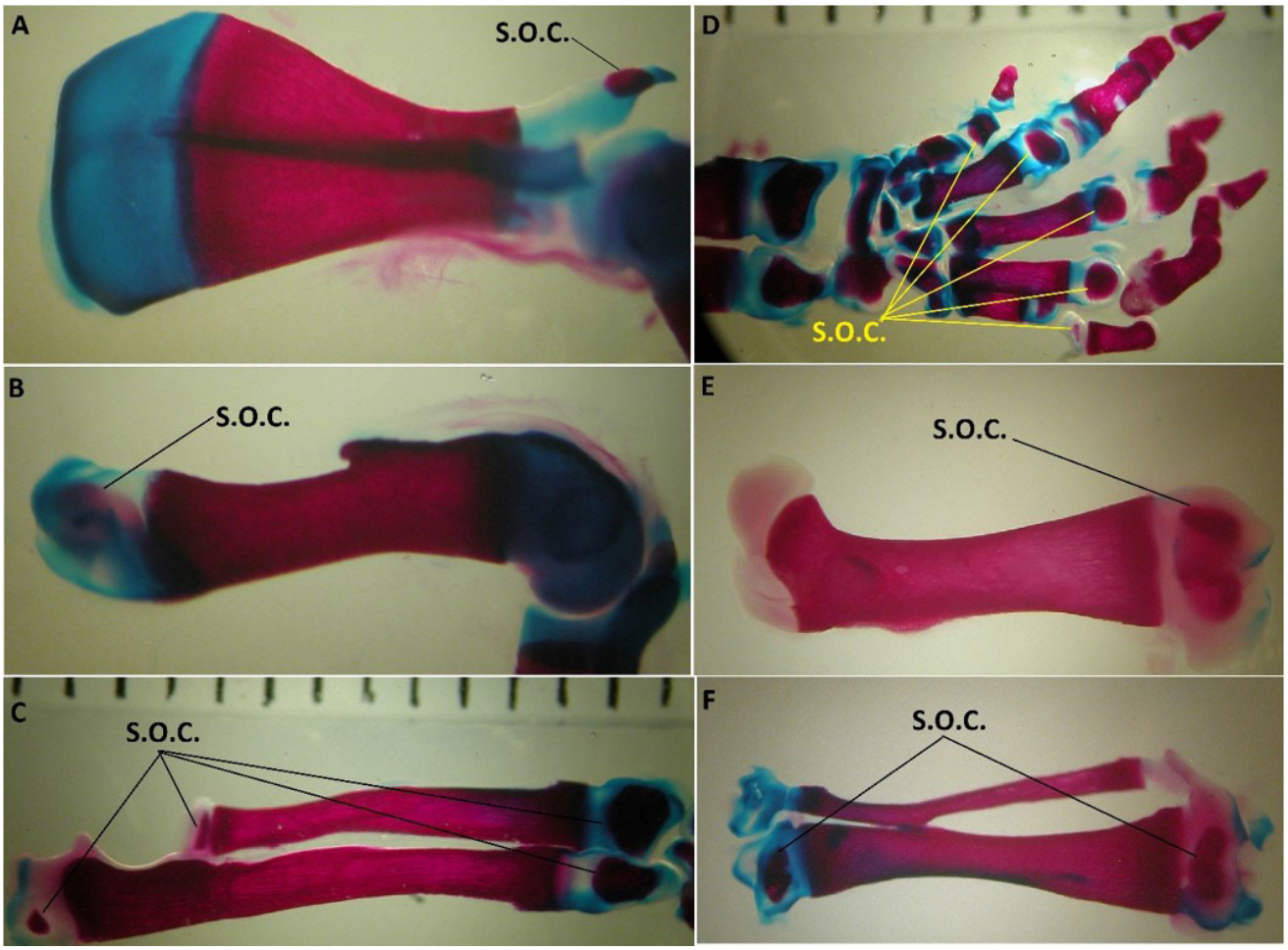

Figure 4. The appearance of secondary ossification centers in the bones of the anterior and posterior extremity. A: Scapula (0-day-old offspring). B: Humerus (0-day-old offspring). C: Radius and Ulna (12-day-old offspring). D: Ossa metacarpi (12-day-old offspring). E: Femur (12-day-old offspring). F: Tibia and fibula (12-day-old offspring).
S.O.C: Seconder ossification center

been exposed to toxic substances [13,15-19]. In our study, unlike other studies, we determined the course of ossification in the postnatal period by measuring the weight and head-stern lengths of fetuses and offspring of both prenatal and postnatal periods.

The formation of the primary ossification center, which is another indicator of bone development, is an indicator of the transition from cartilage tissue to bone tissue. In some studies, it has been reported that the primary ossification center in the clavicle, scapula and humerus is seen between the 15th and 17th days of pregnancy [15,20,21]. In our study, the first cartilage destruction in the clavicula, scapula and humerus was observed on the 16th day of pregnancy, and the first ossification center was observed on the 18th day of pregnancy.

In some studies, it has been reported that the primary ossification center in the radius and ulna is seen between the 15th and 17th days [15,20,21]. In our study, the first cartilage destruction in the radius and ulna started on the 16th day of pregnancy and the first ossification center was seen on the 20th day of pregnancy.

In studies conducted on the day of the first appearance of the primary ossification center in the femur, tibia and fibula from the posterior extremity bones, it has been reported that it occurs between 16 and 17 days of pregnancy [15,20,21]. In our study, the first cartilage destruction in the femur, tibia and fibula was observed on the 16th day of pregnancy, and the first ossification center was observed on the 20th day of pregnancy.

When the primary ossification center first appeared, it was observed that the center of the bone took a spongy appearance due to cartilage destruction and was stained blue with alcian blue, and ossification began in this area in the next period and stained red with alizarin red S for this reason, it was observed that there were differences between the day of cartilage destruction and the first day of ossification.

