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Acute Effect of Kinesiology Taping Applied to Adductor Muscle Group on Endurance, Strenght and Agility in Volleyball Players

Voleybolcularda Addüktör Kas Grubuna Uygulanan Kinezyolojik Bantlamanın Endurans, Güç ve Çeviklik Üzerine Akut Etkisi

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ABSTRACT

Objective: This study was conducted to investigate the acute effect of kinesiology taping (KT) to the hip adductor muscle group (AMG) on endurance, strength and agility in female volleyball players (VP).

Materials and Methods: Thirty female VP, with an average age of 17.53±2.55 years, who played licensed volleyball for at least two years were included in our study. KT was applied longitudinally to the athletes between the pubis and lower border of the tuberositas tibia, including the AMG using the facilitation technique (25% stretching). Evaluations were repeated before taping and 20 minutes after taping. The athletes' Adductor muscle endurance was evaluated using the Copenhagen adduction exercise, vertical jump test for lower extremity strength, and Edgren side step test for agility.

Results: When the results of the study were evaluated, it was found that there was a statistically significant increase in the endurance ($p<0.001$), strength ($p<0.001$) and agility ($p<0.001$) values of the athletes after KT application.

Conclusions: KT applied to the adductor area can be used by physiotherapists during competitions or training due to its positive effects on the performance of VP.

Keywords: Adductor muscles, agility, endurance, kinesiology tape, strength

ÖZ

Amaç: Bu çalışma kadın voleybolcularda, kalça addüktör kas grubuna uygulanan kinezyolojik bant (KB) uygulamasının endurans, güç ve çeviklik üzerine olan akut etkisini araştırmak amacıyla yapılmıştır.

Materyal ve Metot: Çalışmamıza en az iki yıl süre ile lisanslı voleybol oynayan, ortalama yaşı 17,3±2,55 yıl olan, 30 amatör kadın voleybolcu dahil edilmiştir. Sporculara KB, addüktör kas grubunu içine alacak şekilde pubis ile tuberositas tibia alt sınırı arasına, fasilitasyon tekniği (%25 gerilimle) kullanılarak uygulanmıştır. Değerlendirmeler bantlama öncesi ve bantlamadan 20 dakika sonra olmak üzere tekrarlanmıştır. Sporcuların addüktör kas enduransı Kopenhag addüksiyon egzersizi, alt ekstremite gücü dikey sıçrama testi, çevikliği ise Edgren yana adımlama testi kullanılarak değerlendirilmiştir.

Bulgular: KB uygulaması sonrası sporcuların endurans ($p<0,001$), güç ($p<0,001$) ve çeviklik ($p<0,001$) değerlerinde istatistiksel olarak anlamlı artış olduğu saptanmıştır.

Sonuç: Voleybolcularda addüktör bölgeye uygulanacak KB sporcuların performanslarındaki olumlu etkilerinden dolayı müsabaka veya antrenmanlar sırasında kullanılabilir.

Anahtar Kelimeler: Addüktör kaslar, çeviklik, endurans, güç, kinezyolojik bant

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INTRODUCTION

The hip joint is unique in its wide range of motion and high stability through its strong and balanced musculature. The hip muscles, which are approximately two dozen in number and allow the joint to move in three planes, have important functions in producing and transferring forces during activities involving the upper extremity, except athletic activities, including the lower extremities, such as running and jumping.[1]

The adductor muscle group (AMG), responsible for controlling the movements of the femur and pelvis, contributes to the hip joint flexion, extension and internal rotation, and adduction of the hip joint. The torque generated by the AMG on the hip joint in three planes contributes to the performance of repetitive activities such as running or squatting.[1] In addition, the AMG also plays a role in transferring the forces to the ground during sudden changes in direction and speed.[2]

The fundamental skills affecting success in the volleyball branch are jumping performance, the ability to move quickly in the field, and hitting the ball at the required tempo of the volleyball player (VP). These fundamental skills, which are related to various physical fitness components such as strength, endurance, agility, and speed, also require the harmony and synchronisation of the upper and lower extremities.[3]

Kinesiology taping (KT), developed by Dr. Kenzo Kase in 1979, has been an effective method in physiotherapy and rehabilitation in recent years. KT, which positively affects joint range of motion, muscle strength, function and healing processes, is used in athletes to prevent injuries and as a part of the physical therapy and rehabilitation program applied after injury. Apart from all these, the KT is also preferred to improve the performance of athletes from various sports branches.[4]

Our study aimed to investigate the acute effect of KT on endurance, strength, and agility in VP.

MATERIALS AND METHODS

Ethics Committee Approval: To conduct the study, official permission and approval were obtained from the Pamukkale University Non-Invasive Clinical Research Ethics Committee (Date: 12/09/2019, decision no: 60116787-020/62662). During the study, the Helsinki Declaration criteria were complied with to protect the athletes' data, and written consent was obtained from all the athletes participating in the study.

Study Design and Participants: This study was designed as an experimental study. Thirty female VP, aged between 15-23, were included in our study. Inclusion criteria: being a female between the ages of 16 and 25, being a licensed volleyball player for at least 2 years, training at least 3 days a week, and cooperating with the parameters to be applied in the study. Exclusion criteria: having undergone any surgery in the last 6 months, no musculoskeletal injury in the previous 3 months, active inflammation in the participant, open wound on the skin in the KT area.

Data Collection: Before evaluation, the descriptive information of the athletes was recorded. The tests used to evaluate the athletes were performed twice, before and 20 minutes after KT. Before the tests, athletes were asked to warm up for 10 minutes.

Muscular Endurance Evaluation: The athletes' endurance was evaluated using the Copenhagen exercise protocol.[5] The athletes were positioned in the side-lying position with the head, trunk and lower extremities in the same alignment and the forearm at 90° flexion as the support point. The athlete, whose upper lower extremity was supported at the ankle and knee joints by the researcher, was asked to adduct the lower extremity (dominant extremity) and touch the upper foot. The number of adduction exercises was noted regardless of time.

Muscular Strength Evaluation: The vertical jump test was used to evaluate the strength of the athletes. The data from the vertical jump test were recorded using the "My Jump 2" iPhone 7® smartphone application, whose validity and reliability studies were conducted.[6] Athletes were asked to squat and then jump vertically. Athletes were warned not to bend their knees and to keep their hands on the waist during jumping. This process was repeated 3 times. The best jump height was recorded in centimeters (cm).

Agility Evaluation: Edgren side step test was used to evaluate. Five cones were placed in parallel at 1 metre intervals. The athlete was asked to side-step from the rightmost cone to the leftmost cone at the highest speed. One point was given for every 1 metre completed in 10 seconds. A point was not given if the athlete rotated her trunk or crossed her legs during side-stepping.[7]

Kinesiology Tape Application: KT was applied to the AMG of the lower extremity 10 minutes after the first evaluation. Before KT, the area to be taped was cleaned with alcohol. Athletes were positioned in the supine position with hip joint in flexion + abduction. The hip AMG was covered with the I-shaped band using the facilitation technique, with 25% tension applied in the direction of the origo-insertion. The athletes were evaluated for a second time after waiting 20 minutes to adapt and for the effects of the KT to be revealed.[8]

Statistical Analysis: The sample size was calculated using G*Power software (Version 3.1.9.5. Institut der Universit€at Bonn, Bonn, Germany). Type-1

error rate was accepted at 0.05 and power rate at 95%. The effect size was calculated as 0.72 using the data of the reference study.[9] According to the power analysis results, the number of athletes to be included in the study was 27**.** Thirty athletes were included in the study. Statistical analyses were performed using the SPSS 23. The statistical significance level was set at $p<0.05$. All data were presented as mean±standard deviation. The dependent samples t-test was used to analyse data before and after KT.

RESULTS

Thirty athletes participated in the study. The mean age of the athletes was 17.53±2.55 years, their body weight was 57.23±5.35 kg, their height was 169.36±6.89 cm, and their body mass index was 19.93±1.29 kg/m² (Table 1).

When the athletes' endurance before and after KT were examined, the difference was statistically significant ($p<0.001$). It was determined that there was a statistically significant increase in the muscular strength measured with the "My Jump 2" after KT ($p<0.001$). Similarly, the increase in the agility of the athletes evaluated with the Edgren side step test after KT was found to be statistically significant ($p<0.001$) (Table 2).

Table 1. Demographic characteristics of athletes.

Variables	X±SD (n=30)
Age (year)	17.53±2.55
Body Weight (kg)	57.23±5.38
Height (cm)	169.36±6.89
Body Mass Index (kg/m^2)	19.93±1.29

X: mean; SD: Standard deviation.

Table 2. Comparison of athletes' endurance, strength and agility pre and post-kinesiology taping application.

Variables	Pre-KT application (X±SD)	Post-KT application (X±SD)	Variation (%)	p
Endurance (Repetation number)	19.00±5.25	22.66±5.92	20.21	0.000*
Strength (Jump height)	26.75±4.39	27.89±5.00	4.1	0.000*
Agility (Number of cones passed)	20.03±1.58	21.86±1.45	9.4	0.000*

*p<0.05; X: mean; SD: Standard deviation; KT: Kinesiology taping.

DISCUSSION AND CONCLUSION

In our study, in which we examined the acute effect of KT applied to the AMG in VP on the endurance, strength, and agility of the athletes, the acute improvement caused by the KT in the endurance, power and agility of the athletes were found.

In volleyball, an interval sport with both aerobic and anaerobic components, muscular endurance is extremely important to squat, jump and move in different directions.[10,11] In the literature, we found no study showing the effect of KT applied to the adductor region in VP on adductor muscle endurance; it is also seen that there is no consensus on the results of the studies conducted with different muscle groups. Studies evaluating the acute effect of KT on endurance in different muscles such as finger flexors,[12] quadriceps,[13] and gastrocnemius[14] have shown that KT does not increase endurance. In these studies, KT was applied from origin to insertion to facilitate different muscle groups. Contrary to the common view, studies in the literature suggest that KT should be applied from insertion to origin to provide muscle facilitation.[9,15] Therefore, studies investigating KT's effects on muscle endurance with different techniques should be conducted.

In our study, it was found that KT applied to the AMG caused an acute increase in endurance. There are also studies in the literature reporting similar results to the findings of our study and examining different populations and muscle groups. A study investigating KT's effect on abdominal muscle endurance reported that KT acutely increased trunk flexor and left lateral muscle endurance.[16] In another study investigating the acute effects of KT applied to the forearm in tennis players, significant positive effects of KT on wrist flexor muscle endurance were shown.[17] We can explain the positive effect of KT on endurance by the increase in cutaneous afferent inputs and, consequently, facilitation in motor neuron excitability.[18] In addition, we think that one of the potential reasons for a positive effect on endurance may be the placebo effect.[19]

Vertical jumping skills in VP are another important factor for their service, spike, block, and defence performances.[20] Athletes need to generate as much power as possible in the shortest possible time to

optimise their vertical jump performance, which is affected by both muscular and neural mechanisms.[21] A correlation is observed between jump height and lower extremity muscle strength in VP.[20] Different results have been reported concerning the effects of KT on muscle strength. In a study examining the effect of KT applied to quadriceps and gastrocnemius muscles in VP on jump height and strength, it was reported that KT did not cause a significant change in the jump performance of participants.[22] Similarly, another study involving football players found that KT did not cause an acutely significant increase in quadriceps strength.[23]

In our study, we observed that the jumping performance of VP increased acutely after KT. There are also studies in the literature that support our results. In a study involving basketball players, it was observed that the vertical jump performance of athletes increased significantly.[24] Aktaş and Baltacı also reported that the increase in isokinetic knee extension peak torque value and jump performance was significant after KT.[25] We think the different results in the literature may be due to factors such as the selected KT technique, the test method used and the population.

Biomotor skills such as agility, coordination, and reaction became prominent characteristics that athletes should have in sports branches played in narrow spaces, such as volleyball. Agility, which can be defined as a minimal loss of control and the ability to change direction at an average speed, has positive effects on the performance of athletes by contributing to the effective acceleration and deceleration of VP in a short time.[11,26,27] In our study, we found that the agility performance of athletes increased after KT was applied to the adductor region. We think that the positive effect of KT on agility performance is related to both the increase in proprioceptive sensation caused by stimulation of mechanoreceptors[28] and the application of KT to the AMG, which will contribute to stabilising hip and knee joints.[1] Although studies in the literature report that KT increases agility,[8,29] there are also studies reporting the opposite. It has been reported that KT in VP, by supporting the peroneus longus and peroneus brevis muscles and the tibiofibular ligaments of both legs, does not cause a significant change in the agility performance of athletes.[8] Similarly, after KT was applied to both quadriceps muscles in football players, no significant changes were found in the agility performances of the athletes.[30] In these studies, we think that the effect of KT on agility may have been insufficient due to the evaluation of agility with different methods, a small sample size, and low statistical power.

The limitations of our study are that the researchers could not be blinded, the sample size was small, only a female VP was included in the study, the relationship between the dominant and non-dominant extremities of the athletes was not investigated, there was no control group, and examination of only the acute effect of KT on endurance, strength, and agility.

In conclusion, our study will contribute to the literature because no analysis shows the effect of KT applied to the adductor region on performance in VP. We think that the KT can be used to improve performance and prevent injuries in VP. Studies can be conducted to investigate the long-term effects of KT with larger sample sizes, evaluating symptomatic VP and including athletes from different sports branches.

Ethics Committee Approval: To conduct the study, official permission and approval were obtained from the Pamukkale University Non-Invasive Clinical Research Ethics Committee (Date: 12/09/2019, decision no: 60116787-020/62662). During the study, the Helsinki Declaration criteria were complied with to protect the athletes' data, and written consent was obtained from all the athletes participating in the study.

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The Relationship Between Fear of Death and Healthy Lifestyle Behaviors in Individuals Having Percutaneous Coronary Intervention

Perkütan Koroner Girişim Geçiren Bireylerde Ölüm Korkusu ve Sağlıklı Yaşam Biçimi Davranışları Arasındaki İlişki

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ABSTRACT

Objective: The study aims to investigate the relationship between fear of death and healthy lifestyle behaviors in individuals undergoing percutaneous coronary intervention.

Materials and Methods: The sample number was determined to be 109. The study was carried out in the angiography unit and cardiology clinics of a hospital. Data were collected using the Thorson-Powell Death Anxiety and Healthy Lifestyle Behaviors II Scales.

Results: The patients' death anxiety scale score was 55.17±11.52 and their healthy lifestyle behavior scale score was 123.51±14.56. A negative relationship (r = -0.683, p = 0.003) was found between death anxiety and healthy lifestyle behavior scales. As death anxiety increased, healthy lifestyle behaviors decreased. A negative correlation was found between death anxiety level and physical activity (r = -0.720, p=0.002). As physical activity decreased, death anxiety level increased.

Conclusions: In order for patients to experience less death anxiety, the importance of a healthy lifestyle, such as diet, exercise, spiritual development, stress management, and a healthy social life, should be known. Healthy lifestyle education programs should be applied to patients. To ensure permanence, patients should be supported with visuals and brochures, and training should be repeated.

Keywords: Intervention, percutaneous coronary, death, health, lifestyle

ÖZ

Amaç: Perkütan koroner girişim geçiren bireylerde ölüm korkusu ve sağlıklı yaşam biçimi davranışları arasındaki ilişkiyi incelemektir.

Materyal ve Metot: Örneklem sayısı 109 olarak belirlendi. Çalışma, bir hastanenin anjiyo ünitesi ve kardiyoloji kliniklerinde gerçekleştirildi. Veriler, Thorson-Powell Ölüm Kaygısı ve Sağlıklı Yaşam Biçimi Davranışları II ölçekleriyle toplandı.

Bulgular: Hastaların ölüm kaygısı ölçeği puanı 55,17±11,52 ve sağlıklı yaşam biçimi davranışları ölçeği puanı 123,51±14,56 olarak bulundu. Ölüm kaygısı ile sağlıklı yaşam biçimi davranışları ölçekleri arasında negatif ilişki (r = -0,683, p= 0,003) bulundu. Ölüm kaygısı arttıkça sağlıklı yaşam biçimi davranışları azaldı. Ölüm kaygısı düzeyi ile fiziksel aktivite arasında negatif korelasyon (r = -0,720, p=0,002) bulundu. Fiziksel aktivite azaldıkça ölüm kaygısı düzeyi arttı.

Sonuç: Hastaların daha az ölüm kaygısı yaşamaları için diyet, egzersiz, manevi gelişim, stres yönetimi ve sağlıklı sosyal hayat gibi sağlıklı yaşam tarzının önemi bilinmelidir. Hastalara sağlıklı yaşam eğitim programları uygulanmalıdır. Kalıcılığı sağlamak için hastalar görsellerle ve broşürlerle desteklenmeli, eğitimler tekrarlanmalıdır.

Anahtar Kelimeler: Girişim, perkütan koroner, ölüm, sağlık, yaşam tarzı

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INTRODUCTION

Cardiovascular diseases are the leading cause of death in the world and our country. According to the TUIK (Turkish Statistical Institute) 2020 report, when deaths in our country are examined according to their causes, cardiovascular diseases take the first place with 36.8% in 2019.[1] According to the WHO 2016 report, when the causes of death in the world are examined, cardiovascular diseases come first with 32.3% in 2016.[2] Percutaneous coronary intervention (PCI) is considered among the minimally invasive surgical interventions.[3,4] Necrosis in the heart muscle is prevented by balloon technique or stenting in narrowed or occluded coronaries.[5] Patients feel less pain in PCI.[6] PCI offers less limitation of motion and the possibility of bleeding compared to other surgical interventions of the heart. In addition to all these advantages, PCI still carries risks. The location and degree of obstruction, the skill of the physician, and the quality of the materials used are risk factors.[7]

Patients resort to PCI in critical situations such as chest pain and heart attack. In this process, patients experience an intense emotion between life and death. After experiencing the first myocardial infarction, patients tend to change their behavior.[8] Understanding the seriousness of the clinical situation is associated with fear of death.[9,10] Myocardial ischemia is associated with exercise intolerance that limits patients' activities of daily living.[6] The antiischemic effect of exercise reduces myocardial oxygen demand by lowering resting heart rate and systolic blood pressure.[11]

One out of every five people living with CAD also lives with depression and/or anxiety.[12] Perera et al. found that female patients had low adherence to healthy lifestyles after PCI. He stated that anxiety and lack of motivation in women led to this result.[10] Bendig et al. found that depression decreased in CAD as a result of web-based training.[12] The need for PCI and the risk of complications decrease in CAD patients who develop healthy lifestyle behaviors.[8] There are studies in the literature examining the relationship between depression and anxiety and healthy lifestyle behaviors in patients with PCI.[12]

The study aims to examine the relationship between fear of death and healthy lifestyle behaviors in individuals who have undergone PCI.

MATERIALS AND METHODS

Ethical Principles of Research: Before starting the research, ethical approval (Date: 02.11.2021, decision no: 1276) was obtained from the Ethical Principles and Ethics Committee of the Rectorate of Bitlis Eren University. Institutional permission was obtained from the Bitlis Provincial Health Directorate (number 70871440). In accordance with the privacy and confidentiality principle of the Declaration of Helsinki, every precaution has been taken to protect the privacy and confidentiality of the personal information of the patients who volunteered to participate in the study.

Type of Research: Our research was designed to be descriptive and correlational.

Place and time of the research: It was held between 08.11.2021 and 08.07.2022 in Bitlis Tatvan State Hospital's angio unit and cardiology clinics.

Population and Sample of the Research: The universe of the research; It consists of patients who have undergone percutaneous coronary intervention in the last 1 year at Bitlis Tatvan State Hospital. The sample includes patients who met the inclusion criteria and were selected from the population by the nonprobability random sampling method. In this study, statistical power analysis (G-Power version 3.1) was performed by taking the effect size of 0.40 (calculated with η2- eta square), alpha=0.05 and power=0.94, and the total required minimum number of subjects was determined as 109.

Inclusion Criteria: (i) Having had PCI, (ii) Being 18 years or older, (iii) To be able to communicate verbally and not have cognitive problems.

Exclusion Criteria: (i) Desire to leave the research, (ii) Having a communication problem.

Data Collection: Data were collected from patients who were determined using a Patient Information Form created by the researchers, the Thorson-Powell Death Anxiety Scale (TPDAS) and the Healthy Lifestyle Behaviors II Scale (HLBS-II). The forms were filled out using the face-to-face interview method. Each interview lasted 15-20 minutes.

Data Collection Tools

Patient Information Form: The form consists of 11 questions. It was created by researchers by scanning the literature.[8,13-15]

Thorson-Powell Death Anxiety Scale: The scale measures the general death anxiety of patients. The Turkish adaptation of the scale was first done by Karaca and Yıldız. The Cronbach alpha coefficient of the scale was found to be 0.84. Thorson-Powell Death Anxiety Scale consists of 25 items prepared in a 5-point Likert format. A score between 0 and 100 is obtained from the scale, and high scores indicate high death anxiety. Items 4, 10, 11, 13, 17, 21, 23 and 25 in the scale are scored reversely.[16]

Healthy Lifestyle Behaviors Scale II: The scale was developed by Walker et al. and revised again in 1996.[17] The validity and reliability of the scale in Turkey were made by Esin and Akça.[18] The scale measures health-promoting behaviors associated with an individual's healthy lifestyle. The scale consists of 52 items in total and has 6 sub-factors. Subg-

roups: spiritual development, health responsibility, physical activity, nutrition, interpersonal relations and stress management. All items on the scale are positive. The rating is in the form of a 4-point likert. A score between 52 and 208 is obtained from the scale. The alpha reliability coefficient of the scale is 0.94.[18]

Statistical Analysis: The normality of the data according to the groups was checked with the Shapiro-Wilk test. Whether the scale levels differed according to the number of coronary interventions was examined with one-way ANOVA. The Duncan post-hoc test was used for the differences between the groups. Post-hoc test results are symbolized with a lowercase letter. The levels of linear relationship between age, duration of procedure, number of coronary interventions, and scale levels were analyzed using Pearson correlation analysis. In addition, Multiple Correspondence Analysis (MCA) was run to determine the linear and nonlinear relationship structure between age and number of coronary interventions. With MCA analysis, the relationship structure between variables at different measurement levels can be easily interpreted on a diagram by representing them in a two-dimensional space by dimension reduction method.[19,20] IBM SPSS Statistics for Windows 26.0 software was used for statistical analysis, and $p < 0.05$ was considered statistically significant.

RESULTS

According to Table 1, 52.3% of the patients participating in the study were male, 44.0% were primary school graduates, 55.0% were married, 33.9% were housewives, and 67.0% had a middle-income level. The mean age of the patients participating in the study was 65.20±14.36 years. The mean duration of coronary interventions is 36.52±16.32 minutes.

According to Table 2, the mean death anxiety scale score of the patients was 55.17±11.52. High scores obtained from the scale indicate a high death anxiety level. Accordingly, patients experience moderate death anxiety. The mean score of the healthy lifestyle behaviors scale is 123.51±14.56. High scores indicate that patients have healthy lifestyle behaviors. According to this result, the patients have moderate healthy lifestyle behaviors.

Table 1. Descriptive characteristics of the patients (n=109).

		n (%)
Gender	Female	52(47.7)
	Male	57(52.3)
Educational status	İlliterate	40(36.7)
	Primary school graduate	48(44.0)
	High school graduate and above	21(19.3)
Marital status	Married	60(55.0)
	Single	49(45.0)
Job	Housewife	37(33.9)
	Retired	31(28.4)
	Employee	14(12.8)
	Self-employment	14(12.8)
	Officer	4(3.7)
	Other*	9(8.3)
Income rate	Bad	20(18.3)
	Middle	73(67.0)
	Good	16(14.7)
Number of coronary interventions	1	53(48.6)
	2	29(26.6)
	3	18(16.5)
	4 and above	9(8.3)
Information about the operation	I bought	33(30.3)
	I got some information	53(48.6)
	I did not take	23(21.1)
Is there any cardiovascular disease in your family?	Yes	33(30.3)
	No	76(69.7)
Has anyone in the family died from a heart attack?	Yes	32(29.4)
	No	77(70.6)
	Mean±SD	
Age	65.20±14.36	
Operation time (minutes)	36.52±16.32	

*:Unemployed:4; Farmer:3; Artisan:2

Table 2. Descriptive statistics results of Thorson-Powell Death Anxiety Scale and Healthy Lifestyle Behaviors Scale II and its sub-dimensions (n=109).

	Minimum	Maximum	Mean±SD
TPDAS	25.00	93.00	55.17 ± 11.52
HLBS-II	69.00	164.00	123.51 ± 14.56
Health responsibility	10.00	31.00	20.86 ± 3.95
Physical activity	8.00	25.00	15.90 ±4.86
Nutrition	15.00	32.00	22.28 ± 3.30
Spiritual development	12.00	34.00	23.49 ± 3.52
İnterpersonal relations	12.00	31.00	22.89 ± 3.40
Stress management	9.00	27.00	18.06 ± 3.81

TPDAS: Thorson-Powell Death Anxiety Scale; HLBS-II: Healthy Lifestyle Behaviors Scale II; SD: Standard Deviation.

According to Table 3, negative high correlation (r=-0.683, p=0.003) was found between the death anxiety scale and the healthy lifestyle behaviors scale. A negative high correlation (r=-0.720, p=0.002) was found between death anxiety level and physical activity, which is a sub-dimension of healthy lifestyle behaviors. A high positive correlation (r=0.659, p=0.001) was found between stress management and physical activity.

According to Table 4, death anxiety levels of patients with a coronary intervention number (NCI) of 4 and above were found to be significantly lower than those who had a coronary intervention for the first time (p=0.14). It was observed that the death anxiety levels of the patients who had the first three interventions were high, and this anxiety decreased as the NCI increased.

Table 3. Pearson correlation levels of patients' TPDAS, HLBS-II and sub-dimensional scores between processing time and age variables (n=109).

		Age	OT	TPDAS	HLBS II	HR	PA	N	SD	İR
OT	r	-0.033								
	p	0.736								
TPDAS	r	0.095	0.141							
	p	0.324	0.143							
HLBS II	r	-0.048	-0.067	-0.683						
	p	0.624	0.486	**0.003**						
HR	r	-0.006	-0.115	-0.145	0.697					
	p	0.950	0.233	0.134	**0.001**					
PA	r	-0.174	-0.074	-0.720	0.687	0.433				
	p	0.070	0.447	**0.002**	**0.001**	**0.001**				
N	r	0.020	0.118	-0.131	0.616	0.362	0.203			
	p	0.833	0.223	0.173	**0.001**	**0.001**	**0.035**			
SD	r	0.019	-0.062	-0.163	0.498	0.143	-0.009	0.273		
	p	0.847	0.525	0.089	**0.001**	0.139	0.924	**0.004**		
İR	r	0.006	-0.053	0.057	0.597	0.335	0.084	0.395	0.504	
	p	0.950	0.587	0.557	**0.001**	**0.001**	0.386	**0.001**	**0.001**	
SM	r	0.007	-0.042	-0.647	0.694	0.328	0.659	0.248	0.156	0.125
	p	0.945	0.662	**0.001**	**0.001**	**0.001**	**0.001**	**0.009**	0.105	0.194

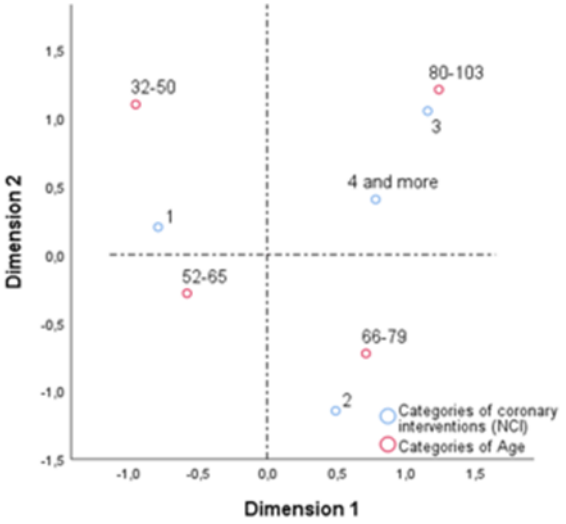
OT: operation time; TPDAS: Thorson-Powell Death Anxiety Scale; HLBS II: Healthy Lifestyle Behaviors Scale II; HR: Health responsibility; PA: Physical activity; N: Nutrition; SD: Spiritual development; İR: İnterpersonal relations; SM: Stress management; r: Pearson correlation coefficient.

Table 4. Comparison of Thorson-Powell Death Anxiety Scale and Healthy Lifestyle Behaviors II Scale and sub-dimension scores of the patient by number of coronary interventions (n=109).

NCI	TPDAS Mean±SD	HLBS II Mean±SD	HR Mean±SD	PA Mean±SD	N Mean±SD	SD Mean±SD	İR Mean±SD	SM Mean±SD
1	57.45^{a}±11.81	123.21±13.82	20.36±3.90	15.43±4.68	22.43±3.53	23.64±3.13	23.51±3.42	17.83±3.81
2	56.55^{a}±12.24	122.34±18.94	20.83±4.42	15.55±5.45	22.03±3.58	23.72±4.38	22.34±4.02	17.86±3.95
3	50.56^{ab}±8.23	125.39±10.19	22.11±3.61	16.83±4.25	22.39±3.13	22.94±3.40	22.50±2.50	18.61±3.62
≥4	46.56^{b}±6.75	125.33±11.27	21.44±3.21	18.00±5.10	22.00±0.71	23.00±3.24	21.89±2.26	19.00±4.15
p	0.014	0.888	0.419	0.400	0.950	0.846	0.322	0.759

NCI: Number of coronary interventions; TPDAS: Thorson-Powell Death Anxiety Scale; HLBS II: Healthy Lifestyle Behaviors Scale II; HR: Health responsibility; PA: Physical activity; N: Nutrition; SD: Spiritual development; İR: İnterpersonal relations; SM: Stress management. P-values represent the results of one-way ANOVA analysis. Duncan's analysis results were symbolized with lowercase letters for differences in death anxiety levels between subgroups.

In addition, when the level of linear relationship between the number of coronary interventions (NCI) that the patients were exposed to and their age was examined, a significant positive correlation level was found (r = 0.470, p=0.030). Accordingly, it was found that there was a moderate linear relationship between age and NCI. However, (according to Figure 1) when the multi-relationship structure between the age variable and the NCI categories is examined by categorizing the age variable, it is seen that there is an increase in the NCI as the age increases. The performance results of the MCA diagram regarding the multi-relationship structure between age categories and NCI categories are summarized in Figure 1. An average of 57.80% confidence level (Cronbach's Alpha level) was observed between dimensions and variables. The average rate of explanation of the variation between the two dimensions between age and NCI categories was 60.00%. Accordingly, the comments on the MCA diagram are sufficient and reliable.



Dimension	Cronbach's Alpha	Variance Disclosure Ratio	
		Total (Eigenvalues)	% of Varyans
1	0.625	1.270	63.485
2	0.531	1.131	56.544
Total		2.401	
Mean	0.578	1.200	60.015

Figure 1. The result of MCA regarding the relationship structure between the age of the patients and the categories of coronary interventions (NCI) to which they were exposed.

DISCUSSION AND CONCLUSION

In this study, the relationship between the level of fear of death and healthy lifestyle behaviors in patients after PCI was examined. The percentage of patients who reported receiving information about the PCI procedure is 78.9%, as shown in Table 1. In the study of Sönmez, in which he examined patients who underwent coronary artery bypass graft surgery, 10.5% of the patients stated that they did not receive any preoperative information.[13] In the study of Afacan, she stated that 97.1% of the patients did not have a history of being informed.[14] In this study, 21.1% of the patients stated that they did not receive information before the procedure. Informing patients before surgery reduces patient anxiety, increases the patient's compliance with treatment, and has an effect on accelerating recovery.[21] Due to the fact that PCI is an acute intervention, it may also suggest that insufficient information can be given to the patients. As a matter of fact, when the literature is examined, very different results are seen regarding informing patients.[5,6]

Our patients experience moderate death anxiety. Our patients have moderate levels of healthy lifestyle behaviors (Table 2). When the average scale levels related to the sub-dimensions of healthy lifestyle behaviors was examined, it was determined that the patients' spiritual development scores were high, physical activity scores were low, and other sub-dimension scores were moderate (Table 2). Buruntekin found in her study that patients experienced moderate levels of death anxiety.[15] Cengizhan found in his study that patients experienced high levels of death anxiety.[22] Doğan determined that patients undergoing PCI have moderate healthy lifestyle behaviors.[23] Afacan reported that patients had moderate healthy lifestyle behaviors.[14] Our scale score averages coincide with the averages of the studies in the literature.[12,24] In addition, HLBS-II sub-dimensions are consistent with the scale mean. Patients expe-

riencing moderate death anxiety; It can be explained by having more than one PCI, high average age and good spiritual development scores. Demonstrating behavior change is a difficult process. It is inversely proportional to the age factor. Patients have moderate healthy lifestyle behaviors; It can be explained by low education levels and high average age (decrease in communication and physical activity, etc.).

In this study, a negative correlation was found between the death anxiety scale and the healthy lifestyle behaviors scale ($r = -0.683$, $p=0.003$, Table 3). As the death anxiety level increases, the patient's healthy lifestyle behaviors decrease. In the examination performed by Ashour et al. immediately after PCI and 6 months later, it was observed that the patients' anxiety decreased and they developed a healthy lifestyle.[25] Gulanick et al. reported that the high level of anxiety in patients after PCI prevented them from developing healthy lifestyle behaviors.[6] Higgins et al. reported that high anxiety about their health after PCI prevents them from developing a healthy lifestyle.[24] In all three studies, it is stated that there is a negative relationship between death anxiety levels and healthy lifestyle behaviors. Similar results are seen in the literature review.[26] The high level of anxiety prevents the development of healthy behavior in patients. This situation can be explained by the psychological aspect of human beings.

In this study, a negative relationship was found between the level of death anxiety and the physical activity subscale (Table 3). Gaudel et al. found that the training program positively affected the physical activity sub-dimension.[26] Qin et al. stated that they found the patients' anxiety levels high, but that the patients had sufficient knowledge about physical activity.[27] In their study, Li et al. found a significant relationship between insufficient exercise capacity and anxiety after PCI.[28] Our results are similar to the literature. After cardiac interventions, patients are expected to create healthy lifestyle changes. In order to regain their health, their compliance with exercise and diet programs can be high.

In this study, no significant linear relationship was found between the age of the patients and their death anxiety levels and the level and sub-dimensions of healthy lifestyle behaviors (Table 3). In Doğan's study, individuals aged 65 and over had low HLBS-II scores.[23] In the study of Cengizhan, no significant relationship was found between the age of the patients and their death anxiety levels.[22] Although there is no significant relationship between age and death anxiety, death anxiety increases as age increases. In addition, it may be more difficult for older age groups to change their behavior.

In this study, it was found that death anxiety levels of patients with a coronary intervention number (NCI) of 4 and above were significantly lower than those who had a coronary intervention for the first time ($p=0.14$, Table 4). It was found that the number of coronary interventions did not create a significant difference between the levels of healthy lifestyle behaviors and the sub-dimensions of the scale ($p>0.05$, Table 4). In their study, Zaru et al. found that multiple coronary interventions increased compliance with healthy lifestyle behaviors.[29] Astin et al., in their study on patients undergoing PCI, found that the number of previous acute infarctions caused high levels of anxiety.[30] The results of our study differ from the literature. Having a coronary intervention for the first time can cause high anxiety. After repeated interventions, it is expected that patients have sufficient information, increase their compliance with treatment and reduce their anxiety levels. Healthy lifestyle changes are expected in individuals who have undergone multiple coronary interventions. The fact that this situation does not occur can be explained by the high age of the patients.

In Conclusion, our patients undergoing PCI have moderate death anxiety and moderate healthy lifestyle behaviors. As the death anxiety increases, the patient's healthy lifestyle behaviors decrease. As physical activity decreases, death anxiety level increases. It was observed that the level of death anxiety increased in patients who could not manage stress. It was found that the death anxiety levels of the patients decreased significantly as the number of coronary interventions increased. The importance of a healthy lifestyle should be explained to patients so that they can experience less death anxiety. Healthy life education programs should be applied to the patients. Experimental studies with large samples can be conducted.

Ethics Committee Approval: Before starting the research, ethical approval (Date: 02.11.2021, decision no: 1276) was obtained from the Ethical Principles and Ethics Committee of the Rectorate of Bitlis Eren University. Institutional permission was obtained from the Bitlis Provincial Health Directorate (number 70871440). In accordance with the privacy and confidentiality principle of the Declaration of Helsinki, every precaution has been taken to protect the privacy and confidentiality of the personal information of the patients who volunteered to participate in the study.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept - ŞO, CÖ, AG, MTH; Supervision - ŞO, CÖ, AG, MTH; Materials - ŞO, MTH; Data Collection and/or Processing - ŞO, CÖ, AG; Analysis and/or Interpretation - ŞO, CÖ, AG, MTH; Writing - ŞO, CÖ, AG, MTH.

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Determining the Landing Error Scoring System after a Jump by Artificial Intelligence

Sıçramadan Sonra Yere İniş Hata Puanlama Sistemi'nin Yapay Zeka ile Belirlenmesi

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ABSTRACT

Objective: The study aims to examine the predictability of the Landing Error Scoring System (LESS) results after the jump with the Adaptive Boosting (AdaBoost) algorithm.

Materials and Methods: A model has been developed by artificial intelligence to shorten the scoring system significantly. In the data preprocessing stage, 17 different items contained in the original dataset were reduced to 13. A total of 3790 data items were included in the dataset used in the study, and the dataset was divided into 4 different sub-datasets. AdaBoost was chosen to give the highest accuracy tested in five different machine learning used for regression. The model's reliability was evaluated by testing the proposed AdaBoost model with performance metrics.

Results: The error score given by the clinician in the LESS was in the range of 0-86.6%. Recommended AdaBoost model for Sub_1, Sub_2, Sub_3, and Sub_4 respectively 98%, 87%, 88%, 89% accuracy has been achieved.

Conclusions: The score given to the LESS's 8^{th}, 10^{th}, 16^{th}, and 17^{th} items can be predicted with high accuracy, and the total score can be reached through the model proposed in the research.

Keywords: AdaBoost model, artificial intelligence, dataset, jump, Landing Error Scoring System

ÖZ

Amaç: Çalışmada, Adaptive Boosting (AdaBoost) algoritması ile Sıçramadan Sonra Yere İniş Hata Puanlama Sistemi (SSYİ-HPS) sonuçlarının öngörülebilirliğinin incelenmesi amaçlanmıştır.

Materyal ve Metot: Puanlama sistemini daha da kısaltmak için yapay zeka yardımıyla bir model geliştirilmiştir. Veri ön işleme aşamasında, orijinal veri setinde yer alan 17 farklı madde 13'e düşürülmüştür.

Çalışmada kullanılan veri setinde toplam 3790 veri yer almış ve veri seti 4 farklı alt veri setine ayrılmıştır. Regresyon için kullanılan beş farklı makine öğrenim modelinden en yüksek doğruluğu veren AdaBoost seçilmiştir. Modelin başarısı, önerilen AdaBoost modelinin performans metrikleri ile test edilmesiyle değerlendirilmiştir.

Bulgular: SSYİ-HPS'de klinisyen tarafından verilen hata puanı %0-86,6 aralığındaydı. Önerilen AdaBoost modelinde sırasıyla Sub_1, Sub_2, Sub_3 ve Sub_4 için %98, %87, %88, %89 doğruluk sağlanmıştır.

Sonuç: Araştırmada önerilen model ile SSYİ-HPS'nin 8., 10., 16. ve 17. maddelerine verilen puan yüksek doğrulukla tahmin edilebilmekte ve toplam puana ulaşılabilmektedir.

Anahtar Kelimeler: AdaBoost modeli, Sıçramadan Sonra Yere İniş Hata Puanlama Sistemi, veri seti, yapay zeka, sıçrama

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INTRODUCTION

Assessment of biomechanical risk factors plays a key role in protecting against sports injuries.[1-3] Although three-dimensional (3D) motion analysis systems are shown as the gold standard the development of 2D motion analysis systems has been brought to the agenda. The widespread use of digital video cameras and software has also popularized the use of 2D motion analysis systems.[1,3-5] In addition, Padua et al.[6] has found that the results obtained in 2D motion analysis systems are valid and reliable with 3D motion analysis systems, which also increases confidence in these systems.[7,8]

Following the Landing Error Scoring System (LESS) protocol, the test sequence is asked to land on the ground by making a bilateral 'drop vertical jump' at the determined length.[6,9-11] From the images at the front and side camera angles where the landing on the ground is recorded after the jump, the error status of movements can be scored.[6]

The LESS: users risk analysis, neuromuscular training, post-development monitoring, etc.[7] in conjunction with the offering, this system for motion analysis in the analysis of each athlete in the image of an experienced evaluator, and there is a need for at least 30 minutes. On the other hand, it is predicted that this scoring process can be achieved in a much shorter time and independent of experience with artificial intelligence (AI) techniques. It is thought that AI methods[12-15] in the field of health and sports can be used to make this system more practical.

According to the information we have obtained from the previous research studies, AI methods are not used to estimate the LESS scoring. The purpose of this study is to examine the predictability of the LESS score with AI methods.

MATERIALS AND METHODS

Ethics Committee Approval: The study was approved by the Isparta University of Applied Sciences Ethics Committee (Date: 23.03.2021, decision no: 3). The study was planned under the Helsinki Principles. The results of 112 people (21.7±1.2 years, 54.5% male, 45.5% female) were evaluated. To evaluate the results of the LESS with AI techniques and to develop a model, they were applied.

Data Preprocessing: Seventeen different items contained in the original dataset[6] were reduced to 13. This inference on the dataset is determined by the following inference.

- S7. and S8. substance affects the response to each other.
- S9. and S10. substance affects the response to each other.
- The outcome of substance S12., S13. and S14. determines the outcome of substance 16.
- The outcome of substance S5. and S16. determines the outcome of substance S17.

Thirteen input and 4 output parameters were determined in the dataset (3790 items) with feature extraction. Since the number of items affected by the determined inferences is different, the dataset is divided into 4 different sub-datasets. Sub_1 dataset was 224 counts. Sub_1 dataset's classification was Substance 8, and the classification type was 0-1-Null. The Sub_2 dataset was 224 counts. Sub_2 dataset's classification was Substance 10, and the classification type was 0-1-Null. Sub_3 dataset was 502 counts. Sub_3 dataset's classification was Substance 16, and the classification type was 0-1-2. The Sub_4 dataset was 336 counts. The Sub_4 dataset classified Substance 17, and the classification type was 0-1-2. As a result of this partitioning, 1286 data items were extracted for training and testing the model. Of these four sub-models, 80% of the dataset was used for training, and 20% was used for testing.

Development of the Model: According to two statistical concepts, model selection begins with predicting the performance of different models to choose the best model. According to the results, the generalization error is estimated, and the best model is evaluated.[16,17] Adaptive Boosting (AdaBoost) from ensemble learning algorithms was used in the proposed model (Figure 1). Four subsets of data are sent to the model separately. The AdaBoost model is trained

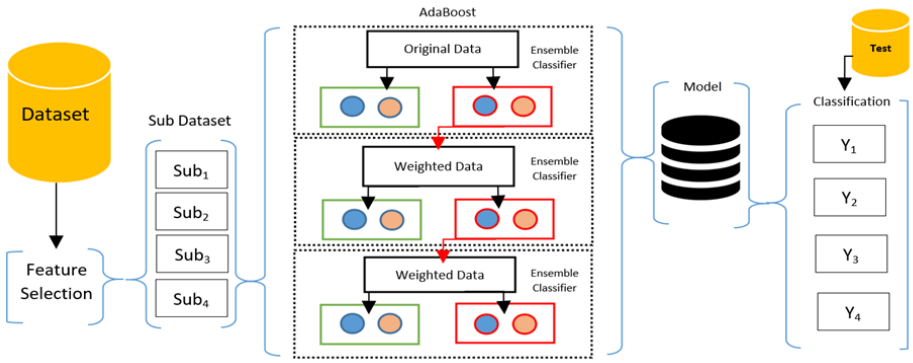

Figure 1. The proposed model.

and classified with initial training data. It then transfers the relative weight of misclassified training data to the next training. The second classifier model is trained with increased weights and classified again. In the third step, the weight is updated this way, and the consequences are created for the final model. In the last stage, the classification is completed by giving the model test data.[18]

First of all, in the mathematical structure of the model, the dataset is represented as . Where N is the size of the real numbers or the number of attributes in the dataset. X is the set of scoring data. Y is a target variable of 0, 1, or 2 because it is a triple classification problem. The same weights are used to train all data in the initial training phase of the model. The addition of weighted samples is always 1, as shown in Equation 1. For this reason, the value of each weight is between 0 and 1 in the first stage.

$$w = \frac{1}{n} \in [0,1] \quad (1)$$

In the second step, using Equation 2 for this classifier, its actual effect on the classification of the scoring data is calculated. ε_t is the numerical value of how effective this step will be in the final classification. is the total number of incorrect classifications for the current training set divided by the training set size.

$$\varepsilon_t = \frac{1}{2}\ln\frac{(1-\sum error)}{\sum error)} \quad (2)$$

After entering the actual values for each classification step, the weights, initially taken as 1/N for each data point, are updated according to Equation 3. Here, two cases occur for ε as plus and minus. The ε is positive when the predicted score and actual output match. In this case, the weight update does not occur. The ε value is negative when the predicted output does not match the actual score. In this case, the sample weight should be increased so that the same incorrect classification is not repeated in the next training. This process is repeated until the error function changes or the maximum limit of the classifier number is reached. The classification steps of the proposed model are shown in the rough code (Table 1).

$$w_i = w_{i-1}e^{\pm\varepsilon} \quad (3)$$

Performance metrics for machine learning are used to evaluate the developed model. Performance metrics are used to evaluate training and test data estimation results. The ratio of correctly identified sam-

Table 1. Pseudocode of the classification algorithm of the model.

Input: Initial training dataset $N = \{x_i, y_i\}, y_i \in \{0,1,2\}$

(1) $w_i = \frac{1}{m}$ Initialize the sample weights

(2) for u=1,2, 3,…., U do

$h_t = L(N, N_t)$ Train a sample from D with Dt $\varepsilon_t = p_x(h_t(x) \neq f(x))$ Evaluate the errors of ht

if $\varepsilon_t > 0.5$ go to (2)

(3) $\alpha_t = \frac{1}{2}\ln(\frac{(1-\varepsilon_t)}{\varepsilon_t}$ determine the weight of ht

end

(4) $\begin{Bmatrix} \exp(-\alpha_t) & h_t(x) = f(x) \\ \exp(\alpha_t) & h_t(x) \neq f(x) \end{Bmatrix}$

Output:

$$H(x) = sign\sum_{t=1}^{T} 1(y = h_t(x))$$

ples to total samples is considered by many academics to be the most plausible performance metric. By definition, accuracy (ACC) also functions in situations when there are more than two labels.[19-22] However, accuracy loses its reliability when the dataset is unbalanced, leading to an overly optimistic estimate of the classifier's performance on the majority class. The Matthews correlation coefficient (MCC) offers a useful remedy for the class imbalance problem.[19,21,22]

For the performance evaluation of the proposed model, ACC (Equation 1), Precision (Equation 2), Recall (Equation 3), and F1-score (Equation 4) are measured. Pseudocode of the classification algorithm of the model is below:

$$(1) \quad Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$(2) \quad Precision = \frac{TP}{TP + FP}$$

$$(3) \quad Recall = \frac{TP}{TP + FN}$$

$$(4) \quad F1_{score} = 2 \times \left(\frac{Precision \times Recall}{Precision + Recall}\right)$$

Abbreviation in the formulas above: TP: True Positives; FP: False Positives; FN: False Negatives; TN: True Negatives.

Statistical Analysis: The SPSS v.23 package program was used for the analysis. Clinician' data were presented as frequency (n), percentile (%), mean±standard deviation.

RESULTS

The score of the LESS determined by the clinician was calculated as 6.8±2.1. The error score rate of item 1 (knee flexion angle at initial contact) was 86.6%. The error score rate of item 2 (hip flexion angle at initial contact) was 0%. The error score rate of item 3 (trunk flexion angle at initial contact) was 48.2%. The error score rate of item 4 (ankle plantarflexion angle at initial contact) was 9.8%. The error score rate of item 5 (knee valgus angle at initial contact) was 14.3%. The error score rate of item 6 (lateral trunk flexion angle at initial contact) was 7.1%. The error score rate of item 7 (stance width–wide) was 0%. The error score rate of item 8 (stance width–narrow) was 70.5%. The error score rate of item 9 (foot position–toe in) was 0%. The error score rate of item 10 (foot position–toe out) was 17%. The error score rate of item 11 (symmetric initial foot contact) was 25.9%. The error score rate of item 12 (knee flexion displacement) was 33%. The error score rate of item 13 (hip flexion at max knee) was 0%. The error score rate of item 14 (trunk flexion at max knee flexion) was 31.3%. The error score rate of item 15 (knee valgus displacement) was 69.6%. The error score rate of item 16 (joint displacement) was 84.8% (35.7%: 1 point, 49.1%: 2 points). The error score rate of item 17 (overall impression) was 98.2% (60.7%: 1 point, 37.5%: 2 points).

The model was developed in Spyder software with Python language. The training and testing of the model were completed on an AI machine with an I9 processor and a 24 GB video card. The confusion matrix of the classification of 4 different scores in different intervals in the dataset is shown in Figures 2a, b, c, and d. The scoring result density in the Sub_1 dataset is 1, so 98% of the model has correctly classified the result 1 (Figure 2a). It is seen that the classification results are close to each other (0-88%, 1-84%) as the scoring result density in the Sub_2 dataset is approximately equal (Figure 2b). The classification success was similar due to the equal distribution of the scoring result density in the Sub_3 data (Figure 2c). It is seen that the scoring result density in the Sub_4 data is almost all 1 and 2, so the result is classified as 1 and 2 (Figure 2d).

ACC and MCC performance criteria were used to evaluate the performance of the classification model.[19,21-23] Accuracy, Precision, Recall, and F1-score values were calculated with TP, TN, FP, and FN values in the confusion matrix shown in Figure 2. Accordingly, Accuracy, Precision, Recall, and F1-score values obtained in each dataset and the average success of the model are given in Table 2.

After the model's training and testing process, test software was developed with the C # programming language. The trained file of the AdaBoost model was saved in Keras software with the h5 format. Then, the model was run by loading it into the test

Table 2. Metric values from scoring classification and comparison.

	AdaBoost				K-Nearest Neighbors	Support Vector Machine	Decision Trees	Gaussian process regression
Dataset	**Accuracy**	**Precision**	**Recall**	**F1score**	**Accuracy**	**Accuracy**	**Accuracy**	**Accuracy**
Sub_1	0.98	0.97	0.92	0.95	0.91	0.85	0.95	0.92
Sub_2	0.87	0.86	0.85	0.86	0.85	0.87	0.86	0.83
Sub_3	0.88	0.88	0.87	0.88	0.87	0.88	0.84	0.83
Sub_4	0.89	0.89	0.86	0.87	0.88	0.82	0.87	0.84
Avg	0.90	0.89	0.87	0.89	0.87	0.85	0.88	0.85

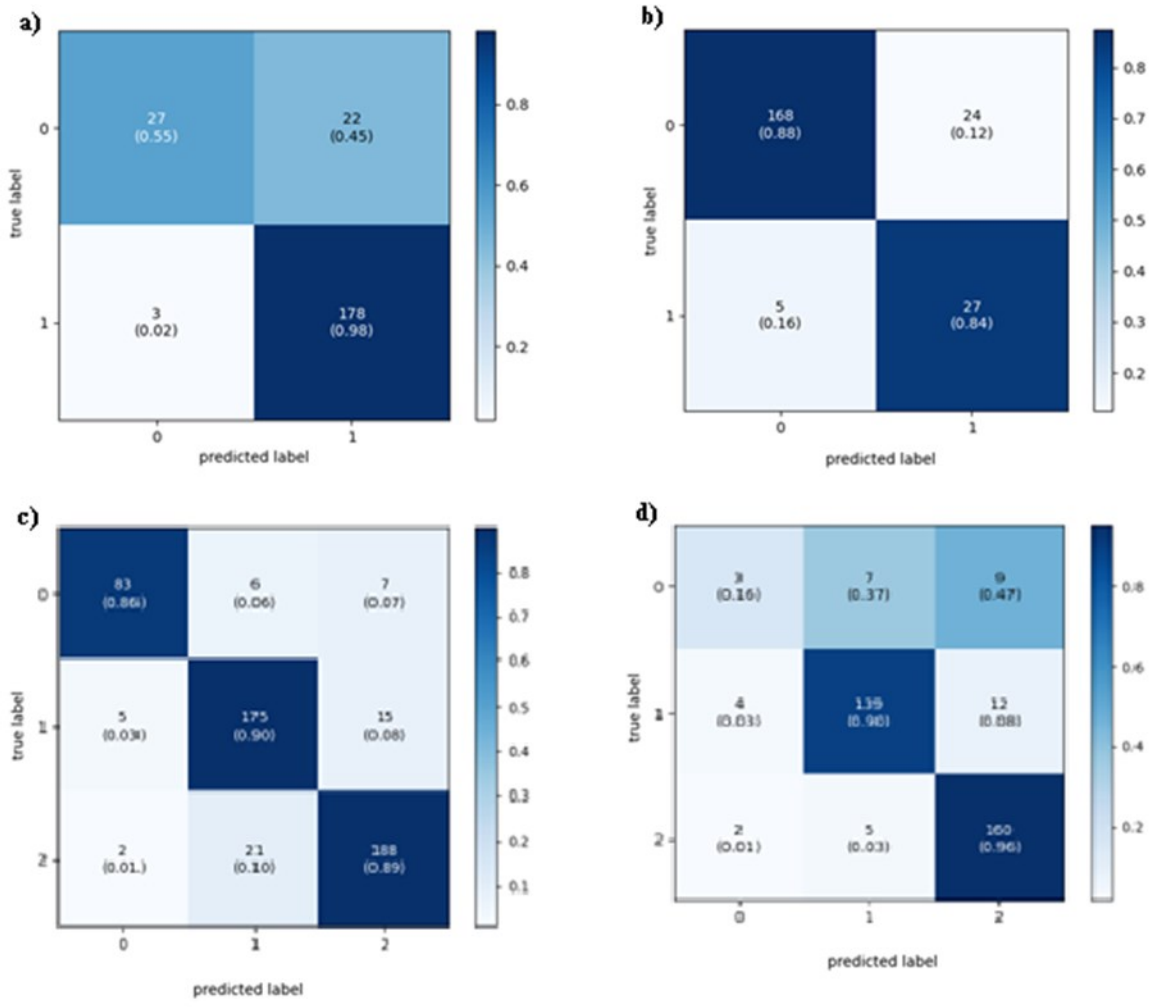

Figure 2. Confusion matrix of the score classification model for 4 datasets.

software. After the data entry of 13 items from the test results, the model estimates for 4 items. After the model estimates, it also calculates the total score for expert evaluation.

DISCUSSION AND CONCLUSION

The original scoring system of 17 items could be shortened to 13 items using AI methods. It was ensured that items 16th and 17th, whose scoring may vary depending on experience, could be scored easily and accurately using AI methods. The score to be given to the LESS's 8th, 10th, 16th, and 17th items can be predicted with high accuracy, and the total score can be reached with the proposed model.

It was observed that an attempt was made to easily develop evaluation methods/tools with the help of automated systems, such as the markerless motion-capture system, to score the LESS.[11,24] But after the jump with automated systems, the 17th item of the LESS (Overall impression item) was excluded from the analysis because it could not be evaluated.[11,24] In our research, the predictability of substances shortened by the model we proposed without any original substances being excluded from the analysis was high. The ability to predict the substances (items 16th and 17th) that experience will come into play with our proposed model has created an advantage.

Technology usage areas of the sports industry cover a wide spectrum, such as health, education, and tourism.[25] Another fact that technological progress has brought into our lives is AI.[26] AI is a system capability that will help to shorten the LESS with its feature of helping motion analysis[27] and supporting decision -making processes[26] without compromising its reliability. As demonstrated in our study, the fact that the motion analysis processes of AI systems provide convenience to the rater in the decision-making process will make the motion analysis systems more common and user-friendly.

In conclusion, the score given to the 8th, 10th, 16th, and 17th items of LESS can be estimated with a high accuracy rate, and the total score can be reached. In this way, in addition to providing ease of use to researchers who will use the LESS, 16th and 17th items can be scored easily and with significant accuracy using AI methods. In addition, the fact that the error scores in the dataset studied were relatively high (3-11 points) was considered a limitation of the study.

Ethics Committee Approval: Our study was approved by the Isparta University of Applied Sciences (Date: 23.03.2021, decision no: 3). The study was carried out following the international declaration and guidelines.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – SE, AAS, FB, ZB; Supervision – FB, ZB; Materials – AAS; Data Collection and/or Processing – SE; Analysis and/or Interpretation – AAS; Writing – SE, AAS.

Peer-review: Externally peer-reviewed.

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Prevalence of HBV, HCV, HIV and Effect on Clinical Course in COVID-19 Patients

COVID-19 Hastalarında HBV, HCV, HIV Prevalansı ve Klinik Seyre Etkisi

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ABSTRACT

Objective: COVID-19 may progress with hepatic exacerbation in viral infections such as hepatitis B. It was aimed to investigate the prevalence and clinical course of HBV, HCV, and HIV in COVID-19 patients.

Materials and Methods: Patients who were hospitalized due to COVID-19 and requested hepatitis and HIV serological tests were included in the study. Demographic data, HBV, HCV, HIV serologies, ALT, and AST results of patients were recorded.

Results: Out of 226 patients included in the study, 118 (52%) were male, 108(48%) were female, and the average age was 63.47±16.09 years. HBsAg positivity was found in six (3%), isolated Anti-HBcIgG positivity in six (3%), and Anti-HCV positivity in seven (3%) patients. Anti-HIV positivity wasn't detected. In two HBsAg-positive patients, HBV-DNA was negative, and in four, it was positive. In anti-HCV-positive patients, HCV-RNA was negative. Although not statistically significant, the median age of HBsAg-positive patients was lower, and the median ALT and AST were higher. However, the length of hospital stay, transfer rate to the intensive care unit, and discharge status were similar in all groups.

Conclusions: Liver enzymes were high in the HBsAg-positive patient group. Therefore, it was considered that COVID-19 may cause hepatic exacerbation in HBsAg-positive patients.

Keywords: COVID-19, HBV, HCV, HIV

ÖZ

Amaç: COVID-19, hepatit B gibi viral enfeksiyonlarda hepatik alevlenmeyle seyredebilir. COVID-19 hastalarında HBV, HCV, HIV prevalansının ve klinik seyrinin araştırılması amaçlanmıştır.

Materyal ve Metot: COVID-19 nedeniyle yatarak takip edilen, hepatit ve HIV serolojik tetkikleri istenen hastalar çalışmaya dahil edildi. Hastaların demografik verileri; HBV, HCV, HIV serolojileri; ALT, AST sonuçları kaydedildi.

Bulgular: Çalışmaya alınan 226 hastanın 118'i (%52) erkek, 108'i (%48) kadındı, yaş ortalaması 63,47±16,09 yıldı. HBsAg pozitifliği altı (%3), izole Anti-HBcIgG pozitifliği altı (%3), Anti-HCV pozitifliği yedi (%3) hastada mevcuttu. Anti-HIV pozitifliği saptanmadı. HBsAg pozitif hastaların ikisinde HBV-DNA negatif, dördünde pozitifti. Anti-HCV pozitif hastalarda HCV-RNA negatifti. İstatistiksel olarak anlamlı olmasa da HBsAg pozitif hastaların yaş ortancası daha düşük; ALT, AST ortancası daha yüksekti. Ancak yatış süresi ile yoğun bakıma devir ve taburcu olma durumu tüm gruplarda benzerdi.

Sonuç: HBsAg pozitif hasta grubunda karaciğer enzimlerinin yüksek olduğu saptanmıştır. Bu nedenle COVID-19'un HBsAg pozitif hastalarda hepatik alevlenmeye sebep olabileceği düşünülmüştür.

Anahtar Kelimeler: COVID-19, HBV, HCV, HIV

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INTRODUCTION

In December 2019, a disease clinically similar to viral pneumonia was observed in Wuhan, China. This disease's causative agent, defined as coronavirus disease 2019 (COVID-19) by the World Health Organization in February 2020, was named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[1] COVID-19 is a disease that can occur with different clinical symptoms, such as mild respiratory infection, severe pneumonia, respiratory failure, or multiple organ dysfunction.[2] While the virus causes more respiratory symptoms through the angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface, it also affects the liver, heart, pancreas and intestines.[3] In the studies, it has been reported that liver enzyme elevation is observed at 16.1-53.1% rates in COVID-19 infection.[4] Especially in studies examining the prevalence of liver disease in COVID -19 patients, severe liver disease is a moderate risk factor for COVID-19 infection.[5,6]

The prevalence of hepatitis C virus (HCV) infection is approximately 1% in the world. Turkey is among the low-prevalence countries with a prevalence rate between 0.6%-0.8%. However, HCV infection is a disease in which 85% of cases can become chronic disease, 20% of chronic hepatitis C cases progress to cirrhosis, and 1-4% of cirrhosis cases progress to hepatocellular carcinoma.[7] Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) is a global problem. According to the United Nations 2021 report, since the beginning of the HIV epidemic, approximately 73 million HIV infections and 36 million deaths from AIDS-related diseases have occurred. In our country, it was reported that there were about 30 thousand people with confirmed HIV and two thousand AIDS cases between 1985-2021.[8] Hepatitis B virus (HBV) infection is an important cause of mortality and morbidity worldwide. Our country is among the middle-endemic countries. The approximate prevalence is 4-6%. There are many causes of acute flare-ups in chronic hepatitis B infection. Immunosuppressive therapy, pregnancy, drug resistance and other infections are some.[9]

Viral or bacterial infections such as COVID-19 may lead to hepatic flare-ups of infections such as HBV, HCV, and HIV. For this reason, in addition to respiratory problems, patients with elevated liver enzymes or chronic HBV, HCV, and HIV infections should also be evaluated primarily in terms of hospitalization. It was aimed to investigate the prevalence and clinical course of HBV, HCV and HIV infection in patients followed up with COVID-19 infection.

MATERIALS AND METHODS

Ethics Committee Approval: Approval for the study was obtained from the Clinical Research Ethics Committee of Düzce University with the ethics committee decision dated 20.06.2022 and numbered 2022/122. All procedures have been carried out following the Helsinki Declaration.

Study Design and Participants: The data of 460 patients who were followed up with the diagnosis of COVID-19 infection in the Infectious Diseases and Clinical Microbiology Clinic of Düzce University Research and Application Hospital between 15.03.2020-15.03.2022 were retrospectively analyzed. However, 226 patients who requested serological tests for hepatitis B, hepatitis C and HIV were included in the study. Demographic data such as age and gender of the patients, HBV, HCV, HIV serologies (HBsAg, Anti-HBs, Anti-HBcIgG, Anti-HCV, Anti-HIV-studied by ELISA method), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) results were recorded. Prevalence of HBV, HCV, HIV and isolated Anti HBcIgG, COVID-19 polymerase chain reaction (PCR) results, length of stay, last clinical status, ALT and AST levels were examined. ALT 0-41U/mL, AST 0-50IU/L (male) and 0-35IU/L (female) values were considered normal. AntiHBs values of 10 mIU/mL and above were considered positive. The effect of COVID-19 infection on elevated liver enzymes and length of hospital stay in patients with HBV, HCV and HIV infection was investigated.

COVID-19 Infection Diagnosis: In our country, the diagnosis of COVID-19 infection is made according to the COVID-19 guidelines published and revised by the Ministry of Health. Therefore, patients with a positive COVID-19 PCR test and/or signs of COVID-19 pneumonia on thorax computed tomography that cannot be explained for any other reason were considered COVID-19 infections during the pandemic.[10]

Study Groups

Group 1: HBsAg, anti-HCV, anti-HIV negative 207 patients were determined as group 1. Of these 207 patients, 28 were naturally immune to HBV (anti-HBcIgG positive, anti-HBs positive), 70 were negative for anti-HBcIgG, and 109 were not tested for anti-HBcIgG, but HBsAg results were negative.

Group 2: HBsAg-positive six patients were determined as group 2. Patients with negative anti-HCV and anti-HIV results but positive HBsAg test were evaluated in this group.

Group 3: Isolated anti-HBcIgG positive six patients were determined as group 3. Patients who have negative HBsAg, anti-HBs, anti-HIV and anti-HCV results but positive anti-HBcIgG test were evaluated in this group.

Group 4: Anti-HCV positive seven patients were determined as group 4. Patients who have negative

HBsAg, anti-HBcIgG and anti-HIV results but positive anti-HCV tests were evaluated in this group.

Group 5: Anti-HIV positive patients were determined. However, this group was excluded from the study because there were no patients with positive anti-HIV tests

Statistical Analysis: Statistical analysis was performed between the groups regarding gender, age, COVID-19 PCR, number and proportion of patients with elevated ALT and AST levels, median of ALT and AST, length of hospital stay, and clinical outcomes. SPSS 23 package program was used for statistical evaluation of the data. Chi-Square and Fisher Freeman Halton Test were used to analyse categorical variables, and the Kruskal-Wallis Test was used to evaluate the relationship between the mean values of numerical values between groups; $p<0.05$ was considered significant.

RESULTS

Out of 226 patients included in the study, 118 (52%) were male, and 108 (48%) were female; the mean age was 63.47±16.09 years. HBsAg positivity in six (3%) patients, isolated anti-HBcIgG positivity in six (3%, from 116 patients) patients, anti-HBs positivity in 74 (33%) patients, anti-HCV positivity in seven (3%) patients were present. Anti-HBcIgG positivity in 42 (19%) patients was present (28 were naturally immune to HBV, six HBsAg positive, six isolated anti-HBcIgG positive, and two anti-HCV positive). Anti-HIV positivity was not detected. Two HBsAg-positive patients had negative HBV DNA results; the other four were positive with 360, 470, 1000 and 5010 IU/mL, respectively. Only one of those with isolated anti-HBcIgG positivity had HBV-DNA tested, and that was negative. HCV-RNA results were negative in all patients who were anti-HCV positive. The number of patients with high ALT levels was 79 (35%), and the number of patients with high AST levels was 89 (39%) in all patients. The overall mortality rate was 2% (4/226), and the rate of admission to the intensive care unit was 14% (31/226). Although it was not statistically significant in the intergroup analysis, the median age of HBsAg-positive patients was lower, and the median of ALT and AST was higher compared to other groups ($p=0.149$, $p=0.177$ and $p=0.229$, respectively). The ALT and AST medians of patients with HBsAg positive were determined respectively as 23.55 and 20.95 before COVID-19 infection. Still, the ALT median was 62.8, and the AST median was 71.4 in COVID-19 infection. ALT (median 27.0) and AST (median 32.8) levels decreased after COVID-19 infection in HBsAg-positive patients with liver enzyme elevation. However, the median length of hospitalization ($p=0.117$), need for intensive care, and discharge status ($p=0.954$) were similar (Table 1).

DISCUSSION AND CONCLUSION

Hepatic damage in COVID-19 can occur by various mechanisms. Some of these are the side effects of the drugs used in the treatment, hepatic immunology and the direct cytotoxic effect of the virus. The virus causes overexpression in hepatocytes via the ACE2 receptor. It initiates replication in the cell with the same receptor, stimulates the synthesis and release of a new viral RNA, and makes protein synthesis.[11] The effect of ACE2 receptors on hepatocytes is limited. Therefore, hepatitis does not develop in every patient; however, patients who developed acute hepatitis due to COVID-19 have also been reported.[12,13] Especially in patients with COVID-19 pneumonia, SARS-CoV-2 infection may trigger hepatic failure.[14] For this reason, even in those who do not

Table 1. Results of patients diagnosed with COVID-19 and requested serological tests (n:226).

Specifications		**Group 1 (n:207)***	**Group 2 (n:6)****	**Group 3 (n:6)*****	**Group 4 (n:7)******	**p**
Age (year)	Median/IQR	65.00 (21.00)	53.00 (17.00)	67.00 (28.25)	70.00 (17.00)	0.149
Gender n (%)	Male	106 (51%)	4 (67%)	5 (83%)	3 (43%)	
	Female	101 (49%)	2 (33%)	1 (17%)	4 (57%)	0.441
COVID-19 PCR,	Positive	179 (86%)	5 (83%)	5 (83%)	6 (86%)	1.000
[a]n(%)	Negative	28 (14%)	1 (17%)	1 (17%)	1 (14%)	
Length of hospitalization (day), Median/IQR		7.00 (6.00)	4.00 (10.00)	7.50 (9.00)	5.00 (5.00)	0.117
Last clinical status,	Healing	174 (84%)	5 (83%)	6 (100%)	6 (86%)	
n (%)	Intensive care	29 (14%)	1 (17%)	0	1 (14%)	0.954
	Discharge status	4 (2%)	0	0	0	
ALT, Median/IQR		32.20 (38.40)	62.80 (54.10)	34.85(36.63)	31.40 (30.40)	0.177
AST, Median/IQR		34.70 (29.40)	71.40 (58.13)	40.00 (21.23)	36.10 (28.80)	0.229
High-level ALT, [b]n (%)		72 (35%)	4 (67%)	2 (33%)	1 (14%)	0.285
High-level AST, [c]n (%)		82 (40%)	4 (67%)	1 (17%)	2 (29%)	0.343

*: HBsAg, anti-HCV, anti-HIV negative patients; **: HBsAg positive patients; ***: Anti-HBcIgG positive, HBsAg and anti-HBs negative patients; ****: Anti-HCV positive patients; [a]: Number of patients with signs of COVID-19 pneumonia on thorax computed tomography; [b]: Number of patients with high ALT level; [c]: Number of patients with high AST level.

have any chronic disease, COVID-19 disease can cause hepatic dysfunction. In studies conducted in China in patients with a diagnosis of COVID-19 and predisposed to hepatic disease, it has been reported that hepatic functions were impaired at a rate of 14-50%.[1,15] In the study of Phipps et al.[16], it was found that baseline and peak ALT levels were higher in those with positive SARS-CoV-2 test than those with negative results. It has been reported that 45% of positive cases have mild, 21% moderate, and 6.4% severe hepatic damage, and severe damage was associated with a higher rate of mortality and intensive care hospitalization. On the other hand, Wang et al.[14] reported that 41% of their patients had elevated hepatic enzymes, and these patients had higher radiology scores. However, they stated no difference in mortality and hospitalization days between those with and without hepatic damage. In our study, 35% of our patients had elevated ALT, and 39% had elevated AST. It was concluded that this rate is low due to the low number of hepatitis B patients and the absence of chronic hepatitis C and HIV-positive patients. Although not statistically significant, the median ALT and AST of HBsAg-positive patients were higher than those of other groups.

In a meta-analysis, Kunutsor and Laukkanen[17] stated that acute hepatic injury, hepatic enzyme abnormality and hypoproteinemia were common hepatic complications in patients hospitalized due to coronavirus disease 2019. They also reported worse outcomes of COVID-19 pneumonia in those with a known hepatic disease. While the most common causes of chronic hepatic disease in Western countries are chronic alcohol consumption and HCV infection, HCV and HBV-related hepatitis are the most common causes in our country.[18] Studies on hepatic symptoms, signs and difficulties in SARS-CoV-2 infection continue. Observations and guidelines are important in multiple viral infections. In an early meta-analysis from China, the prevalence of chronic hepatic disease was reported to be 3% in people infected with COVID-19. However, no specific data was found for HCV and HBV infections. Chronic hepatitis B and C are still common infections worldwide. Therefore, the effect of COVID-19 infection on the course of HBV and HCV raises concerns.[19] In the United States, the rates were lower in 5700 patients hospitalized with the diagnosis of SARS-CoV-2, with the prevalence of HBV infection at 0.1% and HCV <0.1%.[20] In a study conducted with 20.133 inpatients diagnosed with COVID-19 infection in 208 care hospitals in England, Wales and Scotland, among the comorbid diseases, moderate-to-advanced hepatic disease as 1.8%, mild hepatic disease as 1.6% and HIV-positive patient rate as 0.5% were reported.[5] In the study of Jin et al.[21], 25 (3.8%) of 651 COVID-19 patients reported having a previous hepatic disease. In another study conducted in Wuhan, China, 23 (2.1%) of 1099 patients were reported to be infected with HBV, and this rate was 2.4% in mild cases and 0.6% in severe cases.[22] However, in another study conducted in the same country, 15 of 123 (12.2%) patients had HBV infection, and even HBV-positive patients had higher rates of severe COVID-19 (46.7%>24.1%) and death (13.3%>2.8%).[23] Zha et al.[24] reported the prevalence of HBV as 6.5% (2 in 31 patients) in their study and observed that HBV infection also delayed the clearance of the SARS-CoV-2 virus. In our study, our rate of HBsAg-positive patients was lower than the literature with 3%. However, anti-HBcIgG positivity was detected in 42 (19%) patients. Our rate of anti-HCV positive patients (3%) is higher than the literature. Since the HCV-RNA test was negative in all anti-HCV-positive patients, it was observed that there was no patient with a diagnosis of chronic hepatitis C in our study. In addition, immunity to hepatitis C or false positivity could not be differentiated in these patients.

From the studies on HIV and COVID-19 coinfection, Blanco et al.[25] reported that 0.9% (5/543) of hospitalized patients had HIV infection and viral pneumonia developed in three of these patients, but no death was observed. In our country, Altuntas Aydın et al.[26] stated that only four of the 1224 HIV-positive cases they followed were diagnosed with COVID-19. No HIV-positive patient was found in our study. This was thought to be due to the low number of HIV-positive patients followed in our city and some of them being followed in other cities due to social pressure. Docherty et al.[5], in their study with an overall mortality rate of 26%, found that apart from factors such as male gender and advanced age, some other diseases such as non-asthmatic chronic lung disease, obesity, and hepatic disease were associated with increased mortality. In our study, the mortality rate was 2%, lower than the literature, but the rate of admission to the intensive care unit was 14%. Since the intensive care patients were not included in the study, it was concluded that the mortality rate was low. The median length of hospitalization, need for intensive care, and discharge status were similar between the groups.

In conclusion, COVID-19 may cause hepatic flare-ups in HBsAg-positive patients, like other viral or bacterial infections. Hepatic enzymes were found to be high in the HBsAg-positive patient group. However, its insignificance was thought to be related to the low number of patients. Although elevated hepatic enzymes or the presence of chronic diseases such as hepatitis and immunodeficiency syndrome increase the possibility of hospitalization, our HBsAg-positive patient rate was found to be lower

than similar studies. Our anti-HCV positivity rate is higher than that of similar studies, but all of them are HCV-RNA negative. It is noteworthy that there are no anti-HIV-positive patients. In the literature, there are some studies on the presence of hepatic disease with other underlying diseases, but there are few studies on HBV, HCV, HIV and COVID-19 coinfection. More study on this subject is needed. To minimize the risk of hepatic failure in COVID-19 infection, chronic hepatitis B, C and HIV infections of the patient should be investigated, and individuals with the disease should be followed closely.

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Düzce University (Date: 20.06.2022, decision no: 2022/122).

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – DY; Supervision – DY, ARG, Nİ, EÇ; Materials –DY, ARG; Data Collection and/or Processing –ARG, Nİ, EÇ; Analysis and/ or Interpretation – DY, EÇ; Writing – DY, Nİ.

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The Frequency and Role of Urinary Tract Infection in Prolonged Jaundice in Neonates

Yenidoğanlarda Uzamış Sarılıkta İdrar Yolu Enfeksiyonunun Sıklığı ve Rolü

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ABSTRACT

Objective: The study aims to investigate the frequency and role of urinary tract infection (UTI) in prolonged jaundice in preterm and term neonates.

Materials and Methods: This retrospective study occurred at Zeynep Kamil Maternity and Children's Hospital in Istanbul between January 2014 and April 2018. The study involved 391 neonates who presented to our outpatient clinics with prolonged jaundice. UTI was defined as urine culture growth of at least 10.000 colony-forming units per millilitre (CFU/ml) in samples obtained via urethral catheterization. Birth weight, sex, gestational age, chronological age, laboratory results, hospital courses of patients, ultrasound findings and phototherapy history were recorded.

Results: UTI frequency was found to be 2.8% in 391 neonates with prolonged jaundice. Nine of them were male (81.8%), and 2 were female (18.2%). Eight patients with UTI (72.7%) were term and 3 (29.3%) were preterm. History of phototherapy, presence of leukocyturia or nitrituria, white blood cell count, and thrombocyte counts were significantly higher in the UTI group.

Conclusions: Prolonged jaundice may be the first sign of UTI in neonates. Routine urine culture in neonates with prolonged jaundice may be useful, especially in those with a history of phototherapy and high white blood cell and thrombocyte counts.

Keywords: Phototherapy history, prolonged jaundice, urinary tract infection

ÖZ

Amaç: Bu çalışma preterm ve term yenidoğanlarda uzamış sarılıkta idrar yolu enfeksiyonunun (İYE) sıklığını ve rolünü belirlemeyi amaçlar.

Materyal ve Metot: Retrospektif tipteki bu çalışma, Ocak 2014-Nisan 2018 zaman aralığında İstanbul Zeynep Kamil Kadın Doğum ve Çocuk Hastanesi'nde yapıldı. Uzamış sarılık nedenli başvuran 391 yenidoğan incelendi. İYE, üretral kateterizasyonla elde edilen idrar kültürünün en az 10.000 koloni oluşturan ünite/ml pozitifliği olarak tanımlandı. Başvuru yapan hastaların doğum ağırlığı, cinsiyeti, gebelik haftası, yaşı, laboratuvar değerlendirmeleri, hastane seyri, ultrason bulguları ve fototerapi öyküsü kaydedildi.

Bulgular: Uzamış sarılığı olan 391 yenidoğanda İYE sıklığı %2,8 olarak bulundu. Dokuzu (%81,8) erkek, 2'si (%18,2) kız idi. Sekizi (%72,7) term, 3'ü (%29,3) preterm idi. Fototerapi öyküsü, lökositüri veya nitritüri varlığı, beyaz küre sayısı ve trombosit sayısı İYE grubunda anlamlı olarak yüksekti.

Sonuç: Uzamış sarılık yenidoğanlarda idrar yolu enfeksiyonunun ilk belirtisi olabilir. Bu nedenle uzamış sarılığı olan, özellikle fototerapi öyküsü olan, lökosit ve trombosit sayısı yüksek olan yenidoğanlarda rutin idrar kültürü yapılması faydalı olabilir.

Anahtar Kelimeler: Fototerapi öyküsü, idrar yolu enfeksiyonu, uzamış sarılık

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INTRODUCTION

Serum total bilirubin (STB) level ≥10 mg/dl lasting ≥ 14 days in term neonates, and ≥ 21 days in preterm neonates is considered a prolonged jaundice. Breast milk jaundice constitutes a significant portion of prolonged jaundice; however, it is a diagnosis of exclusion, and other pathological causes must be ruled out.[1] Major pathological causes of prolonged jaundice are infections, e.g. urinary tract infection (UTI), hypothyroidism, inborn errors of metabolism, congenital liver diseases and hemolytic disorders.[2]

Urinary tract infection is one of the important causes of prolonged jaundice in neonates. The frequency of UTI varies in studies conducted in newborns with prolonged jaundice. Reported frequencies were between 0.3% and 11% in the literature.[3-4] UTI causes jaundice by inducing erythrocyte hemolysis, reducing liver conjugation and impeding bilirubin excretion.[5] The consequences of a UTI are typically mild; however, in early infancy, there is a possibility of progression to renal scarring, particularly if there is a febrile UTI. Hypertension, proteinuria, renal damage, and even chronic renal failure in many adults are delayed sequela associated with renal scarring.[6]

In the present study, we aimed to investigate the frequency and role of UTI as an etiology of prolonged jaundice in preterm and term neonates, and to define the characteristics of patients with UTI in our prolonged jaundice cohort.

MATERIALS AND METHODS

Ethical Status: The ethical approval was obtained from the Clinical Research Ethics Committee of Zeynep Kamil Maternity and Children's Hospital (Date: 02/12/2020, decision no: 2020-176). The study was carried out in compliance with the Declaration of Helsinki.

Patients: This retrospective study occurred at Zeynep Kamil Maternity and Children's Hospital, Istanbul, between January 2014 and April 2018. The study involved 391 neonates who presented to our outpatient clinics with prolonged jaundice during the study period. Prolonged jaundice was defined as a STB level ≥10 mg/dl detected at ≥14 days of life in full-term neonates or at ≥21 days of life in preterm neonates. UTI was defined as urine culture growth of at least 10.000 colony-forming units per millilitre (CFU/ml) in samples obtained via urethral catheterization. Birth weight, sex, gestational age, chronological age, phototherapy history and hospital course of patients were recorded. Collected laboratory values were direct Coombs test, serum total and direct bilirubin, complete blood count, c-reactive protein (CRP), blood culture, urine analysis and urine culture**.** If available, renal ultrasound findings of patients with UTI were also recorded.