The secondary ossification center is an indicator of the maturation of the bone tissue and the final shape of the

Table 4. Findings of anterior and posterior extremity bones

	Total Bone Length (cm)	Length of Ossified Part (cm)	Ossification Rate	Total Bone Length (cm)	Length of Ossified Part (cm)	Ossification Rate	Total Bone Length (cm)	Length of Ossified Part (cm)	Ossification Rate
		HUMERUS			**RADIUS**			**ULNA**	
	X̄±SD	**X̄±D**	%	**X̄±SD**	**X̄±SD**	%	**X̄±SD**	**X̄±SD**	%
16 day old fetus	0,18±0,01	-	-	0,11±0,01	-	-	0,15±0,01	-	-
18 day old fetus	0,32±0,02	0,07±0,02	15,4	0,23±0,01	-	-	0,30±0,01	-	-
20 day old fetus	0,54±0,01	0,27±0,02	48,9	0,40±0,02	0,22±0,01	53.2	0,52±0,03	0,28±0,02	55.7
0 day old offspring	0,70±0,11	0,40±0,04	52,2	0,56±0,10	0,34±0,02	62.1	0,68±0,03	0,42±0,03	63.9
3 day old offspring	0,76±0,05	0,46±0,05	55,6	0,61±0,03	0,41±0,04	64.5	0,77±0,06	0,52±0,04	66.2
7 day old offspring	0,90±0,06	0,59±0,04	58,5	0,75±0,03	0,56±0,05	67.4	0,94±0,04	0,67±0,04	66.7
12 day old offspring	1,04±0,07	0,68±0,07	69,7	0,89±0,08	0,66±0,07	78.4	1,21±0,12	0,87±0,12	73.3
		FEMUR			**TIBIA**			**FIBULA**	
	XX̄±SD	**XX̄±SD**	%	**X̄±SD**	**X̄±SD**	%	**X̄±SD**	**X̄±SD**	%
16 day old fetus	0,16±0,03	-	-	0,13±0,01	-	-	0,11±0,01	-	-
18 day old fetus	0,35±0,05	0,10±0,16	-	0,29±0,01	0,04±0,01	-	0,26±0,02	0,04±0,01	-
20 day old fetus	0,60±0,07	0,24±0,03	33,6	0,47±0,06	0,22±0,04	43,2	0,44±0,06	0,22±0,04	44,3
0 day old offspring	0,74±0,13	0,41±0,06	44,6	0,70±0,16	0,44±0,11	56,6	0,65±0,16	0,41±0,10	60,1
3 day old offspring	0,83±0,07	0,47±0,04	46,6	0,83±0,11	0,55±0,06	63,6	0,74±0,06	0,52±0,05	67,2
7 day old offspring	1,09±0,08	0,66±0,06	51,3	1,07±0,08	0,73±0,07	69,5	0,96±0,08	0,67±0,06	68,3
12 day old offspring	1,18±0,09	0,81±0,08	63,5	1,39±0,08	0,91±0,07	75,5	1,18±0,08	0,82±0,08	69,2

X̄: Arithmetic Mean, SD: Standard Deviation

bone, so the time of appearance of the secondary ossification center is another indicator of skeletal development [22,23]. However, when we look at the literature, it is seen that the findings about the initial development of the secondary ossification center are limited and are obtained from studies on some specific bones.

In their study, Morini S and et al. [5] reported that ossification that started on the 4th day after birth in the humeral head became evident on the 11th day and the secondary ossification center developed.

Campion S.N. and et al. [24] reported that a secondary ossification center develops at the proximal end of the femur between 15 and 20 days after birth.

In their study on rats, Hedberg A and et al. [25] reported that on the 10th day after birth, secondary ossification center formation started at the distal femur and proximal end of the tibia, and the epiphyseal ossification reached a relatively mature state on the 15th day.

In our study, the secondary ossification center was first seen in the 0-day-old offspring (in offspring) of the scapula and humerus. 7-day-old offspring was seen at the proximal end of the radius and ulna, and at the distal end of the radius and ulna in 12-day-old offspring. In our study, a secondary ossification center was observed at the distal end of the 12-day-old offspring femur and both the distal and proximal ends of the tibia.

Other parameters used to evaluate the effect of the toxic substance are fetal bone length, ossification length and ossification rate. However, it is seen that measurements for these parameters are frequently made in 20-day-old fetus groups [6,7]. When the findings of these parameters belonging to our study are compared with some studies in the literature, it is seen that the results are close to each other. In our study, we measured these values in a total of 7 groups, including prenatal and postnatal, and revealed the course of ossification in a wider time interval.

Evaluation of fetuses after administration of the toxic substance to pregnant animals is common and current practice in developmental toxicology studies [18,19]. However, it is wondered how the postnatal outcome and developmental process of the fetus, which has been exposed to toxic effects in the prenatal period, which has been discussed for a long time, is affected. The postnatal bone formation process continues rapidly and the evaluation of whether the anomaly seen on fetuses collected during the fetal period is reversible and transient (variation) or permanent (malformation) cannot be made without more data in most cases. For this reason, it is necessary to include not only studies covering the fetal period, but also studies covering the postnatal period.

Hofmann T. and et al. [10] suggested that anomalies such as delayed ossification, presence of extra or rudimentary ribs, and twisted long bones can only be evaluated as malformations with more data, therefore the animal exposed to toxic substance should be evaluated in the postnatal period.

Saeidinezhad M. and et al. [26] reported that morphine had a toxic effect on 7-day-old offspring mice in their study examining the effect of morphine, and that morphine reduced the growth of longitudinal bone by reducing the growth of primary and secondary ossification centers.

Carney E.V. and Kimmel C.A. [27] suggested that both delayed ossification and wavy rib findings should be evaluated in a larger time frame in conjunction with other findings.

Hayasaka I and et al. [28] reported that mothers treated with 90 mg/kg/day azosemide had a significant increase in skeletal abnormalities in their fetuses, such as wavy ribs, twisted scapula, and twisted arm bone, in rats, but these observed anomalies resolved in adult offspring.

Limitations of the Study

This study has limitations. In order to follow the comprehensive developmental process, it is necessary to increase the number of postnatal groups and to follow them over a longer period of time.

Conclusion

Considering that the development of the skeletal system begins in fetal life with intramembranous and endochondral ossification and continues after birth, the normal development of the skeleton in both prenatal and postnatal periods should be well known. For this reason, in our study, we revealed the normal morphological developmental course of the bones of the anterior and posterior extremities during rat skeletal development in both prenatal and postnatal periods. We think that our study will be a source for developmental toxicology studies covering prenatal and postnatal periods and will contribute to a more comprehensive evaluation of the findings to be obtained from these studies in the light of the findings we have presented.