Statistical Analysis: Statistical Package for Social Sciences for Windows 22.0 program (IBM Corporation, USA SPSS) was used for statistical analysis. The normal distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Descriptive statistical tests were used to present baseline demographic and clinical data. Numbers and percentages were used to present categorical variables. The mean and standard deviation were reported for normally distributed continuous variables. The median and minimum-maximum values were presented for non-normally distributed continuous variables. The Mann-Whitney U test was used for binary comparisons of non-normally distributed continuous variables. The comparison of categorical variables between groups was carried out using the Pearson Chi-Square or the Fischer Exact test. A p-value of less than 0.05 was considered as the threshold for determining statistical significance.

RESULTS

In this study, 391 neonates with prolonged jaundice were admitted to our outpatient clinics during the study period: 246 (62.9%) male and 145 (37.1%) female. Of 391 neonates, 315 (80.6%) were term and 76 (19.4%) were preterm. Phototherapy history was found to be in 129 (33.0%) neonates. Urine cultures were sent from all 391 neonates with prolonged jaundice. Complete urine analysis was obtained from 313 neonates. Leukocyturia (≥5 cells/area) was detected in 34 (10.9%) neonates. Nitrituria was found to be in 6 (1.9%) neonates (Table 1).

Table 1. Characteristics and baseline laboratory values of study cohort (n: 391).

Characteristics	Data
Age at presentation, days, median (Min-Max)	22 (14-52)
Birth weight, gram, median (Min-Max)	3180 (1750-4650)
Total bilirubin, mg/dl, median (Min-Max)	12.6 (10.0-20.8)
Direct bilirubin, mg/dl, median (Min-Max)	0.52 (0.24-1.44)
Hemoglobin, g/dl, median (Min-Max)	13.8 (7.4-20.2)
White blood cell, count/microL, median (Min-Max)	7,400 (3,900-16,800)
Thrombocyte, count/microL, median (Min-Max)	310,000 (140,000-690,000)

Table 1. Continue.

Gestational age, n (%)	Term	315 (80.6)
	Preterm	76 (19.4)
Gender, n (%)	Male	246 (62.9)
	Female	145 (37.1)
Phototherapy history, n (%)	Yes	129 (33.0)
	No	262 (67.0)
Direct Coombs, n (%)	Positive	12 (3.1)
	Negative	379 (96.9)
Leukocyturia ≥5 Cells/Area*, n (%)	Yes	34 (10.9)
	No	279 (89.1)
Nitrituria*, n (%)	Yes	6 (1.9)
	No	307 (98.1)

*: Complete urine analysis was obtained from 313 of the newborns.

Eleven neonates with prolonged jaundice had urine culture growth consistent with UTI; 9 were males (81.8%), and 2 (18.2%) were female. Eight patients with UTI (72.7%) were term and 3 (29.3%) were preterm. A history of phototherapy was found in 8 (72.7%) newborns with UTI. All direct Coombs tests were negative in patients with UTI. Median STB level was 11.8 (min-max 10.1-16.4) mg/dl. The median hemoglobin, white blood cell and thrombocyte counts were 14.7 g/dl (min-max=11.5-17.6), 10.600 count/microL (min-max=6.780-14.200) and 407.000 count/microL (min-max=246.000-566.000), respectively. Ten of the newborns with UTI had a complete urinalysis. Leukocyturia and nitrituria were found in 6 (60%) and 3 (30%) newborns with UTI, respectively. There were statistically significant differences between UTI and no-UTI groups regarding urine analysis results. Leukocyturia (60% vs 9%) and nitrituria (30% vs. 1%) were significantly more common in the UTI group compared to the no-UTI group (both p<0.001). The median white blood cell 10.600 count/microL (min-max=6.800-14.200), vs 7.400 count/microL (min-max=3.900-16.800) and thrombocyte 407.000 count/microL (246.000-566.000), vs 296.000 count/microL (min-max=140.000-690.000) were also significantly higher in UTI group compared to non-UTI group (p=0.001 and p=0.006, respectively). A history of phototherapy was found in 8 (72.7%) of 11 newborns with UTI and 121 (31.8%) of 380 newborns without UTI (p=0.007) (Table 2).
Pelvicalyceal ectasia was found in 3 (27%) of 11 newborns with UTI on urinary ultrasonography.

Table 2. Comparison of study characteristics and laboratory values between UTI and No-UTI groups.

Parameters		**UTI (n=11)**	**No-UTI (n=380)**	**p-value**
Total bilirubin, mg/dl, median (Min-Max)		11.8 (10.1-16.4)	12.7 (10.0-20.8)	0.3
Hemoglobin, g/dl, median (Min-Max)		14.7 (11.5-17.6)	13.6 (10.4-20.2)	0.1
White blood cell, count/microL, median (Min-Max)		10.600 (6.800-14,200)	7.400 (3.900-16.800)	0.001
Thrombocyte, count/microL, median (Min-Max)		407.000 (246.000-566.000)	296.000 (140.000-690.000)	0.006
Gestational age, n (%)	Term	8 (72.7)	307 (80.8)	0.4
	Preterm	3 (29.3)	73 (19.2)	
Gender, n (%)	Male	9 (81.8)	237 (62.4)	
	Female	2 (18.2)	143 (37.6)	0.2
Phototherapy history, n (%)	Yes	8 (72.7)	121 (31.8)	
	No	3 (29.3)	259 (68.2)	0.007
Direct Coombs, n (%)	Positive	0 (0)	12 (3.2)	
	Negative	11 (100.0)	368 (96.8)	0.9
Leukocyturia ≥5 Cells/Area*, n(%)	Yes	28 (9.2)	6 (60.0)	
	No	275 (90.8)	4 (40.0)	0.001
Nitrituria*, n (%)	Yes	3 (30.0)	3 (1.0)	
	No	7 (70.0)	300 (99.0)	0.001

*: Complete urine analysis was obtained from 313 of the newborns.

None of the neonates with UTI had high CRP values (>1 mg/dL). Blood culture growth was not detected in any of the patients with UTI. Pathogens detected in urine cultures were *Escherichia coli* (n=4, 36%), *Enterobacter* spp. (n=3, 27%), *Klebsiella* spp. (n=2, 18%), group b *Streptococcus* (n=1, 9%) and *Enterococcus* spp (n=1, 9%). Treatment regimens were ampicillin-gentamicin in 6 patients, ampicillin-amikacin in 3 patients, ampicillin-cefotaxime in 1 patient and meropenem in 1 patient. The median length of hospital stay in newborns with UTI was 7 (min-max 7-10) days (Table 3).

Table 3. Ultrasound findings, some laboratory values, treatments and length of hospital stay in newborns with UTI (n=11).

Characteristics		UTI
Ultrasound findings, n (%)	Normal	8 (73)
	Pelvicalyseal ectasia	3 (27)
High c-reactive protein (CRP)	Yes	0 (0)
value (>1 mg/dL), n (%)	No	11 (100)
Blood culture growth, n (%)	Yes	0 (0)
	No	11 (100)
Pathogens, n (%)	Escherichia coli	4 (36)
	Enterobacter spp.	3 (27)
	Klebsiella spp.	2 (18)
	Group B Streptococcus	1 (9)
	Enterococcus spp.	1 (9)
Treatment, n (%)	Ampicillin-gentamicin	6 (55)
	Ampicillin-amikacin	3 (27)
	Ampicillin-cefotaxime	1 (9)
	Meropenem	1 (9)
Length of hospital stay, day, median (Min-Max)		7 (7-10)

DISCUSSION AND CONCLUSION

Urinary tract infection is one of the significant factors of prolonged jaundice in neonates. The frequency of UTI varies in studies conducted on neonates with prolonged jaundice. In a systematic review and meta-analysis study involving 1750 Iranian infants with prolonged jaundice, the total prevalence of UTI was found to be 11%.[4] In a study conducted in Türkiye in 2020, UTI was detected in 8% of all infants with prolonged jaundice.[5] Eleven (2.8 %) neonates had urine culture growth consistent with UTI in our neonate cohort with prolonged jaundice. This significant difference in UTI rates was likely related to the definition of jaundice (STB levels ≥10 mg/dl in our study vs 5 mg/dl in those studies). UTI may cause mild jaundice (STB levels between 5 mg/dl and 10 mg/dl) rather than more severe jaundice (≥10 mg/dl). Although not statistically significant, the incidence of UTI in male newborns with prolonged jaundice was higher in our study, similar to a study conducted by Tola, et al.[4] In a study by Chowdhury T et al. with 319 infants with prolonged jaundice, only 1 infant had UTI confirmed by recurrent culture.[3] In this study, urine samples were collected using a urine pad, not urethral catheterization.[3] In our study, UTI was defined as a positive urine culture with at least 10.000 colony-forming units/ml in urine samples obtained by urethral catheterization under sterile conditions. According to our study, this difference may be due to racial variations or alterations in obtaining the urine sample.

Neonates with UTI with prolonged jaundice are more frequently accompanied by a history of previous phototherapy.[7] In a study by Ozcan et al. in Türkiye, 155 newborns who received phototherapy for jaundice in the first 10 days of their lives were evaluated for UTI.[7] In this study, UTI was detected in 16.7% of neonates who received phototherapy for jaundice in the first 10 days of life.[7] In another study conducted by Bilgin et al. in Türkiye, it was found that 51% of infants with prolonged jaundice had a previous history of phototherapy.[8] In our study, the history of phototherapy rates in neonates with prolonged jaundice was 33%. When we compared the proportions of phototherapy history between UTI and no-UTI groups, a statistically significant difference was found (p=0.007). Considering the high rate of UTI in neonates with jaundice in the first 10 days of life in a study conducted by Ozcan et al., we speculate that UTI was the undiagnosed reason for phototherapy in patients who had both UTI and a history of phototherapy in our study.[7] In another study in Taiwan in 2018 by Weng et al., a history of phototherapy was found in 67% of those with prolonged jaundice and 42% of those

without prolonged jaundice.[9] Similar to our study result, previous jaundice and a history of phototherapy are significant risk factors for prolonged jaundice in a study conducted by Weng et al.[9]

Total serum bilirubin levels, white blood cell counts, hemoglobin levels and thrombocyte counts may differ in newborns with and without UTI.[10] In a study conducted by Nickavar et al. in 2015, a significant difference was found between the mean levels of STB (mg/dl) between those with and without urine culture growth.[10] In our study, no significant difference in the median levels of STB was found between those with and without UTI. In this study by Nickavar et al., patients with UTI had lower mean hemoglobin levels than those without UTI.[10] However, in our study, no significant difference was found in median hemoglobin level (g/dl) between those with and without UTI (14.7 vs 13.6, p=0.1). We found a statistically significant difference in white blood cell count between UTI and no-UTI groups (10.600 vs 7.400 count/microL, p=0.001). In the study by Nickavar et al., white blood cell counts were significantly different between those with and without UTI (10.970 vs 9.292 count/microL, p=0.014).[10] A high white blood cell count can be a warning sign of a UTI in neonates with prolonged jaundice. In the study by Nickavar et al., no significant difference was found in thrombocyte counts between those with and without UTI (333.215 vs 298.580 count/microL, p=0.2).[10] We found a statistically significant difference in thrombocyte count. In the study by Nickavar et al., no significant difference was found regarding thrombocyte between those with and without UTI (407.000 vs 296.500 count/microL, p=0.006). In another study by Kahraman et al., the white blood cell count and the platelet count were significantly higher in patients with a positive urine culture (p=0.004 and p=0.015, respectively).[11] High platelet counts may be an indicator of infections such as UTI in prolonged jaundice since thrombocytes, like white blood cells, are acute phase reactants. None of the newborns in our cohort had increased CRP or blood culture growth; however, CRP value was found to be high in 17% of infants with UTI, and no culture growth was detected in the study by Nickavar et al.[10] This difference in proportions of increased CRP may be related to timing of sample collection (early vs late in disease course) or severity of the clinical disease.

In the study by Ünsal et al., leukocyturia was found to be positive with a frequency of 75% and nitrite at 30% in pediatric patients with UTI.[12] In our study, leukocyturia and nitrituria were found in 6 (60%) and 3 (30%) newborns with UTI, respectively. When the presence of leukocyturia or nitrituria in the complete urine analysis was compared according to the presence of growth in the urine culture, it was found that there was a statistically significant difference between the groups (p<0.001 and p<0.001, respectively). Leukocyturia or nitrituria may be significant findings of a UTI.

Abnormal findings in the urinary system may contribute to a UTI. In the study by Bahat Özdoğan et al., all jaundiced infants with UTI were evaluated with urinary ultrasonography; abnormal findings such as pelvicalyceal ectasia and increased echogenicity in the renal parenchyma were detected in 28.1% of infants.[13] In terms of urinary ultrasonography findings, pelvicalyceal ectasia was found in 3 (27%) of the 11 infants with prolonged jaundice and UTI in our study. UTI detection for prolonged jaundice evaluation may be an opportunity to detect congenital urinary anomalies.

In conclusion, our study had important limitations with its retrospective design, such as missing important variables. We could not extract potentially impactful parameters like weight loss, nutritional status (breastfeed vs formula) and maternal blood group. Our study was single-centered and had a low event (UTI) number, which makes its statistical power and generalizability low. In the present study, we reported 391 newborns with prolonged jaundice. UTI was detected in 2.8% as a presumed etiology of prolonged jaundice. Although not statistically significant, likely because of the small sample size, the proportion of UTIs in males and preterm newborns was higher. History of phototherapy, presence of leukocyturia or nitrituria, white blood cell count, and thrombocyte count were significantly higher in the UTI group. Although we reported a low incidence of UTI in newborns with prolonged jaundice, UTI should be considered a potentially treatable etiology in newborns with prolonged jaundice.

Ethics Committee Approval: Ethical approval was obtained from the Clinical Research Ethics Committee of Zeynep Kamil Maternity and Children's Hospital (Date: 02/12/2020, decision no: 2020-176).

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Determination of Reasons for the Exclusion of Kidney Transplant Candidates from the Organ Offer List

Böbrek Nakli Adaylarinin Organ Teklif Listesinden Dışlanma Nedenlerinin Belirlenmesi

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ABSTRACT

Objective: The study aims to determine why kidney transplantation candidates are excluded from the organ offer list.

Materials and Methods: The study was conducted as a retrospective screening of archived records. The data of 228 patients who met the study criteria were included. Evaluations were made concerning sociodemographic characteristics, blood group, dialysis type and time, panel reactive antibody results, duration of waiting for an organ, and the recipient's current status (on the active waiting list, transplanted, or deceased).

Results: Of the candidates on the organ transplantation waiting list, 14.9% could not be contacted at the telephone number in the records, and 6.1% could not attend the centre because of transport problems. A statistically significant difference was determined between the age range, the time since starting dialysis, and the candidate's current status according to the waiting duration.

Conclusions: Through collaboration with dialysis and transplantation centres and the Regional Health Authority, nurses can update the contact telephone numbers and resolve transplant candidates' transport problems, thereby allowing those receiving dialysis treatment to be added to the organ transplantation waiting list without losing time.

Keywords: Kidney transplantation, nursing care, organ waiting list

ÖZ

Amaç: Çalışmamız böbrek nakli adaylarının teklif listesinden dışlanma nedenlerini tespit etmek için planlanmıştır.

Materyal ve Metot: Çalışmamız retrospektif arşiv taraması şeklinde yapılmıştır. Örneklem kriterlerine uyan 228 adayın dosyası çalışmaya dahil edilmiştir. Adayların sosyo-demografik özellikleri, kan grubu, diyaliz türü ve zamanı, panel reaktif antikorlar (PRA) sonuçları, organ bekleme süresi, alıcının güncel durumu (aktif bekleme listesinde, nakil olan ve yaşamını kaybeden) bilgileri elde edilmiştir.

Bulgular: Organ teklif listesinden dışlanan adayların % 14,9'una sistemde kayıtlı olan telefon numarasından ulaşılamadığı, %6,1'inin ulaşım sorunları nedeni ile merkeze gelemediği saptanmıştır. Bekleme süresi ile yaş aralığı, diyalize girme süresi, adayın güncel durumu arasında istatistiksel olarak anlamlı farklılık saptanmıştır ($p<0,05$).

Sonuç: Hemşireler diyaliz, nakil merkezleri ve İl Sağlık Müdürlükleri ile iş birliği yaparak adayların iletişim numaralarını güncelleyebilir, ulaşım sorunlarına çözüm bulabilir, diyaliz tedavisi alan adayların vakit kaybetmeden bekleme listesine alınmasına olanak sağlayabilir.

Anahtar Kelimeler: Böbrek nakli, hemşirelik bakımı, organ bekleme listesi

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INTRODUCTION

Kidney transplantation is the best treatment option for patients with end-stage renal failure who have been accepted onto the waiting list. However, as there are insufficient organs available to meet the needs, there is an increasing number of candidates waiting for transplantation. In the USA, there are more than 109,000 candidates on the organ waiting list.[1] According to the 2022 statistical data, there are 26,757 candidates registered on the kidney transplantation waiting list in Türkiye.[2]

The organ waiting list in Türkiye is managed by the National Coordination Centre (NCC). For organ transplantation from a cadaver, candidates must be registered on the organ waiting list of a transplantation centre and wait until there is a suitable organ available. Organs are presented to the transplantation centres through the Regional Coordination Centres (RCC).[3] The details of each organ are sent electronically to the organ transplantation coordinator physician/nurse. Then, when the coordinator nurse has reviewed the characteristics of the organ, it is presented to a transplantation surgeon. After the necessary evaluations, the organ is accepted.[4] After the transplantation centre accepts the organ, the NCC sends an organ offer list to the centres. Candidates are then invited to the transplantation centre according to the order of the organ offer list sent by the NCC, and a suitable recipient is determined.[3]

Transplantation centres make a comprehensive evaluation concerning the suitability of the candidate for transplantation and complications that may develop following transplantation.[5,6] In addition, the organ transplant coordination nurse prepares a detailed report for candidates who cannot undergo transplantation according to the order of the organ offer list. The status of the candidates and reasons that they are not suitable for transplantation are addressed in this report, which is then presented to the NCC.[7]

Many candidates registered on the waiting list have a complex medical history and comorbidities which can prevent transplantation. There are several protocols which are recommended for the evaluation of candidate recipients. Still, very few recipient studies in the literature have shown the reasons for the exclusion of candidates from transplantation. [8]

This study aimed to determine the efficacy of the organ offer list evaluation process and the reasons for excluding the candidates from the kidney transplantation offer list.

MATERIALS AND METHODS

Ethics Committee Approval: Approval for the study was granted by the Clinical Research Ethics Committee of the Medical Faculty and the Medical Director of the hospital (Date: 08.02.2021, decision no: 06). All the procedures in this study were applied in accordance with the ethical requirements of the National Research Committee and the 1964 Helsinki Declaration and revisions or comparable ethical standards.

Study Design: This study was conducted as a descriptive, retrospective archive screening study.

Place and Date of the Study: The study was conducted in a public hospital's organ transplantation coordination unit between March 15 and April 15, 2021.

Patients who develop renal failure apply to the organ transplantation coordination unit to be able to have a transplant from a cadaver and are registered on the kidney transplant waiting list. The organ transplantation coordinator nurses undertake the tests necessary for registration (tissue type, blood group, hepatitis, etc). The coordinator nurse also obtains the contact information to reach the candidate when a cadaver organ becomes available, invites the candidate to the centre at certain intervals and performs the tests necessary for transplantation so that the candidate is waiting and ready for transplantation at any time.

By educating the candidates about what needs to be done while waiting, the coordinator nurse also contributes to the process of the Coordination system. After receiving the offer list sent by the NCC, the organ transplantation coordinator nurse invites the candidates to the transplantation centre in the order in which they appear on the list and coordinates the preparation of the candidate for transplantation.

Population and Sampling: Between January 2009 and December 2020, 350 recipient candidates were invited to our centre according to the order of the offer list for cadaver kidney transplantation. Since the file information of (n=122) candidates could not be accessed, the candidates were excluded from the research. The files of (n=228) candidates were included in the study.

All the patients meeting the following criteria were included in the study;

- Age >18 years,
- A diagnosis of kidney failure,
- Undergoing dialysis,
- Registered on the waiting list,
- Having received an offer of kidney transplantation,
- Data available in the electronic patient records system.

Data Collection Tools: The hospital information system data, the organ offer list, and the information in council reports were examined for all the candidates included. For each candidate, a record was made of sociodemographic characteristics such as age, gender, marital status, blood group, dialysis

type and time, panel reactive antibody (PRA) results, and the time spent on the waiting list. The information was also examined on the candidates who remained actively on the waiting list and those who had undergone transplantation and died.

Statistical Analysis: Data obtained in the study were analyzed statistically using SPSS vn. 20.0 software (IBM Corp., Armonk, NY, USA). The conformity of the data to normal distribution was evaluated with histograms, q-q graphs, and the Shapiro-Wilk test. Categorical data were analyzed using the Chi-square test and were stated as number (n) and percentage (%). A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

The transplantation candidates included in the study comprised 51.3% males and 48.7% females, 58.8% were aged ≤50 years, 78.9% were married, and body mass index (BMI) was determined to be ≤25 in 83.8% (Table 1).

The transplant offer list and some clinical findings of the candidates are shown in Table 2. Of the total candidates, 61.4% had been receiving dialysis for less than 10 years, and 54.8% had been waiting for a kidney transplant for less than 5 years. Of the organs offered to the candidates, 5.9% were presented with full compatibility. In the order of the organ offer list, 60.1% of the candidates were placed 1-5, of which 64.5% were excluded for other reasons (a candidate placed higher on the list was found to be suitable, PRA positivity, high BMI). Of the 80.3% of the candidates invited to the centre once for transplantation, 89% were determined to be PRA negative. According to the offer list, 40.8% of the candidates were determined to be compatible with 2 HLA, 44.7% were blood group A, and 14.5% had died while waiting for a transplant (Table 2).

Table 1. Sociodemographic characteristics of the candidates (n=228)**.**

Candidate characteristics		n (%)
Age	≤50 years	134 (58.8)
	≥51 years	94 (41.2)
Gender	Female	111 (48.7)
	Male	117 (51.3)
Marital status	Married	180 (78.9)
	Single	48 (21.1)
BMI	≤25	191 (83.8)
	≥26	37 (16.2)

Table 2. The offer list and some clinical findings of the candidates (n=228)**.**

Characteristics		n (%)
Time in dialysis	≤10 years	140 (61.4)
	≥11 years	88 (38.6)
Dialysis type	Hemodialysis	215 (94.3)
	Peritoneal dialysis	13 (5.7)
Waiting time	≤5 years	125 (54.8)
	6-10 years	89 (39.0)
	≥11 years	14 (6.1)
Reason for organ offer*	Centre order	29 (85.3)
	Treatment order	2 (8.8)
	Full match	3 (5.9)
Place in organ offer list	1-5th place	137 (60.1)
	6-10th place	91 (39.9)
Reasons for exclusion	HCV infection	3 (1.3)
	Not attending the centre/not wishing to have a transplant	14 (6.1)
	LCM positivity	17 (7.5)
	Active infection	1 (0.4)
	Cardiac problems	2 (0.9)
	Patient could not be contacted	34 (14.9)
	Patient did not wish to have a transplant	10 (4.4)
	Other reasons**	147 (64.5)
Number of invitations to the centre	1 time	183 (80.3)
	2 times	40 (17.5)
	3 times	5 (2.2)
PRA	Negative	203 (89.0)
	Positive	25 (11.0)

*: Calculated according to the number of organs offered; **: Other reasons (a candidate in a higher position on the list found to be a suitable recipient, PRA positivity, high BMI), (LCM) Lymphocyte Cross-match.

Table 2. Continue.

Number of HLA matches	1 match	50 (21.9)
	2 matches	93 (40.8)
	3 matches	72 (31.6)
	4 matches	10 (4.4)
	6 matches	3 (1.3)
Blood group	0	97 (42.5)
	A	102 (44.7)
	B	28 (12.3)
	AB	1 (0.4)
Candidate cuuurrent status	Transplantation performed	51 (22.4)
	Waiting	144 (63.2)
	Deceased	33 (14.5)

*: Calculated according to the number of organs offered; **: Other reasons (a candidate in a higher position on the list found to be a suitable recipient, PRA positivity, high BMI), (LCM) Lymphocyte Cross-match.

Comparisons of the offer list characteristics and the offer list order of the candidates are shown in Table 3. No significant difference was determined between the age and gender of the candidates in the order of the offer list (p>0.05). It was determined that 75% of the candidates in dialysis for longer than 11 years were placed 1-5 on the organ offer list, and a highly significant difference was determined between the ordering of the organ offer list and the time of starting dialysis (p=0.000). Of the candidates in dialysis for longer than 11 years, 85.7% were placed 1-5 on the organ offer list, and a statistically significant difference was determined between the ordering of the organ offer list and the time of waiting for an organ (p=0.030). When the ordering of the organ offer list was examined according to the number of HLA matches, it was determined that all the candidates with 6 matches, 60% of those with 4 matches, and 63.9% of those with 3 matches were placed 1-5 on the offer list, but no statistically significant difference was determined between the number of HLA matches and the offer list order (p>0.05). No statistically significant difference was determined between the current status of the candidates and the organ offer list order (p>0.05) (Table 3).

Comparisons of the PRA test results and the offer list characteristics and gender of the candidates are shown in Table 4. No statistically significant difference was determined in the PRA test results according to the gender of the candidates, time in dialysis, waiting time and current status (p>0.05) (Table 4).

Table 3. Comparisons of the offer list characteristics of the candidates according to the place on the organ offer list (n=228).

Offer list characteristics		**Place on organ offer list**		**χ2**	**p**
		1-5th place n (%)	**6-10th place n (%)**		
Age	≤50 years	83 (61.9)	51 (38.1)	0.465	0.495
	≥51 years	54 (57.4)	40 (42.6)		
Gender	Female	60 (54.1)	51 (45.9)	3.284	0.070
	Male	77 (65.8)	40 (34.2)		
Time in dialysis	≤10 years	71 (50.7)	69 (49.3)	13.289	0.000**
	≥11 years	66 (75.0)	22 (25.0)		
Waiting time	≤5 years	67 (53.6)	58 (46.4)	6.986	0.030*
	6-10 years	58 (65.2)	31 (34.8)		
	≥11 years	12 (85.7)	2 (14.3)		
Number of HLA matches	1 match	29 (58.0)	21 (42.0)	2.890	0.576
	2 matches	53 (57.0)	40 (43)		
	3 matches	46 (63.9)	26 (36.1)		
	4 matches	6 (60.0)	4 (40.0)		
	6 matches	3 (100)	0 (0)		
Candidate current status	Transplantation performed	33 (64.7)	18 (35.3)	1.471	0.479
	Waiting	87 (60.4)	57 (39.6)		
	Deceased	17 (51.5)	16 (48.5)		

*: p<0.05; **: p<0.001; χ2: Ki-kare.

Table 4. Comparisons of the candidate gender and offer list characteristics according to the PRA test results (n=228).

Characteristic		PRA test Negative n (%)	PRA test Positive n (%)	χ2	p
Gender	Female	99 (89.2)	12 (10.8)	0.966	0.617
	Male	104 (88.9)	13 (11.2)		
Time in dialysis	≤10 years	123 (87.9)	17 (12.1)	0.966	0.617
	≥11 years	80 (90.9)	8 (9.1)		
Waiting time	≤5 years	111 (88.8)	14 (11.8)	1.045	0.903
	6-10 years	79 (88.8)	10 (11.2)		
	≥11 years	13 (92.9)	1 (7.1)		
Candidate current status	Transplantation performed	45 (88.2)	6 (11.8)	3.624	0.459
	Waiting	128 (88.9)	16 (11.1)		
	Deceased	30 (90.9)	3 (9.1)		

χ2: Ki-kare.

Comparisons of some candidate characteristics according to waiting time are shown in Table 5. Of the candidates aged ≤50 years, 62.7% had been waiting for kidney transplantation for ≤5 years and a significant difference was determined in waiting time according to age (p=0.016). Of the patients in dialysis for longer than 11 years, 33% had been waiting for a kidney transplant for less than 5 years, and a highly significant difference was determined between the duration of dialysis and the waiting time (p=0.000). No significant difference was determined in the waiting time according to the number of invitations to the centre and the number of HLA matches (p>0.05). Of the patients who had developed mortality, 57.6% had been waiting for an organ transplant for ≤5 years and a significant difference was determined between current status and waiting time (p=0.017) (Table 5).

DISCUSSION AND CONCLUSION

From a literature screening, a limited number of studies related to patients excluded from the cadaver organ offer list were determined.[8,9] Therefore, the results of this study are discussed with those of studies conducted with similar patient groups.

In Türkiye, organ offers are made on a points basis. As age increases, the candidate points decrease.[7] Stewart et al. [10] reported no difference between patients waiting for kidney transplantation concerning sociodemographic factors such as age, gender, and education level. In a study by Holley et al., it was reported that patients who underwent organ transplantation were younger than those excluded from the offer list. [8] In the current study, no significant difference was determined in the organ offer list ordering according to age and gender (p>0.05). While access to organs was allocated to young candidates, this decreased for older adults, but in the comparison of other conditions required for equitable organ offers (blood group, number of HLA matches, time in dialysis, etc.), the inequality decreased.[10,11]

According to the scoring table for cadaver kidney distribution in Türkiye, 3 points are given every

Table 5. Comparisons of candidate characteristics according to waiting time (n=228)

Candidate characteristics		Waiting time ≤5 years n (%)	Waiting time 6-10 years n (%)	Waiting time 11≥ years n (%)	χ2/p
Age	≤50 years	84 (62.7)	44 (32.8)	6 (4.5)	8.328/**0.016***
	≥51 years	41 (43.6)	45 (47.9)	8 (8.5)	
Time in dialysis	≤10 years	96 (68.6)	43 (30.7)	1 (0.7)	36.329/**0.000****
	≥11 years	29 (33.0)	46 (52.3)	13 (14.8)	
Number of invitations to the centre	1 time	104 (56.8)	70 (38.3)	9 (4.9)	4.991/ 0.288
	2 times	20 (50.0)	16 (40.0)	4 (10.0)	
	3 times	1 (20.0)	3 (60.0)	1 (20.0)	
Number of HLA matches	1 match	27 (54.0)	19 (38.0)	4 (8.0)	12.967/0.113
	2 matches	44 (47.3)	40 (43.0)	9 (9.7)	
	3 matches	49 (68.1)	22 (30.6)	1 (1.4)	
	4 matches	4 (40.0)	6 (60.0)	0 (0)	
	6 matches	1 (33.3)	2 (66.7)	0 (0)	
Candidate current status	Transplantation performed	38 (74.5)	11 (21.6)	2 (3.9)	4/**0.017***
	Waiting	68 (47.2)	65 (45.1)	11 (7.6)	
	Deceased	19 (57.6)	13 (39.4)	1 (3.0)	

*: p<0.05; **:p<0.001; χ2: Ki-kare.

month in dialysis.[12] Of the candidates in the current study, 38.6% had been in dialysis for ≥11 years, of which 6.1% had been waiting for an organ for ≥11 years, and 75.0% of the candidates in dialysis for ≥11 years were in the top places of the offer list ($p<0.05$). A statistically significant difference was determined between the time since starting dialysis and the duration of being registered on the kidney transplantation waiting list ($p<0.05$). It was also determined that 85.7% of those waiting for kidney transplantation for ≥11 years were placed 1-5 on the offer list ($p<0.05$). Being in the top places on the organ offer list was due to the candidates having been in dialysis for a long time and the high points awarded associated with that. The difference between the time in dialysis and the duration of waiting for a kidney transplant shows that the candidates were registered on the waiting list a long time after having started dialysis. This finding suggests that there is no information about the registration of patients who have developed kidney failure at any transplantation centre for a cadaver organ transplant. Therefore, much time is lost for registration on the waiting list.

Any negative condition that may develop during the organ waiting time can cause a candidate to be excluded from the list or can have a negative effect on follow-up after transplantation.[9,11,13] In a previous study, it was reported that candidates on whom transplantation could not be performed for non-clinical reasons (the candidate could not be contacted on the telephone number recorded in the system or could not attend the centre because of transport problems) were temporarily suspended from the waiting list. The 5-year survival of patients suspended from the list for 2 years was found to be extremely low.[14] Among the reasons for exclusion from the offer list determined in the current study, it was found that 14.9% of the candidates could not be contacted on the telephone number recorded in the system, 10% did not wish to have transplantation, and 6.1% could not or did not wish to attend the centre because of transport problems. It appears that some patients failed to show up for their transplantation appointments, and there were cases where necessary information was missing after they had been added to the waiting list.

Lymphocyte cross-match (LCM) positivity is a significant problem that prolongs the waiting time on the list and causes exclusion from the offer list.[9] Oruç et al. reported that cross-match positivity was among the reasons for the exclusion of candidates from the offer list. [9] In the current study, LCM positivity was determined to be the reason for exclusion from the offer list in 7.5% of the candidates. These findings in the current study support the results of previous research showing that LCM positivity causes graft rejection after transplantation. In another study that evaluated liver offer acceptance models, it was determined that adult candidates for liver transplantation received an offer of a liver a mean of 5 times while on the waiting list. [15] In the current study, it was determined that 80.3% of the candidates were offered a kidney once. This difference between the studies can be attributed to the difference in transplantation types and the waiting list scoring of the candidates.

Various risk factors, including blood transfusions, pregnancy, and previous organ transplantation, have been defined as related to HLA antigen sensitivity.[16,17] These risk factors can cause graft rejection after transplantation.[18] In a study by Oruç et al., the PRA positivity rate was determined to be high in females, and therefore there were more females in the group excluded from the offer list. [9,19] This finding could be linked to cross-match and PRA positivity that can emerge in pregnancy. In the current study, 48.7% of the candidates were female, but no significant difference was determined between the groups concerning gender and PRA positivity. The difference between these study results can be due to the differences in the birth status or the number of births of the female candidates.

The tissue group is recorded on the list before the patient characteristics to ensure the organ transplantation to the appropriate candidate and avoid serious problems after transplantation.[20,21] When creating the organ offer list in Türkiye, 150 points are assigned for each DR antigen match, 50 points for B antigen, and 25 points for A antigen. When there is a complete match (2A 2B 2DR match), the donor's kidney is presented directly to a recipient with a full match, without conditions. [7,12]In a study by Holley et al., tissue incompatibility (28%) was determined among the medical reasons for exclusion from transplantation.[8] In the current study, a kidney was offered to 3 candidates with full compatibility, and it was determined that patients moved up the offer list as the tissue compatibility increased. This was due to the high points obtained by the candidates according to the tissue matching.

Approximately 15%-20% of patients in dialysis die each year while waiting for an organ.[16,22] Sokas et al.[23] reported that 49.4% of patients removed from the transplantation list died within the first 5 years. In the current study, a significant difference was determined between the current status of the patients and the waiting time ($p<0.05$), and 57.6% of those waiting for ≥5 years were determined to have died while waiting. It was thought that these findings could be associated with increased age and dialysis complications.

In conclusion, the results of this study demonstrated the inability to contact the patient, that the patient

did not wish to undergo transplantation, and that PRA positivity was among the reasons for the exclusion of candidates from the organ offer list. It can be considered that the problems determined in this study could be resolved with information and education of candidates through regular follow-up and evaluation of patients with the collaboration of the transplantation and dialysis centres with the Regional Health Authorities. Coordinator nurses who can monitor the waiting list can improve collaboration between centres. They can also make necessary updates and provide patient education to prevent any loss of patient rights that may occur due to non-clinical reasons. There were some limitations to this study, primarily that as it was a retrospective records study, the data were restricted to the data that could be accessed from the records. A second limitation was a lack of detail in the recorded data, which limited the understanding of the causes behind the events leading to exclusion from the organ offer list. Finally, it was not possible to fully explain why patients did not attend the transplantation centre or declined transplantation.

Ethics Committee Approval: Approval for the study was granted by the Clinical Research Ethics Committee of the Medical Faculty and the Medical Director of the hospital (Date: 08.02.2021, decision no: 06). All the procedures in this study were applied in accordance with the ethical requirements of the National Research Committee and the 1964 Helsinki Declaration and revisions or comparable ethical standards.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept –DS, AS, FYZB; Supervision – DS, AS, FYZB; Materials – DS; Data Collection and/or Processing – DS, AS; Analysis and/ or Interpretation – DS, AS, FYZB; Writing – FYZB, DS.

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Examination of Healthy Lifestyle Behaviors of Athletes

Sporcuların Sağlıklı Yaşam Biçimi Davranışlarının İncelenmesi

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ABSTRACT

Objective: Athletes must lead healthy lifestyles to maintain their health and performance. This study aimed to reveal the levels of healthy lifestyle behaviors among athletes.

Materials and Methods: A total of 338 athletes were included in the study. The participants were administered a Demographic Form and the Healthy Lifestyle Behavior Scale-II. The demographic form provided information on participants' gender, age, nationality, and sports experience.

Results: The study found a significant difference in the nutrition sub-dimension of the Healthy Lifestyle Behavior Scale-II according to the participants' gender ($p<0.05$). Furthermore, there was a significant difference in the sub-dimensions and total score of the scale according to nationality ($p<0.05$). In addition, a significant difference was observed in all sub-dimensions and the scale's total score according to the participants' sports experience ($p<0.05$).

Conclusions: Athletes often display healthy lifestyle habits and understand the significance of health in sports. Notably, national athletes exhibit higher healthy lifestyle habits than non-national athletes. Moreover, their healthy lifestyle behavior tends to increase as they progress towards higher levels in their sports career. To ensure that athletes maintain their performance, it is crucial to implement plans and policies that encourage the adoption of health-improving behaviors.

Keywords: Athlete health, health behaviors, healthy lifestyles, sports

ÖZ

Amaç: Sporcuların sağlıklarını ve performanslarını sürdürebilmeleri için sağlıklı yaşam stilleri benimsemeleri gerekmektedir. Bu çalışmada sporcuların sağlıklı yaşam biçimi davranışlarının düzeylerinin belirlenmesi amaçlamıştır.

Materyal ve Metot: Çalışmaya 338 sporcu dahil edilmiştir. Katılımcılara Demografik Form ve Sağlıklı Yaşam Biçimi Davranışları Ölçeği-II (SYBDÖ-II) uygulanmıştır. Katılımcıların demografik form cinsiyet, yaş, millilik durumu, spor yılı bilgileri elde edilmiştir.

Bulgular: Katılımcıların cinsiyetlerine göre SYBDÖ-II beslenme alt boyutunda anlamlı bir farklılık bulunmuştur ($p<0,05$). Bunun yanı sıra ölçeğin alt boyutları ve toplam puanında millilik durumuna göre anlamlı bir farklılık tespit edilmiştir ($p<0,05$). Ayrıca katılımcıların spor deneyimlerine göre ölçeğin tüm alt boyutlarında ve toplam puanında anlamlı bir farklılık gözlenmiştir ($p<0,05$).

Sonuç: Sporcular büyük çoğunlukla sağlıklı yaşam tarzı alışkanlıkları sergilemekte ve sporda sağlığın önemini anlamaktadır. Özellikle milli sporcular, milli olmayan sporculara kıyasla daha yüksek düzeyde sağlıklı yaşam tarzı alışkanlıkları sergilemektedir. Bunun yanı sıra, spor kariyerlerinde daha üst seviyelere doğru ilerledikçe sağlıklı yaşam tarzı davranışları da artma eğilimindedir. Sporcuların performanslarını sürdürebilmelerini sağlamak için sporcularda sağlığı geliştirici davranışların benimsenmesini teşvik eden plan ve politikaların uygulanması büyük önem taşımaktadır.

Anahtar Kelimeler: Sağlık davranışları, sağlıklı yaşam tarzı, spor, sporcu sağlığı

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INTRODUCTION

The World Health Organisation has defined the concept of "health" as "not only the absence of disease and disability but also a state of complete physical, mental and social well-being".[1] One of the key points for health protection is individuals' lifestyle behaviours.[2, 3]

Healthy lifestyle behaviours are a set of behaviours that enable individuals to maintain a healthy lifestyle and improve their health positively.[4] Today, it is known that lifestyle behaviours of individuals directly affect their health and unhealthy lifestyle behaviours lead to the development of many chronic diseases. With healthy lifestyle behaviours, these chronic diseases can be prevented and mortality rates can be reduced.[5,6] Besides, thanks to these behaviours, individuals protect and increase their health and quality of life.[7,8] For these reasons, teaching healthy lifestyle behaviours to individuals and practices for the development and maintenance of these behaviours gain importance.[9,10] Examples of healthy lifestyle behaviours include taking responsibility for one's health, increasing the level of physical activity, maintaining healthy eating habits, establishing healthy interpersonal, and providing stress and providing stress management.[11-13] There are many factors affecting healthy lifestyle behaviours such as disease, exercise addiction, etc.[14,15]

The athletes' health should be protected and improved, and their behaviors that may negatively affect their health must be regularly evaluated for the athletes to keep and maintain their sportive performance high.[3,16] To protect and improve health, the importance of healthy lifestyle behaviours emerges in sports. By providing athletes with healthy lifestyle behaviours, sports injuries and sudden deaths in sports can be prevented and sportive success can be increased.

This study aimed to reveal the healthy lifestyle behaviours of athletes. In this context, this study will enlighten the plans and policies to be implemented to gain and increase these behaviour levels on the way to sportive success by determining the healthy behaviour levels of athletes. Since current and periodic evaluation of the risks of lifestyle behaviours in terms of health is important, this study will reveal the current healthy lifestyle behaviours of athletes.

MATERIALS AND METHODS

Ethical Approval: The study adhered to the Declaration of Helsinki. In this study, ethical approval was obtained from the ethics committee before starting the study. Approval was obtained from Yalova University Human Research Ethics Committee (Date: 29.11.2022, decision no: 2022/129). Participants were informed about the study and signed a consent form for their participation.

Study Design: The study was conducted as a descriptive study to evaluate athletes' Healthy Lifestyle Behaviours according to their sociodemographic characteristics.

Research Questions: Is there a significant difference in the Healthy Lifestyle Behaviours of athletes according to sociodemographic characteristics (gender, national athlete status, sports experience years)

Setting and Sample: The research was a descriptive study and the sample of the study consisted of 338 athletes living in Bursa province. Participants were included in the study on a voluntary basis. The participants invited to the study were informed about the study with the Informed Consent Form. The form took approximately 15-20 minutes to fill out.

Research Inclusion Criteria: Athletes residing in Bursa who participated in the research voluntarily and filled out the informed consent form were included in the study.

Research Exclusion Criteria: In the study, athletes who did not reside in Bursa, who did not voluntarily participate in the research, and who did not fill out the informed consent form were excluded.

Data Collection Tools: Demographic Information Form and Healthy Lifestyle Behaviours Scale II were used to collect the data. Demographic Information Form was used to obtain information about the participants' age, gender, nationality status, and sports experience. The Healthy Lifestyle Behaviours Scale II (HLSBS-II) was developed by Walker et al.,[17] and a Turkish validity and reliability study was conducted by Bahar et al. The HLSBS-II consisted of a total of 52 questions and 6 sub-dimensions. The sub-dimensions of the scale consisted of health responsibility, physical activity, nutrition, mental development, interpersonal relationships, and stress management. The behaviours of the participants in the direction of improving their health were measured with the HLSBS-II. The questions in the scale measure the health-promoting behaviours of individuals about healthy lifestyles. The sub-dimensions of the HLSBS-II include mental development, nutrition, physical activity, health responsibility, and stress management. The scale had a 4-point Likert scale including "never", "sometimes", "frequently", and "regularly". Scale scoring varies between 52-208 points.

Statistical Analysis: The evaluation of the data was conducted through SPSS-26 package programme. Shapiro-Wilk test, Skewness and Kurtosis values, descriptive statistics, Independent sample T-test, one -way ANOVA, and multiple comparison tests were used to analyse the data. The statistical significance value was accepted as $p<0.05$. In the study, Cronbach Alpha reliability analysis was performed to test

the reliability of the scale. As a result of the analysis, using the total score of the scale in this study,[19] the reliability of the HLSBS-II was found to be 0.925.

RESULTS

Table 1 shows the demographic information of 338 athletes included in the study.

The mean total score of the Healthy Lifestyle Behaviours Scale (HLSBS-II) administered to the participants was 137.10±22.73 (Table 2). The mean scores related to the sub-dimensions of the HLSBS-II are given in Table 2.

It was determined that the nutrition subscale score was statistically higher in male athletes than in female athletes (p=0.003). No statistical difference was found in the other sub-dimensions (p>0.05) (Table 3).

Table 1. Frequency distribution of participants' demographic data.

Variables		n (%)
Gender	Male	223 (66)
	Female	115 (34)
Age	18-25	298 (88.2)
	25 and older	40 (11.8)
National Athlete Status	Yes	50 (14.8)
	No	288 (85.2)
Sports Experience	1-5 Years	83 (24.6)
	6-10 Years	164 (48.5)
	10 years and more	91 (26.9)

Table 2. Participants' HLSBS-II subscale scores and total scores.

Participants	Sub-dimension/total	Questions Number	Mean±SD	Min-Max.
Athlete	Mental Development	6,12,18,24,30,36,42,48, and 52	23.37±4.29	9-36
	Nutrition	2,8,14,20,26,32,38,44, and 50	24.63±4.52	9-36
	Physical Activity	4,10,16,22,28,34,40, and 46	21.07±4.28	8-32
	Health Responsibility	3,9,15,21,21,27,33,39,45, and 51	23.99±5.01	9-36
	Interpersonal Relationship	1,7,13,19,25,25,31,37,43, and 49	22.49±5.07	9-36
	Stress Management	5,11,17,17,23,29,35,41, and 47	21.57±4.18	8-32
	HLSBS-II Total	1-52	137.10±22.73	52-208

HLSBS-II: Healthy Lifestyle Behaviours Scale; Min-Max.: Minimum-Maximum; Mean±SD: Mean±Standard Deviation.

Table 3. Differences in the HLSBS-II sub-dimension scores and total scores of the participants according to gender.

Scale	Sub-dimensions/ total	Gender	n	Mean±SD	t	df	p
HLSBS-II	Mental Development	Male	223	23.69±4.38	1.939	336	0.053
		Female	115	22.74±4.07			
	Nutrition	Male	223	25.15±4.43	3.012	336	**0.003***
		Female	115	23.61±4.53			
	Physical Activity	Male	223	21.20±4.42	0.791	336	0.430
		Female	115	20.81±4.00			
	Health Responsibility	Male	223	24.02±5.19	0.122	336	0.903
		Female	115	23.95±4.66			
	Interpersonal Relationship	Male	223	22.72±5.09	1.197	336	0.232
		Female	115	22.03±5.01			
	Stress Management	Male	223	21.66±4.15	0.576	336	0.565
		Female	115	21.38±4.25			
	HLSBS-II Total	Male	223	138.44±23.2	1.508	336	0.133
		Female	115	134.51±21.65			

HLSBS-II: Healthy Lifestyle Behaviours Scale; Mean±SD: Mean±Standard Deviation; *: p<0.05.

It was determined that the national athletes scored statistically higher than the non-national athletes in the mental development, nutrition, interpersonal relations sub-dimensions and HLSBS-II total score ($p<0.05$) (Table 4). However, no statistical difference was found in other sub-dimensions ($p>0.05$).

When the sub-dimensions and total scores of the HLSBS-II were examined according to the sports experience of the participants, a significant difference was observed in all sub-dimensions and total scores of the scale according to the sports experience ($p<0.05$) (Table 5). Post-hoc tests were carried out to determine where this difference originates from. In post hoc tests, a statistically significant difference was found in the Mental Development, Nutrition, Physical Activity, Health Responsibility, Stress Management subscale scores and HLSBS-II Total score between the athletes with 1-5 years of sports experience and the athletes with 6-10 years of sports experience ($p<0.05$). A statistically significant difference was detected in the Mental Development, Nutrition, Physical Activity, Health Responsibility, Interpersonal Relationship, Stress Management subscale scores and HLSBS-II Total score between the athletes with 1-5 years of sports experience and the athletes with more than 10 years of sports experience ($p<0.05$). However, no significant difference was found between the athletes with 6-10 years of sports experience and those with more than 10 years of sports experience ($p>0.05$).

Table 4. Differences in HLSBS-II sub-dimension scores and total scores according to the national athlete status of the participants.

Scale	Sub-dimensions/ total	National athlete status	n	Mean±SD	t	df	p
HLSBS-II	Mental Development	Yes	50	24.58±4.37	2.177	336	**0.030***
		No	288	23.16±4.25			
	Nutrition	Yes	50	25.98±4.39	2.309	336	**0.022***
		No	288	24.39±4.50			
	Physical Activity	Yes	50	21.34±4.49	0.492	336	0.623
		No	288	21.02±4.25			
	Health Responsibility	Yes	50	25.22±4.95	1.881	336	0.061
		No	288	23.78±5.00			
	Interpersonal Relationship	Yes	50	23.92±4.86	2.181	336	**0.030***
		No	288	22.24±5.07			
	Stress Management	Yes	50	22.18±4.19	1.127	336	0.260
		No	288	21.46±4.18			
	HLSBS-II Total	Yes	50	143.22±22.25	2.072	336	**0.039***
		No	288	136.04±22.68			

HLSBS-II: Healthy Lifestyle Behaviours Scale; Mean±SD: Mean±Standard Deviation; *: $p<0.05$.

Table 5. Differences in HLSBS-II sub-dimension scores and total scores according to participants' sports experience.

Scale	Sub-dimensions	Sports Experience	n	Mean±SD	F	p	Differences
HLSBS-II	Mental Development	1-5 years (1)	83	21.83±3.99	7.650	**0.001***	1 and 2, **p=0.003***; 1 and 3, **p=0.001***
		6-10 years (2)	164	23.71±4.11			
		10 and more (3)	91	24.15±4.58			
	Nutrition	1-5 years (1)	83	22.76±4.84	9.905	**0.000***	1 and 2, **p=0.000***; 1 and 3, **p=0.001***
		6-10 years (2)	164	25.24±4.22			
		10 and more (3)	91	25.23±4.31			
	Physical Activity	1-5 years (1)	83	19.82±4.06	5.309	**0.005***	1 and 2, **p=0.034***; 1 and 3, **p=0.005***
		6-10 years (2)	164	21.27±4.35			
		10 and more (3)	91	21.84±4.15			
	Health Responsibility	1-5 years (1)	83	22.30±5.33	6.737	**0.001***	1 and 2, **p=0.006***; 1 and 3, **p=0.002***
		6-10 years (2)	164	24.38±4.89			
		10 and more (3)	91	24.84±4.60			
	Interpersonal Relationship	1-5 years (1)	83	21.29±5.04	3.666	**0.027***	1 and 3, **p=0.025***
		6-10 years (2)	164	22.63±5.07			
		10 and more (3)	91	23.32±4.94			
	Stress Management	1-5 years (1)	83	20.42±4.20	4.199	**0.016***	1 and 2, **p=0.023***; 1 and 3, **p=0.043***
		6-10 years (2)	164	21.92±4.07			
		10 and more (3)	91	21.97±4.20			
	HLSBS-II Total	1-5 years (1)	83	128.42±23.49	8.678	**0.000***	1 and 2, **p=0.001***; 1 and 3, **p=0.000***
		6-10 years (2)	164	139.15±21.68			
		10 and more (3)	91	141.34±22.01			

HLSBS-II: Healthy Lifestyle Behaviours Scale; Mean±SD: Mean±Standard Deviation; *: $p<0.05$.

DISCUSSION AND CONCLUSION

Healthy lifestyle behaviours are behaviour models that protect and improve the health of individuals. Health has a great share in building the basis of sportive success. Therefore, it is important to determine the level of healthy lifestyle behaviours of athletes. This study aimed to evaluate the healthy lifestyle behaviours of athletes residing in Bursa province. When the literature was examined, a limited number of studies examining the healthy lifestyle behaviours of athletes were observed. Most of the studies conducted in this direction were conducted on samples such as health personnel and university students. This situation constitutes a limitation in analysing the results of the study with the literature.

When the total score of the healthy lifestyle behaviours scale of the participants was evaluated, it was seen that the participants frequently maintained healthy behaviours. This may be because that the participants were athletes and were aware that health was a prerequisite for sportive success. When the literature was examined, there were studies indicating that the health behaviours of physical education teachers, coaches, and athletes were moderate.[20] In another study conducted on athletes, it was observed that the total score of the HLSBS-II of team and individual athletes and national athletes was in a similar range.[21] Besides, in the study conducted by Ertop et al.,[8] it was determined that healthy lifestyle behaviours were higher in individuals who did sports compared to those who did not. It can be said that the result of the study was parallel to the literature.

In the study, no significant difference was found between the total scores of healthy lifestyle behaviours according to the gender of the athletes ($p<0.05$). Differences were determined only between the scores obtained in the nutrition sub-dimension according to gender. It can be said that male athletes showed healthier eating habits than female athletes. In the literature reviews, similar to the results of the study, there were studies that healthy lifestyle behaviours did not differ according to gender.[15,19,22-24] When analysed in terms of sub-dimension, contrary to the study, some studies found that women had higher scores than men in the nutrition sub-dimension.[25] This difference may be because the participants in the study were athletes.

In the study, it was found that the mental development, nutrition, and interpersonal relationships sub-dimension scores of national athletes were statistically significantly higher than those of non-national athletes ($p<0.05$). This indicated that national athletes exhibit healthy lifestyle behaviours more in terms of mental development, nutrition, and interpersonal relationships. Moreover, it was found that healthy lifestyle behaviours were more common in general. The status of national athletes, their focus on success, their desire to win, and their sportive careers may enable them to maintain behaviours that improve their health. For this reason, it can be said that their healthy eating behaviours were more intense. Parallel to this result in the literature reviews, studies showed that national athletes had higher nutritional knowledge levels than non-national athletes.[26,27] National athletes are in contact with more athletes in international competitions in addition to national competitions, which may contribute to the development of healthier behaviours in terms of interpersonal relationships. Furthermore, it is known that national athletes have high levels of sportsmanship orientation.[28] These orientations may contribute to the high level of healthy lifestyle behaviours regarding mental development and interpersonal relationships in athletes.

In the study, based on the scores of mental development, nutrition, physical activity, health responsibility, stress management sub-dimensions, and the total score of the scale, it was found that athletes with 1-5 years of sports experience had higher levels of healthy lifestyle behaviours than athletes with more than 5 years of sports experience. In the dimension of interpersonal behaviours, it was determined that athletes with more than 10 years of sports experience showed significantly healthier behaviours than athletes with 10 years or less of sports experience. It was revealed that healthy lifestyle behaviours did not differ significantly between athletes who have been doing sports for 6-10 years and athletes who have been doing sports for more than 10 years. In general, athletes' healthy lifestyle behaviors, including mental development, nutrition, physical activity, health responsibility, and stress management, significantly improved after five years of their sporting life. However, healthy lifestyle behaviours of athletes, including interpersonal relationships, significantly differentiate and improve after the tenth year of their sports experience. Based on this result, it can be said that athletes can behave more experienced, stable, and goal-oriented after 5 years of experience in their sports life. Therefore, they increase their health-enhancing behaviours by fully realising that the concept of health is important for sports. In addition to this, the existence of rules in sports, compliance of athletes with these rules, respect for the opponent, and development of tolerance are among the characteristics gained by sports in athletes.[29] Based on this study, there are noticeable differences in healthy lifestyle behaviours in the dimension of interpersonal behaviour after 10 years of experience. This may be due to the fact that athletes tend to reach a certain level of maturity, which leads to an increase in their self-esteem. Moreover, their levels of ambition and anxiety are likely to decrease over time. To clearly demonstrate these results, we re-

commend that studies in which anxiety levels and self-esteem are also examined, in addition to healthy lifestyle behaviours in athletes according to their sports experience, should be conducted.

In conclusion, it has been observed that athletes tend to maintain healthy lifestyle habits, and the levels of these habits vary depending on their nationality and the number of years they have been involved in sports. It can be inferred that athletes understand the importance of health in sports. It is crucial to highlight the significance of "health" in the policies and plans for athletes' performance enhancement in the future. Athletes should be educated and supported in various aspects, including nutrition, psychosocial, and health responsibility. Health behaviors need to be developed in this context. The limitations of our study include not examining other parameters besides healthy lifestyle habits. Future studies should focus on exploring whether healthy lifestyle habits, which increase with being a national athlete, are related to interpersonal relations and moral behaviors. Additionally, it is recommended that future studies investigate whether healthy lifestyle behavior levels, which differ according to sports year, are related to athletes' conscious awareness, anxiety levels, and self-esteem.

Ethics Committee Approval: The study was carried out by the Helsinki Declaration and approved by the Yalova University Human Research Ethics Committee (Date: 29.11.2022, decision no: 2022/129), and written consent was obtained.

Conflict of Interest: No conflict of interest was declared by the authors.

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Investigation of the Effect of Vaccination Status on the Prognosis of Covid-19 Patients Hospitalized in Pandemic Service

Pandemi Servisinde Yatan Kovid-19 Hastalarında Aşılanma Durumunun Prognoza Etkisinin Araştırılması

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ABSTRACT

Objective: We aimed to evaluate the effect of vaccination status on the prognosis of COVID-19 patients with varying vaccine combinations during hospitalization

Materials and Methods: The study was conducted with the data of 854 COVID-19 patients, of which 457 were female. The dependent variable in the comparisons was the need for intensive care, and the independent variables were gender, risk score, severity score, and vaccination status.

Results: The mean age of the patients was 57.7±15.71 with standard deviation (SD); 49.2% had never been vaccinated, and 18.3% needed intensive care. Three logistic regression models were developed using different vaccine combinations. Three doses of CoronaVac or two doses of CoronaVac and one dose of Comirnaty vaccine in the first model, three doses of CoronaVac vaccine in the second model, and receiving the vaccine solely (at least one dose in the 6 months before the disease) in the last model were found to reduce the risk of intensive care unit (ICU) admission by 82%, 77%, and 42%, respectively.

Conclusions: The effectiveness of vaccination in preventing the need for intensive care in inpatients was once again demonstrated, and it was observed that this effectiveness increased even more with booster vaccine doses.

Keywords: COVID-19, COVID-19 vaccines, inpatients, intensive care

ÖZ

Amaç: Hastanede yatış sırasında değişen aşı kombinasyonları ile aşı durumunun COVID-19 hastalarının prognozuna etkisini değerlendirmeyi amaçladık.

Materyal ve Metot: Araştırma 457'si kadın olmak üzere 854 COVİD-19 hastasının verileriyle gerçekleştirildi. Karşılaştırmalarda yer alan bağımlı değişken yoğun bakım ihtiyacı, bağımsız değişkenler; cinsiyet, risk skoru, şiddet skoru, aşılanma durumlarıdır.

Bulgular: Hastaların yaş ortalaması 57,7±15,71 standart sapma (SS) yıl olup, %49,2'si hiç aşı olmamış ve % 18,3'ününde yoğun bakım ihtiyacı olmuştur. Hastaların. Farklı aşı kombinasyonları kullanılarak üç lojistik regresyon modeli oluşturulmuştur. İlk modelde üç doz CoronaVac ya da iki doz CoronaVac bir doz Comirnaty aşısı, ikinci modelde üç doz CoronaVac aşısı, son modelde sadece aşı olmanın (hastalık öncesi 6 ayda en az bir doz) yoğun bakım ünitesi (YBÜ)'ne yatış riskini sırasıyla %82, %77 ve %42'lik azalma sağladığı saptanmıştır.

Sonuç: Aşılamanın yatan hastalarda yoğun bakım ihtiyacını önlemedeki etkinliği bir kez daha ortaya kondu ve rapel aşı dozlarıyla bu etkinliğin daha da arttığı gözlendi.

Anahtar Kelimeler: COVID-19, COVID-19 aşıları, yatan hastalar, yoğun bakım

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INTRODUCTION

COVID-19 disease has a wide spectrum ranging from asymptomatic course to multiorgan dysfunction and septic shock.[1] The clinical characteristics of COVID-19 are similar to other viral upper respiratory tract diseases, including nonspecific symptoms such as fatigue, cough, shortness of breath, and fever. The most common symptoms observed in patients are fever, fatigue, dry cough, and diarrhea.[2] Loss of taste and smell may be observed, and typical loss of smell is observed at the beginning of the disease.[3,4] Dyspnea tends to occur between days four and eight after the onset of symptoms in most patients but can also occur after ten days.[2]

The presence of co-morbidities such as coronary heart disease, pulmonary diseases including chronic obstructive pulmonary disease (COPD), liver diseases, obesity (body mass index >30), immune deficiencies, and malignancies increases the risk of death from COVID-19.[5]

In the guideline prepared by the Ministry of Health, poor prognosis markers (blood lymphocyte count <800/ µl or CRP>50mg/l upper limit value, ferritin>500ng/ml or D-DIMER>1000ng/ml) have been determined for admission blood in patients with COVID-19 pneumonia and should be evaluated for hospitalization in the presence of these criteria.[6]

Radiological imaging is an essential part of the management of COVID-19 patients. Chest radiography is usually the first choice in COVID-19 pneumonia. Although it is possible to observe some abnormalities on chest X-ray imaging, the presence of normality in X-ray results is not used to rule out disease. As the disease progresses, the likelihood of chest X-ray findings increases. COVID-19 involvement is bilateral, peripherally localized, with lower lobe involvement of the basal segments.[7]

There is no antiviral treatment with proven efficacy and safety for COVID-19. Thus, changes are experienced in treatment schemes as a result of studies and drug trials conducted by different countries and centers.[7]

The main goal of vaccines is to create immunity so that if the causative agent is reencountered, the infection can be managed asymptomatically or with mild symptoms. In various countries, mRNA, protein-based, and viral vector-based vaccine studies have been conducted. After demonstrating the effectiveness and safety of these vaccines, they have started to be administered worldwide against COVID-19. The primary goal of the COVID-19 pandemic is to reduce the severity of the disease and thus reduce mortality rates. Rapidly increasing vaccination rates are thought to be an essential part of ending the pandemic.

This study aimed to investigate how the vaccination status of patients with different vaccine combinations hospitalized in the COVID service affected the referral processes to the intensive care unit (ICU).

MATERIALS AND METHODS

Ethics Committee Approval: The study was initiated with the approval of Sakarya University Faculty of Medicine Non-Interventional Research Ethics Committee (date: 25.10.2021, decision no: E-71522473-050.01.04-74700-489), and the study group was expanded and determined as a thesis subject with the approval of the same ethics committee (date: 30.11.2021 decision no: E-71522473-050.01.04-835562-536).

The Type of Study: The present study was conducted with the data of inpatients 854 hospitalized in the COVID Service of a training and research hospital between 01.07.2021 and 31.10.2021 and is a cross-sectional descriptive study.

Data Collection Tools:

The data collection material consists of three parts: disease risk score and assessment form, disease severity score and assessment form, and vaccination process assessment form. The disease risk score and assessment form and the disease severity score and assessment form were developed based on the guidelines prepared by the Ministry of Health, General Directorate of Public Health.[8] The vaccination status of the patients was accessed through the "E-Nabız" or "Aşıla" application of the Ministry of Health.

Disease Risk Score and Assessment Form: The parameters used in disease risk assessment are as follows: age, smoking, body mass index (BMI), immunosuppression status (Bone marrow or organ transplantation, primary immunodeficiencies, use of corticosteroids equivalent to prednisolone >20 mg/ day for at least 14 days in the last 30 days, use of biologics such as infliximab in the previous 90 days, use of immunomodulators such as methotrexate, chemotherapy for cancer), comorbid conditions (Hypertension, Type 1 and Type 2 Diabetes, COPD, history of cardiovascular disease, asthma requiring daily medication, cancer). The parameters evaluated were scored as 1 point for age >60 years, smoking, BMI >30, immunosuppression status, and 1 point for each comorbidity. The risk assessment score for each patient was determined as a value between 0-9 points. Higher scores indicate an increased risk level for the patient.

Disease Severity Score and Assessment Form: The disease severity score form prepared by the researchers included parameters directly related to disease prognosis during COVID-19 disease follow-up. These parameters include lymphocyte count, CRP, ferritin, D-Dimer, respiratory count, Spo2 value, and

AC radiology imaging findings. Blood lymphocyte count <800/µl, CRP >50 mg/L, ferritin >500 ng/ml, D-Dimer >1000 ng/ml, respiratory rate 24 and above, and room air Sp02 93 and below were scored as 1 point, bilateral diffuse (>50%) involvement on AC radiology imaging was scored as 2 points, and non-diffuse involvement was scored as 1 point. The disease severity score is given a value in the range of 0-8.

Vaccination Process Assessment Form: This form records the vaccination status of the patients, if vaccinated, how many doses of which vaccine they received, the date of the last dose of vaccine, the date when the diagnosis of COVID-19 was confirmed by RT-PCR method, and their hospitalization in ICU during the disease process.

Statistical Analysis*:* The conformity of continuous variables to normal distribution was evaluated using the Kolmogorov-Smirnov Test and graphs. Descriptive statistics are given as mean ± standard deviation for normally distributed variables; median, 1st, and 3rd quartiles for non-normally distributed variables; and numbers and percentages for categorical variables. Categorical independent variables that might be associated with the dependent variable of ICU admission were analysed using Pearson's chi-square and Fisher's chi-square tests. Continuous variables that were not normally distributed were evaluated with the Mann-Whitney U Test. Predicting possible risks for ICU admission was evaluated with Binary Logistic Regression Analyses. In the regression model, p<0.25 was considered as the criterion for inclusion in the model related to intensive care hospitalization
with variables related to intensive care hospitalization in univariate analyses. Risk score and Severity score are also included in the model as variables. Hosmer Lemeshow goodness-of-fit statistics were used to assess model fit. P < 0.05 was considered statistically significant.

RESULTS

The study included 854 participants, 457 of whom were female, with a mean age of 57.7±15.71 Standard Deviation (SD). It was determined that 49.2% of the patients had never been vaccinated, 27.0% had two doses of Corona Vac, 6.4% had one dose of Comirnaty, 6% had three doses of Corona Vac, 4.8% had one dose of Corona Vac, 3.7% had two doses of Comirnaty, and 2.8% had two doses of Corona Vac and one dose of Comirnaty (Table 1).

Male gender and higher disease risk and severity scores were found to be statistically significant in the need for an ICU (p 0.01, 0.001, 0.001 and 0.001, respectively). Smoking status (p<0.001) and body mass index of 30 and above (p=0.006), which are disease risk score components, were found to increase the need for intensive care at a statistically significant level. The data that we found effective in the need for intensive care are given in Table 2.

Table 1. Distribution of disease risk score and components and vaccination status of the patients included in the study.

Specifications		n (%)
Age	≥60	370 (43.3)
	<60	484 (56.7)
Smoking status	Smoker	231 (27.0)
	Non-smoker	623 (73.0)
Body Mass Index	≥30	246 (28.8)
	<30	608 (71.2)
Immunosuppression	Yes	11 (1.3)
	No	843 (98.7)
Number of comorbid diseases	No	350 (41.0)
	1	149 (17.4)
	2	178 (20.8)
	3	145 (17.0)
	4 and >4	32 (3.7)
	unvaccinated	420 (49.2)
	1 dose of CoronaVac	41 (4.8)
	2 doses of CoronaVac	231 (27.0)
	3 doses of CoronaVac	51 (6.0)
	1 dose of Comirnaty	55 (6.4)
	2 doses of Comirnaty	32 (3.7)
	2 doses of CoronaVac and 1 dose of Comirnaty	24 (2.8)
The median risk score		2.00 (1.00-4.00)

Table 2. Comparison of disease risk score components and disease severity score components of patients included in the study based on intensive care needs.

Specifications			Intensive Care Need Yes n (/%)	Intensive Care Need No n (/%)	p
Risk Score (Median, 1-3rd quartile)			3.00 (1.0-4.0)	2.00 (1.0-3.0)	**0.001**[a]
Severity Score (Median, 1-3rd quartile)			6.00 (6.0-7.0)	4.00 (3.0-5.0)	**0.001**[a]
Gender		Male	87 (21.9)	310 (78.1)	**0.01***
		Female	69 (15.1)	388 (84.9)	
Disease Risk Score	**Age**	≥60	75 (20.3)	295 (79.7)	0.185*
		<60	81 (16.7)	403 (83.3)	
	Smoking status	Smoker	67 (29.0)	164 (71.0)	**0.001***
		Non-smoker	89 (14.3)	534 (85.7)	
	Body Mass Index	≥30	59 (24.0)	187 (76.0)	**0.006***
		<30	97 (16.0)	511 (84.0)	
	Immunosuppression	Yes	1 (9.1)	10 (90.9)	0.699**
		No	155 (18.4)	688 (81.6)	
	Number of comorbid diseases	No	52 (14.9)	298 (85.1)	0.187*
		1	28 (18.8)	121 (81.2)	
		2	37 (20.8)	141 (79.2)	
		3	30 (20.7)	115 (79.3)	
		4 and >4	9 (28.1)	23 (71.9)	
Disease Severity Score	**Lymphocyte**/µl	<800	102 (29.4)	245 (70.6)	**0.001***
		≥800	54 (10.7)	453 (89.3)	
	CRP ng/ml	>50	127 (21.4)	466 (78.6)	**0.001***
		≤50	29 (11.1)	232 (88.9)	
	Ferritin ng/ml	≥500	101 (30.1)	235 (69.9)	**0.001***
		<500	55 (10.6)	463 (89.4)	
	D dimer ng/ml	≥1000	61 (30.3)	140 (69.7)	**0.001***
		<1000	95 (14.5)	558 (85.5)	
	Respiratory Rate	≥24	143 (48.1)	154 (51.9)	**0.001***
		<24	13 (2.3)	544 (97.7)	
	SpO2	≤93	155 (26.4)	432 (73.6)	**0.001***
		>93	1 (0.4)	266 (99.6)	
	Pulmonary Infiltration	No	1 (2.9)	34 (97.1)	**0.01***
		Non-diffuse	1 (0.4)	237 (99.6)	
		Diffuse	154 (26.5)	427 (73.5)	
Need for intensive care		18.3%			

In COVID-19 patients, a one-unit increase in disease risk score increases the risk of ICU admission by 1.33-fold, and a one-unit increase in disease severity score increases the risk of ICU admission by 2.85-fold. Examination of vaccination status in the model in which all patients were included showed an 82% reduction in the risk of ICU admission with three vaccinations (three doses of CoronaVac or two doses of Corona Vac and one Comirnaty vaccine). A one-unit increase in the disease risk score resulted in a 1.26-fold increase in the risk of ICU admission, and a one-unit increase in the disease severity score resulted in a 2.79-fold increase in the risk of ICU admission. Detailed information is given in Table 3.

Table 3. Intensive care risk situations according to vaccination status.

		B	S.E.	Sig.	Exp(B)	95% C.I.for EXP (B) Lower	95% C.I.for EXP (B) Upper
Risk Score		0.287	0.073	0.000	1.332	1.154	1.538
Severity Score		1.049	0.091	0.000	2.854	2.386	3.415
Vaccination status*	**Vaccination (1)**	-0.271	0.406	0.504	0.763	0.344	1.689
	Vaccination (2)	-0.838	0.645	0.194	0.433	0.122	1.532
	Vaccination (3)	-1.712	0.494	0.001	0.180	0.068	0.476
	Vaccination (4)	-0.504	0.290	0.082	0.604	0.342	1.067
	Constant	-7.428	0.573	0.000	0.001	-	-
Increasing the risk score by one unit**	**Vaccination status**	-0.540	1	0.027	0.583	0.361	0.942
	Risk Score	0.236	1	0.001	1.266	1.106	1.450
	Severity Score	1.029	1	0.000	2.798	2.349	3.331
	Male gender	-0.082	1	0.713	0.921	0.596	1.425
	Constant	-7.280	1	0.000	0.001	-	-

*: Having one Sinovac or One Biontech vaccine (1); Having two Biontech vaccines (2); Three Sinovac or Two Sinovac One Biontech vaccines (3); Receiving two Sinovac vaccines (4); **: The omnibus test result in the model is X2 272,500 p 0.001, and the model is statistically significant. The Hosmer Lemeshow test, which evaluated the model fit, also shows that the model is compatible (p=0.305). The correct classification rate of the model is %85.9.

Patients vaccinated with at least one dose of the Comirnaty vaccine at least one week before and at least one dose of the Corona Vac vaccine at least two weeks before were considered effectively vaccinated. Those with more than 6 months between the date of last vaccination and the date of admission to hospital due to COVID-19 infection were considered unvaccinated. When considered in this way, 21.4% of the unvaccinated needed intensive care, while only 14.6% of the vaccinated needed intensive care, and it was found that vaccination statistically significantly reduced the need for intensive care (p=0.01) (Figure 1).

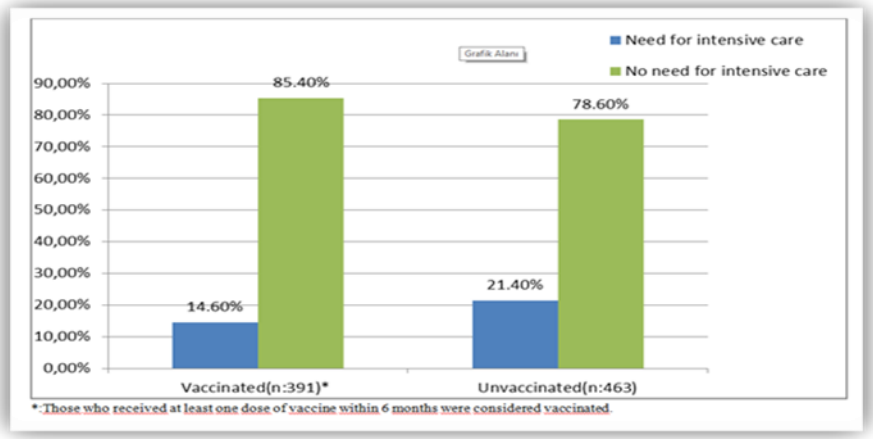

Figure 1. Comparison of intensive care needs and vaccination status of patients included in the study.

DISCUSSION AND CONCLUSION

We assessed the impact of COVID-19 vaccines on the need for an ICU in inpatients with COVID-19 and compared the prognosis of patients according to different vaccination statuses. Three doses of the vaccine resulted in an 82% reduction in the risk of ICU admission in the model in which all patients were analyzed. In an analysis excluding patients with more than one type of vaccine, a 77% reduction in the risk of ICU admission was found in patients who received 3 doses of the CoronaVac vaccine. Patients vaccinated with at least one dose of the Comirnaty vaccine at least one week before and at least one dose of the Corona Vac vaccine at least two weeks before were considered effectively vaccinated. Those with more than 6 months between the date of last vaccination and the date of admission to hospital due to COVID-19 infection were considered unvaccinated. When analyzed, it was discovered that 21.4% of unvaccinated individuals required intensive care, while only 14.6% of vaccinated individuals required the same, proving that vaccination significantly reduces the need for intensive care. Among the disease risk score components, smoking status and BMI of 30 and above were found to increase the need for intensive care at a statistically significant level. Disease severity score was found to statistically significantly increase the need for intensive care in the presence of components. Intensive care need was increased dramatically in patients with higher disease risk and severity scores. In COVID-19 patients, a one-unit increase in the disease risk score increases the risk of intensive care admission 1.33-fold, and a one-unit increase in the disease severity score increases the risk of intensive care admission 2.85-fold.

In our study, we found that 14.6% of patients who had received at least one dose of any COVID-19 vaccine needed intensive care, while 21.4% of unvaccinated patients required the same level of care. The risk of intensive care unit admission was reduced by 42% in patients vaccinated compared to those who had not. However, it's important to note that our study did not evaluate the number and type of vaccines administered, and it was conducted solely among hospitalized patients, so the relatively low rate of intensive care unit admissions may be subject to hospitalized patients. As a result of a study investigating the effectiveness of the Comirnaty vaccine against COVID-19, 92% protection was found for severe COVID-19.[9] In the study conducted with the Comirnaty and Oxford vaccines, the Comirnaty vaccine was found to be 93.7% and 88.0% protective against alpha and delta variants.[10] In the present study, the fact that vaccination showed significant protection against severe disease, while protection appeared to be relatively low, is an expected situation as the study was conducted only in COVID-19

patients receiving inpatient treatment. One factor that prevents the rate from being higher may be the high mortality rate of the disease in the early stages of the pandemic.

We found that the need for intensive care was significantly higher in male patients than in female patients. In studies, male gender was found to be associated with mortality as well as posing a high risk for COVID-19 disease.[11,12] Our study supports the literature data.

Considering the disease risk score parameters in our study individually, smoking and high BMI were found to be associated with the risk of having severe disease. Each point increase in disease risk scoring increased the risk of ICU admission by approximately 33% in inpatients with COVID-19. Xie et al. reported that patients with comorbidities and/or over 60 years of age are at high risk.[9] Docherty et al. evaluated 16749 COVID-19 patients receiving inpatient treatment in the UK and concluded that increasing age and comorbid conditions, including obesity, are associated with high mortality.[13] In our study, when risk parameters such as age, immunosuppression status, and comorbid diseases were compared individually, no statistically significant difference was found in the risk of severe disease. This could be attributed to the different vaccination status of patients in different age groups, the fact that our study was conducted on patients already receiving inpatient treatment and therefore had indications for hospitalization, the low number of immunosuppressive patients, the different age distribution of patients with comorbidities, and the different vaccination status.

Considering the changes in the parameters included in the disease severity score one by one, the changes were found to be statistically significant in increasing the risk of ICU admission in inpatients with COVID-19. Each one-point increase in the severity of the disease score was associated with a more than 2.8-fold increase in the need for ICU admission. Many studies reported that leukopenia, lymphopenia, prothrombin time, and D-dimer elevation are commonly observed in COVID-19 patients in need of intensive care. In another meta-analysis, it was reported that high CRP, PCT, D-dimer, and serum ferritin levels are associated with severe COVID-19 and increased need for intensive care.[13-16] Wu et al. evaluated the relationship between chest computed tomography imaging findings and the clinical course of the disease. In that study, lesions were scored based on whether they occupied more than 50% of the lung segment volume, and a significant correlation was found between the degree of pulmonary inflammation and clinical symptoms and laboratory results.[6]

It was conducted in a single center with data from inpatients over four months. Therefore, the number of patients included in the study was limited. As it was conducted only in inpatients, it does not provide insight into what extent patients protect vaccines against infections or hospitalization. As a cross-sectional descriptive study, the results cannot be generalized to the universe, and we do not claim to establish any cause-and-effect relationship.

In conclusion, it was recommended that it should not be disregarded that the increase in the disease severity score parameters used in the follow-up of COVID -19 patients receiving inpatient treatment increases the risk of severe disease and that the necessary interventions should be made and intensive care should be considered. Three doses of vaccination were found to be effective in preventing disease progression and reducing ICU admission in this study. The current situation serves as another reminder of the importance of vaccination and the significance of booster doses. Amid ongoing discussions about the effectiveness and potential side effects of COVID-19 vaccines, our study adds valuable insights to this discourse.

Ethics Committee Approval: The study was initiated with the approval of Sakarya University Faculty of Medicine Non-Interventional Research Ethics Committee (Date: 25.10.2021, decision no: E-71522473-050.01.04-74700-489), and the study group was expanded and determined as a thesis subject with the approval of the same ethics committee (Date: 30.11.2021, decision no: E-71522473-050.01.04-835562-536).

Conflict of Interest: No conflict of interest was declared by the authors.

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Reason for Discontinuing the Drug in Patients Using Statins

Statin Kullanan Hastalarda İlacın Kesilme Nedeni

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ABSTRACT

Objective: In recent years, there has been a lot of news about the negative effects of statin use on patients. Our study aimed to investigate the reasons for drug discontinuation in patients with indications for statin use.

Materials and Methods: 180 patients who were indicated to start statin treatment for any reason were included in the study. Demographic and clinical characteristics of the patients, cardiovascular disease risk factors, and lipid levels at admission were determined.

Results: It was determined that 81.1% of the patients were started on statin treatment. It was observed that patients using regular medication had lower LDL (low-density lipoprotein) and total cholesterol levels than those who did not use medication regularly ($p<0.05$). It was suggested that the most common reason for patients who had previously started statin treatment to stop taking the medication was discontinuation by the doctor.

Conclusions: Hyperlipidemia is still a significant cause of coronary artery disease today. Our study has shown that treatment compliance in patients, contrary to popular belief, is due to the influence of the physician on the patient rather than the influence of the media.

Keywords: Cardiovascular disease risk factors, hyperlipidemia, statin, statin treatment to stop

ÖZ

Amaç: Son yıllarda statin kullanımının hasta üzerindeki olumsuz etkisine dair birçok haber yapılmaktadır. Çalışmamızda, statin kullanma endikasyonu olan hastalarda ilacın bırakılma nedenlerinin ne olduğunun araştırılması amaçlandı.