Conflict of Interest: The authors declare no conflict of interest related to this article.

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Ethics Committee Approval: Erciyes University Local Animal Ethics Committee (Date:10.08.2011 No:11/86)

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Research Article

Araştırma

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Which Scapula Fractures Should We Operate on and What Are the Functional Outcomes?

Hangi Skapula Kırıklarını Opere Etmeliyiz ve Fonksiyonel Sonuçları Nelerdir?

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ABSTRACT

Aim: In this study we reported the functional results and complications of patients with scapula fractures who underwent open reduction and internal fixation.

Methods: The study included 16 patients with scapula fractures who were treated with open reduction and internal fixation(ORIF) between September 2015 and March 2022. Radiologic examination (AP in the scapular plane, lateral and axillary radiography) and computed tomography (CT) scans were performed in all patients. Fractures were classified according to the revised (AO/OTA) classification system. The patients underwent deltopectoral and posterior approaches described by Judet. Functional outcomes were measured using Constant-Murley scores.

Results: Ten patients had a scapular neck fracture, or glenoid fossa fracture, five patients had a scapular trunk fracture affecting the glenohumeral joint, and one patient had a scapula process fracture. It was accompanied by clavicle shaft fracture in five patients. The mean follow-up period after injury was 42 months (6-92 months). The mean Constant-Murley score (CMS) for the shoulder with scapula fracture was 93.8 (±8.93).

Conclusion: Open reduction and internal fixation of displaced scapular fractures is an effective treatment option in terms of union rate and functional outcome.

Key Words: Scapula Fracture, Constant-Murley Score, Judet Approaches

ÖZET

Amaç: Bu çalışmada, skapula kırığı nedeniyle, açık redüksiyon ve internal tespit yapılan hastaların fonksiyonel sonuçlarını ve komplikasyonları araştırdık.

Yöntem: Çalışma, Eylül 2015 ile mart 2022 arasında skapula kırığı olan açık redüksiyon ve internal tespit (ARIF) ile tedavi edilen 16 hasta değerlendirildi.Radyolojik inceleme skapular planda AP,lateral,aksiller grafi ve bilgisayarlı tomografi (BT) ile yapıldı.Kırıklar revize edilmiş (AO/OTA) sınıflandırma sistemine göre sınıflandırıldı.Hastalara cerrahi teknik olarak deltopektoral ve Judet tarafından tarif edilen posterior yaklaşım uygulandı. Fonksiyonel sonuçlar, Constant-Murley skorları kullanılarak değerlendirildi.

Bulgular: On hastada skapula boyun kırığı,glenoid fossa kırığı, beş hastada glenohumeral eklemi etkileyen skapular gövde kırığı ve bir hastada skapula proçes kırığı vardı.Ayrıca beş hastada klavıkula cisim kırığı varidi.Takip süresi ortalama 42 ay (6-92 ay). Omuz Constant-Murley skoru(CMS)ortalama93.8(±8.93)di.

Sonuç: Yer değiştirmiş skapular kırıkların açık redüksiyonu ve internal fiksasyonu , kaynama oranı ve fonksiyonel sonuç açısından etkili bir tedavi seçeneğidir.

Anahtar Kelimeler: Skapula Kırığı, Constant -Murley Skoru, Judet Yaklaşımı

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Introduction

Fractures of the scapula are rare injuries due to the thick muscle cover and account for an average of 0.5% of all fractures [1]. In the past, the most common treatment options were immobilization and rehabilitation. Scapular fractures are usually caused by high-energy trauma; therefore, they are associated with other multiple injuries [2]. The majority of extra-articular scapular fractures can be treated conservatively. Indications for surgical treatment of scapular fractures are controversial. Surgical treatment is recommended for severe displacement fractures, especially those involving the lateral column. Surgical indications include intra-articular glenoid fractures with 2-10 mm displacement, glenoid neck fractures with more than 10-25 mm medial/lateral displacement, angulation deformity greater than 25-45°, shortening greater than 25 mm and glenopolar angle less than angulation 22° and floating shoulder (more than 10 mm displacement with clavicle, acromion or coronoid process fracture or acromioclavicular dislocation) [3,4]. Insufficient bone stock, complex three-dimensional anatomy, and difficult surgical incisions create problems with open reduction and internal fixation [5]. Complications vary. Treatment of extra-articular scapula fractures is due to the paucity of information on treatment outcomes and relative unfamiliarity with the treatment of these injuries. Therefore, it has historically been non-operative. However, studies are showing that intra-articular fractures and some types of highly displaced fractures have better outcomes when treated operatively. These fractures require a well-designed diagnostic study and a properly implemented rehabilitation plan [6].

This study aims to describe the surgical technique after open reduction and internal fixation in patients with scapular fractures and to report the functional results and complications on a patient basis.

Materials and Methods

~~The study includes 16 patients with scapula fractures who~~ were treated with open reduction and internal fixation (ORIF) between September 2015 and March 2022. The clinical and demographic findings of the patients were obtained from the hospital records with the decision of the ethics committee. Patients' age, gender, side, anesthesia type, waiting time to start the operation, operation time, patient satisfaction with anesthesia and surgery were examined from medical records. Our surgical indication criteria are the following: medial/lateral displacement greater than 20 mm, shortening more than 25 mm, angular deformity greater than 40°,intra-articular deviation greater than 4 mm, fracture of the clavicle, coracoid or displaced acromion greater than 10 mm. All patients underwent radiological examination (AP in the scapular plane, lateral and axillary radiography) and computed tomography (CT) scans. Fractures were classified according to the revised (AO/OTA) classification system. The patients underwent deltopectoral and posterior approaches described by Judet. Functional outcomes were measured using Constant-Murley scores (Figure1,2,3).

Statistical Analysis

Normal distribution analysis was evaluated using 5 parameters (skewness-kurtosis, histogram, Standard Deviation/MEAN, histogram, and Q-Q plots). The frequency of demographic data was expressed as numbers and percentages. Data are shown as median (minimum-maximum). Spearman's Rho correlation analysis was performed between clinical aspects. Biserial correlation analysis was performed between the fracture site and clinical angles. Frequency analysis between patient classifications and CMS values was evaluated using the Fisher's exact test (minimum expected value<5). The SPSS 28.00 package program was used in the statistical analysis of the study.

Results

The mean age of the patients included in the study was 42.50±13.25 (95% CI: 35.18-49.31). The mean follow-up time after injury was 42 months (6-92 months). Sixteen patients with scapular neck fractures and glenoid fossa fractures in nine patients, scapular body fractures affecting the glenohumeral joint in five patients, and scapula process fracture in one patient were included in the study. Clavicle shaft fracture was accompanied in five patients. The injury mechanism was considered high-energy in all patients. Nine patients had more than one injury. Associated injuries in these multiple injured patients include rib fractures (n=3), pelvis fractures (n=1), thoracic vertebral fractures (n=1), pneumothorax (n=2), and ipsilateral clavicle fractures (n=5).

Union problem was seen in one patient and infraspinatus muscle atrophy was seen in two patients and no other complications were observed. Frequency analysis of the data is available in Table 1.

MLD (Medial Lateral Displacement) median value is 17.15 (0-32.60), 25% and 75% percentile values are 12.55 and 23.92, respectively.

GPA (Glenopolar Angle) median value is 34.35 (0-64.50), and 25% and 75% percentile values are 20.20 and 46.85, respectively.

AD (Angular Deformity) sagittal median value is 30.7 (0-63.00), 25% and 75% percentile values are 8.32 and 38.92, respectively,

APD (Anterior posterior displacement) median value is 13.85 (0-28.20) and 25% and 75% percentile values are 7.25 and 16.15, respectively (Table 2).

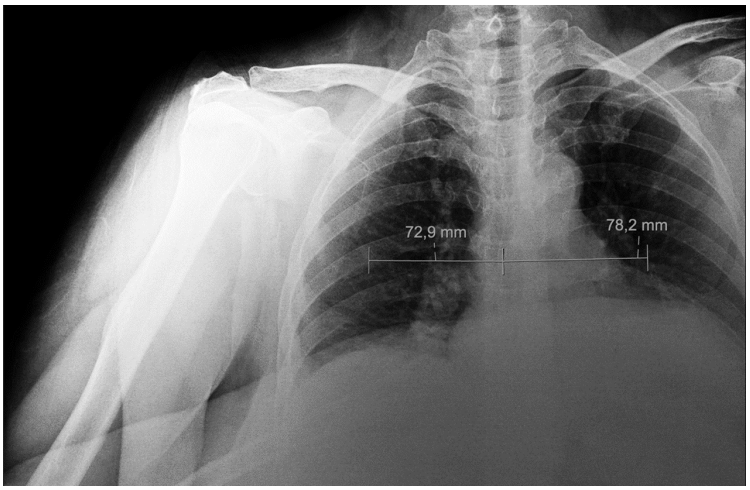

Figure 1. This is an AP radiograph of a 49-year-old female patient who has a scapula fracture.

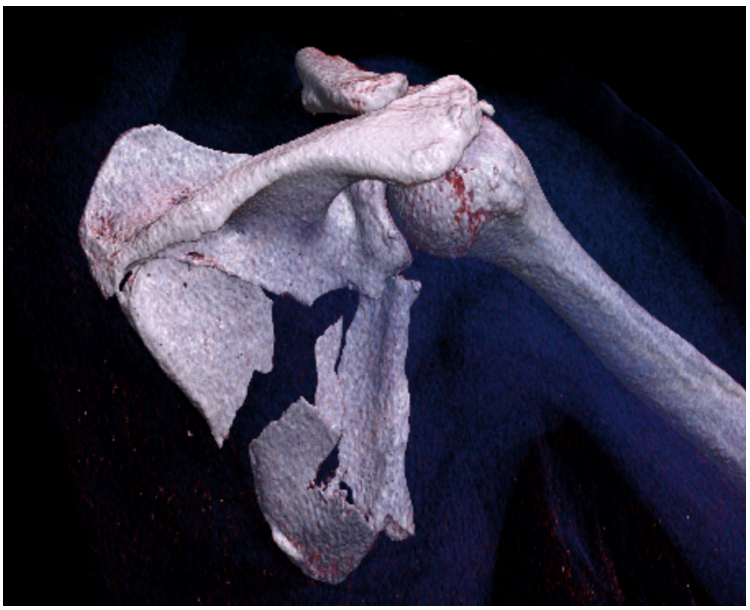

Figure 2. Comminuted fracture of the scapular body, extra-articular with a neck fracture, 3D CT reconstruction, posterior view in the same patient

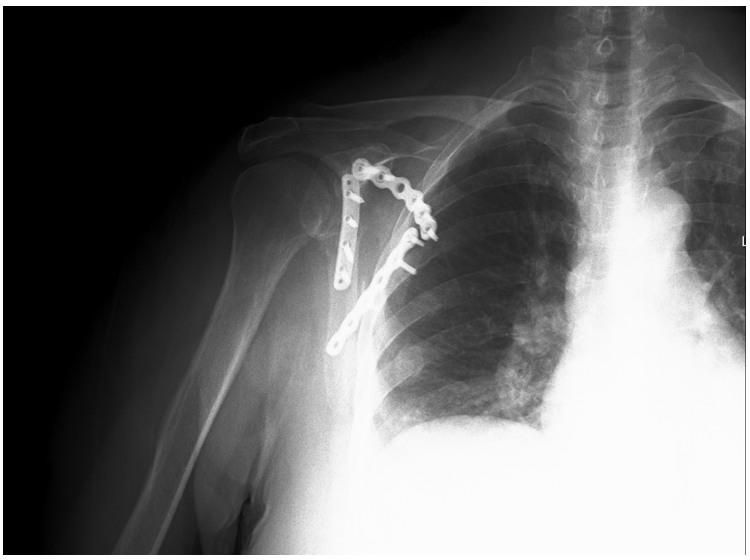

Figure 3. Post-op AP radiograph of the same patient 3 months later

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Table 1. Frequency analysis of the data