Materyal ve Metot: Çalışmaya herhangi bir nedenle statin tedavi başlama endikasyonu konulan 180 hasta dahil edildi. Hastaların demografik ve klinik özellikleri, kardiyovasküler hastalık risk faktörleri, başvuru esnasındaki lipid düzeyleri belirlendi.

Bulgular: Hastaların %81,1'ine statin tedavisi başlandığı saptandı. Düzenli ilaç kullanan hastaların düzenli ilaç kullanmayanlara göre daha düşük LDL (düşük dansiteli lipoprotein) ve total kolesterol düzeylerine sahip olduğu görüldü ($p<0,05$). Daha önce statin tedavisi başlanan hastaların en sık ilaç bırakma gerekçesi olarak doktor tarafından kesilmesi ileri sürüldü.

Sonuç: Hiperlipidemi halen günümüzde önemli bir koroner arter hastalığı sebebidir. Çalışmamız, hastalardaki tedavi uyumunun sanılanın aksine medya etkisinden çok hekimin hasta üzerindeki etkisinden kaynaklandığını göstermiştir.

Anahtar Kelimeler: Kardiyovasküler hastalık risk faktörleri, hiperlipidemi, statin, statin tedavisinin durdurulması

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INTRODUCTION

Cardiovascular diseases remain one of the leading causes of death globally. Recently, lipid metabolism has been adversely affected due to deteriorating eating habits and food additives. Elevated levels of cholesterol lead to coronary artery disease, which directly affects multiple vascular systems. Hyperlipidemia is among the preventable risk factors for the development of cardiovascular diseases. Tight lipid control has significant contributions to mortality and morbidity in individuals with risk factors.[1]

Dyslipidemia has negative effects on the vascular system. Despite this, compliance with treatment is still inadequate in most patients.[2] In addition to diet and lifestyle changes, lifelong statin treatment is recommended, especially in patients diagnosed with coronary artery disease.[3] In a study conducted in Turkey, it was shown that the use of statins for secondary protection in very high-risk group patients with an indication for statin use due to coronary artery disease was not at an effective dose, and the target cholesterol levels of the patients were not reached.[4] There are many reasons for this. Mainly due to some news in the written and visual media and news on social media, such as the negative effects of statin treatments, the treatment is either not started at all or is interrupted before the target value is reached. This situation increases the likelihood of new coronary events and the risk of stent restenosis, especially in individuals with coronary artery disease. It has been observed that the rate of remyocardial infarction in patients with previous coronary artery disease and using statins is reduced by 20%.[5-6] In the cardiovascular disease prevention guide published by the European Society of Cardiology (ESC), the target low-density lipoprotein (LDL) level in individuals at very high risk was reduced from 70 mg/dl to 55 mg/dl.[3] This shows that patients at risk of experiencing vascular events should have a strict LDL target value.

Our study aimed to investigate the factors affecting treatment compliance in patients with indications for receiving statin therapy in line with the recommendations of current guidelines.

MATERIALS AND METHODS

Ethical Statement: The study was conducted in our hospital's cardiology outpatient clinic. Ethics Committee approval was received from Necmettin Erbakan University for the study (Date: 2/4/2021, decision no: 221-3186). The study was carried out following the international declaration, guidelines, etc.

Subject and Study Design: According to the SCORE risk classification, patients with a risk of ≥ 10% were evaluated in the "very high risk" group. Patients with an LDL value of ≥55 mg/dl were considered as an indication for drug initiation. Patients who were prescribed statins anytime between 2015 and 2020 took them regularly in the past year, stopped using them in the previous five years and were diagnosed. Patients with hearing and vision problems advanced Alzheimer's, or dementia who refused to participate or missed regular check-ups were excluded from the study.

Evaluation of Data: For the study, which was conducted in an observational, descriptive style, the current European Society of Cardiology Dyslipidemia guidelines were taken as an example.[7] In the study, patients were grouped based on their demographic and clinical characteristics. The participants were asked about their medical history and whether they had any conditions, such as smoking, diabetes mellitus, hypertension, peripheral artery disease, or cerebrovascular accident, which could be associated with cardiovascular disease. Patients who had been taking regular statin therapy for the past year were considered compliant with their treatment. Those who stopped taking the medication were asked about the reasons behind their decision. After fasting for 12 hours, the patients' blood lipid levels were measured.

Statistical Analysis: Data analysis was done using the SPSS 20 statistical program. Chi-square and Student's t-test were used with descriptive statistics. Results with a P value <0.05 were considered significant.

RESULTS

A total of 180 patients, 79 (43.9%) women and 101 (56.1%) men, with an average age of 62.9±12.3 years, were included in the study. It was determined that 81.1% of the patients had previously been diagnosed with dyslipidemia and started statin treatment. All patients participating in the study were literate. 78.3% of the patients had primary school-level education. While 85% of the patients continued statin treatment for any reason, 27 did not use any medication. 18.9% of the patients did not have any additional risk factors. While 20% of the patients using statins had a reason for using statins other than a history of coronary artery disease, 80% of the patients were using statins due to coronary artery disease. The average LDL cholesterol levels of the patients participating in the study were 93±46 mg/dl, and total cholesterol levels were 169±59 mg/dl. Table 1 shows the patients' clinical, demographic and laboratory characteristics grouped by gender.

Table 1. Grouping of clinical, demographic and laboratory characteristics by gender.

Characteristics		Female	Male	General
Age, Mean± SD		64,.3±10.8	61.9±13.3	62.9±12.3
Gender, n **(%)**		79 (43.9)	101 (56.1)	180 (100)
Educational background, n **(%)**	Primary school	72 (91.1)	69 (68.3)	141 (78.3)
	Middle school	3 (3.8)	5 (5)	8 (4.4)
	High school	3 (3.8)	21 (20.8)	24 (13.3)
	University	1 (1.3)	6 (5.9)	7 (3.9)
Treatment duration, n (%)	Not using	16 (20.3)	11 (10.9)	27 (15)
	<3 months	9 (11.4)	8 (7.9)	17 (9.4)
	3-6 months	2 (2.5)	5 (5)	7 (3.9)
	6-12 months	1 (1.3)	5 (5)	6 (3.3)
	12-24 months	11 (13.9)	20 (19.5)	31 (17.2)
	>24 months	40 (50.6)	52 (51.5)	92 (51.1)
Risk factors, n (%)	No	14 (17.7)	20 (19.8)	34 (18.9)
	Diabetes mellitus	4 (5.1)	7 (6.9)	11 (6.1)
	Hypertension	11 (13.9)	30 (29.3)	41 (22.8)
	Chronic renal failure	1 (1.3)	0 (0)	1 (0.6)
Coronary artery disease, n (%)	No	22 (27.8)	14 (13.9)	36 (20)
	Acute coronary syndrome	1 (1.3)	3 (3)	4 (2.2)
	Stent applied	23 (29.1)	45 (44.6)	68 (37.8)
	Medical follow-up	16 (20.3)	10 (9.9)	26 (14.4)
	Coronary bypass surgery	17 (21.5)	29 (28.7)	46 (25.6)
Lipid level, mg/dl, Mean± SD	Triglyceride	185±104	181±137	183±123
	Total cholesterol	182±63	159±53	169±59
	LDL	101±53	86±39	93±46
	HDL	43±11	39±10	41±10

HDL: Density Lipoprotein.

While the LDL cholesterol level was 80±32 mg/dl in patients using regular medication, it was 120±59 mg/dl in patients not ($p<0.001$, t value t:-4.902). While the total cholesterol value was 155±46 mg/dl in patients using regular medication, it was 199±70 mg/dl in patients not using regular medication ($p<0.001$, t:-4.396). Table 2 shows the distribution of cholesterol parameters according to drug use.

A total of 58 of the patients stopped using medication, and among them, the most common reason for quitting was discontinuation by the doctor. The doctor stopped the drug of 14 patients (24.1%). Among those who stopped taking the medication, one patient (1.7%) was influenced by the news in the media and stopped taking the drug. Table 3 shows the reasons for stopping the medication.

Table 2. Distribution of cholesterol parameters according to drug use.

Characteristics	Regular user	Not regular user	p	t
LDL, mg/dl, Mean± SD	80±32	120±59	**0.001**	**-4.902**
HDL, mg/dl, Mean± SD	40±11	41±10	0.799	-0.255
Triglyceride, mg/dl, Mean± SD	167±90	217±170	0.041	-2.082
Total cholesterol, mg/dl, Mean± SD	155±46	199±70	**0.001**	**-4.396**

LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein.

Table 3. Reasons for stopping the drug.

Reasons for quitting	Data
I think the medicine is unnecessary, n (%)	6 (10.3)
I think the medicine is damaging my kidneys, n (%)	4 (6.9)
I think diet and exercise are enough, n (%)	6 (10.3)
I used too much medication, that's why I quit, n (%)	12 (20.7)
I am having trouble obtaining the medicine, n (%)	9 (15.5)
My doctor cut it off, n (%)	14 (24.1)
I am affected by the news in the media, n (%)	1 (1.7)
Other reasons, n (%)	6 (10.3)

While 88.5% of patients using regular medication came for regular check-ups, 11.5% did not come for regular check-ups. While 67.3% of the patients who did not use regular medication came for a check-up within one year, 29.3% did not come for a routine check-up. While 82.8% of the patients came for regular check-ups, 17.2% did not come for regular check-ups. Table 4 shows the relationship between medication use and frequency of check-ups.

When we look at the reasons for stopping medication by gender, the most common reason for stopping medication in women is seen as discontinuation by the doctor. In contrast, the most common reason for stopping medication in men is seen as the difficulty in obtaining the drug. Among men, no patient is affected by the news in the media and stops taking the medication. Figure 1 shows the reasons for stopping medication by gender.

Table 4. The relationship between medication use and frequency of check-ups.

Frequency of check-up	Regular medication use	No regular medication use	General
0-3 months, n (%)	27 (22.1)	9 (15.5)	36 (20)
3-6 months, n (%)	22 (18)	16 (27.6)	38 (21.1)
6-12 months, n (%)	12 (9.8)	2 (3.4)	14 (7.8)
Once a year, n (%)	41 (33.6)	12 (20.7)	53 (29.4)
Every 2 years, n (%)	6 (4.9)	2 (3.4)	8 (4.4)
Irregular, n (%)	14 (11.5)	17 (29.3)	31 (17.2)

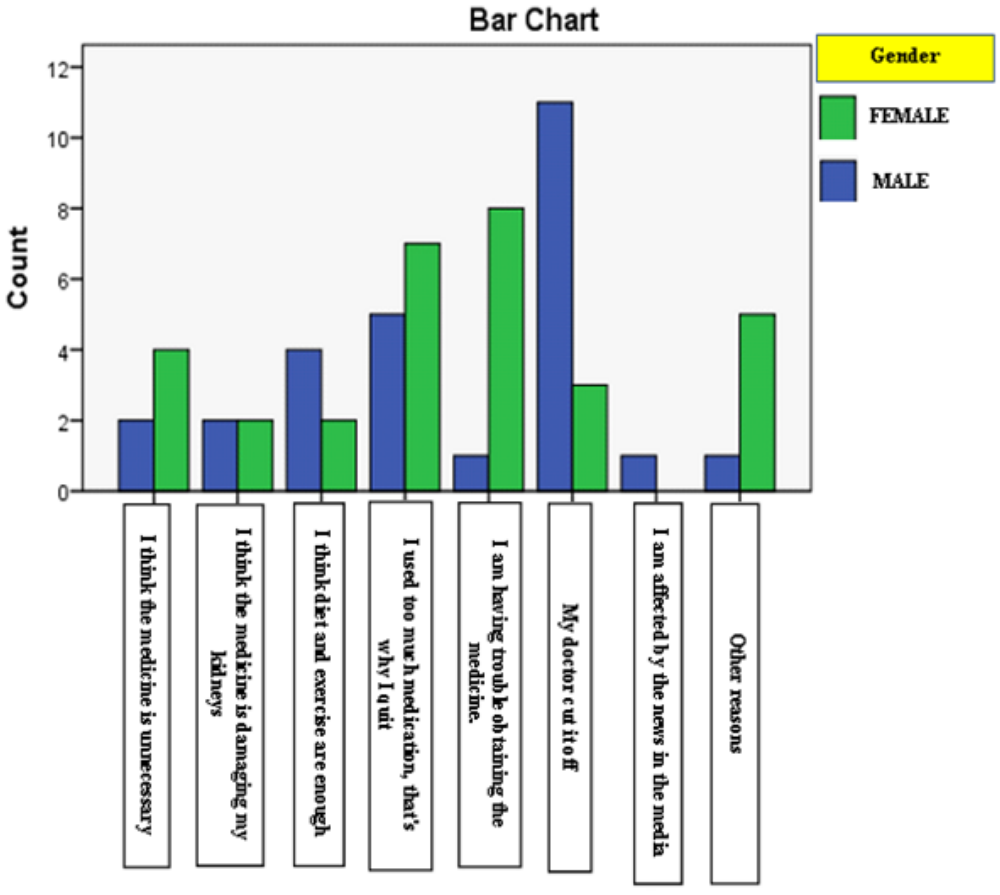

Figure 1. Reasons for stopping medication by gender.

DISCUSSION AND CONCLUSION

Atherosclerosis is an important problem in developing cardiovascular diseases and other vascular diseases. Many studies are highlighting the importance of statin treatments in preventing coronary artery disease. Although many studies have emphasized reducing LDL cholesterol levels, the desired treatment targets have still not been achieved in real-life data.[5-8] Dyslipidemia continues to be an important health problem in our country. Kayıkçıoğlu et al[9] In a meta-analysis conducted by et al., in the adult patient group, hypercholesterolemia was found in 3 out of every 10 people, hypertriglyceridemia in 1 out of every 3 people, and low HDL cholesterol in 1 out of every 2 people. Although scientific data on the necessity of using cholesterol-lowering drugs is increasing daily, some patients still do not understand its importance. Even in patients with a history of coronary artery disease and acute coronary syndrome who underwent stenting, noncompliance with the use of cholesterol-lowering drugs continues. A study by Kahraman et al.[10] showed that the rate of statin use was lower, and the incidence of cardiovascular events was higher in patients with stable coronary artery disease. Cardiovascular mortality and morbidity rates that may develop in patients with high medication compliance are reduced, and the costs imposed on the healthcare system are also reduced. In a study conducted by Dinçer et al.,[11] they claimed that many of the patients who were previously prescribed statins discontinued the drug due to the influence of written and visual media. In a study conducted by Golder et al.[12], due to the news on social media, patients claimed that the harm of statin use outweighed its benefits. However, our study differs from the results of this study. In our study, a very small number of patients stated that the news influenced them in the media as a reason for stopping the medication. Most patients in our patient group stated that the doctor stopped their medication. Due to the high literacy rate of our patient group, we think that the media effect in previous studies is ineffective in our patient group.

In a study conducted by Yeğiner et al.[13] in 2010 on compliance with statin use and reaching the target LDL value, it was observed that 56.2% of the patients discontinued the drug at any stage of the treatment. The main reason for discontinuing treatment was shown to be reaching target LDL values. In our study, 48.9% of the patients stopped using medication within two years, which was similar to the results of this study. In addition, it was observed that 15% of the patients participating in our study did not use medication. 65.6% of the patients with a history of coronary artery disease in our patient group also have a history of acute coronary syndrome, stent, or coronary bypass. According to these results, we can state that patients' medication compliance with a history of cardiac intervention is high. This is especially important in preventing events such as recurrent stent restenosis and stent thrombosis in patients with risk factors such as coronary artery disease.[14-16] In a study conducted by Yeğiner et al.,[13] patients using regular medication were found to have a higher rate of reaching the target LDL value. In our study, the rate of reaching LDL cholesterol and total cholesterol levels in patients using regular medication was more elevated than in patients who did not use medication regularly.

A study conducted by Ingersgaard et al.[14] investigating the reasons for non-adherence to statins showed that high income and education levels positively affected statin use. The results of our study are different from those of this study. While 5.1% of the patients participating in our study had a high school education or above, 26.7% of men had a high school education or above. These results showed us that medication compliance is independent of education level. It was also observed that 50.6% of the female patients participating in the study received regular statin treatment for more than 2 years. Considering that 94.9% of female patients have primary and secondary school education, it can be seen that regular drug use is independent of education level. In a study conducted by Hope et al.[15], they showed that medication compliance was higher as the income level of men increased. Our study is similar to this study. In our study, it is suggested that the main reason for the difficulty in obtaining medication, which is among the reasons why male patients stop taking medication, is financial difficulties. It has been suggested that financial difficulties are the main reason for difficulty in obtaining medication, which is the most common reason for male patients to quit medication. The main reasons for this situation can be listed as the low income level of many families in our country, the fact that men play a major role in financial gain, and the fluctuations in drug prices. A study conducted by Lowenstern et al.[16] showed that clinicians' belief in statin use and adherence to current guidelines were significantly associated with target cholesterol levels. When the results of our study are examined, it is seen that the medication compliance of patients who come to regular physician check-ups is high. While 88.5% of the patients came for regular check-ups, 11.5% did not come for regular check-ups. This shows that the physician's influence on the patient is very high in patients requiring statins and that the physician plays the primary role in the continuity of treatment.

In conclusion, hyperlipidemia is still an important cause of coronary artery disease today. Our study is important in terms of treatment compliance of patients who need to receive statin therapy. Our study

has shown that treatment compliance in patients, contrary to popular belief, is due to the influence of the physician on the patient rather than the influence of the media. Informative training is recommended to ensure continuity of treatment and possible risk assessment in patients with inadequate treatment compliance. Our most important limitations are the low number of patients, the fact that it is a single center, and the nutritional culture of the patients is not clearly known. One of our limitations is that the subgroups of statin drugs used in our patient group are unknown. One of our limitations is that the number of patients using ezetemibe, which has been among the current cardiology guideline recommendations in recent years, was not included in the study.

Ethics Committee Approval: Ethics Committee approval was received from Necmettin Erbakan University for the study (Date: 2/4/2021, decision no: 221-3186). The study was carried out following the international declaration, guidelines, etc.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – ST, YA; Supervision – YEY; Materials – IO, ALS, MC; Data Collection and/ or Processing – ST, YA; Analysis and/or Interpretation – ST, IO; Writing –ST.

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Evaluation of Inflammation Markers in Elderly Patients Undergoing Hip Fracture Surgery

Kalça Kırığı Cerrahisi Geçiren Yaşlı Hastalarda İnflamasyon Belirteçlerinin Değerlendirilmesi

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ABSTRACT

Objective: High inflammatory markers are thought to be important in evaluating poor postoperative outcomes in older orthopedic patients. We aim to investigate the effect of inflammatory markers in patients over 80 who have undergone a hip fracture surgery regarding 30-day mortality and preoperative evaluation.

Materials and Methods: Patients over 80 who had undergone hip fracture surgery were included. Age, gender, type of anesthesia, comorbidities, anticoagulant, intensive care unit (ICU) admission, hospitalization period, 30-day mortality rates, were recorded and platelet/lymphocyte ratio (PLR), neutrophil/lymphocyte ratio (NLR) and systemic immune inflammation index (SII) values were calculated.

Results: The mean age was 85.48±4.07 years old. Postoperative ICU admission was found to be 41.1%, and the 30-day mortality rate was 3.3%. The PLR value was 191±105, the NLR value was 6.4±4.5, and the SII value was 1410±1210. A significant correlation was found between ICU hospitalization and mortality, preoperative and postoperative hospital stay.

Conclusions: While there was a positive correlation between intensive care hospitalization and mortality, although no statistically significant correlation was found between the PLR, NLR and SII values and mortality rates, high values close to those stated in the literature were found.

Keywords: Anesthesia, hip fracture, inflammation marker, intensive care unit admission, mortality

ÖZ

Amaç: İleri yaş ortopedik hastalarda yüksek inflamatuar belirteçlerin kötü postoperatif sonuçların değerlendirilmesinde önemli olduğu düşünülmektedir. Amacımız 80 yaş üstü kalça kırığı ameliyatı geçirmiş hastalarda 30 günlük mortalite ve ameliyat öncesi değerlendirme açısından inflamatuar belirteçlerin etkisini araştırmaktır.

Materyal ve Metot: Kalça kırığı ameliyatı geçiren 80 yaş üstü hastalar çalışmaya dahil edildi. Yaş, cinsiyet, anestezi türü, komorbiditeler, antikoagülan kullanımı, yoğun bakım ünitesinde (YBÜ) yatış süresi, hastane yatış süresi, 30 günlük mortalite oranları kaydedilerek trombosit/lenfosit oranı (PLR), nötrofil/lenfosit oranı (NLR) ve sistemik immun inflamasyon indeksi (SII) değerleri hesaplandı.

Bulgular: Yaş ortalaması 85,48 ± 4,07 idi. Postoperatif YBÜ yatışı % 41,1 idi ve 30 günlük mortalite oranı ise % 3,3 idi. PLR değeri 191 ± 105, NLR değeri 6,4 ± 4,5, SII değeri ise 1410 ± 1210 olarak saptandı. YBÜ yatışı ile mortalite, preoperatif ve postoperatif yatış süreleri arasında anlamlı korelasyon saptandı.

Sonuç: Yoğun bakıma yatış ile mortalite arasında pozitif bir ilişki bulunurken, PLR, NLR ve SII değerleri ile mortalite oranları arasında istatistiksel olarak anlamlı bir ilişki bulunmamasına rağmen literatürde belirtilen değerlere yakın yüksek değerler bulunmuştur.

Anahtar Kelimeler: Anestezi, inflamasyon belirteci, kalça kırığı, mortalite, yoğun bakım yatışı

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INTRODUCTION

A hip fracture is a serious orthopedic problem that becomes more prevalent with advancing age, and it is associated with both short-term and long-term mortality in affected individuals.[1,2] In elderly patients who underwent hip fracture surgery, the mortality rate was found to be 24.7% during the postoperative follow-up period.[3] In a systematic review, it has been shown that the postoperative one-year mortality rate ranges from 8.4% to 36%, and this condition has been associated with arrhythmia, pneumonia, cardiac disease, elevated leukocyte count, and low albumin levels.[4] In another study, it has been revealed that elevated inflammatory marker levels are associated with 30-day mortality following a hip fracture.[5]

Platelets (PLT) are blood cells that have important roles in clotting and have also been shown to have functions in the inflammation process.[6,7] The PLT/ lymphocyte ratio (PLR) and the neutrophil/ lymphocyte ratio (NLR) are calculated values based on hemogram parameters and are used in studies as indicators of inflammation.[7,8] It is believed that elevated NLR values in elderly orthopedic patients may moderately predict adverse postoperative outcomes such as in-hospital mortality.[9] Systemic immune inflammation index (SII) is another inflammatory marker used as a prognosis-determining factor in many diseases.[10-12] Additionally, it has been demonstrated that elevated SII values serve as a good risk indicator in distinguishing fracture risk in postmenopausal osteoporosis patients.[13]

In this study, we aim to investigate the effect of inflammatory markers on 30-day mortality and the development of the need for an intensive care unit (ICU) in preoperative evaluation in patients over 80 who have undergone a hip fracture surgery.

MATERIALS AND METHODS

Ethics Committee Approval: In the study, after obtaining approval from the Local Ethics Committee of the Sakarya University Faculty of Medicine (Date: 02/02/22, Decision no: E-71522473-0.50.01.04-102134-26), patients aged 80 and over who underwent a hip fracture surgery between January 2017 and January 2022 were screened. The study was carried out by the Helsinki Declaration.

Data on 160 consecutive patients over 80 who underwent hip fracture surgery were obtained from the hospital's electronic data program. Nine patients with preoperative ICU admission were excluded from the study. A total of 151 patients were included in the study. Age, gender, type of anesthesia, comorbidities, anticoagulant use, need for preoperative ICU admission, postoperative ICU admission, preoperative hospitalization period, postoperative hospitalization period and 30-day mortality rates of the patients included were recorded. Biochemical parameters were recorded, including neutrophil, lymphocyte, platelet, and monocyte count. The PLR, NLR, and SII values were calculated using these parameters. SII is calculated as platelet count x neutrophil count/lymphocyte count. It has been shown that the normal NLR value is between 0.37 and 2.87, and the PLR value is between 36.63 and 172.68 in healthy patients.[14]

Patients aged 80 and over, with an American Society of Anesthesiologists I–III, who underwent hip fracture surgery were included in the study. Patients under 80 and those monitored in the ICU during the preoperative period were excluded from the study. Preoperative anesthesia evaluations were conducted within the last 72 hours before the operation and the preoperative laboratory parameters used during this evaluation were utilized.

Statistical analysis: Statistical analysis of the data was conducted using the SPSS 20 software package. Qualitative data were presented as counts and percentages. Quantitative data were indicated as mean and standard deviation (SD). The correlation of quantitative data was assessed using the Pearson correlation test. A statistical significance level (p) of 0.05 was adopted for all tests.

RESULTS

In this study, when patients over 80 who underwent a hip fracture surgery were scanned, the data of 151 patients who met the study criteria were obtained. While the average age was 85.48 ± 4.07 (elder: 96 years, youngest: 80 years), it was observed that 70.2% (n = 106) were female and 29.8% (n = 45) were male. Regional anesthesia was preferred for 93.38% (n=141) of the patients, while general anesthesia was administered to 6.62% (n=10) of them. The regional anesthesia techniques comprised 72.0% spinal anesthesia, 26.3% combined spinal-epidural anesthesia, and 0.7% peripheral nerve blocks. The most common comorbidities were hypertension (58.9%), neurological diseases (27.2%), and diabetes mellitus (22.5%), with acetylsalicylic acid being the most frequently used medication for anticoagulant and antiplatelet therapy at 27.2%, the second most common being clopidogrel at 6%. Postoperative ICU admission was present in 41.1% of the patients. While the preoperative stay length averaged 4.8 days, the postoperative stay length was 4.5 days. The 30-day mortality rate was 3.3% (Table 1).

Table 1. Demographic and operational characteristics of patients.

Specifications		Data
Age		85.48±4.07
Gender, n (%)	Female	106 (70.2%)
	Male	45 (29.8%)
Anesthesia Type, n (%)	Spinal anesthesia	103 (68.2%)
	Combined spinal-epidural anesthesia	37 (24.5%)
	General anesthesia	10 (6.6%)
	Peripheral Block	1 (0.7%)
Comorbidity, n (%)	Coronary Artery Disease	24 (15.9%)
	Diabetes Mellitus	34 (22.5%)
	Hypertension	89 (58.9%)
	Neurological	41 (27.2%)
	Renal	7 (4.6%)
	Malignancy	3 (2.0%)
Anticoagulant, anti-aggregant, n (%)	Acetylsalicylic Acid	41 (27.2%)
	Warfarin	5 (3.3%)
	Clopidogrel	9 (6.0%)
	New-generation oral anticoagulant	4 (2.6%)
Intensive care unit admission, n (%)		62 (41.1%)
Preoperative length of hospital stay, days		4.8 ± 3.0
Postoperative length of hospital stay, days		4.5 ± 2.8
Mortality, n (%)		5 (3.3%)

The calculated PLR value was recorded as 191 ± 105, the NLR value was 6.4 ± 4.5, and the SII value was 1410 ± 1210 (Table 2).

A significant correlation was detected between ICU admission and mortality, and the preoperative and postoperative stay length (p=0.006, p=0.017, p=0.006, respectively). However, no significant correlation was found between ICU admission and NLR, PLR, and SII. Similarly, no significant correlation was found between mortality, preoperative and postoperative stay length, NLR, PLR, and SII (Table 3).

Table 2. PLR, NLR, and SII scores.

Parameters	Data
PLR, Mean±SD	191 ± 105
NLR, Mean±SD	6.4 ± 4.5
SII score, Mean±SD	1410 ± 1210
	1120 (667-1768)*

*: Interquartile range (IQR); PLR: Platelet/lymphocyte ratio; NLR: Neutrophil/lymphocyte ratio; SII: Systemic immune inflammation index.

Table 3. Correlation of infection parameters with ICU admission and mortality.

Specifications		Intensive Care Unit Admission	Mortality	Preoperative Admission Duration	Postoperative Admission Duration	NLR	PLR	SII
Intensive care unit admission	r	1	0.222	0.194	0.222	0.092	0.053	0.115
	p	-	0.006*	0.017*	0.006*	0.262	0.522	0.161
Mortality	r	0.222	1	0.050	0.136	0.021	0.050	0.040
	p	0.006*	-	0.545	0.097	0.796	0.540	0.622

*: p<0.005; PLR: Platelet/lymphocyte ratio; NLR: Neutrophil/lymphocyte ratio; SII: Systemic immune inflammation index.

DISCUSSION AND CONCLUSION

Our study, which evaluated the 30-day mortality and ICU admission after a hip fracture surgery, found no correlation between inflammatory parameters and mortality or ICU admission. However, a significant correlation was found between ICU admission and preoperative and postoperative hospital stays.

The association of systemic inflammation with cardiovascular disease, abdominal disease, surgical site infections, and orthopedic disease has been revealed.[8,10-13] The relationship between several inflammatory markers and 30-day mortality in hip fractures has been demonstrated.[5] An experimental animal study has shown that hip fracture causes significant systemic inflammation and acute lung injury, and elderly rats were found to experience more pronounced acute lung injury compared to young rats.[15] A 30-day postoperative mortality rate was 9.9% in hip fracture patients.[5] Another study reported that the 30-day mortality rate in patients was 6%.[16] In a previous study conducted in our clinic, Palabiyik et al.[7] found a 30-day postoperative mortality rate of 10.4% in patients operated on for hip fractures. However, our current study's 30-day mortality rate was 3.3%. Peroperative conditions and medical comorbidities are important in determining risk factors for developing complications after hospitalization. These conditions, if managed well, have the potential to reduce postoperative morbidity and mortality.

In a comprehensive meta-analysis examining elderly hip fracture patients, it has been reported that a low lymphocyte count is valuable data in predicting increased mortality.[17] On the other hand, a high PLT count is a risk factor for the development of postoperative pressure ulcers in patients with hip fractures.[18] A PLR value exceeding 189 is associated with mortality in elderly hip fractures.[19] In our study, similarly to other studies, the mean PLR value was found to be 191; however, a correlation was not found between PLR and mortality.

Fisher et al.[9] have shown a significant association between high NLR values and hip fractures. Furthermore, it has been demonstrated that elevated NLR at the time of admission is not only associated with the presence of fractures and comorbidities but also closely linked to significant adverse outcomes such as postoperative myocardial injury, high inflammatory response, and in-hospital mortality. In patients with fractures, the reasons for elevated NLR at the time of admission can be multifactorial. It may be related to various pre-existing comorbid conditions, concurrent clinical or subclinical infections, and the process of responding to the fracture. Forget et al.[20] have reported that preoperative high NLR values, while insufficient as a standalone predictor for mortality, can be considered as one of the risk factors. Although the NLR value was 6.4 in our study, no significant correlation was observed between mortality and ICU admission. In our study, NLR and PLR rates were significantly higher than in normal healthy individuals. Mortality rates are lower than other studies. The decision-making process for admitting patients to intensive care after surgery may vary between physicians. Many factors, in addition to inflammatory markers, have an impact on intensive care unit admission and mortality of patients with hip fractures in the elderly. For such reasons, no significant difference was detected between inflammatory markers and mortality.

A high SII value has been identified as a good predictor factor for osteoporotic fractures.[13] Wang et al.[19] demonstrate that in elderly adults who experience and undergo surgery for hip fractures, the SII is significantly associated with mortality and could be a good indicator for predicting prognosis. Wang et al.[21] reported an association between the SII and mortality in patients undergoing hip fracture surgery in another study where they examined 290 patients. It has been indicated that the SII can be easily applied in routine clinical practice due to its simplicity and cost-effectiveness.[19] In this study, while the SII value was approximately 1410, no correlation or significant difference was found in mortality and ICU admission.

The most significant limitation of this study is the small sample size. The use of short-term mortality rates has posed challenges in interpreting the data. The absence of preadmission laboratory parameters for patients has limited a thorough assessment of the baseline impact of inflammation caused by a hip fracture.

In conclusion, a positive correlation was observed between ICU admission and mortality. Statistically, no significant correlation was found between the PLR, NLR, SII values and mortality rates. However, the inflammatory markers of the patients were found to be above normal levels, similar to those stated in the literature. Larger-scale studies with a higher number of patients are needed for a more comprehensive investigation.

Ethics Committee Approval: Our study was approved by the Ethics Committee at the Sakarya University Faculty of Medicine (Date: 02/02/22, Decision no: E-71522473-0.50.01.04-102134-26). The study was carried out following the international declaration and guidelines.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – FŞ, OP; Supervision – OP, HK, AK; Materials – FŞ, MHT; Data Collection and Processing – FŞ, MHT; Analysis and Interpretation – HK, AK; Writing –FŞ, HK, MHT.

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Construction of Mesenchymal Stem Cell-Derived Artificial Human Urinary Bladder: A Preliminary Study

Mezenkimal Kök Hücre Kaynaklı Yapay İnsan Mesanesi Geliştirilmesi: Bir Ön Çalışma

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ABSTRACT

Objective: The present study aimed to obtain the required cells and select a suitable scaffold material for constructing an artificial bladder using the tissue engineering approach.

Materials and methods: The convenience of obtaining human adipose tissue-derived stem cells (hADMSCs) was used in this study. It was attempted to differentiate these cells into smooth muscle cells (SMC), which are present along the wall of the bladder. Urothelial cells were enzymatically isolated from tissue biopsies. Synthetic (polylactide co-glycolic acid, PLGA) and natural (chitosan) polymers were used in scaffold fabrication using a tissue engineering approach.

Results: In the cellular experiments, urothelial cells couldn't be cultured in polystyrene culture vessels *in vitro* and required a support material to maintain viability. Better results were obtained with the feeder layer. The hADMSCs exhibited the expected morphological changes in the serum-rich medium content in the SMC differentiation experiments. Chitosan, biocompatible and biodegradable, was mixed with PLGA as an alternative scaffold combination.

Conclusions: This study indicated that hADMSCs-derived smooth muscle cells and biopsy-isolated urothelial cells cultured on hybrid chitosan–PLGA scaffolds with appropriate physical properties could serve as a suitable model for tissue-engineered artificial bladder construction.

Keywords: Mesenchymal stem cell, tissue engineering, urinary bladder, urothelial cells

ÖZ

Amaç: Bu çalışmada, doku mühendisliği yaklaşımıyla yapay mesane yapımı için gerekli olan hücrelerin elde edilmesi ve uygun iskele malzemesinin seçilmesi amaçlanmıştır.

Materyal ve Metot: Bu çalışmada kolaylıkla elde edilebilen insan yağ doku kökenli kök hücreler (hADMSCs) kullanılmıştır. Bu hücrelerin mesane duvarı boyunca yerleşmiş olan düz kas hücrelerine (SMC) farklılaştırılmasına çalışılmıştır. Ürotelyal hücreler ise doku biyopsi örneklerinden enzimatik aktivite ile izole edilmişlerdir. Doku iskelesi yapımında, doku mühendisliği yaklaşımı kullanılarak, sentetik (Poli-laktid-ko-glikolik asit, PLGA ve doğal (kitosan) polimerler kullanılmıştır.

Bulgular: Hücresel deneylerde, ürotelyal hücreler polistiren kültür kaplarında in vitro olarak kültüre edilememiş ve canlılıklarını sürdürmek için bir destek malzemesine ihtiyaç duydukları belirlenmiştir. Bu aşamada, besleyici hücre tabakası ile iyi sonuçlar elde edilmiştir. Ayrıca hADMSC'ler, SMC farklılaşma deneylerinde yüksek serum içeriğine sahip ortamda beklenen morfolojik değişiklikleri sergilemiştir. Biyouyumlu ve biyolojik olarak parçalanabilen kitosan alternatif iskele kombinasyonu olarak PLGA ile karıştırılmıştır.

Sonuç: Bu çalışma, uygun fiziksel özelliklere sahip hibrit kitosan-PLGA yapı iskeleleri üzerinde çoğaltılmış hADMSC türevli düz kas hücreleri ve mesane biyopsisinden izole edilmiş ürotelyal hücrelerin, doku mühendisliği ile yapay mesane üretimi için iyi bir model olabileceğini göstermiştir.

Anahtar Kelimeler: Doku mühendisliği, mesane, mezenkimal kök hücre, ürotelyal hücreler

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INTRODUCTION

The bladder is an important organ in the human body and is not directly involved in affecting vital activities. Any major problems in the bladder, such as urinary incontinence, congenital urological anomalies, and cancer formation, could reduce the quality of life of an individual and even result in death in some instances.[1]

In the cases where bladder repair cannot be performed, such as cancers, congenital anomalies (bladder exstrophy, posterior urethral valve stenosis, etc.) or dysfunctions, and organ losses, organ transplantation is considered a solution. However, difficulty finding a suitable donor and the development of complications after transplantation limits the application of this approach. Moreover, complications are encountered upon curating certain critical urinary system problems, such as inflammation, neoplasm formations, immune rejection, etc. Therefore, it is imperative to search for novel treatment methods for the above conditions related to bladder issues.[2,3]

The development of various biomaterials and tissue engineering methods in recent years has led to increased use of alternative techniques, achieving considerable success in eliminating bladder dysfunctions.[4]

In the present study, biocompatible and biodegradable chitosan[1,5] was mixed with another FDA-approved PLGA polymer to create an alternative tissue scaffold.[1,6] Thus, an alternative tissue scaffold was created for this study. Chitosan was selected as previous studies have demonstrated that this biocompatible and biodegradable material[7] stimulated angiogenesis during artificial tissue/organ production.[1,8] The other biodegradable and FDA-approved polymer PLGA was selected for similar reasons.[1,9,10]

Another important factor determining success in tissue engineering applications is the use of cells with appropriate properties. In various tissue engineering applications, cells are obtained from different sources depending on the specific requirements.[11] Stem cells are present in various tissues of an adult organism as undifferentiated cells with self-renewing abilities and the potential to differentiate into several cell types.[12] These stem cells formed the cellular basis of the present study.[13,14]

Since smooth muscle cells are one of the main cell types in the structure of the bladder, it was decided to obtain these cells by differentiating the human adipose tissue mesenchymal stem cells (hADMSCs).[15] Urothelium cells were the other type of cells required in the present study and were attempted to be isolated from bladder biopsy samples.[1,16,17]

The present study aimed to obtain the required cells for constructing a complete artificial bladder using the tissue engineering approach and then assemble these cells on a suitable scaffold material to achieve the appropriate bladder anatomy/physiology.

MATERIALS AND METHODS

Ethics Committee Approval: The tissue biopsy samples required for the isolation of urothelial cells, which were to be used in the cellular experiments in the study, were obtained from the surgical interventions performed at the Department of Pediatric Surgery, Faculty of Medicine, Manisa Celal Bayar University. The protocols used in the study were approved by the Ege University Clinical Research Ethics Committee (Date: 11.01.2010, decision no: 09–12/1). All experimental studies were conducted at the Animal Cell Culture and Tissue Engineering Laboratories, Department of Bioengineering, Faculty of Engineering, Ege University. The study was carried out by the Declaration of Helsinki.

Construction of Tissue Scaffolds: In the first stage of the tissue scaffold experiments conducted within the scope of the present study, scaffolding was performed using the polymer PLGA (poly-lactide co-glycolic acid)[18] and chitosan,[19] which have been used for constructing artificial bladder in previous studies as well. First, a chitosan scaffold was constructed to be used as the control. Porous tissue scaffolds were prepared by freeze-drying a 10 mg/mL chitosan solution (C3646, Sigma, USA) in 0.2 M acetic acid. The solution of PLGA (503H, 50:50, Boehringer Ingelheim Chemicals, USA) was prepared in 15% dichloromethane. Afterwards, the above chitosan solution was mixed with 4% PVA (Polyvinyl alcohol, 363138, Sigma, USA) in the ratios of 3:1 and 10:1, forming two different mixtures. PVA was used to support homogenization by forming an interface between chitosan and the PLGA solution, which are present in two separate phases under normal conditions and, therefore, cannot form a homogeneous mixture in such conditions. Hybrid scaffolds were prepared by freeze-drying the solution formed by mixing chitosan and PLGA with PVA. All prepared tissue scaffolds were examined under a scanning electron microscope (SEM, Jeol JSM-5200, Japan).