		Number(n=16)	Percent (%)
	Male	13	81.3
Gender	Female	3	18.7
	A3	5	31.3
	B1	1	6.3
	C1	1	6.3
	C2	1	6.3
Classification	C3	8	50
	No	2	12.5
Extra-articular	Yes	14	87.5
	No	6	37.5
Intra-articular	Yes	10	32.5
	No	12	75
Neck	Yes	4	25
	No	2	12.5
Body	Yes	14	87.5
	No	11	68.8
Clavicula Shaft	Yes	5	31.2
	No	15	93.8
Floating Shoulder	Yes	1	6.2
	No	0	0
Union	Yes	16	100
	poor	1	6.3
	good	3	18.8
	fair	2	12.5
CMS	very good	10	62.5

Table 2. Clinically significant angle values

	Minimum	%25 percentiles	Median	75% percentile	Maximum
MLD	0	12.55	17.15	23.92	32.6
GPA	0	20.2	34.35	46.85	64.5
AD sagittal	0	8.32	30.7	38.92	63
AP displacement	0	7.25	13.85	16.15	28.2

Medial Lateral Displacement (MLD)
Glenopolar Angle (GPA)
Angular Deformity (AD)
Anterior posterior displacement (APD)

There is a moderate positive correlation between MLD value and AD sagittal values (0.5<r<0.7, p<0.05)

There is a high positive correlation between MLD value and AP placement values (0.7<r<0.9, p<0.001).

There is a high positive correlation between AD sagittal and AP displacement values (0.7<r<0.9, p<0.01).

A double-point correlation was made between the presence of fracture direction and clinical values. There is a moderate positive correlation (0.5<r<0.7, p<0.5) between the presence of extra-articular fracture and the increase in the AD sagittal angle.

There is a moderate correlation between the incidence of extra-articular fractures and the increase in the AD sagittal angle.

There is a moderate positive correlation between the presence of neck fracture and increased GPA angle (0.5<r<0.7, p<0.1).

There is a moderate positive correlation between neck fracture and increased GPA angle in the cases. There is a moderate positive correlation (0.5<r<0.7, p<0.5) between the presence of body fracture and the increase in the AD sagittal angle.

There is a moderate correlation between the occurrence of fracture in the cases and the increase in the AD sagittal angle (Table 3).

Cross-tabulation was made according to CMS classification. In this test, where Fischer's Exact test (minimum expected value<5) was applied, the same letters show that they belong to the same group, and different letters show that they belong to a different group.

Discussion

Surgical treatment of patients with scapula fractures has recently been better recognized as a treatment option with a predictable outcome. Conservative treatment, used predominantly in the past, has often resulted in reduced range of motion and functional outcomes. Displaced fractures of the scapula can alter shoulder girdle function due to malalignment, dysfunction of the rotator cuff, arthrosis, impingement, and scapulothoracic dyskinesia.

Esenkaya et al. in a prospective study in which anatomic plate osteosynthesis was performed in nine patients with a mean follow-up period of 39.8 (12-77) months and Miller Type 2 or 4 scapula fractures, the results were evaluated using the Herscovici score, and it was found to be a safe method allowing an early range of motion [7]. Similar results were found in our study.

Anavian et al. thirty-three patients with displaced intra-articular glenoid fractures were treated surgically. Five patients had extra-articular involvement of the scapula. A posterior approach was used in 21 patients, an anterior approach in seven patients, and a combined approach in five patients. Functional results including Arm, Shoulder, and Hand Disabilities (DASH) and Short Form-36 (SF-36) scores, shoulder motion and strength, and return to work and/or activities were achieved in thirty patients (91%). Surgical treatment of complex, displaced intra-articular glenoid fractures with or without scapular neck and body involvement has been shown to have good functional outcomes and a low complication rate [8]. They observed brachial plexus damage in one patient and suprascapular nerve damage in 8 patients. In our study, there were two suprascapular nerve lesions.

Table 3. Correlation between the presence of fracture and clinical angles

		MLD	GPA	AD sagittal	AP displacement
Extra-articular					
	r	0.472	0.185	0.513	0.472
	p	0.065	0.494	0.042	0.065
Intra-articular					
	r	-0.225	-0.084	-0.112	-0.042
	p	0.403	0.757	0.679	0.877
Neck					
	r	-0.298	0.689	0	-0.125
	p	0.262	0.003	1	0.643
Body					
	r	0.472	0.185	0.513	0.472
	p	0.065	0.494	0.042	0.065

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61 patients with extra-articular fractures of the scapula were surgically treated within 20 days of injury. 49 of 61 patients (80%) were followed for ≥1 year (mean 33 months) after surgery. After follow-up, 100% union was observed, and mean Arm, Shoulder, and Hand Disabilities (DASH) score was 12.1 points (0-54 points). Complications and/or secondary surgery were performed in eight patients (16%). Displaced scapular body and glenoid neck fractures treated with ARIF had good functional outcomes [9].

Of the 250 patients operated for scapula fracture, 16 geriatric patients aged 65 and older were identified. Dash, Short Form Health Questionnaire versions 1 and 2 (SF-36), Range of Motion (ROM), and strength at the last follow-up of 1 year or longer were evaluated. Minor perioperative complications were seen in three patients (transient delirium in 2 patients, and urinary tract infection in 1 patient). One patient required subsequent removal of the intra-articular screw and one patient required resection of the heterotopic ossification and the implant was requested to be removed. The mean ROM ranged from 78% to 96%, and the mean strength ranged from 76% to 92%. Displaced in patients aged 65 and over operative treatment for fractures has been reported to be safe and yield good functional results [10]. No heterotopic ossification case or minor perioperative complications, urinary tract infection (delirium) were observed in our study.

Strengths and limitations of the study: When compared with the literature, there was no significant difference between the results of surgical treatment applied in such fractures and the results we found. However, the limitation of this study is the lack of a sufficient number of patients, and the follow-up period is relatively short. However, scapula fractures are rare and are mostly treated conservatively. Indications for surgical treatment are limited. On the other hand, there is no clear comparative evidence on the outcomes of fractures treated by surgical and non-surgical methods [11,12]. Therefore, there are few studies on the results of surgical treatment of scapula fractures. The results of our study may contribute to national data and/or systematic reviews and meta-analyses [13] or clinical practice guidelines [14], at least together with other studies on the subject from our country.

Conclusion

Open reduction and internal fixation of displaced scapular fractures is an effective treatment option in terms of union rate and functional outcome.

Conflict of Interest: The authors declare no conflict of interest related to this article.

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Ethics Committee Approval: This study was approved by the Ethics Committee of Antalya Training and Research Hospital with Approval No:12/8 and Date: 07/09/2023

ORCID and Author contribution: İ.E. (*0000-0002-0469-2062):* The author contributed to the research design and interpretation of data and the drafting and revising of the manuscript.

Peer-review: Externally peer-reviewed.

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Case Report

Olgu Sunumu

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A Diagnosis that Is Probably Missed: Rubeola Lymphadenitis, an Epidemic that Causes a Renewed Alarm

Muhtemelen Atlanılan Bir Tanı: Rubeola Lenfadeniti, Yeniden Alarm Veren Bir Salgın

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ABSTRACT

Because Rubeola (measles) infection is not observed in our country after eradication and is especially on the agenda again after migrations, pathologists may find it challenging to histopathologically recognize cases of Rubeola lymphadenitis and establish an accurate diagnosis. Here we describe the histopathological features of a rare Rubeola case. A 15-year-old Syrian migrant male patient was admitted with a complaint of lymph node swelling in the postauricular region for 2 months. Lymph node excision was performed with a preliminary diagnosis of lymphoma. The excised lymph node was subjected to a routine pathological examination in our clinic. Histopathological examination revealed that the basic structure was preserved under the thick fibrous capsule in the lymph node. Warthin-Finkeldey-type giant cells attracted attention in the interfollicular areas. The appearance compatible with lymphoproliferative neoplasia was not detected. The relevant clinic was informed that there were histological signs of Rubeola lymphadenitis upon detection of Rubeola IgM positivity in the examination, the case was evaluated as Rubeola infection. Rubeola was a common deadly infectious disease in the past century before the vaccine was developed. Today, there has been an epidemic again due to vaccine hesitancy, migration, and sociocultural conditions. Because it is exceptionally rare, we hope that the case we present will provide insights to pathologists for recognizing cases of Rubeola lymphadenitis and making precise diagnoses.

Key Words: Warthin-Finkeldey Cells, Measles, Rubeola, Hematopathology

ÖZET

Rubeola (Kızamık) enfeksiyonu, eradikasyon sonrası ülkemizde görülmediğinden ve özellikle göçler sonrası tekrar gündeme gelmesinden dolayı Rubeola lenfadenit vakalarını histopatolojik olarak tanımak ve doğru tanı koyabilmek patologlar için zorlayıcı olabiliyor. Burada nadir rastladığımız bir Rubeola vakasının histopatolojik özelliklerini tanıtmayı amaçladık. İki aydır postauriküler bölgede lenf nodlarında şişlik şikayeti olan 15 yaşındaki Suriyeli göçmen erkek hastanın lenfoma ön tanısı ile lenf nodu ekzisyonu yapıldı. Ekzisyon yapılan lenf nodu, kliniğimizde rutin patolojik sürece tabi tutuldu. Histopatolojik incelemede lenf nodunda kalın fibröz kapsül altında temel yapının korunmuş olduğu görüldü; ancak interfoliküler alanlarda Warthin-Finkeldey-tip dev hücreler dikkati çekti. Lenfoproliferatif neoplazi ile uyumlu görünüm saptanmadı. Olguda histopatolojik olarak Rubeola lenfadeniti bulgularının olduğu hastayı takip eden kliniğe bildirildi. Yapılan incelemede Rubeola IgM pozitif olarak saptanması üzerine olgu, Rubeola enfeksiyonu olarak değerlendirildi. Rubeola, geçmiş yüzyılda aşı geliştirilmeden önce yaygın bir ölümcül enfeksiyöz hastalıktı. Günümüzde aşılama karşıtı tutumdan, göçlerden ve sosyokültürel şartlardan dolayı tekrar epidemi söz konusu oldu. Oldukça nadir görüldüğünden Rubeola lenfadenit vakalarını tanımak ve doğru tanı koyabilmek adına sunduğumuz vakanın patologlara ışık tutmasını umuyoruz.

Anahtar Kelimeler: Warthin-Finkeldey Hücreleri, Kızamık, Rubeola, Hematopatoloji

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Introduction

Rubeola (measles) was a common infectious disease worldwide before vaccination, which began in 1963. Before the introduction of vaccination, approximately 3 to 4 million people were infected annually in the United States [1-3]. In Turkey, the Extended Immunization Program, which consists of rules that must be followed for managing vaccination services, was implemented in 1981. Rubeola vaccination programs were initiated in our country in 2003 and 2005. Between 2007 and 2011, the number of infected cases remained below five, whereas no cases were followed up from 2008 to 2010. However, after migrants arrived in Turkey because of the civil war in Syria in 2011 and the increase in the percentage of unvaccinated individuals, an epidemic broke out in Turkey in 2013 [4,5].

Anti-vaccination campaigns have also had a great impact on the outbreaks of eliminated diseases such as Rubeola. Vaccination rates have decreased worldwide, particularly because of the attitude adopted by parents who have been misinformed through platforms such as the Internet, social media, and television [6]. For example, the Measles, Mumps, and Rubella (MMR) vaccination rate in the United Kingdom fell from 92% in 1996 to 84% in 2002, and by 2003, in some parts of the country, this rate fell to 61% [7].

Most pathologists have little experience with Rubeola's histopathology because it is still relatively rare in our country We here aim to present the clinicopathologic features of Rubeola lymphadenitis, which is an unusual diagnosis for pathologists, and hope that these findings will shed some more light on the recognition of Rubeola infection.