Cell Culture Experiments

Differentiation of hADMSCs into Smooth Muscle Cells: Smooth muscle cells are one of the basic cell types in the structure of the bladder. Therefore, in the present study, these cells were obtained by differentiating hADMSCs. The hADMSCs population from the cell culture stocks available at the Animal Cell Culture and Tissue Engineering Laboratories, Department of Bioengineering, Faculty of Engineering, Ege University was used. The details regarding

the isolation, culture, and characterization methods of the hADMSCs are available in the previous report published by our research group.[15] The differentiation of hADMSCs, which formed another basis of the present study, into smooth muscle cells (SMCs) was attempted using different methods. DMEM containing 5% horse serum and 50 µM hydrocortisone (H4001, Sigma, USA) was used to differentiate adipose tissue-derived mesenchymal stem cells into smooth muscle cells (SMCs).[20] In this process, various surface coatings (0.1–2% gelatin, FBS) were also utilized to support the differentiation. The hADMSCs were plated on these surface coatings at an initial cell concentration of 3000 cells/cm^2, followed by incubation in the differentiation medium for eight weeks. Afterwards, the cells were labelled with the α-smooth muscle actin antibody (ab15267, Abcam, USA) using immunocytochemistry for characterization.

Isolation and Culture of Urothelial Cells: The urothelial cells lining the inner surface of the bladder were isolated from the ureter or bladder biopsy samples using 25% (w/v) dispase (17105, Invitrogen GIBCO, USA) and Trypsin-EDTA solution (L2163, Biochrom AG, Germany).[21,22] The isolated cells were then cultured in serum-free keratinocyte medium (KSFM,17005034, Invitrogen GIBCO, USA) enriched with the epidermal growth factor (EGF), bovine pituitary extract (BPE) (37000-015, Invitrogen GIBCO, USA) and 30 ng/ml cholera toxin (227036, Calbiochem, Germany). In the above culture process, the urothelial cells were supported with epithelial characteristics using the feeder cell layer formed by 3T3 mouse fibroblast cells, which were generated using mitomycin C (A2190, Applichem, Germany).[22,23] The isolated and successfully cultured urothelial cells, as described above, were characterized using immunocytochemistry by the anti-cytokeratin7 antibody (ab9098, ABCAM, USA).

RESULTS

The SEM image of the porous tissue scaffold prepared through freeze-drying from the 10 mg/mL solution of chitosan in 0.2 M acetic acid is depicted in Figure 1a. The examination of the SEM images obtained after the formation of the porous tissue scaffold from the chitosan–PLGA solution mixed with PVA in a ratio of 3:1 using the freeze-drying method revealed that chitosan and PLGA did not mix up homogeneously with each other (Figure 1b).

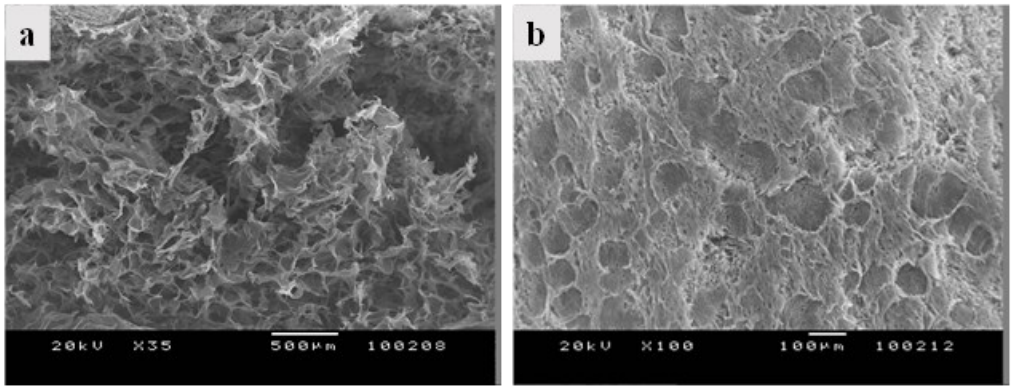

Figure 1. SEM images of the tissue scaffolds prepared using the freeze-drying method. a-Chitosan scaffold; b-Chitosan–PLGA scaffolds.

In the present study, different surface coatings were used to differentiate hADMSCs into smooth muscle cells. The control and experimental wells, with 0.1% gelatin coating, presented differences in the differentiation of hADMSCs into SMCs (Figures 2a-2d). In the differentiation (exploratory) wells, the cells were longer and closer to the SMC morphology (Figures 2b, 2d, and 2f). In addition, after eight weeks, a slight labelling with the α-smooth muscle actin antibody was observed in the differentiated cells (Figures 2d and 2f).

Additionally, the various surface coatings did not produce a significant difference in differentiation but instead resulted in almost equal levels of antibody labelling.

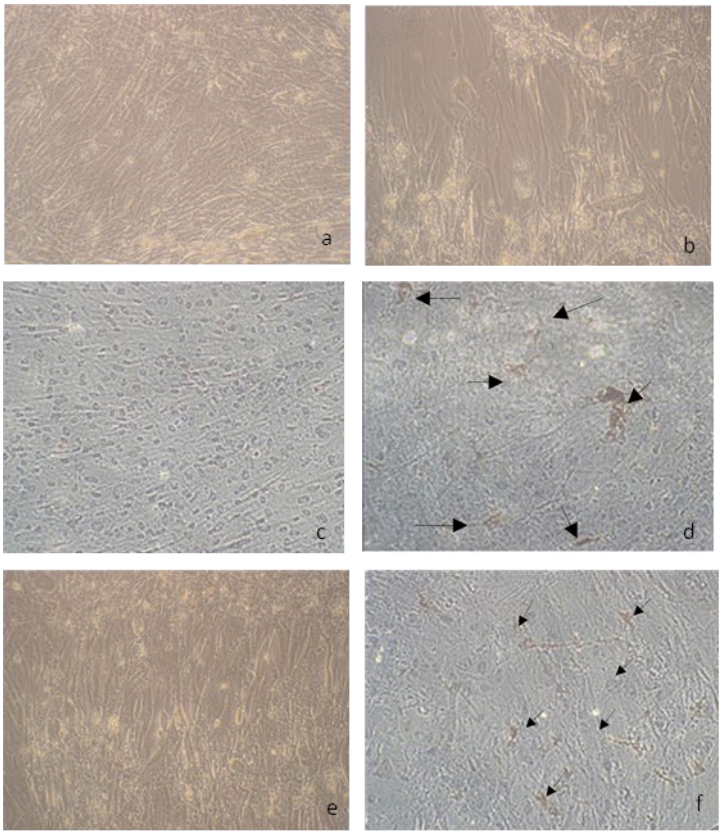

Figure 2. Inverted light microscopy images of ADMSCs in the SMC differentiation medium in the polystyrene wells with 0.1% gelatin coating (a, b, c, and d) and without the uncoating (e and f). The left column and the right column depict the control and the differentiation wells, respectively. The micrographs presented in d and f depict the actin-labelled α-smooth muscle cells (the arrows indicate the antibody-labelled regions).

To isolate urothelial cells, dispase was used for tissue digestion, and after nearly seven days, the resulting culture contained both epithelial and fibroblast cells. It was expected that epithelial cell colonies would be formed with regular medium change (serum-free keratinocyte medium). However, it was observed that as time progressed, at approximately the 9th day, both cell types had lost their viability in the culture. This indicated that the urothelial cells required a supporting layer to survive and proliferate in the culture dish. Therefore, a feeder cell layer was formed, resulting in success in the cell culture using this modified method. The urothelial cells obtained using the above procedure were characterized using the anti-cytokeratin 7 antibody. As depicted in Figure 3, the isolated urothelial cells were positive for the antibody.

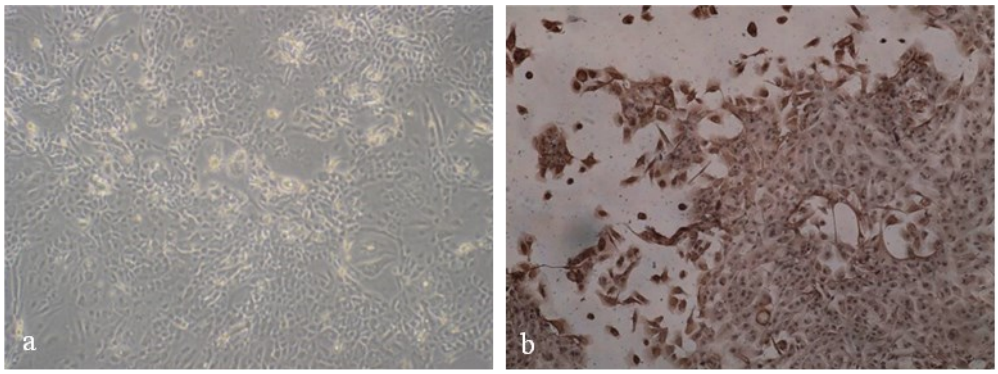

Figure 3. Cultured urothelial cells. a- The urothelial cells cultured on the feeder cell layer; b- The cells labelled with the anti-cytokeratin 7 antibody.

DISCUSSION AND CONCLUSION

The present study involved using cell culture and scaffolding methods to construct the mesenchymal stem cell-derived artificial human bladder. Alternative cell culture and scaffolding experiments were conducted to resolve issues such as fibrotic tissue formation,[24] scaffold rejection, and difficulty reaching the autologous somatic cells,[3] of which have been encountered in previously reported studies on artificial bladder development.

Three different types of biomaterials are generally used for modelling the extracellular matrix to repair or reconstruct the bladder using tissue engineering techniques – natural materials, decellularized tissue matrices, and synthetic polymers. Several scholars have reported using decellularized tissue matrices such as bladder submucosa (BS) and small intestine submucosa (SIS). However, considerably negative effects of using cell-free matrices were stated.[24] Therefore, SIS or BS were not used in the present study. In 1998,[25] new-generation synthetic polymers entered the phase of clinical trials, owing to great advancements in the field of biomaterials. In this study, a three-dimensional structure prepared by placing the urothelial cells and smooth muscle cells isolated from the dog bladder on a bladder-shaped PGA tissue scaffold was implanted in dogs. The success of the afore-stated study also accelerated the human trials, and a pilot study was conducted with seven patients.[26] In this trial, the bladder wall piece was prepared by placing the urothelial cells on the inner surface of the collagen-PGA composite and smooth muscle cells on the outer surface were implanted back into the defective bladder region in the patients. Successful results were achieved in this human study as well. Different polymers have since been used to construct artificial bladder tissues, with proven effectiveness. In this context, the present study aimed to use the biocompatible and biodegradable chitosan, which was demonstrated to exert an angiogenesis-stimulating effect by Drewa and his research group[27] in a mixture with PLGA, which is another biodegradable and FDA-approved polymer, to construct an alternative scaffold for artificial tissue formation. In addition, the present study aimed to select a suitable tissue scaffold type with appropriate properties for artificial human bladder studies. A hybrid scaffold type was selected for use in the study as it could provide support to different cell types in terms of different aspects, such as flexibility, durability, etc., for the repair of structures containing various cell types, such as the bladder. A current study in the literature also supports the accuracy of this choice.[9] In this publication, PLGA-based fibrous tissue scaffolds were evaluated in terms of biodegradability and mechanical strength and their suitability for the bile duct was examined. In this context, information that may support that PLGA is suitable for the bladder, which has a multilayer feature similar to the bile duct.

Mesenchymal stem cells are undifferentiated cells with self-renewal abilities and the potential to differentiate and transform into different cell types. These cells formed the cellular basis of the present study. It is reported that these multipotent cells present in various tissues of an adult organism, such as adipose, bone marrow, liver, and skin, are capable of rapid multiplication in a suitable culture medium.

Adipose tissue is used widely as an effective source of mesenchymal stem cells owing to its widespread presence in an organism and easy accessibility without requiring highly invasive techniques. In addition, MSCs are often preferred to be obtained from the adipose tissue owing to the high efficiency of the process (i.e., numerous stem cells are obtained from a small amount of tissue). Since the present study aimed to establish a scientific basis for the construction of the artificial bladder using adipose tissue-derived mesenchymal stem cells, subcutaneous and visceral adipose tissue biopsy samples ($\approx$1 cm^3) from children were used as the source of stem cells in the present study.[15] Consequently, the result data were consistent with those reported in the literature.[28]

Smooth muscle cells (SMCs) formed an important part of the present study and were preferred to be obtained from hADMSCs through differentiation using specific media rather than the SMCs isolated from a biopsy sample obtained from a donor. Previous studies have demonstrated that the SMCs obtained from a healthy somatic tissue have limited cleavage capacity. However, it is also reported that SMCs may be differentiated and reproduced in the desired amount *in vitro*, which is quite advantageous for tissue engineering studies. It is also reported that using the SMCs derived from hADMSCs in tissue engineering applications minimizes or could even eliminate the possibility of immune rejection, and this was one of the main reasons for preferring this approach in the present study.[18] The literature reports different approaches for the *in vitro* differentiation of hADMSCs into smooth muscle cells. However, different from the general literature, in the present study, which involved the use of tissue engineering, a smooth muscle differentiation experiment was conducted by referring to the study reported by Yoon Ghil and his research group.[29] In the present study, a medium with high serum content and 10% FBS was used, together with horse serum and hydrocortisone, which have been reported to induce myoblast and myotube formation in several previous studies. The enzymatic method was used in this study to isolate the urothelial cells lining the inner surface of the bladder. Specifically, the dispase en-

zyme was used as it is reported widely in the relevant literature.[16,17] However, it was observed that the urothelial cells isolated using this method retained their viability only for a short duration in the traditional polystyrene culture plates, and the sustainability of the culture could not be ensured. Therefore, a feeder cell layer was introduced in the present study to support the culture of the isolated urothelial cells. This modification of dispase and the supporting layer resulted in urothelial cells that exhibited a higher growth rate and a healthier morphology in the culture medium. This observation was consistent with previously reported studies.[22,23] The urothelial cells in the culture were then characterized using immunohistochemistry and the anti-cytokeratin 7 antibody, and results consistent with previous studies were obtained.[30]

In conclusion, hADMSCs-derived smooth muscle cells and biopsy-isolated urothelial cells cultured on hybrid chitosan–PLGA scaffolds with appropriate physical properties could serve as a suitable model for tissue-engineered artificial bladder construction. However, various optimisation studies should be carried out to achieve higher success during the differentiation of stem cells to SMC. If necessary, the microenvironment should be supported with various growth factors. Similarly, to increase the cultivation success of biopsy-isolated urothelial cells and ensure their survival in culture for a more extended period, extra surface modifications should be made, and the process must be supported mechanobiologically. It should be noted that culture conditions need to be improved for both cell types to better contribute to artificial bladder construction, which requires dynamic conditions. On the other hand, optimization studies need to be continued for hybrid chitosan–PLGA tissue scaffolds to gain mechanical properties more suitable for the multilayer bladder structure. In this context, improving all processes and disseminating stem cell-based artificial organ designs is recommended.

Ethics Committee Approval: Ethical committee approval was received from the Ege University Clinical Research Ethics Committee (Date: 11.01.2010, decision no: 09-12/1). Written informed consent was obtained from patients who participated in this study.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept - SET, AŞ, SİDG; Supervision - SET, AŞ, SİDG, EM; Materials - SET, AŞ, SİDG, EM; Data Collection /or Processing: SET, AŞ, SİDG; Analysis and/or Interpretation-SET, AŞ, SİDG; Writing - SET, AŞ, SİDG, EM.

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COX-mediated Regulation of Multiple Organ Damage by Betulin Treatment in Okadaic Acid-induced Alzheimer Rat Model

Okadaik Asitle İndüklenen Alzheimer Sıçan Modelinde Betulin Tedavisi ile Çoklu Organ Hasarının COX Aracılığıyla Düzenlenmesi

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ABSTRACT

Objective: Alzheimer's Disease (AD) is a progressive neurodegenerative disease. Cyclooxygenases (COXs) are essential in the inflammatory and regenerative processes of AD. This study aims to show that Betulin, a natural phytochemical (triterpene), is a candidate for COX-mediated correction of multiple organ damage of AD.

Materials and Methods: In this study, the effects and treatment potential of Betulin were investigated in the kidney, heart, and small intestine tissue in genetic, and histological contexts in an okadaic acid-induced rat AD model. A total of 36 Wistar albino male rats were included in the study. Cyclooxygenase 1 (COX-1) and Cyclooxygenase 2 (COX2) gene expressions were investigated by quantitative real-time PCR (qRT-PCR) in kidney, heart, and small intestine tissues. COX-1 and COX-2 proteins in tissues were analyzed by immunohistochemistry.

Results: COX-1 and COX-2 genes were detected to be overexpressed in the AD model. The expression of both genes was increased in the AD model and decreased after betulin treatment. Histological scores showed a strong positive effect of Betulin on the kidney, while it was relatively less effective on the heart and small intestine tissue.

Conclusions: In treating organ damage in AD, COXs can be inhibited by Betulin and may be effective in functional recovery.

Keywords: Alzheimer's disease, Cyclooxygenase 1, Cyclooxygenase 2, betulin, organ damage

ÖZ

Amaç: Alzheimer Hastalığı (AH) ilerleyici bir nörodejeneratif hastalıktır. Siklooksijenazlar (COX'ler), AH 'nin inflamatuar ve rejeneratif süreçlerinde gereklidir. Bu çalışma, doğal bir fitokimyasal (triterpen) olan Betulin'in, AH 'nin çoklu organ hasarının COX aracılı düzeltilmesi için aday olduğunu göstermeyi amaçlamaktadır.

Materyal ve Metot: Bu çalışmada okadaik asit ile indüklenen sıçan AH modelinde betulin'in böbrek, kalp ve ince bağırsak dokusundaki genetik ve histolojik bağlamdaki etkilerini ve tedavi potansiyeli araştırılmıştır. Çalışmaya 36 adet Wistar albino erkek sıçan dahil edildi. Böbrek, kalp ve ince bağırsak dokularında Cyclooxygenase 1 (COX1) ve Cyclooxygenase 2 (COX2) gen ekspresyonları kantitatif gerçek zamanlı PCR (qRT-PCR) ile araştırılmıştır. Dokulardaki COX-1 ve COX2 proteinleri immünohistokimya ile analiz edildi.

Bulgular: AH modelinde COX1 ve COX2 genlerinin aşırı eksprese edildiği tespit edildi. Her iki genin ekspresyonu AH modelinde artmış ve betulin tedavisinden sonra azalmıştır. Histolojik skorlar Betulin'in böbrek üzerinde güçlü bir olumlu etkisi olduğunu, kalp ve ince bağırsak dokusu üzerinde ise nispeten daha az etkili olduğunu gösterdi.

Sonuç: AH'de organ hasarının tedavisinde COX'lar betulin tarafından inhibe edilebilir ve fonksiyonel iyileşmede etkili olabilir.

Anahtar Kelimeler: Alzheimer hastalığı, Cyclooxygenase 1, Cyclooxygenase 2, betulin, organ hasarı

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INTRODUCTION

Alzheimer's Disease (AD) is an increasingly common neurodegenerative disease that generally affects older people and impairs the quality of life of patients.[1] In today's world, with the aging population, there is also an increase in age-related, non-communicable diseases such as dementia and its most common cause, Alzheimer's.[2] AD initially appears with memory loss and is accompanied by other cognitive functional impairments such as orientation difficulties and speech disorders. There are many questions about the etiopathogenesis of this disease.[3] Betulin is a pentacyclic triterpene metabolite and is found in large amounts in the outer bark of birch trees (Betula, Betulaceae).[4] Recent studies conducted with Betulin also show that it has various properties useful in treating metabolic, cardiovascular, and neurological disorders.[5] Betulin also reduces diet-induced obesity by inhibiting cholesterol and fatty acid biosynthesis. It also reduces the size of atherosclerotic plaques and increases their stability.[6] COX-1 and COX-2 enzymes play a role in maintaining many physiological functions in living things, as well as appearing in pathological events.[7] COXs are expressed in at least two isoforms: COX1 is expressed in most tissues, while COX2 is primarily an inducible enzyme. COX2 expression increases rapidly in many tissues in response to tissue damage or the presence of proinflammatory cytokines.[8] In the gastrointestinal tract, it has led to the idea that COX1 is critical for the cleansing action in the gastrointestinal mucosa. Therefore, COX2 appears to be responsible for inflammation.[9] In light of this information, determining the activity of two enzymes and possible treatment in case of multiorgan dysfunction may pave the way for systemic recovery.

In this study, the potential of Betulin to improve systemic organ dysfunctions and delay/prevent the occurrence of AD by regulating COX1 and COX2 enzymes that contribute to the inflammatory processes of AD was evaluated with genetic and immunohistochemical markers.

MATERIALS AND METHODS

Ethics Committee Approval: The study protocol was approved by Gaziantep University Animal Experiments Local Ethics Committee (Decision Number: 2023/29, Protocol Number: 323).

Animals: The animals were obtained from the Gaziantep University Experimental Animals Research and Application Center. All procedures respected the Guidelines of the European Union (86/609/EU). A total of 35 male Wistar Albino rats (8-12 weeks old, 250-300 gr) were included in the study.

Experimental groups:

1. Control Group (C) (n=6); The control group was not treated.

2. DMSO Group (n=5): Dimethyl Sulfoxide (DMSO) is a Betulin solvent and was applied intraperitoneally (i.p.) once a day (between 9.00-11.00) for 4 weeks as 5 mg/ml (Thermo Fisher, D12345).

3. *Betulin Group (n=6):* The treatment agent Betulin (Selleckchem, Sylvanfield Drive, Houston, TX 77014 USA, Cat no. S4754) was dissolved in DMSO according to the commercial protocol. It was administered as 20 mg/kg/day i.p. every day for 4 weeks.[1]

4. *Phosphate Buffer Saline (PBS) Group (n=6):* The animals were placed in a stereotaxic chamber, and a total of 10 µl of PBS was administered under anesthesia, 5 µl to one side of the brain and 5 µl to the other side.

5.Okadaic Acid (OKA) Group (n=6): Animals in this group were placed in the stereotaxic chamber under anesthesia. OKA was dissolved in DMSO according to the manufacturer's instructions. Animals received 5ml bilateral OKA once (200 ng/kg) (BioVision, Waltham, MA, USA, 78111-17-8in PBS[2] and were kept for 30 days for the AD model to form. According to the bregma coordinates (0.8mm posterior to bregma, 1.8mm lateral, and 3.6mm beneath the cortical surface), bilateral holes were drilled (OmniDrill35, 124 World Precision Instrument, Hertfordshire, UK) into the skull (ICV).

6. Okadaic Acid+Betulin Group (n=6): Animals were placed in the stereotaxic chamber under anesthesia. They received 5 ml bilateral OKA once (200 ng/kg) and were kept for 30 days for the model to form. At the end of the period, Betulin was administered once a day i.p.[3]

Tissue Preparation: The kidney, heart, and small intestine tissues of the animals were removed under xylazine + ketamine (5 mg/kg and 75 mg/kg) anesthesia, washed with PBS, divided into two, and stored in 10% formalin (for immunohistochemistry analyses) and RNA solution (for gene expression analyses).

Gene Expression Differences: Total RNA isolation from a 50 mg tissue sample was performed using the Triazole Method.[10] RNA concentration was measured (260 nm) with a spectrophotometer (MultiSkan® Go, Thermo ScientificÔ), and samples were diluted (10 ng of RNA in 10 ml of complementary DNA (cDNA) reaction). Reverse Transcriptase PCR (RT-PCR) reactions were prepared according to the commercial kit (Abm Good, G236) protocol. The cDNA samples were incubated in a thermal cycler (Veriti, Thermo Fisher) (16°C, 30 minutes, 1 cycle; 42°C, 30 minutes, 1 cycle; 85°C, 5 minutes 1 cycle). At the end of the process, the products were immediately placed on ice and stored at -80°C until analysis. A 20 ml PCR reaction was prepared from

this cDNA library to analyze the expression changes of *COX-1* (Qiagen, QT00187859) and *s* (Qiagen, QT02486701) genes. The reaction contained 2X SYBR Green Reaction Mix (Qiagen), 10X Gene Expression Assay, and ddH_2O. Expression data were normalized to the rat endogenous control beta-actin (ACTB) gene (Qiagen, QT00193473) and a universal rat reference RNA (Thermo Fisher, QS0641). The samples were incubated in the Real-Time PCR (Qiaegen, Rotor-Gene Q) device under two-step incubation conditions (95°C, 15 min, 1 cycle; 94°C-15 sec, 60°C-30 sec, 40 cycles). All measurements were analyzed in triplicate. The results were analyzed for DDCt. Fold change values (Fc) were calculated with $2^{-\Delta\Delta Ct}$ [11].

Immunohistochemical Analysis: Tissues were removed from 10% formalin, dehydrated, embedded in paraffin, cooled, and 3 mm thick sections were cut. Samples were inhibited with endogenous peroxidase solution and then treated with antigen retrieval (Abcam, ab970). They were then kept in a normal blocking solution and blocked with avidin-biotin (Santa Cruz, sc-516217), incubated for 24 hours for primary antibody staining for COX-1 (Elabscience, E-AB-61656) and COX-2 (Elabscience, E-EL-R0792). In the next step, they were incubated with a biotin-labelled secondary antibody at the end of the incubation, and the samples were kept in streptavidin-HRP (Abcam ab7403). Finally, they were stained with a DAB Substrate Kit (Abcam, ab64238) and observed under a light microscope (Primo Star, Zeiss). In evaluating COX-1 and COX-2 in each tissue, scoring was done based on the staining intensities and percentages of the stained area.[12]

Statistical Analysis: All analyses were performed in SPSS 22.0 (Release 22.0, SPSS Inc, Chicago, IL, USA). The normality of the groups was tested with Shapiro-Wilk. Tukey was used for post-hoc analyses, and One-Way ANOVA was used for the differences between the groups. The direction of significance was determined by descriptive statistics and multiple comparative analyses. Correlations were analyzed for the expression of the genes in each tissue. The Kruskal-Wallis Test was used to analyze the variances. $p < 0.05$ was considered statistically significant.

RESULTS

In kidney tissue analysis, the difference between the groups was statistically significant for COX-1 (df (5.29), MS=204.294, F=31.612, P<0.05) and COX-2 (df (5.29), MS=133.187, F=28.218, P<0.05). Two genes were increased in AD rats, and they decreased after the Betulin treatment. For heart tissue, COX-1 (df (5.29), MS=163.943, F=55.585, P<0.05) and COX-2 (df (5.29), MS=164.509, F=123.060, P<0.05) expressions were found to be increased in AD and decreased in treatment. Small intestinal tissue was also statistically significant for COX-1 (df (5.29), MS=117.409, F=96.362, P<0.05) and COX-2 (df (5.29), MS=118.809, F=43.899, P<0.05) genes.

Table 1. Descriptive statistics for COX-1 and COX-2 gene expressions in three tissues.

Tissues	Genes	Experimental Groups	n	Mean	SD	SE	95% CI for Mean	P-value
Kidney	COX-1	Control	6	37.787	2.066	0.843	35.619-39.954	**0.001***
		DMSO	5	36.288	3.864	1.728	31.490-41.086	
		Betulin	6	22.888	0.307	0.126	22.566-23.211	
		PBS	6	35.675	0.844	0.344	34.790-36.560	
		OKA	6	37.907	4.040	1.650	33.666-42.147	
		OKA+ Betulin	6	37.510	2.034	0.830	35.375-39.645	
	COX-2	Control	6	38.283	1.116	0.456	37.112-39.455	**0.001***
		DMSO	5	34.552	1.264	0.565	32.983-36.121	
		Betulin	6	25.896	2.197	0.897	23.591-28.202	
		PBS	6	35.452	3.859	1.575	31.402-39.501	
		OKA	6	38.472	0.372	0.152	38.081-38.862	
		OKA+ Betulin	6	37.215	2.235	0.913	34.869-39.561	
Heart	COX-1	Control	6	39.467	0.326	0.133	39.124-39.809	**0.001***
		DMSO	5	35.468	0.478	0.214	34.874-36.062	
		Betulin	6	25.208	0.714	0.292	24.459-25.958	
		PBS	6	36.580	2.788	1.138	33.654-39.506	
		OKA	6	38.730	1.270	0.519	37.397-40.063	
		OKA+ Betulin	6	37.337	2.631	1.074	34.576-40.098	
	COX-2	Control	6	39.015	2.301	0.939	36.601-41.429	**0.001***
		DMSO	5	33.232	0.337	0.151	32.814-33.650	
		Betulin	6	24.510	0.365	0.149	24.127-24.893	
		PBS	6	35.043	0.692	0.283	34.317-35.770	
		OKA	6	37.462	0.339	0.138	37.106-37.817	
		OKA+ Betulin	6	37.057	1.282	0.523	35.712-38.402	

*: P<0.05; SD: Standard deviation; SE: Standard error; CI: Confidence interval; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

Table 1. Continue.

Small Intestine	COX-1	Control	6	35.352	0.921	0.376	34.385-36.319	**0.001***
		DMSO	5	33.942	0.554	0.248	33.254-34.630	
		Betulin	6	25.140	0.720	0.294	24.385-25.895	
		PBS	6	35.783	1.598	0.653	34.106-37.461	
		OKA	6	35.468	0.298	0.121	35.156-35.781	
		OKA+ Betulin	6	37.505	1.905	0.778	35.506-39.504	
	COX-2	Control	6	35.683	1.365	0.557	34.251-37.116	**0.001***
		DMSO	5	32.938	0.511	0.228	32.304-33.572	
		Betulin	6	25.110	1.080	0.441	23.977-26.243	
		PBS	6	35.583	2.380	0.972	33.085-38.081	
		OKA	6	36.387	0.979	0.400	35.359-37.415	
		OKA+ Betulin	6	36.875	2.416	0.986	34.340-39.410	

*: P<0.05; SD: Standard deviation; SE: Standard error; CI: Confidence interval; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

Descriptive statistics for gene expressions in each tissue are given in Table 1.

Differences in group averages were tested with the Independent-samples Kruskal Wallis Test. Genes were detected as significant for all 3 tissues (Figure 1).

Multiple comparative analysis of groups is given in Table 2.

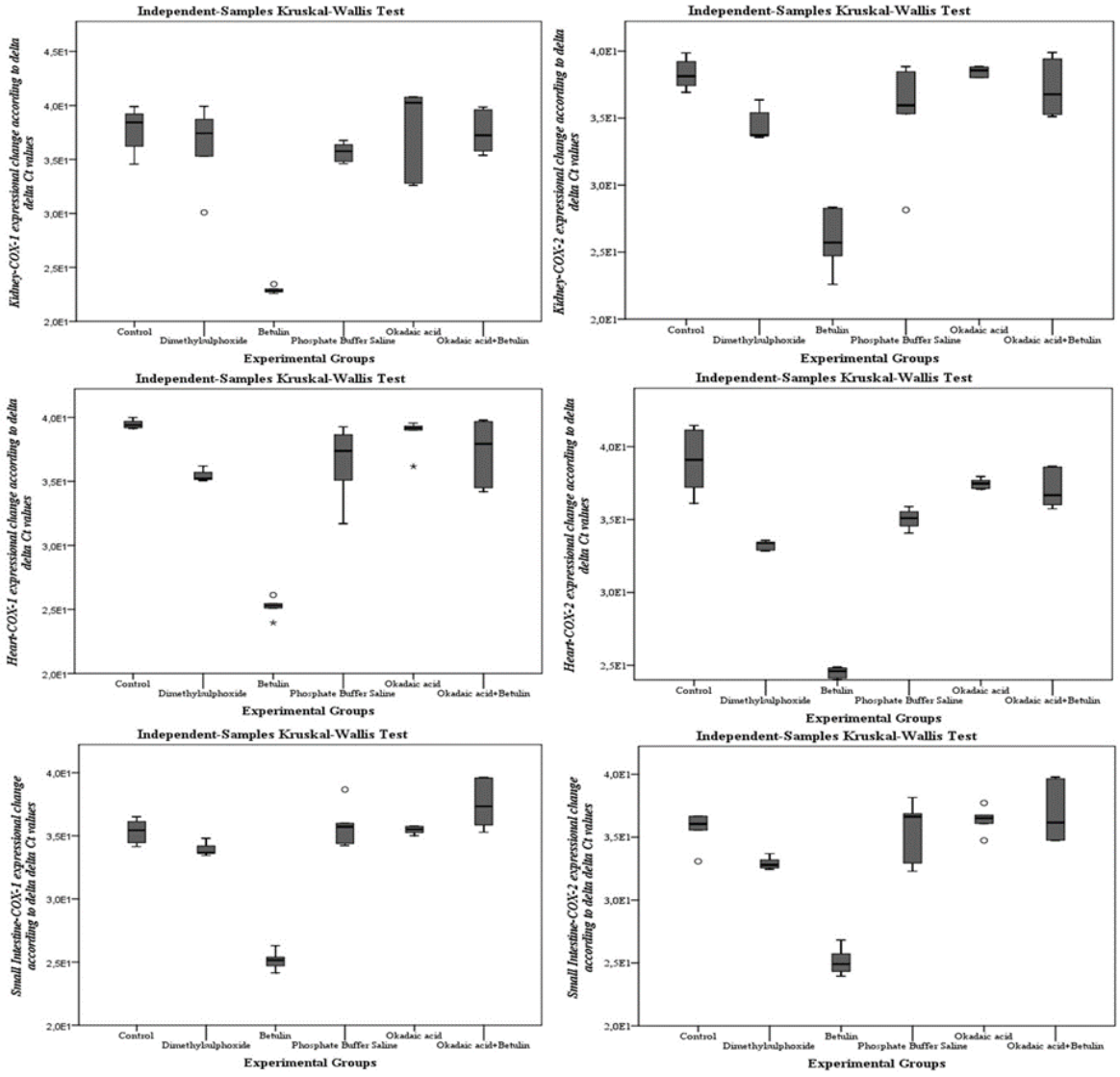

Figure 1. The box-plot graph represents gene expression differences between the three tissues of group means. Comparative analyses were performed using the Independent-Samples Kruskal-Walli's test.

Table 2. Multiple comparative analyses for groups in different tissues.

Tissues	Genes	Experimental Groups (I)	Experimental Groups (II)	Mean	SE	95% CI for Mean	P-value
Kidney	COX1	Control	DMSO	1.499	1.539	-3.194-6.191	0.923
			Betulin	14.898	1.468	10.421-19.327	**0.001***
			PBS	2.112	1.468	-2.363-6.586	0.704
			OKA	-0.120	1.468	-4.594-4.354	1.000
			OKA+ Betulin	0.277	1.468	-4.198-4.751	1.000
		DMSO	Control	-1.499	1.539	-6.191-3.194	0.923
			Betulin	13.400	1.539	8.707-18.093	**0.001***
			PBS	0.613	1.539	-4.080-5.306	0.999
			OKA	-1.619	1.539	-6.311-3.074	0.896
			OKA+ Betulin	-1.222	1.539	-5.915-3.471	0.966
		Betulin	Control	-14.898	1.468	-19.373--10.424	**0.001***
			DMSO	-13.400	1.539	-18.092--8.707	**0.001***
			PBS	-12.787	1.468	-17.261--8.312	**0.001***
			OKA	-15.018	1.468	-19.493--10.544	**0.001***
			OKA+ Betulin	-14.622	1.468	-19.096—10.147	**0.001***
		PBS	Control	-2.111	1.468	-6.586-2.363	0.704
			DMSO	-0.613	1.539	-5.305-4.080	0.999
			Betulin	12.787	1.468	8.312-17.261	**0.001***
			OKA	-2.232	1.468	-6.706-2.247	0.654
			OKA+ Betulin	-1.835	1.468	-6.309-2.639	0.809
		OKA	Control	0.120	1.468	-4.354-4.594	1.000
			DMSO	1.619	1.468	-3.074-6.311	0.896
			Betulin	15.018	1.468	10.544-19.492	**0.001***
			PBS	2.231	1.468	-2.243-6.706	0.654
			OKA+Betulin	0.397	1.468	-4.078-4.871	1.000
		OKA+Betulin	Control	-0.277	1.468	-4.751-4.197	1.000
			DMSO	1.222	1.539	-3.471-5.914	0.966
			Betulin	14.622	1.468	10.147-19.095	**0.001***
			PBS	1.835	1.468	-2.639-6.309	0.809
			OKA	-0.397	1.468	-4.871-4.077	1.000
	COX2	Control	DMSO	3.731	1.315	-0.279-7.741	0.080
			Betulin	12.387	1.254	8.563-416.210	**0.001***
			PBS	2.832	1.254	-0.992-6.655	0.955
			OKA	-0.188	1.254	-4.012-3.635	1.000
			OKA+ Betulin	1.068	1.254	-2.755-4.892	0.955
		DMSO	Control	-3.761	1.315	-7.741-0.279	0.080
			Betulin	8.655	1.315	4.645-12.666	**0.001***
			PBS	-0.899	1.315	-4.910-3.111	0.982
			OKA	-3.920	1.315	-7.930-0.091	0.058
			OKA+ Betulin	-2.663	1.315	-6.673-1.247	0.354
		Betulin	Control	-12.387	1.254	-16.211--8.563	**0.001***
			DMSO	-8.656	1.315	-12.666--4.645	**0.001***
			PBS	-9.555	1.254	-13.379--5.731	**0.001***
			OKA	-12.575	1.254	-16.399--8.751	**0.001***
			OKA+ Betulin	-11.318	1.254	-15.143--7.495	**0.001***
		PBS	Control	-2.832	1.254	-6.655-0.992	0.244
			DMSO	0.900	1.315	-3.111-4.910	0.982
			Betulin	9.555	1.254	5.731-13.379	**0.001***
			OKA	-3.020	1.254	-6.846-0.803	0.187
			OKA+ Betulin	-1.763	1.254	-5.587-2.060	0.723
		OKA	Control	0.188	1.254	-3.635-4.012	1.000
			DMSO	3.919	1.315	-0.091-7.930	0.058
			Betulin	12.575	1.254	8.752-16.399	**0.001***
			PBS	3.020	1.254	-0.804-6.843	0.187
			OKA+Betulin	1.257	1.254	-2.567-5.080	0.914
		OKA+Betulin	Control	-1.068	1.254	-4.892-2.755	0.955
			DMSO	2.663	1.315	-1.347-6.673	0.354
			Betulin	11.318	1.254	7.495-15.142	**0.001***
			PBS	7.763	1.254	-2.060-5.587	0.723
			OKA	-1.257	1.254	-5.080-2.567	0.914

*: P<0.05; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

Table 2. Continue.

Tissues	Genes	Experimental Groups (I)	Experimental Groups (II)	Mean	SE	95% CI for Mean	P-value
		Control	DMSO	3.400	1.040	0.828-1.469	**0.007***
			Betulin	14.258	0.991	11.236-17.281	**0.001***
			PBS	2.887	0.991	-0.136-5.909	0.068
			OKA	0.737	0.991	-2.286-3.759	0.975
			OKA+ Betulin	2.130	0.991	-0.893-5.153	0.292
		DMSO	Control	-3.999	1.039	-7.169--0:825	**0.007***
			Betulin	10.260	1.039	7.089-13.430	**0.001***
			PBS	-1.112	1.039	-4.282-2.058	0.889
			OKA	-3.262	1.039	-6.432--0.092	**0.041***
			OKA+ Betulin	-1.869	1.039	-5.039-1.301	0.483
		Betulin	Control	-14.258	0.991	-17.281--11.236	**0.001***
			DMSO	-10.260	1.039	-13.430--7.089	**0.001***
			PBS	-11.372	0.991	-14.394--8.349	**0.001***
			OKA	-13.522	0.991	-16.544--10.499	**0.001***
			OKA+ Betulin	-12.128	0.991	-15.151--9.106	**0.001***
	COX1	PBS	Control	-2.887	0.991	-5.909-0.136	0.068
			DMSO	1.112	1.040	-2.059-4.282	0.899
			Betulin	11.372	0.991	8.349-14.394	**0.001***
			OKA	-2.150	0.991	-5.173-0.873	0.283
			OKA+ Betulin	-0.757	0.991	-3.779-2.266	0.972
		OKA	Control	-0.737	0.991	-3.759-2.286	0.975
			DMSO	3.262	1.040	-0.872-5.172	**0.041***
			Betulin	13.523	0.991	10.499-16.5442	**0.001***
			PBS	2.150	0.991	-0.873-5.173	0.283
			OKA+Betulin	1.393	0.991	-1.629-4.416	0.724
		OKA+Betulin	Control	-2.130	0.991	-5.153-0.893	0.292
			DMSO	1.869	1.040	-1.301-5.040	0.483
			Betulin	12.128	0.991	-.106-15.151	**0.001***
			PBS	0.757	0.991	-2.266-3.779	0.972
			OKA	-1.393	0.991	-4.416-1.629	0.724
		Control	DMSO	5.783	0.700	3.649-7.917	**0.001***
			Betulin	14.505	0.667	12.470-16.540	**0.001***
			PBS	3.972	0.667	1.937-6.007	**0.001***
			OKA	1.553	0.667	-0.482-3.588	0.216
			OKA+ Betulin	1.958	0.667	-0.077-3.993	0.065
		DMSO	Control	-5.783	0.700	-7.917--3.649	**0.001***
			Betulin	8.722	0.700	6.588-10.856	**0.001***
			PBS	-1.811	0.700	-3.946--2.095	**0.001***
			OKA	-4.230	0.700	-5.959--1.690	**0.001***
			OKA+ Betulin	-3.825	0.700	-5.959-1.690	**0.001***
		Betulin	Control	-14.505	0.667	-16.540--12.470	**0.001***
			DMSO	-8.722	0.700	-10.856--6.588	**0.001***
			PBS	-10.533	0.667	-12.568--8.498	**0.001***
			OKA	-12.952	0.667	-14.897--10.971	**0.001***
Heart			OKA+ Betulin	-12.547	0.667	-14.582--10.512	**0.001***
	COX2	PBS	Control	-3.972	0.337	-6.007--1.937	**0.001***
			DMSO	1.811	0.700	-0.323-3.946	0.133
			Betulin	10.533	0.667	8.498-12.568	**0.001***
			OKA	-2.418	0.667	-4.453--0.383	**0.013***
			OKA+ Betulin	-2.013	0.667	-4.048-0.022	0.054
		OKA	Control	-1.553	0.667	-3.588-0.482	0.216
			DMSO	4.230	0.700	2.095-6.364	**0.001***
			Betulin	12.952	0.667	10.917-14.987	**0.001***
			PBS	2.418	0.6670	0.383-4.453	**0.013***
			OKA+Betulin	0.405	0.667	-1.630-2.244	0.990
		OKA+Betulin	Control	-1.958	0.667	-3.993-0.076	0.065
			DMSO	3.825	0.700	1.690-5.959	**0.001***
			Betulin	12.547	0.667	10.512-14.582	**0.001***
			PBS	2.013	0.667	-0.022-4.048	0.054
			OKA	-0.405	0.667	-2.440-1.630	0.990

*: P<0.05; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

Table 2. Continue.

Tissues	Genes	Experimental Groups (I)	Experimental Groups (II)	Mean	SE	95% CI for Mean	P-value
		Control	DMSO	1.410	0.706	-0.743-3.562	0.368
			Betulin	10.212	0.673	8.159-12.264	**0.001***
			PBS	-0.432	0.673	-2.484-1.620	0.987
			OKA	-0.117	0.673	-2.169-1.935	1.000
			OKA+ Betulin	-2.153	0.673	-4.205—0.101	**0.035***
		DMSO	Control	-1.410	0.706	-3.562-0.743	0.368
			Betulin	8.802	0.706	6.650-10.954	**0.001***
			PBS	-1.841	0.706	-3.994-0.311	0.127
			OKA	-1.526	0.706	-3.679-0.626	0.286
			OKA+ Betulin	-3.563	0.706	-5.715—1.411	**0.001***
		Betulin	Control	-10.212	0.673	-12.264—8.159	**0.001***
			DMSO	-8.802	0.706	-10.954—6.650	**0.001***
			PBS	-10.643	0.673	-12.695—8.591	**0.001***
			OKA	-10.328	0.673	-12.380—8.276	**0.001***
			OKA+ Betulin	-12.365	0.673	-14.417—10.312	**0.001***
	COX1	PBS	Control	0.432	0.673	-1.620-2.483	0.987
			DMSO	1.841	0.706	-0.311-3.994	0.127
			Betulin	10.643	0.673	8.591-12.696	**0.001***
			OKA	0.315	0.673	-1.737-2.367	0.997
			OKA+ Betulin	-1.722	0.673	-3.774-0.330	0.141
		OKA	Control	0.117	0.673	-1.935-2.169	1.000
			DMSO	1.526	0.706	-0.626-3.679	0.286
			Betulin	10.328	0.673	8.276-12.380	**0.001***
			PBS	-0.315	0.673	-2.367-1.737	0.997
			OKA+Betulin	-2.0367	0.673	-4.089-0.015	0.053
		OKA+Betulin	Control	2.153	0.673	0.101-4.205	**0.001***
			DMSO	3.563	0.706	1.411-5.715	**0.001***
			Betulin	12.365	0.673	10.312-14.417	**0.001***
			PBS	1.722	0.673	-0.330-3.774	0.141
			OKA	2.037	0.673	-0.015-4.089	0.053
		Control	DMSO	2.745	0.996	-0.291-5.782	0.094
			Betulin	10.573	0.950	7.678-13.469	**0.001***
			PBS	0.100	0.950	-2.795-2.995	1.000
			OKA	-0.703	0.950	-3.599-2.192	0.975
			OKA+ Betulin	-1.192	0.950	-4.087-1.704	0.806
		DMSO	Control	-2.745	0.996	-5.782-0.291	0.094
			Betulin	7.820	0.996	4.791-10.865	**0.001***
			PBS	-2.645	0.996	-5.682-0.391	0.116
			OKA	-3.449	0.996	-6.485--0.485	0.019
			OKA+ Betulin	-3.937	0.996	-6.974--0.900	**0.006***
		Betulin	Control	-10.573	0.950	-13.469--7.678	**0.001***
			DMSO	-7.828	0.996	-10.865--4.791	**0.001***
			PBS	-10.473	0.950	-13.369--7.578	**0.001***
			OKA	-11.277	0.950	-14.172--8.381	**0.001***
			OKA+ Betulin	-11.765	0.950	-14.660--8.869	**0.001***
*Small Intestine	COX2	PBS	Control	-0.100	0.950	-2.995-2.795	1.000
			DMSO	2.645	0.996	-0.391-5.682	0.116
			Betulin	10.473	0.950	7.578-13.369	**0.001***
			OKA	-0.803	0.950	-3.699-2.092	0.956
			OKA+ Betulin	-1.292	0.950	-4.187-1.604	0.750
		OKA	Control	0.703	0.950	-2.192-3.599	0.975
			DMSO	3.449	0.996	0.7412-6.485	**0.019***
			Betulin	11.277	0.950	8.381-14.172	**0.001***
			PBS	0.803	0.950	-2.092-3.699	0.956
			OKA+Betulin	-0.488	0.950	-3.384-2.407	0.995
		OKA+Betulin	Control	1.192	0.950	-1.704-4.087	0.806
			DMSO	3.937	0.950	0.900-6.974	**0.006***
			Betulin	11.765	0.950	8.869-14.660	**0.001***
			PBS	1.292	0.950	-1.604-4.187	0.750
			OKA+Betulin	0.488	0.950	-2.407-3.384	0.995

*: P<0.05; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

The distribution of hippocampal COX proteins was tested in the AD rat model. The results showed that Betulin had different histochemical scores in different tissues (H-score = (0 x P_0) + (1 x P_1) + (2 x P_2) + (3 x P_3)). The results showed that the most significant effect of COX1 was in the heart, moderately in the kidney, and to a lesser extent in the small intestine. COX2 showed the most significant effect in the kidney, a moderate impact on the heart, and finally, a low effect in the small intestine tissue. H-scores for AD and treatment group were as follows. In the structural histological evaluation of kidney tissue, a high expression of COX1 was observed in tubular, mesangial cells and podocytes (Figure 2A), and a relatively lower rate of uptake for COX2 was observed (Figure 2G). While protein retention was not observed for COX1 after Betulin treatment (Figure 2B), it was found to decrease for COX2 (Figure 2H). Heart tissue analyses showed focal myonecrosis and degeneration in the AD model for COX1 (Figure 2C) and COX2 (Figure 2I). Low protein levels were detected after Betulin treatment (Figure 2D, 2J). Mild and moderate defects observed in AD heart tissue could not be seen after Betulin treatment. In the small intestine tissue, expression of COX1 and COX2 in the ileum was detected in the AD group (Figure 2E, 2K). A decrease in protein expression

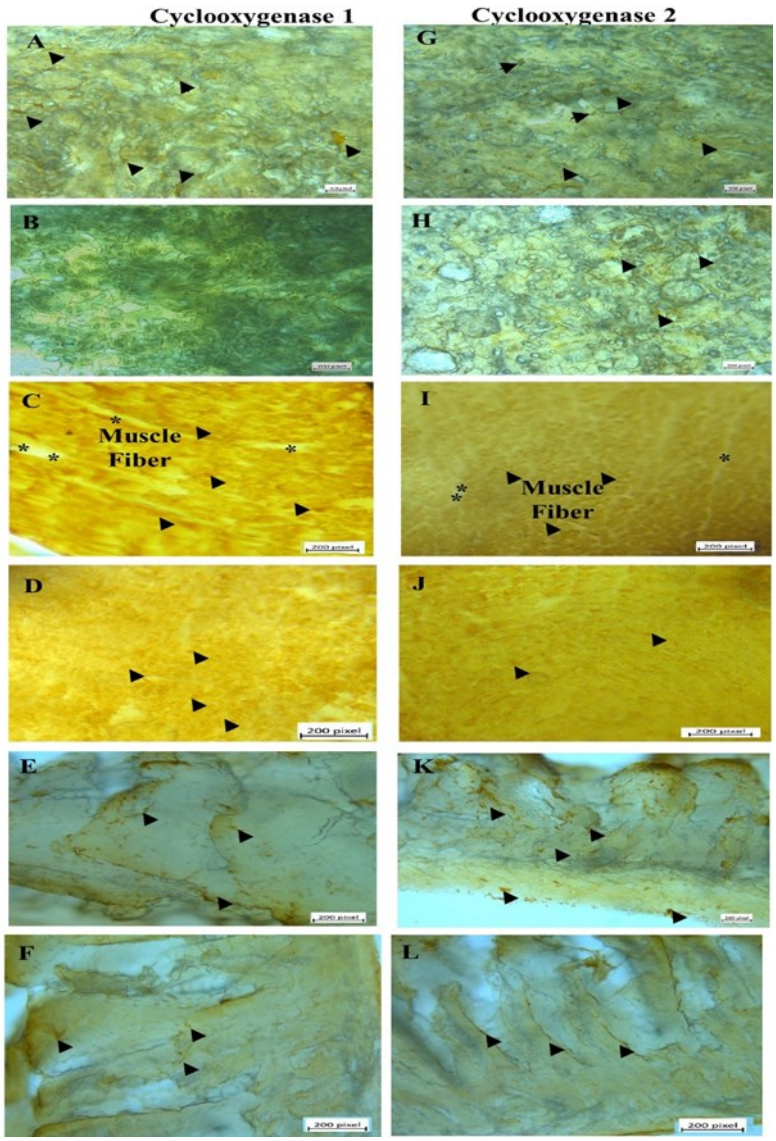

Figure 2. Immunohistochemical staining of COX1 and COX2 in rat kidney, heart, and small intestine (100×). A: Kidney- Okadaic acid showing high expression of COX1 in tubular cell, mesangial cell, and podocytes (black arrow); B: Kidney- Okadaic acid + Betulin group this group showing no expression for COX1; C: Heart- Okadaic acid showing high expression in heart muscle cells for COX1 (black arrow); D: Heart- Okadaic acid + Betulin group after Betulin treatment, COX1 expression was observed in low amounts in heart muscle cells; E: Small intestine-Okadaic acid showing high expression for COX1 in ileum section; F: Small intestine-Okadaic acid + Betulin group, COX1 expression was observed in low amounts in ileum, G. Kidney-Okadaic acid showing high expression of COX2; H: Kidney-Okadaic acid + Betulin group, showing low expression for COX2 in kidney section; I: Heart-Okadaic acid showing high expression in heart muscle cells for COX2; J: Heart- Okadaic acid + Betulin group, COX2 expression was observed in low amounts in heart muscle cells; K: Small intestine-Okadaic acid showing high expression for COX2 in ileum section; L: Small intestine-Okadaic acid + Betulin group, COX2 expression was observed in low amounts in the ileum; MF: muscle fiber; *: mild defect; **: moderate defect.

was observed after Betulin treatment (Figure 2F, 2L).

DISCUSSION AND CONCLUSION

The relationship between dementia and acute organ dysfunction threatens the patient's life and affects mortality. In chemically OKA-induced AD, excessive COX1 and COX2 gene expressions in kidney, heart, and small intestine tissues indicate their structural and functional integrity is in danger. The literature mostly mentions the predominant effect of COX2 dysfunction in the kidney. However, there is no definitive comment for COX1. When the function of COX-1 in the kidney is examined, it is seen that it plays a role in hemodynamic regulation.[12] Therefore, afferent and efferent COX1 dysfunction may disrupt renal hemodynamics and unbalance the glomerular filtration rate. The improvement of COX1 and COX-2 levels under the influence of Betulin indicates that this metabolism can be regulated by targeting COXs. The possibility of damage via COXs is supported by a mouse study. The fact that gene expressions can be decreased by Betulin indicates that the COX pathway may be Betulin's target. Ensuring homeostasis related to the absorption of ions in renal functions is regulated by many physiological mechanisms. Studies have shown that protein phosphatase 2 (PP2A) is responsible for maintaining ion channels and homeostasis.[13] In addition to its function in Na-Cl-dependent transporter systems, PP2A accelerates the flow of Na-K-ATPase from the intracellular system to the basal-lateral membrane in human adenocarcinoma cells. In this mechanism, okadaic acid inhibits the increase in Na-K-ATPase activity as a selective inhibitor of PP2A.[14] Two features observed in the initial pathology of chronic kidney disease, glomerulosclerosis, and tubulointerstitial fibrosis, are associated with microvascular endothelial cell dysfunction.[15] Okadaic acid reduces the effect of PP2A on the endothelial cell remodelling process. Thus, okadaic acid harms kidney homeostasis, both structurally and functionally. In our study, it is possible to regulate okadaic acid-induced kidney damage during the AD process by managing COX enzymes with the effect of Betulin. A study supports the accuracy of this idea. The study showed that the left ventricle in AD patients was thicker than in patients without the disease. The reason for this thickness is that Ab plaques in the AD brain accumulate in the same form in the ventricle. Ventricular thickening impedes blood flow, which can result in cardiovascular problems and a higher risk of heart attack and stroke.[16] PP2A function is also present in the heart. It is a phosphatase that modulates Ca^{2+} utilization as a channel regulator. In our study, heart tissue analyses show that cellular COX protein accumulations in the okadaic acid AD model are partially reduced by the effect of Betulin and that damage can be reduced by regulating COX metabolism with Betulin treatment. Therefore, it indicates that partial recovery of heart functions is possible. The most well-known effect of okadaic acid on the intestinal system is that it stimulates the phosphorylation of proteins that control Na release in cells, increases protein phosphorylation due to solute permeability, and therefore causes fluid loss. The data we obtained in our study may indicate that structural differentiation and permeability in intestinal cells may change as a result of overexpression of COXs, causing them to become partially or completely dysfunctional. Reducing the amount of protein with the effect of Betulin can restore this function.

Peripheral inflammation outside the CNS in AD is a risk factor for the disease.[17] The contribution of these processes to the neurology of AD is unclear. However, acute inflammation creates a temporal immune challenge that will cause tissue damage.[18] COXs may have a role in AD's blood-brain barrier and neuro-immune connection.[19,20] The impact of peripheral inflammation on brain function in the neuro-immune connection is associated with increased inflammatory proteins in the blood.[21] These proteins activate endothelial cells, resulting in vascular inflammation.[22] Proinflammatory transcription factors that are subsequently activated cause the expression of the same molecules in the brain.[23] At the same time, peripheral inflammatory proteins can be transported to the brain by age-dependent caveolar transcytosis.[24] The vagus nerve also plays a role in this event.[25] It transmits inflammation signals from the intestine, liver, lung, and other organs to the brain. These signals can activate inflammatory proteins and receptors in glial cells via the solitary nucleus.[17] A sudy shows that sepsis following infection threatens acute organ failure, aggravates AB accumulation and triggers systemic inflammation that causes AD to progress.[26] Damage to the vascular system is associated with an increased risk of AD. Neuroinflammation and systemic overstimulation are among the etiologies of AD and lead to neuronal death due to synaptic dysfunction. There is increasing evidence that infection and organ disorders cause peripheral AB and neurovascular dysfunction.[27]

In conclusion, Betulin may be a COX inhibitor candidate like other potent NSAIDs. Therefore, it may have a role in AD and treating specific damage to each other's organs. Most importantly, it can be mentioned that it has a potential effect on all mechanisms under the COX effect. The idea that organ damage causes AD may change direction towards the idea that AD can also cause organ damage. The limitation of this study is the analysis of structural changes and cellular damage in 3 tissues. Additional studies may help elucidate the extent of textural

damage and its association with genes and histology. In addition, all organ functions are affected by AD, which involves inflammatory processes, and not only is the brain the target of treatment. The COXs expressed in this study have significant potential as therapeutic targets. Although many alternatives exist, gene-level editing tools can be guiding in this regard.

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Evaluation of The Relationship Between the Selected Particle Size and Procedure Success and Complications in Bronchial Artery Embolisation

Bronşiyal Arter Embolizasyonunda Kullanılan Partikül Boyutu ile İşlem Başarısı ve Komplikasyonlar Arasındaki İlişkinin Değerlendirilmesi

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ABSTRACT

Objective: Hemoptysis refers to the discharge of blood, which originates from the lungs or bronchial system through the mouth. This study aimed to examine the importance of the particle size used in patients undergoing bronchial artery embolisation (BAE) regarding procedure success and complications.

Materials and Methods: Data from patients who underwent BAE using polyvinyl alcohol (PVA) particles were collected retrospectively. Hemoptysis etiology and localisation, lesion type, the size of the embolising agent used, and postoperative complications were recorded. Thirty-day follow-up results were evaluated.

Results: Fifty-six patients were included in the study. The size of the PVA particles used was 300-500 microns in 30 patients (53.6%) and 500-700 microns in 26 patients (46.4%). Bleeding completely stopped in 92.9% of the cases within 30 days after the procedure. Procedure success or complications did not significantly differ between the patient groups in which 300-500 micron and 500-700 micron particles were used during BAE.

Conclusions: BAE is a safe, effective, and minimally invasive method that can be performed repeatedly to treat hemoptysis. This study showed that regardless of size, PVA particles were effective and safe agents that could be used during this procedure.

Keywords: Embolization, hemoptysis, particular agent

ÖZ

Amaç: Hemoptizi pulmoner veya bronşiyal vasküler sistemden kaynaklanan kanın ağızdan gelmesidir. Bu çalışmanın amacı bronşiyal arter embolizasyonu (BAE) yapılan hastalarda kullanılan partikül boyutunun işlem başarısı ve komplikasyonlar açısından önemini incelemekti.

Materyal ve Metot: Polivinil alkol partikülleri (PVA) kullanılarak BAE yapılan hastaların verileri retrospektif olarak toplanmıştır. Hemoptizi etiyolojisi, lokalizasyonu ve lezyon tipi, kullanılan embolize edici ajan boyutu ve postoperatif komplikasyonlar kaydedildi. 30 günlük takip sonuçları değerlendirildi.

Bulgular: Çalışmaya 56 hasta dahile dildi. 30 hastada (% 53,6) 300-500 mikron, 26 hastada (%46,4) ise 500-700 mikron boyutlu partiküler ajanlar kullanıldı. İşlem sonrası hastaların %92,9'unda kanama durdu ve 30 gün içerisinde tekrarlamadı. 300-500 mikron ile 500-700 mikron boyutlu partikül kullanılan hastalar karşılaştırıldığında başarısı ve komplikasyonlar açısından anlamlı farklılık saptanmadı.

Sonuç: BAE, hemoptizi tedavisi için tekrar tekrar yapılabilen güvenli, etkili ve minimal invaziv bir yöntemdir. PVA partiküllerinin BAE'nda kullanılabilecek etkin ve güvenli bir ajan olduğu gösterildi.

Anahtar Kelimeler: Embolizasyon, hemoptizi, partiküler ajan

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INTRODUCTION

Hemoptysis refers to the expectoration of blood from the respiratory system due to pulmonary or tracheobronchial disorders, such as bronchiectasis, respiratory tract infections, asthma, chronic obstructive pulmonary disease (COPD), and malignancy.[1] Hemoptysis is classified as mild, moderate, and massive based on the quantity of expectorated blood. It is considered mild if 24-hour bleeding is less than 100 ml, moderate if 100-600 ml, and massive if greater than 600 ml.[2] Bleeding often stops spontaneously with supportive treatment. However, especially in patients with massive bleeding, the escape of blood elements into the respiratory tract may require urgent intervention since it can cause asphyxia and death.[3]

This study aimed to compare different particle sizes utilised in bronchial artery embolisation (BAE) regarding their efficacy and potential complications.

MATERIALS AND METHODS

Ethics Committee Approval: The study was approved by the Ethics Committee of the Sakarya University Faculty of Medicine (Date: 02.02.2022, decision no: 102126_23) and performed by the Helsinki Declaration.

Subjects: Fifty-six patients who underwent BAE at our clinic due to hemoptysis from January 2019 to January 2022 were included in the study. Patient data were retrospectively screened from the hospital's electronic system. Causes of hemoptysis, bleeding sites, pathological vascular findings on digital subtraction angiography, embolising agents used, and postoperative complications were recorded. The procedure was assumed to be technically successful if pathological vascular findings disappeared after embolisation. No recurrence of bleeding during the 30-day follow-up was considered clinical success. During the embolisation process, the arteries from which the bleeding was thought to originate were catheterised using 5-Fr Cobra 2 or Simmons 1 diagnostic catheters (Terumo, Tokyo, Japan). Bronchial arteries were carefully examined to detect possible branches and critical collateral circulations. A 3-Fr microcatheter (Renegade, Boston Scientific, USA) was used to reach and embolise the more distal portion. Polyvinyl alcohol (PVA) particles (Contour; Boston Scientific, Cork, Ireland) were used as embolising agents in all patients. Possible bleeding etiologies and enlargement of bronchial arteries were considered when determining the particle size planned to be used. While small-sized particles were preferred to ensure more distal penetration in patients bleeding due to malignant lesions, large-sized particles were preferred in patients with tortuous bronchial arteries. Upon the disappearance of the pathological vascularisation and findings, the procedure was deemed successful and subsequently terminated.

Statistical Analysis: MedCalc (version 12, Ostend, Belgium) was used for statistical analyses. Descriptive statistics are presented as median (minimum-maximum) and mean ± standard deviation values. Categorical variables were expressed as frequencies and percentages. The Fisher, Pearson chi-square, and Yates' corrected Pearson chi-square tests were used to compare categorical variables. The independent-sample t-test was used to compare continuous variables with a normal distribution according to the Kolmogorov-Smirnov test. In contrast, the Mann-Whitney U test was used for data that did not comply with a normal distribution. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 56 patients were included in the study. Forty-four patients were male (78.6%), and 12 (21.4%) were female. The mean hemoglobin value was 11.4 ±1.8 g/dL. The mean platelet count was 271±81K/uL. The mean international normalised ratio (INR) was 1.2±0.12. The etiology of bleeding was evaluated as malignancy in 18 patients (32.1%), COPD in 14 (25%), tuberculosis in 12 patients (21.4%), bronchiectasis in eight patients (14.3%), and COVID-19 pneumonia in four (7.2%) (Table 1).

Table 1. Bleeding etiologies.

Etiology	n (%)
Malignancy	18 (32.1)
COPD	14 (25)
Tuberculosis	12 (21.4)
Bronchiectasis	8 (14.3)
COVID-19 pneumonia	4 (7.2)

COPD: chronic obstructive pulmonary disease; COVID-19: Coronavirus disease 19.

The right bronchial artery was embolised in 26 patients (46.4%), the left bronchial artery in 24 patients (42.9%), and both bronchial arteries in six patients (10.7%). The PVA particles used as an embolising agent were 300-500 or 500-700 microns in size (Contour; Boston Scientific, Cork, Ireland). PVA particles of 300-500 microns in size were used in 30 patients (53.6%), and 500-700 micron PVA particles were employed in 26 patients (46.4%). The success criterion of the procedure was accepted as the absence of hemoptysis recurrence.

The procedure successfully stopped bleeding in 52 (92.9%) patients (Figure 1). Hemoptysis persisted following embolisation in the remaining four (7.1%) patients. Patients whose bleeding did not stop or recurred within 24 hours were defined as recurrent bleeding. Embolisation was repeated in three of these patients, and it was observed that the bleeding was from a different artery. The bleeding stopped after the second embolisation. One patient died due to systemic reasons, while the second procedure was being planned.

The most common complication after the procedure was chest pain, which developed in nine (16%) patients. This complication was relieved with supportive medical treatment. No significant difference was detected between the 300-500 micron and 500-700 micron PVA particle groups regarding pain development.

There were no significant differences between the 300-500 micron and 500-700 micron PVA particle groups concerning age, gender, hemoglobin value, platelet count, INR, procedure success, or complications (Table 2).

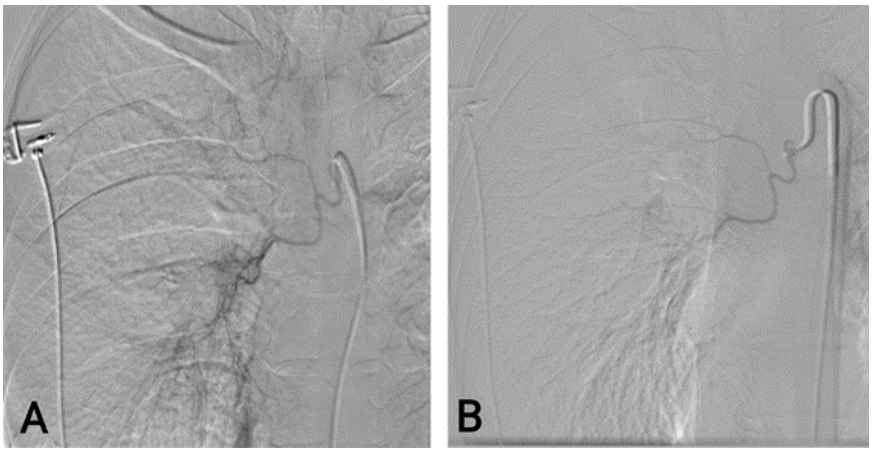

Figure 1. Increased vascularity originates from the right bronchial artery due to the tumor (A). Increased vascularity disappeared after a successful embolisation (B).

Table 2. Comparison of 300-500 micron and 500-700 micron particle groups.

	300-500 micron	500-700 micron	p
Age	61.4	60.7	0.847
Gender, (M/F)	23/7	21/5	0.752
Hemoglobin, (g/dL)	11.6	11.2	0.705
Platelet count, (K/uL)	277	264	0.574
INR	1.29	1.23	0.935
Procedure success	93.3%	92.3%	0.644

M: male; F: female; INR: International normalised ratio.

DISCUSSION AND CONCLUSION

Hemoptysis is the expectoration of blood originating from the pulmonary or bronchial vascular system.[4,5] Malignancies and COPD were prominent in the bleeding etiology of the patients included in our study.

Transarterial embolisation plays a crucial role in intervening in bleeding that does not stop with supportive treatment. Despite several variations, there are generally two main bronchial arteries, one on the right and two on the left.[6,7]

Various agents can be used for embolisation, with the most commonly utilised materials being PVA particles, shaped microspheric particles, absorbable gelatin sponges, N-Butyl 2-Cyanoacrylate (Glue), liquid embolising agents, and coils. The choice of material depends on the clinician's experience and the etiology of the bleeding.[8-10] As shown in the studies, there is no relationship between the selected embolisation material and the procedure's success.

Unlike PVA, shaped microspheric particles are smaller in diameter and tend to accumulate less in

the catheter due to their uniform structure. Therefore, for safe embolisation, microspheric particles that are larger should be selected compared to PVA particles.[11]

In our study, we aimed to evaluate whether there is a relationship between the selected particle sizes and procedure success and complications. In the study conducted by Nilpatrewar et al.,[12] the overall success rate of the procedure was 88%. In the study conducted by Soylu et al.,[13] bleeding stopped entirely in 94.2% of the cases in the first 30 days after the procedures. Our study determined that bleeding stopped after embolisation in 52 of 56 patients (92.8%), which is compatible with the literature. Furthermore, when evaluating the procedure outcomes, we detected no significant difference in procedure success between the cases where BAE was performed using 300-500 micron and 500-700 micron PVA particles.

The most common complications of BAE are groin hematoma, chest pain, and focal neurological deficits. The study by Panda et al.[14] shows us a rate of major complications remained negligible and stable over time with a median incidence of 0.1% (0%–6.6%), and the other study conducted by Tom et al.[15] shows us major complications less than 1%. In our study, there were no major complications. Nine (16%) patients developed chest pain, which was relieved with short-term analgesic support, and these patients were discharged without any symptoms. There was no difference in the rate of patients with chest pain according to the PVA particle size.

In conclusion, BAE stands out as a very effective treatment method with low complications and high clinical success rates in the treatment of hemoptysis. This study showed that regardless of size, PVA particles were effective agents for embolisation. However, the lack of long-term follow-up results can be considered a study limitation.

Ethics Committee Approval: Our study was approved by the Sakarya University Ethics Committee (Date: 02.02.2022, decision no: 102126_23). This study was conducted following the principles of the Declaration of Helsinki.

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Potential Role of Laurus nobilis Essential Oil in Reducing Indomethacin-Induced Gastric Ulcer in Rats

Laurus nobilis Esansiyel Yağının Sıçanlarda İndometasin Kaynaklı Mide Ülserinin Azaltılmasında Potansiyel Rolü

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ABSTRACT

Objective: This study aimed to investigate the gastroprotective effects of Laurus nobilis leaves essential oil (LANO) against indomethacin (INDO)-induced gastric ulcers in rats.

Materials and Methods: In this study, an indomethacin-induced gastric ulcer model was employed. 30 Sprague-Dawley rats were divided into five groups (n=6): Control, LANO, INDO, INDO with famotidine (FAM), and INDO with LANO. Indomethacin (25 mg/kg) induced ulcers, while LANO and FAM were administered by oral gavage at 200 mg/kg and 40 mg/kg, respectively. Gastric tissues underwent histopathological examination for ulceration, and biochemical assays measured total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and nitric oxide (NO) levels.

Results: Compared to the INDO group, treatment with LANO significantly decreased the number of gastric ulcer foci. Biochemically, LANO moderated TOS and OSI levels and preserved TAS, indicating reduced oxidative stress. Additionally, LANO appeared to stabilize NO levels. These biochemical findings were corroborated by histopathological examination.

Conclusions: The study's results indicate that LANO may be beneficial in protecting against NSAID-induced gastric damage. LANO's observed modulation of oxidative stress markers and NO levels suggests its potential role in managing gastric ulcers.

Keywords: Gastric ulcer, indomethacin, Laurus nobilis, nitric oxide, oxidative stress

ÖZ

Amaç: Bu çalışmanın amacı, sıçanlarda indometasin (INDO) ile indüklenen gastrik ülserlere karşı Laurus nobilis yaprakları uçucu yağının (LANO) gastroprotektif etkilerini araştırmaktır.

Materyal ve Metot: Bu çalışmada, INDO ile indüklenen gastrik ülser modeli kullanıldı. 30 adet Sprague-Dawley sıçan beş gruba ayrıldı (n=6): Kontrol, LANO, INDO, famotidin (FAM) ile INDO, LANO ile INDO. INDO (25 mg/kg) ülserleri indüklerken, LANO ve FAM oral gavaj yoluyla sırasıyla 200 mg/kg ve 40 mg/kg olarak uygulandı. Gastrik dokular ülserasyon açısından histopatolojik incelemeye tabi tutuldu ve biyokimyasal analizlerle toplam oksidan durum (TOS), toplam antioksidan durum (TAS), oksidatif stres indeksi (OSI) ve nitrik oksit (NO) seviyeleri ölçüldü.

Bulgular: İndometasin grubuyla karşılaştırıldığında, LANO ile tedavi gastrik ülser odaklarının sayısını önemli ölçüde azalttı. Biyokimyasal olarak, LANO uygulaması TOS ve OSI seviyelerini düşürdü ve TAS seviyesini korudu, bu da oksidatif stresin azaldığını göstermektedir. Ayrıca, LANO'nun NO seviyelerini stabilize ettiği görüldü. Bu biyokimyasal bulgular histopatolojik inceleme ile desteklendi.

Sonuç: Çalışmanın sonuçları, LANO'nun INDO kaynaklı mide hasarına karşı korunmada faydalı olabileceğini göstermektedir. LANO'nun oksidatif stres belirteçleri ve NO seviyelerinde gözlenen modülasyonu, mide ülserlerinin yönetiminde potansiyel rolünü ortaya koymaktadır.

Anahtar Kelimeler: İndometasin, Laurus nobilis, mide ülseri, nitrik oksit, oksidatif stres

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INTRODUCTION

Gastric ulcers, common in peptic ulcer disease, occur due to an imbalance between mucosal defense and aggressive factors like acid and pepsin and are caused by various factors, including *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drugs (NSAIDs), stress, smoking, and genetics.[1] The pathogenesis of gastric ulcers involves disrupted mucosal defense, increased gastric acid secretion, and impaired epithelial repair mechanisms.[2]

Indomethacin (INDO), an NSAID, is known to inflict more significant damage on the gastric mucosa in rats than other similar medications. This damage includes vascular harm, ulcerations, and cell necrosis within the gastric lining. The INDO-induced gastric ulcer model serves as a key tool for exploring the mechanisms of gastric ulcer development and testing new treatments. Central to the damage caused by INDO is the increased production of reactive oxygen species, leading to oxidative stress. This process depletes key antioxidants like superoxide dismutase and glutathione in the stomach's defense system, resulting in harmful oxidation products that damage the gastric mucosa, underscoring the need for targeted therapeutic strategies to mitigate these effects[3].

Laurus nobilis (LANO), an evergreen tree native to the Mediterranean region, has been used traditionally for its medicinal properties. The leaves and fruits of LANO are known for their antibacterial, antioxidant, and gastroprotective properties.[4,5] These effects are primarily attributed to their rich composition of bioactive compounds such as flavonoids, tannins, and essential oils.[6] LANO oil's therapeutic potential in gastric ulcers is hypothesized to stem from its ability to enhance mucosal defense, reduce oxidative stress in gastric tissues, and possibly modulate gastric acid secretion.[7] The antioxidant properties of LANO, containing numerous compounds such as eucalyptol, α-terpinyl acetate, sabinene, α-pinene, and α-terpineol, may play a vital role in neutralizing free radicals, thus protecting the gastric mucosa from oxidative damage.[8,9] Additionally, the anti-inflammatory properties of LANO can mitigate inflammation, which is a key component in the pathogenesis of gastric ulcers.[10]

The study aimed to investigate the gastroprotective effects of LANO in treating gastric ulcers, focusing on the possible underlying mechanisms.

MATERIALS AND METHODS

Experimental Animals and Ethics

Ethics Committee Approval: The study was sanctioned by the Erzincan Binali Yıldırım University's Experimental Animals Local Ethics Committee (Date: 28.12.2023, decision no: 322655). The study strictly adhered to the ARRIVE guidelines and was conducted following the national regulations for the ethical use and care of laboratory animals.

Animals: For this research, we procured thirty Sprague–Dawley female rats, each weighing between 200-250 grams and aged around 10 to 12 weeks, from the Experimental Research and Application Center of Erzincan Binali Yıldırım University. During the experiment, the rats were allowed to consume standard pellet food and water without any restrictions. They were accommodated in an environment with controlled humidity and ambient temperature suitable for their well-being.

Experimental Design Models and Groups: In the study, thirty rats were divided into five groups (n=6). The groups were as follows: Control, *Laurus nobilis* (LANO), indomethacin (INDO), INDO+Famotidine (FAM), and INDO+LANO. The rat received oral administration of INDO at 25 mg/kg,[11] FAM at 40 mg/kg,[11] and LANO at 200 mg/kg[7].

LANO was administered prophylactically via gavage to the rats for seven days before the ulcer induction[7]. After this period, the rats fasted for 24 hours. On the seventh day, targeted groups received LANO and FAM. One hour after this administration, a 25 mg/kg dose of INDO was given to all groups except the Control and LANO groups to induce ulcers. Six hours after the INDO administration[11], rats were euthanized using an intraperitoneal injection of thiopental at 50 mg/kg, and their stomachs were excised for macroscopic examination and photography of ulcers.

Plant Materials and Essential Oil Extraction: LANO leaves from Hatay-Türkiye were cleaned and dried at 40 °C in a vacuum oven. Then, 5 grams were extracted using an ultrasonic bath at 18 kHz with 50 mL of 50:50 ethanol-water for 20 minutes at 40 °C, as per Tometri et al. The extract was filtered and concentrated using a rotary evaporator, and the essential oil was stored at −18 °C for experimental use.[12]

Tissue Preparation and Histopathological Examination: Gastric tissue samples were divided for analyses: one-third for biochemical assays and two-thirds fixed in 10% formalin for 24 hours. The fixed tissues were sectioned into 2 mm strips and processed with the Sakura Tissue-Tek VIP 6 AI system. Post-processing, they were embedded in paraffin, cut into 3 micrometer sections, and stained using hematoxylin and eosin for microscopic analysis, focusing on ulceration or necrosis. Histopathological examination was performed using an Olympus B70 light microscope. Tissue areas (mm^2) were calculated by measuring the size of the examined areas under the light microscope, employing standardized methods. In histopathological evaluation, ulceration/

necrotic foci were evaluated, and the number of ulcerated/necrotic foci was determined for each case. The Lesion Index was determined for each parameter to quantify the extent of histopathological changes. As defined by Natale et al., it was calculated using the formula: Lesion Index=[Length of the damaged area/total mucosal length]×100, expressing the percentage of mucosal damage.[13]Additionally, the number of foci exhibiting histopathological changes was recorded. To standardize the assessment, the focus score was calculated as the number of foci with changes per square centimeter of the sampling area (Focus Score=[Number of foci with change/ Sampling area mm^2]×100).

Biochemical Analyses: To determine oxidant and antioxidant parameters (TAS, TOS, OSI, and NO), a 100 mg sample of gastric tissue was weighed for each rat. These samples were homogenized on ice using a tissue homogenizer in pH 7.4 phosphate buffer.

Total Antioxidant Status (TAS) Analysis: The assessment of TAS in gastric tissues was performed following the established Erel method.[14] TAS in tissue was measured with a Rel Assay Diagnostics kit, converting ABTS radical to colorless, indicating antioxidant capacity, at 660 nm. Calibration used a Trolox equivalent, ensuring assay accuracy. CV% was <10%, standard range of 1.20-1.50 mmol/L. Results expressed in mmol/g protein.

Total Oxidant Status (TOS) Analysis: To quantify the TOS in gastric tissue, a methodology was developed by Erel[15] utilizing a kit from Rel Assay Diagnostics in Gaziantep, Türkiye. The method oxidized the iron ion-o-dianisidine complex to ferric form using tissue oxidants, enhanced by glycerol and measured under acidic conditions with xylenol orange by spectrophotometry, indicating oxidant levels. CV% was <10% for 4-6 µmol/L, calibrated with hydrogen peroxide, with results in µmol H2O2 equivalent per gram of protein.

Oxidative Stress Index (OSI) Calculation: OSI was computed using the formula OSI = (TOS/TAS*100).

Evaluation of Nitrate and Nitrite Concentrations: In the study, the measurement of nitrate (NO_3^-) and nitrite (NO_2^-) concentrations was crucial due to the transient nature of NO. NO quickly reacts within biological systems, forming NO_2, which equilibrates to N_2O_4 and subsequently yields NO_2^- and NO_3^- alongside water. These reactions culminate in the formation of N_2O_3, which also produces NO_2^- and water. Recognizing the variability of NO2 and NO3 levels, we assessed the total NO production by measuring both compounds' concentrations using the Cayman Nitrate/Nitrite Colorimetric Assay Kit (Item No: 780001). This kit converts NO_3^- to NO_2^- through nitrate reductase, allowing for comprehensive NO_2^- analysis, with a noted precision in its coefficient of variation (CV) at 2.7% for intra-assay and 3.4% for inter-assay variability.

Statistical Analysis: The normality assumption of the parameters was examined by the Shapiro-Wilk test, and the homogeneity of group variances was checked by Levene's test. The distribution of all parameters in the groups met the normality assumption. One-way ANOVA analysis was used to compare the measurement results of the parameters between the control and experimental groups. After one-way ANOVA analysis, pairwise differences between subgroups were analyzed by Tukey HSD (when the population variances of the groups were equal) or Games-Howell (when the population variances of the groups were not equal) post-hoc tests. The distribution of parameters in the groups was summarized with box-plot graphs. Box-plot graphs were drawn considering minimum, first quartile, median, third quartile and maximum values. SPSS (version 26.0, SPSS Inc., Chicago) package program was used for statistical data analysis. $p<0.05$ was considered statistically significant.