Case Presentation

A 15-year-old Syrian migrant male patient with no previous medical history, was admitted to our hospital with a complaint of regional swelling in the postauricular region. Swelling had been present for 2 months. He had no complaints such as B symptoms. The patient's family history and medical and surgical history were unremarkable. No information about the patient's vaccination history was available. Physical examination revealed enlarged lymph nodes in the postauricular region. Routine laboratory tests revealed leukopenia and lymphopenia. Viral load was not detected by real-time Epstein– Barr virus (EBV) PCR or cytomegalovirus (CMV) PCR. Enzyme-linked immunosorbent assay (ELISA) was used to detect IgM and IgG antibodies in serum, including Herpes simplex type 1/2, Brucella, Varicella Zoster Virus, Mumps, Anti-HBc, Toxoplasma, and HBsAg. The ELISA test was negative for all. In the evaluation of peripheral blood smears, a normochromatic normocytic erythroid series was observed; however, atypia did not attract attention. One of the largest lymph nodes was excised and sent to our pathology department.

Histological sections revealed samples of lymph nodes containing a thick fibrous capsule. Hyalinized bands were present between secondary follicles with large and small germinal centers. Apoptotic activity was evident in lymphoid follicles. There were no signs of malignancy in the lymphocytes. In the interfollicular areas, multinucleated giant cells (Warthin-Finkeldey cells) were observed [Figure-1].

Histologically and immunohistochemically, there were no findings consistent with lymphoproliferative neoplasia.

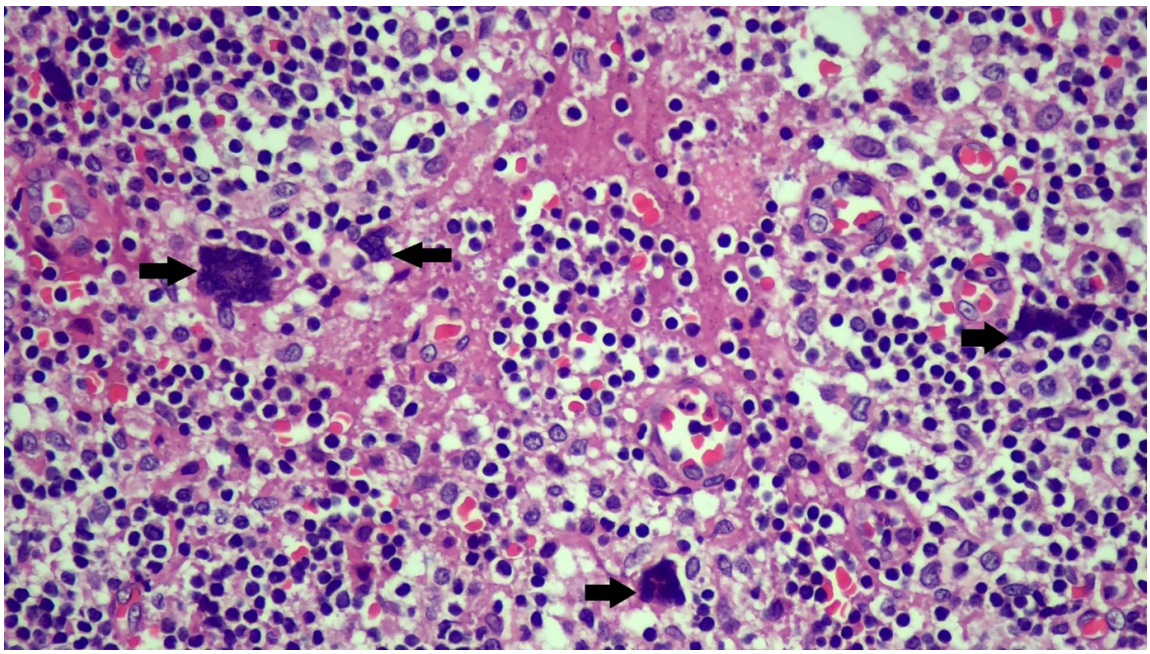

Figure 1. Warthin-Finkeldey cells (black arrows) with nonneoplastic lymphoid tissue on the background (Hematoxylin & Eosin x400)

Immunohistochemical staining for other diseases considered in the differential diagnosis (e.g., EBV infection, HIV lymphadenopathy, Hodgkin lymphoma, other B and T cell lymphomas) (CD3, CD20, PAX5, CD15, CD30, EBV) did not show significant results. The Ki67 proliferation index was within normal limits [Figure-2].

When clinical and histological findings were evaluated together, it was reported to the relevant clinic that there may be Rubeola lymphadenitis in the case, and the clinic was requested to conduct tests for Rubeola. The ELISA test was positive for Rubeola IgM, and the case was considered as "Rubeola lymphadenitis". Subsequently, the patient was isolated, and because there was no specific treatment, the patient was given supportive treatment such as antipyretics, vitamin A, and fluids. No complications have developed.

Discussion

Warthin-Finkeldey-type giant cells were first described by Dr. A. Warthin and Dr. W. Finkeldey in 1931. They named these cells in the lymphoid tissues of children with Rubeola. Warthin-Finkeldey cells (WFCs) have numerous closely packed overlapping nuclei with or without eosinophilic nuclear inclusions [8,9]. At first, WFCs were thought to be pathognomonic for Rubeola. However, these cells have been identified in many other conditions such as Kimura disease, HIV lymphadenopathy, Hodgkin lymphoma, B- and T-cell lymphomas, and nonneoplastic lymph node disorders. It is still unclear whether the cause of the formation of WFCs is intercellular fusion caused by the Rubeola virus, and whether the origin of the cells is lymphoid or dendritic cells. WFCs can be seen in malignant diseases such as various lymphomas, as well as in benign conditions such as HIV lymphadenopathy and Kimura's disease [10]. For example, in the Figure-3 cited in a study by Aladily et al., WFCs attracted attention in this case with signs of follicular lymphoma. Malignant lymphocytes on the ground were also observed in this case [11]. However, in this case, the presence of ordinary lymphocytes (morphologically recognized and immunohistochemically proven) ruled out malignant conditions.