RESULTS

According to the histopathological analysis, as illustrated in Figure 1, the number of ulcerative lesions across the different treatment groups showed marked variation. The control group had no lesions, serving as a baseline. The INDO group showed significantly more lesions, indicating greater gastric damage. The INDO+FAM group had fewer lesions, suggesting famotidine's protective effect with INDO. The LANO group's lesion count was similar to the control, highlighting LANO's effectiveness. The INDO+LANO group had fewer lesions than INDO alone, but more than INDO+FAM.

The macroscopic and histopathological appearance of the gastric resection material of one case from each group is presented in Figure 2.

A comparison between LANO and INDO groups shows marked differences in ulcerative/necrotic foci burden and variations in lesion index and focus scores (Table 1). Notably, the INDO+LANO group exhibited significantly lower values, suggesting a potentially attenuated severity of lesions.

In the biochemical analyses, oxidative stress parameters and nitrate+nitrite values, highlighted in Figure

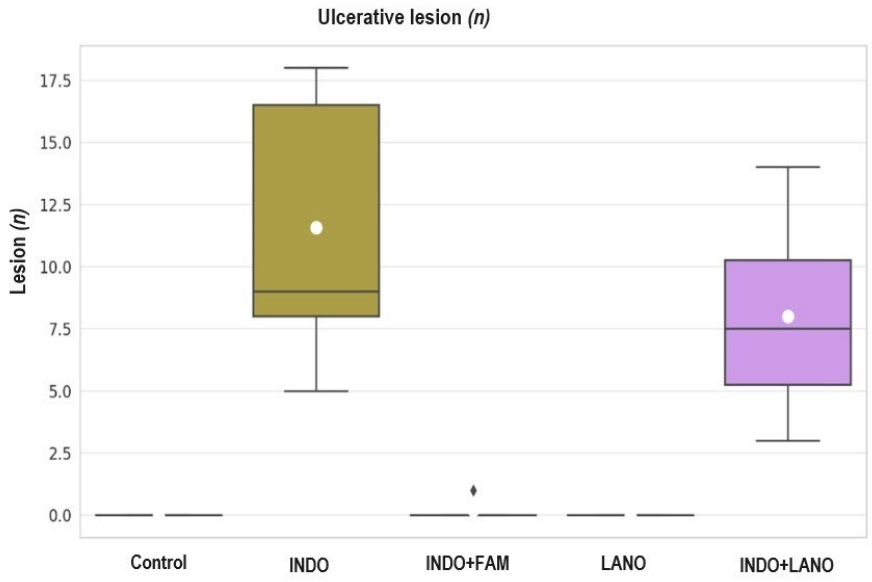

Figure 1. Comparing the number of lesions across various treatment groups. The box plots illustrate the distribution of lesion counts in the groups.

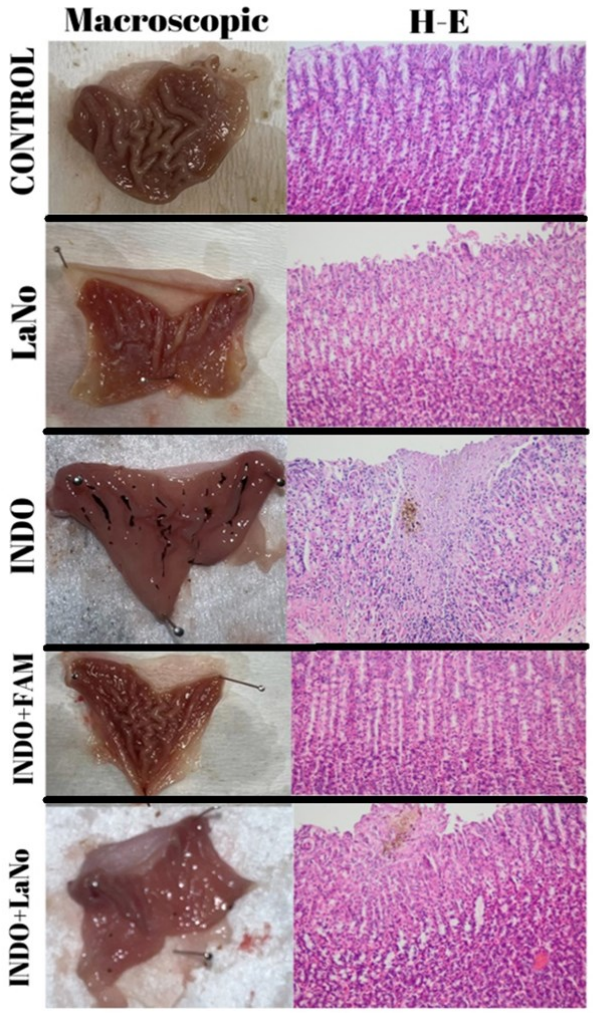

Figure 2. Macroscopic views and H&E staining findings in stomach tissue. Normal-appearing gastric mucosa with regular folds seen in Control, LANO and INDO+FAM groups. Extensive ulcerated areas of hemorrhagic gastric mucosa seen in INDO group. Focally ulcerated gastric mucosa seen in LANO group. Normal epithelial lining and underlying lamina propria seen in Control, LANO and INDO+FAM groups (H&E x200). Superficial mucosal ulcers in INDO+LANO groups (H&E x200). Deep and broad-based ulcer in INDO group (H&E x200).

Table 1. Ulcerative/Necrotic Focus, Lesion Index, and Focus Score in experimental groups.

Groups	Ulcerative/Necrotic Focus (n)	Lesion Index	Focus Score
Control	0	0	0
LANO	0	0	0
INDO	11.57	40	3.8
INDO+FAM	0.2	0.5	0.09
INDO+LANO	8	21	2.7

3, illustrate notable differences across the experimental groups. As shown in Figure 3A, TOS levels were significantly elevated in the INDO group compared to the control ($p<0.01$). However, the INDO+FAM ($p<0.01$) and INDO+LANO ($p<0.01$) groups demonstrated a marked reduction in TOS, suggesting the mitigating effects of famotidine and LANO when administered with indomethacin. Notably, there was no significant difference between the INDO+FAM and INDO+LANO groups ($p>0.05$). The LANO group showed TOS levels comparable to the control group, indicating no significant oxidative stress increase. Conversely, TAS experienced a significant decrease in the INDO group, indicative of depleted antioxidant reserves due to oxidative stress. Treatments with INDO+FAM ($p<0.01$) and INDO+LANO ($p<0.01$) presented a partial restoration of TAS levels, with no significant difference between them ($p>0.05$). The LANO group's TAS levels remained on par with the control group, highlighting its antioxidative potential (Figure 3B). For OSI, which represents the ratio of TOS to TAS, a substantially higher level was observed in the INDO group ($p<0.01$). This level significantly decreased in the INDO+FAM ($p<0.01$) and INDO+LANO ($p<0.01$) groups, with no significant difference between them ($p>0.05$), underscoring an improved oxidative stress regulation with these combination therapies. The OSI of the LANO group was similar to that of the control, further reinforcing its role in

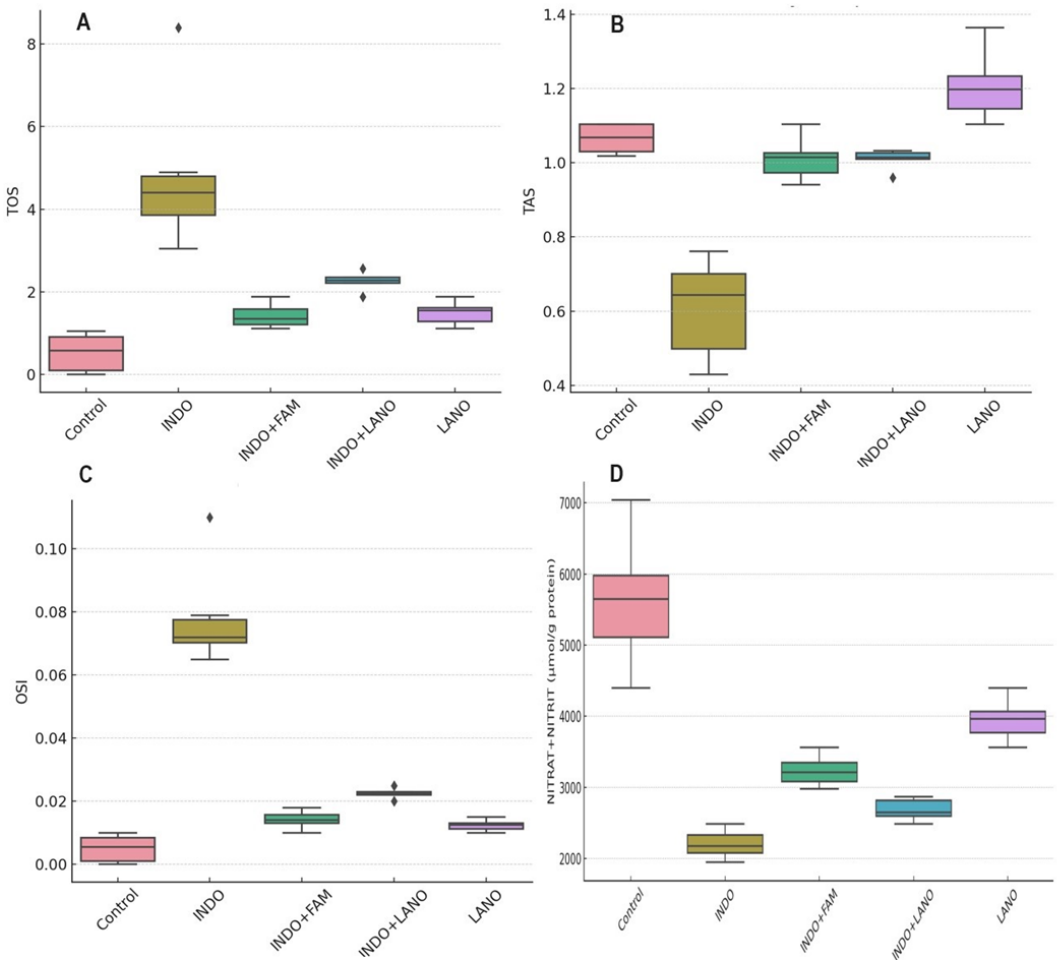

Figure 3. Oxidative stress parameters and Nitrat+Nitrit (NO) values across treatment groups. The box plots display the distribution of Total Oxidant Status (TOS), Total Antioxidant Status (TAS), and Oxidative Stress Index (OSI) among the groups.

protecting against oxidative stress (Figure 3C). Figure 3D delineates the nitrate and nitrite concentrations among the different treatment groups. In the INDO group, a significant decrease in median values of these markers was observed ($p<0.01$ for INDO vs. INDO+FAM; $p<0.05$ for INDO vs. INDO+LANO), suggesting a potential suppression in the synthesis or an increased turnover of nitrate and nitrite due to INDO treatment. However, the treatment groups receiving INDO+FAM and INDO+LANO presented nitrate and nitrite levels with median values approaching those of the control group. Specifically, the INDO+FAM group exhibited nitrate and nitrite levels notably closer to the control group's median ($p<0.01$ compared to the INDO group), indicating a significant mitigation of indomethacin's effect. No substantial difference in nitrate and nitrite levels was observed between the INDO+FAM and INDO+LANO groups ($p>0.05$), suggesting comparable efficacy in normalizing these parameters.

DISCUSSION AND CONCLUSION

In this study, the anti-ulcerative potential of LANO was assessed in a rat model with INDO-induced gastric ulcers. Through histopathological and biochemical methodologies, the study scrutinized LANO's therapeutic impact.

Stress-induced gastric ulceration involves complex etiopathological factors, leading to inconsistent pharmacotherapy and exploring new strategies.[16] Gastric distress and ulceration result from dietary imbalances, NSAID misuse like INDO, and altered gastric acid. Gastrointestinal damage stemming from inflammatory, oxidant, and cytotoxic activities overpowers mucosal defenses. It's linked to disrupted gastric defenses like acid/enzyme secretion, tissue integrity, and prostaglandin-aided mucosal protection.[17,18] In experimental gastroenterology, the INDO-induced stress ulcer model is commonly used to mimic NSAID-related ulceration for research.

Nitric oxide, a widely distributed signalling molecule and free radical, is naturally produced in the body, and its release from certain non-steroidal anti-inflammatory drugs helps mitigate gastrointestinal toxicity linked to traditional NSAID use.[19] Gastric ulcers are related to disrupted NO synthesis and activity, exacerbated by INDO's inhibition of prostaglandin synthesis, crucial for gastric lining protection.[19,20] Prostaglandins and NO are considered to work synergistically to maintain gastric integrity. Indeed, in our study, we observed that INDO administration significantly down-regulated nitric oxide levels, which aligns with the suppression of endogenous prostaglandin production.[21-23] This finding mirrors the literature, where reduced NO availability is closely associated with increased gastric vulnerability and ulceration.[21,24] Our findings indicate LANO application may counteract indomethacin's negative effects, supporting gastric protection via the NO pathway. This is evidenced by reduced lesion formation, highlighting a potential therapeutic strategy for NSAID-induced gastropathy.

The hallmark of INDO-induced gastric ulceration is the presence of macroscopic ulcerative foci within the gastric mucosa, a phenomenon well-documented in the literature.[11,25] Histopathological analysis showed significant ulcer foci in the gastric tissues of the INDO group rats, but treatments with famotidine and LANO notably reduced these ulcers. Additionally, a study by Stefanova et al. found that LANO essential oil demonstrated antimicrobial activity against pathogenic and spoilage microorganisms, potentially relevant to its therapeutic effects.[26] Furthermore, in a study, Yazıcı et al. observed that 0.3% laurel oil in the diet of Nile tilapia didn't cause histopathological changes but rather improved liver and intestinal tissues.[27] The reduction in ulceration with LANO treatment may be attributable to its anti-inflammatory constituents,[7] which could exert protective effects similar to those of prostaglandin-mediated mucosal defense mechanisms disrupted by INDO.

The intricate relationship between oxidative stress and the pathophysiology of gastric ulcers has been increasingly recognized in gastroenterological research.[11] Oxidants, such as reactive oxygen species (ROS), are pivotal in exacerbating gastric mucosal damage, often overwhelming the natural antioxidant defenses and leading to cellular injury and ulcer formation.[27,28] Our study revealed that INDO administration significantly increased TOS and OSI while decreasing TAS, thereby indicating elevated oxidative stress in the gastric environment. This aligns with previous studies that have established the role of oxidants in developing NSAID-induced gastric ulcers.[22,28] LANO showed promising effects with diverse phytochemicals. It displayed significant antioxidant, anti-diabetic, and antimicrobial effects at low concentrations, therapeutic potential.[29] The LANO-treated groups showed decreased TOS and preserved TAS, resulting in a lower OSI than the INDO group. This suggests LANO's potential to enhance mucosal defense by boosting antioxidant capacity or scavenging ROS, thereby mitigating oxidative damage in gastric tissue. This is corroborated by a study by Mssillou et al., which found that essential oil from LANO flowers exhibited significant antifungal and antioxidant activities, attributed to high levels of 1,8-cineole, further underscoring the importance of antioxidants in managing gastric ulcers.[30]

In conclusion, our study revealed that LANO mitigates INDO-induced gastric ulcers, reducing ulcer foci, normalizing TOS, TAS, and OSI levels, and stabilizing NO dynamics, suggesting its potential for

clinical use. However, further research is needed to understand its mechanisms.

Ethics Committee Approval: The work described in this article has been carried out by the Erzincan Binali Yıldırım University's Experimental Animals Local Ethics Committee (Date: 28.12.2023, decision no: 322655).

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept - HÜ, FDK; Supervision – HÜ, GI; Materials- HÜ, NK, SK; Data Collection and/or Processing- HÜ, NGT, NK, FDK; Analysis and/or Interpretation - NK, GI, SK; Writing - HÜ, GI.

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The Effect of Thymoquinone on the Protein Levels of PLA2G7, UCP2, and NEDD4L Genes Associated with Lipid Droplets Formation in Prostate Cancer

Prostat Kanserinde Lipid Damlacık Oluşumu ile İlişkili PLA2G7, UCP2 ve NEDD4L Genlerinin Protein Seviyeleri Üzerine Timokinonun Etkisi

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ABSTRACT

Objective: Prostate cancer (PCa) patients suffer severe side effects of standard treatment beside the resistance to castration. PCa cells shows increased lipogenesis. Thymoquinone (TQ) inhibits cell proliferation, metastasis, and invasion. However, there was no study on the effect of TQ on the levels of NEDDL4, PLA2G7, and UCP2 lipid droplets (LD) related proteins. Hence, the study aims to investigate the impact of TQ on PLA2G7, UCP2, and NEDD4L proteins on DU145 and PC3 cell lines.

Materials and Methods: Cells were cultured and treated with TQ with a IC_{50} of 60 µM and 80 µM for DU145 and PC3, respectively. PLA2G7, UCP2, and NEDD4L levels were measured using the ELISA.

Results: TQ has significantly increased the level of NEDD4L ($p<0.01$ for DU145 and $p<0.001$ for PC3) and decreased the level of UCP2 proteins ($p<0.05$).

Conclusions: Our preliminary findings suggest that TQ may impact NEDD4L and UCP2, indicating a potential role in repressing LD. Further investigations are needed to confirm the efficacy of TQ and explore its potential utility as a therapeutic agent for PCa treatment.

Keywords: NEDD4L, PLA2G7, prostate cancer metabolism, thymoquinone, UCP2

ÖZ

Amaç: Prostat kanseri (PCa) hastalarına yönelik standart tedavide, kastrasyona dirençli tipin gelişmesinin yanı sıra ciddi yan etkiler de yaşanmaktadır. PCa hücrelerinin esas olarak artmış lipogenez ile karakterize olduğu bilinmektedir. Timokinonun (TQ) hücre proliferasyonunu, metastazı ve invazyonu inhibe ettiği gösterilmiştir. Ancak literatürde TQ'nun NEDDL4, PLA2G7 ve UCP2 lipit damlacığı ile ilişkili proteinlerin düzeylerine etkisine ilişkin bir çalışma bulunmamaktadır. Bu çalışmanın temel amacı, TQ'nun PLA2G7, UCP2 ve NEDD4L proteinleri üzerindeki DU145 ve PC3 hücre hatları üzerindeki etkisini araştırmaktır.

Materyal ve Metot: Hücreler çoğaldı ve DU145 ve PC3 için sırasıyla 60 µM ve 80 µM IC_{50} ile TQ ile tedavi edildi. İnkübasyondan sonra, PLA2G7, UCP2 ve NEDD4L seviyeleri ELISA yöntemi kullanılarak ölçüldü.

Bulgular: TQ, NEDD4L düzeyini önemli ölçüde artırmıştır (DU145 için $p<0,01$ ve PC3 için $p<0,001$). Ayrıca, TQ'nun UCP2 protein düzeyini azalttığı da gösterilmiştir ($p<0,05$).

Sonuç: Çalışmamızın başlangıç bulguları, TQ'nun öncelikle NEDD4L ve UCP2 üzerinde etkisi olabileceğini göstermektedir. Bu, TQ'nun LD üzerinde baskılayıcı bir etkiye sahip olabileceğini ve daha fazla araştırma ile PCa tedavisi için faydalı bir molekül olabileceğini öne sürmektedir

Anahtar Kelimeler: NEDD4L, PLA2G7, prostat kanser metabolizması, timokinon, UCP2

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INTRODUCTION

The increasing incidence of PCa over the past century has resulted in-substantial alterations in diagnosis and therapy.[1,2] Despite these breakthroughs, total cure remains elusive, and a considerable number of men continue to suffer from castration resistance and metastasis, which often necessitate a combination of therapies.[3] Thus far, PCa therapy, mainly Androgen deprivation therapy (ADT) and Docetaxel (DTX),[4] induced side effects and the development of castration resistance underline the necessity of treatment alternatives to enhance the overall survival as well as patient's quality of life.

TQ, derived from Nigella sativa seeds, is a promising biopharmaceutical with potent anticancer properties, enhancing treatment efficacy while reducing toxicity. Its synergistic effects with existing drugs further underscore its potential in cancer therapy.[6]

Lipid metabolism rewiring enables PCa cells to grow and increase in aggressiveness and metastasise. Therefore, new means of resistance related to lipogenesis reprogramming have recently risen.[7] Subsequently, LD accumulation is positively associated with an increased Gleason score of PCa.[8] In this study, we focused on three proteins related to LD synthesis and regulation.

First, aside from the role of Phospholipase A2 (PLA2s) in LD synthesis, they may act as providers of free fatty acids, generators of metabolite that may control LD formation and direct controllers of LD.[9]

Furthermore, another study confirmed the effect of Uncoupling protein 2 (UCP2) downregulation in Promoting Phospholipid Synthesis and Increasing Expression of Lipogenic Enzymes, thus lipid accumulations and a potential promotion of LD formation.[10]

Thirdly, in PCa, NEDD4L levels decrease with progression toward malignancy, which is shown to correlate with an increased Gleason score.[11] It was also reported that E3 ligase (NEDD4 proteins family) plays a ubiquitination role of adipophilin on LD, which suggests its potential regulatory role.[12]

Targeting specific cancer therapeutics is of great interest in current cancer therapy; therefore, specific molecular targets for TQ should also be investigated. [9, 10, 12,13, 14]

Our study seeks to conduct a preliminary investigation into the impact of TQ on the protein levels of NEDD4L, PLA2G7, and UCP2, which are implicated in the formation of LD. Through this initial exploration, we aim to elucidate any potential modulatory effects of TQ on these key proteins, laying the groundwork for further comprehensive investigations.

MATERIALS AND METHODS

Ethics Committee Approval: Ethics committee approval is not required for studies to be conducted on commercially available human cadavers, cadaver parts, and other biological materials. Ethics committee approval was not needed since a commercially available cell line was used in this study.

Cell Culture: The Human Prostate Carcinoma Cell lines used in the study, PC3 and DU145, were supplied by Mugla Sıtkı Kocman University and Yeditepe University, respectively. DU145 and PC3 cell lines were cultured in a complete growth media containing % 10 FBS (Capricorn, CP17-1756), % 1 Penicillin-Streptomycin (Capricorn Scientific), % 1 L-Glutamine in DMEM (Capricorn Scientific). Our cell lines were incubated at 37 ◦C with 5% CO2.

Thymoquinone Preparation and Treatment: For this study, 14 mg of Thymoquinone powder (BLD pharm, BD233118, 2-Isopropyl-5-methylcyclohexa-2,5-diene-1,4-dione, 99%, China) was dissolved into 1 ml of DMSO to get the stock solution with a final concentration of 85 mM. This stock was later diluted in DMEM to produce concentrations of 60 µM and 80 µM to treat DU145 and PC3 cell lines, respectively.[15] After successive dilutions, the concentration of DMSO was brought down to less than 0.1 %, which is favourable since DMSO concentrations up to 1% are considered safe and non-cytotoxic.[16] To prepare cell lysates, a freeze-thaw procedure was followed by incubating the cells at -80 °C for 5 min, then at 37°C for the same period for three cycles. This process was followed by centrifugation at +4°C at 1000 rpm for 5 min, after which the supernatant was collected into sterile and labelled Eppendorf tubes.

ELISA Test: ELISA (Enzyme-Linked Immunosorbent Assay) was used to determine the levels of NEDD4L (Shanghai Coon Koon Biotech, 20522), UCP2 (Shanghai Coon Koon Biotech, 13901) and PLA2G7 (Shanghai Coon Koon Biotech, 12314) proteins in both cell types. The kits used in this study were based on sandwich ELISA, which has the advantage of high sensitivity and specificity. The measurement was made at 450 nm absorbance in the Epoch Reader Spectrophotometer device.

Statistical Analysis: GraphPad Prism software is used at this step. The testing for normality was done with the Shapiro-Wilk-normality test. Statistical differences were evaluated using a two-tailed Student's t-test. Differences at $p < 0.05$ were considered as significant.

RESULTS

The analysis of PLA2G7 protein expression revealed notable differences between the experimental groups. In PC3 cells, the expression level was measured at 2.52 ng/mL in the absence of TQ (PC3(TQ-)) and reduced to 1.98 ng/mL in the presence of TQ (PC3(TQ+)). In DU145 cells, the expression level was 2.40 ng/mL without TQ (DU145(TQ-)) and slightly lower at 2.35 ng/mL with TQ (DU145 (TQ+)). In DU145 cells, there was no significant alteration in PLA2G7 protein expression between the treated and control groups, as depicted in (Figure 1a). Conversely, in PC3 cells, a subtle yet statistically insignificant reduction in PLA2G7 protein expression was observed with TQ treatment, as illustrated in (Figure 1b). These findings underscore the differential effects of TQ on PLA2G7 protein expression in the two cell lines studied.

As illustrated in Figure 2, the expression levels of the UCP2 protein were notably distinct between the DU145 (TQ-) and DU145 (TQ+) cells, measuring 98.1 pg/mL and 16.7 pg/mL, respectively. In the PC3 cell lines, the expression levels of UCP2 were similarly varied, with 159 pg/mL in the TQ- group and 62.4 pg/mL in the TQ+ group. The analysis presented in Figure 2 reveals a significant reduction in UCP2 protein levels upon treatment with TQ in both DU145 ($p < 0.05$) (Figure 2a) and PC3 cells ($p < 0.05$) (Figure 2b). This suggests an inhibitory effect of TQ on the level of UCP2 protein, underscoring its potential as a modulator of UCP2 activity in these cell lines.

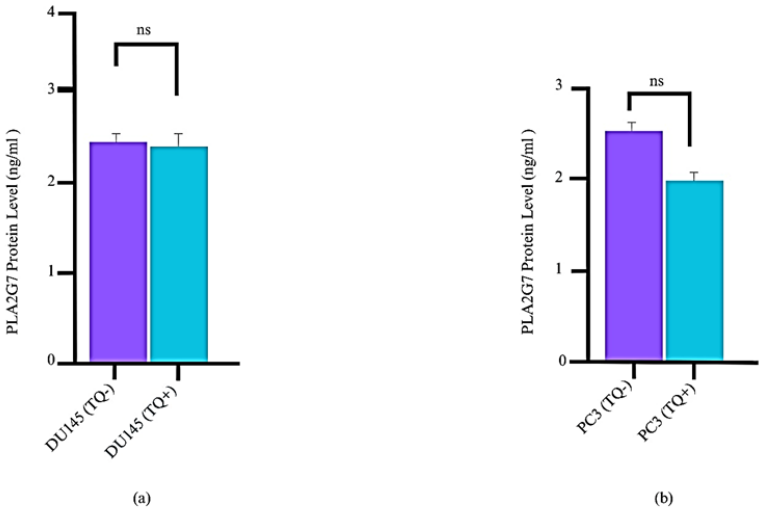

Figure 1. The effect of TQ on PLA2G7 levels in DU145 and PC3. (a) and (b) compared to the control group; ns: $p > 0.05$; Error bars indicate standard ±mean error.

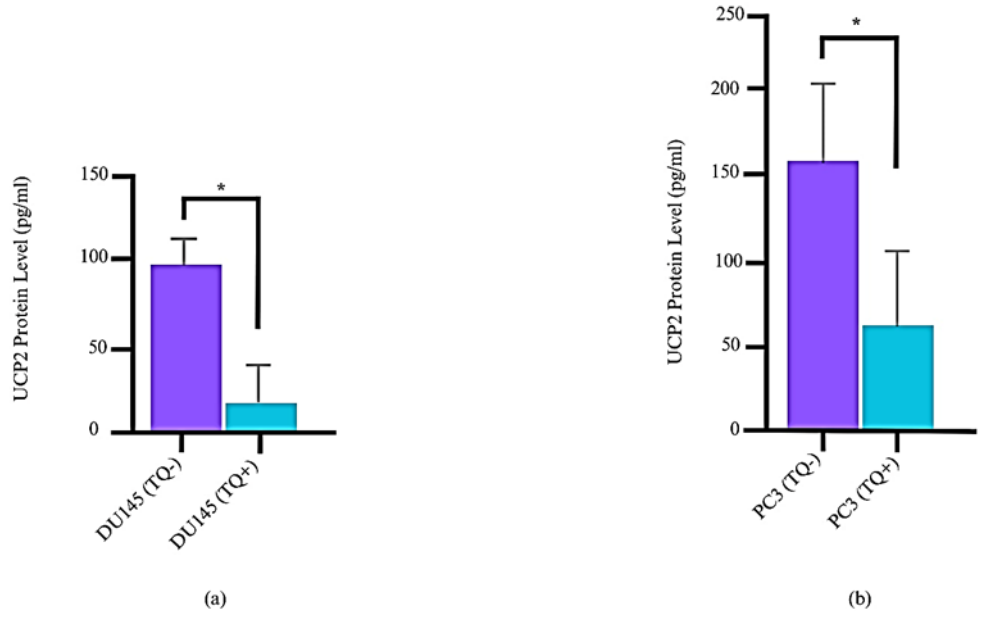

Figure 2. The effect of TQ on UCP2 levels in DU145 and PC 3. (a) and (b) compared to the control group; *: $p < 0.05$; Error bars indicate standard ±mean error.

The expression levels of NEDD4L protein exhibited notable variations across different cell lines and treatment conditions. In PC3 cells, the expression level of NEDD4L protein was 1.30 ng/mL in the untreated group (TQ-), which increased to 2.11 ng/mL in the TQ-treated group (TQ+). Similarly, in DU145 cells, the expression level of NEDD4L protein was 0.52 ng/mL in the untreated group, rising to 1.65 ng/mL in the TQ-treated group. Interestingly, the impact of TQ on NEDD4L protein expression was particularly pronounced in DU145 cells. TQ led to a significant increase in the NEDD4L protein level compared to the untreated group ($p < 0.01$) (Figure 3a). Remarkably, in PC3 cells, this effect was even more substantial, with TQ resulting in a highly statistically significant elevation of NEDD4L protein levels ($p < 0.001$) (Figure 3b), as illustrated in the provided figure.

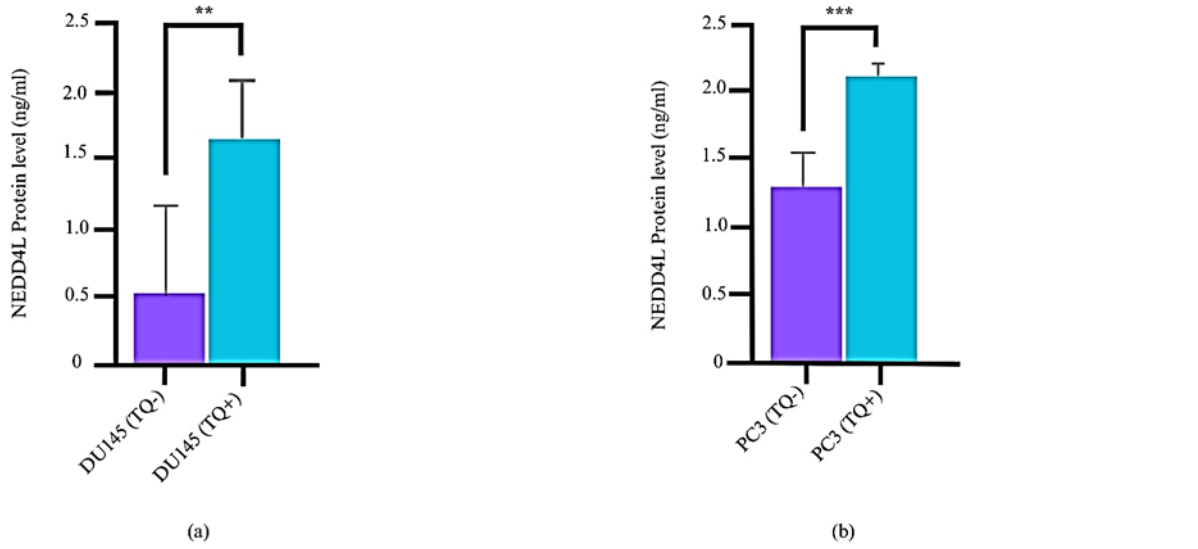

Figure .3 The effect of TQ on NEDD4L levels in DU145 and PC3. (a) and (b) compared to the control group; **: $p < 0.01$; ***: $p < 0.001$; Error bars indicate standard ±mean error.

DISCUSSION AND CONCLUSION

Cancer cells exhibit metabolic alterations that promote tumor growth. And facilitate coping with new growth rate demands during malignancy.[17,18] Intensive research is being conducted on methods to modify the metabolic reprogramming of cancer cells in PCa.[17] Consistently, an expanding body of research has demonstrated a strong positive correlation between upregulated lipogenesis and PCa pathogenesis.[19] An increased number of LD is now considered a hallmark of aggressive PCa.[20] Therefore, studies indicate that an increase in LD is positively linked with a higher Gleason Score of PCa. This may justify the role of LD in the progression of PCa to a more aggressive and malignant form.[8]

These findings give rise to new possibilities for using lipid metabolomics to find therapeutic targets. The use of natural and synthetic drugs that modify the lipogenic phenotype in cancer is a fast-expanding topic of study.[21] Following that, in an unprecedented attempt, we have selected TQ to investigate possible effects on LD in PCa.

At first, and as the ultimate purpose is to find new treatments for men with hormone-refractory PCa, cell lines were chosen to address a broad spectrum of PCa with distinct hormonal dependency and aggressiveness.[22]

Our research is the first attempt to look at the effect of TQ on PLA2s, yet very few studies have addressed the PLA2s using natural products.[23] Contrary to expectations, our study showed no significant differences between the treated and control groups. One possible explanation for such a result is that TQ may have a time-dependent effect, and the incubation period may have been insufficient to cause an inhibitory effect. As such, further investigations should be held to address these limitations. Despite that, Patel et al. have reported increased levels of PLA2 in DU145 and PC3 cell lines, and that siRNA knockdown or specific inhibition of PLA2 by Wyeth -1 resulted in a decrease in cell growth and proliferation both in-vitro and in-vivo.[24]

Our study's originality lies in preliminarily describing the potential effect of TQ on UCP2, which has yet to be previously reported, providing new insights into the underlying mechanism. Our findings match those of a recent study where Genipin, a plant chemical extract, by decreasing UCP2 expression in PC-3 cells, reduced cell migration and growth in contrast to control,[25] which, as hypothesized, supports the idea that TQ could also be a potential inhibitor of UCP2. Additionally, a growing body of literature

shows that cells expressing elevated amounts of UCP2 are more chemoresistant;[26] thus, lowering the UCP2 levels as an attempt to increase chemosensitivity sounds valid, as depicted by Hua et al.[27] TQ may exert its effects on LD through modulation of oxidative stress pathways acting on UCP2 protein levels. Oxidative stress can influence lipid metabolism by altering enzyme activities and lipid oxidation rates. Additionally, TQ might influence mitochondrial function, which plays a crucial role in lipid metabolism. By affecting mitochondrial activity, TQ could indirectly impact LD dynamics.

Moreover, a discrepancy in the expression level of NEDD4L was reported as it decreases in well-differentiated PCa[11] and increases in poor-differentiated PCa.[28] This indicated that NEDD4L protein level regulation may play a role in PCa and give insights into novel treatment strategies.[29] Therefore, given the fact that the cell lines we used are of an advanced aggressive phenotype, we projected a downregulation of NEDD4L before treatment and that TQ to increase the levels of NEDD4L, which is a potential effect on the ubiquitination of LD.

As expected, our preliminary results by ELISA might suggest that TQ has significantly increased the levels of NEDD4L in both cell lines. Remarkably, TQ exhibited a notably more significant impact on NEDD4L in the PC3 cell line, suggesting a promising efficacy against a potentially more aggressive form of PCa. In the same fashion, Wogonin, a non-synthetic compound, has been shown to increase the expression of NEDD4L, the ubiquitin E3 ligase of Pik3ca, inducing the degradation of Pik3ca which in turn hinders PI3K/Akt pathway.[30] Indeed, inferring from the findings described above, we suggest that TQ is more likely to induce LD degradation similarly. TQ might directly interact with LD formation, stabilization, or degradation proteins. For instance, it could bind to proteins that coat the surface of LD and regulate lipolysis, thereby influencing their stability and turnover.

The integration of these studies with our findings suggests that NEDD4L and UCP2 could emerge as promising targets in drug discovery endeavours aimed at combating PCa, given their implication in tumorigenesis. However, further investigations, using western blot method for instance, are imperative to confirm the efficacy of TQ on the aforementioned proteins and to elucidate its precise mechanism of action. Such studies should encompass a comprehensive set of in vivo and in vitro investigations, advancing our understanding of TQ's potential therapeutic utility in PCa treatment.

Ethics Committee Approval: An ethical approval for the study is not required. In this study, cell culture was used.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – MEE, EŞ; Supervision – EŞ; Materials – AH, HY; Data Collection and/or Processing – AH, HY; Analysis and/or Interpretation – AH, BEÖB; Writing – AH.

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Broadening the Discourse on Death Anxiety: Unexplored Dimensions and Holistic Perspectives

Ölüm Kaygısının Geniş Bir Perspektiften Değerlendirilmesi: Keşfedilmemiş Boyutlar ve Bütünsel Bakış Açısı

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ABSTRACT
Understanding death anxiety among patients is crucial for healthcare professionals to provide personalized care and support strategies. Cultural and social norms play a significant role in shaping an individual's perception of death. While some cultures accept death as a natural and cyclical process, others tend to avoid it. Therefore, healthcare professionals must consider these factors to develop effective assistance strategies. This our discourse emphasizes the importance of comprehensively evaluating death anxiety to contribute to the study of Özpancar Şolpan et al. on this subject.
Keywords: Death anxiety, cultural and social norms, healthcare professionals

ÖZ
Hastalar arasındaki ölüm kaygısını anlamak, sağlık çalışanlarının kişiselleştirilmiş bakım ve destek stratejileri sunması açısından çok önemlidir. Kültürel ve sosyal normlar bireyin ölüm algısının şekillenmesinde önemli rol oynamaktadır. Bazı kültürler ölümü doğal ve döngüsel bir süreç olarak kabul ederken bazıları bundan kaçınma eğilimindedir. Bu nedenle sağlık çalışanlarının etkili yardım stratejileri geliştirmek için bu faktörleri dikkate alması elzemdir. Bu söylemimiz, Özpancar Şolpan ve arkadaşlarının bu konudaki çalışmasına katkı sağlamak amacıyla ölüm kaygısının kapsamlı bir şekilde değerlendirilmesinin önemini vurgulamaktadır.
Anahtar Kelimeler: Kültürel ve sosyal normlar, ölüm kaygısı, sağlık çalışanları

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Dear Editor,

I have read with great interest the article titled "Death Anxiety in Patients with Hypertension and ST-Elevation Acute Myocardial Infarction and the Affecting Factors" authored by Özpancar Şolpan et al., and published in the third issue of your journal in 2023.[1] I would like to express my gratitude to the authors and editorial board for this insightful article that sheds light on death anxiety in cardiac patients for clinicians. However, I would like to contribute to the discussion by mentioning a few aspects of death anxiety in patients that have not been addressed in the article. Ethics committee approval is not required for Letter Writing to the Editor.

The first important point is that the level of death anxiety an individual experiences is directly influenced by their cultural and social norms. Some cultures view death as a natural and cyclical process, while others perceive it as an event to avoid. This perception affects the individual's acceptance of death and their approach to seeking support. Therefore, it is crucial to examine cultural and social norms to understand different levels of death anxiety among individuals. Healthcare professionals and counsellors should consider these factors when developing personalized care and support strategies to provide more effective assistance.[2]

The second important point is to consider the impact of death anxiety on quality of life and daily functioning. This includes how it affects compliance with treatment regimens and overall well-being. Understanding these practical effects is crucial for developing interventions that can improve the emotional and general functioning of patients.[3]

The third point to consider is how health systems can help reduce death anxiety. Factors like the accessibility of mental health services, the effectiveness of counselling, and the collaboration between medical and psychological care teams can greatly influence patients' experiences.[4] A thorough examination of health system dynamics can provide valuable insights into the practicality and efficacy of com-

prehensive care strategies.

Finally, it is important to consider the patient's anxiety from a longitudinal perspective. This means understanding how their fear of death changes over time. By studying how these concerns evolve during diseases and treatments, we can gain a better understanding of the nuances involved.[5] Longitudinal studies provide insights into patterns of death anxiety over time, which in turn allow the development of targeted interventions at different stages of the disease journey.

In conclusion, the existing discussion on death anxiety among hypertension and acute myocardial infarction patients provides a solid foundation. However, there are various unexplored dimensions that highlight the multifaceted nature of this anxiety. To gain a more comprehensive understanding, it is necessary to incorporate cultural, functional, systemic, and longitudinal perspectives, along with the inclusion of patient narratives. This holistic approach ensures a more nuanced comprehension of death anxiety, paving the way for patient-centric interventions and improved overall care.

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