According to Fenner's model, Rubeola is considered to develop in four stages: primary invasion, proliferation of lymphoid tissue, secondary viremia, and florid disease. Rubeola infection begins when the virus attacks the respiratory system, and then undergoes replication. Primary viremia causes the virus to spread in lymphoid tissues throughout the body. Replication of the virus in lymphoid tissue causes hyperplasia in the lymphoid tissue, and WFC

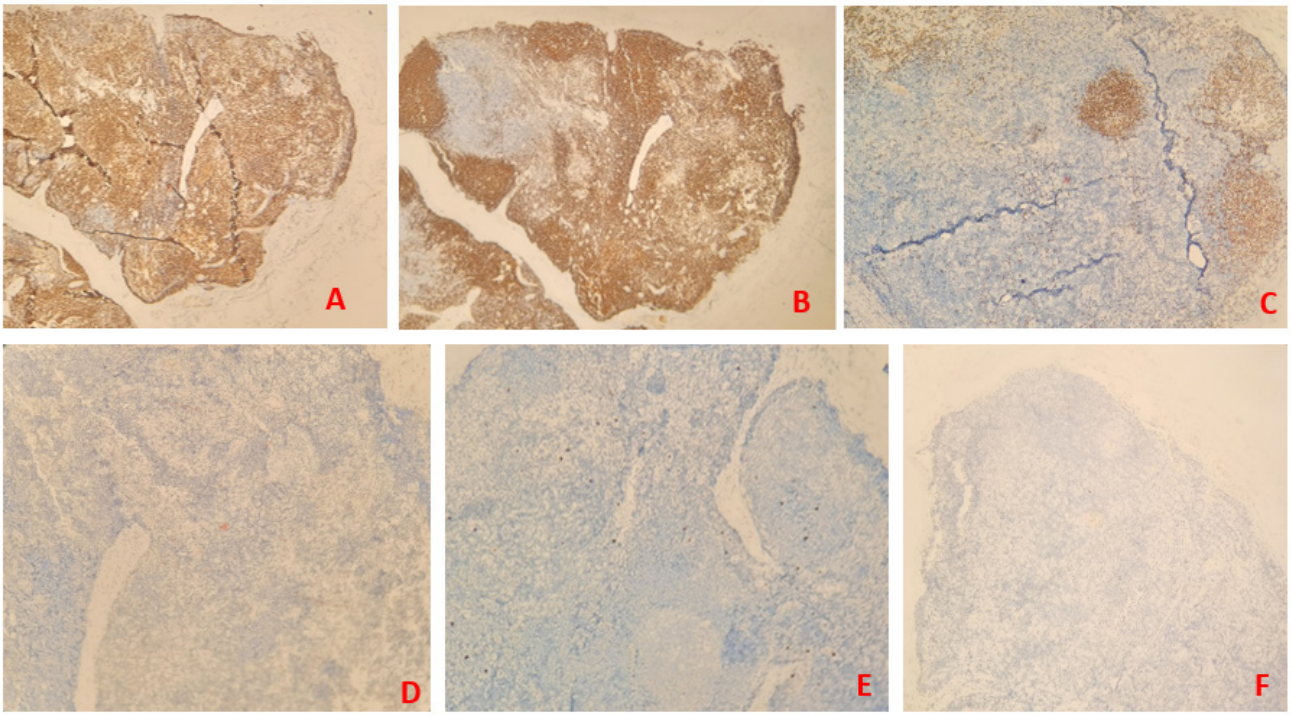

Figure 2. Immunohistochemical expression of CD3, CD20, PAX5, EBV, CD30, and CD15, respectively (A, B, C, D, E, F). A: CD3 staining was observed in nonneoplastic T lymphocytes (magnification, x100). B: CD20 staining was observed in nonneoplastic B lymphocytes (magnification, x100). C: PAX5 staining was observed in nonneoplastic B lymphocytes (magnification, x100). D, E, F: EBV, CD30, and CD15, respectively; no significant staining was observed with EBV, CD30, and CD15 (magnification, x100) (there were artificial deposits).

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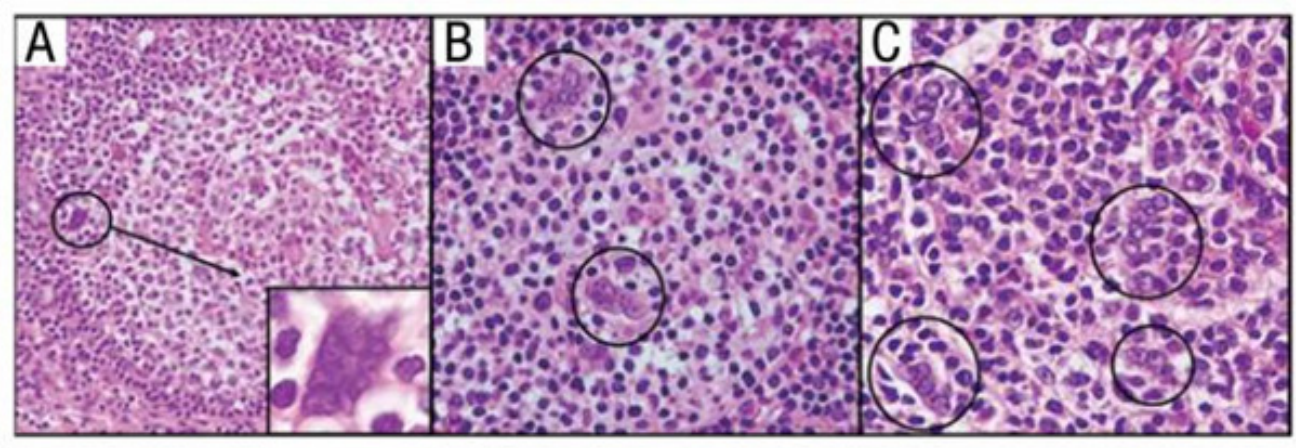

Figure 3. A: Warthin-Finkeldey cells, located on the periphery and central of the follicles (Hematoxylin & Eosin staining, magnification x200; inset at x1000). B & C: neoplastic follicles composed of small, cleaved cells of follicular lymphoma, (magnification x600) [11].

formation is induced. Immediately after this, the second stage of viremia begins. At this stage, the virus is spread to other organs by infected lymphocytes and monocytes [12]. In the prodromal and early stages of Rubeola, WFCs are often observed in germinal centers or interfollicular areas in lymph nodes [13].

Nowadays, the incidence of Rubeola is increasing, and the recognition of WFCs in routine samples by pathologists can alert clinicians to suspect Rubeola, thereby perhaps offering the potential for earlier detection. Thus, the spread of the disease can be prevented, and this re-emerging disease can be prevented from becoming an epidemic.

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