ARAŞTIRMA MAKALELERİ

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İÇİNDEKİLER
CONTENTS

ARAŞTIRMA MAKALELERİ
RESEARCH ARTICLES

Smoking, drinking, and food addiction among youth in Sivas, Türkiye

Türkiye'nin Sivas kentindeki gençler arasında sigara, içki ve yeme bağımlılığı

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ABSTRACT

Aim: Substance abuse is a major issue. The change in eating habits worldwide and the increasing interest in convenience foods are also important issues. The aim of this study is to investigate the relationship between the degree of nicotine dependence, alcohol consumption, and food addiction in young people. It also aimed to assess the role of impulsivity as a mediating variable in this relationship.

Materials and Methods: The study was conducted with 533 students. Data were collected online using the questionnaire form, Fagerström Test for Nicotine Dependence Test (FTND), Alcohol Use Disorders Identification Test (AUDIT-C), UPPS Impulsive Behaviour Scale, and Yale Food Addiction Scale. Correlation, MANCOVA, and mediated regression analysis (Hayes, Model 4) were used to analyze the data.

Results: The study involved 79% female and 21% male participants. The prevalence of smoking and alcohol consumption among the participants was 21.2% and 15.8%, respectively. The prevalence of severe food addiction was estimated at 5.4%. The findings of this study indicate that sex has a significant effect on alcohol consumption and degree of nicotine dependence, with these effects varying depending on impulsive behavior characteristics. The results of the mediated regression analysis also demonstrate that the degree of nicotine dependence has a significant effect on food addiction depending on impulsive behaviors, although this effect is not significant for alcohol use.

Conclusion: The findings of our study indicate that legal substance use and food addiction are prevalent among young people. Furthermore, our results demonstrate a significant correlation between the degree of nicotine dependence and food addiction, which can be attributed to impulsive behaviors. Further research, including the use of different techniques, is required in order to obtain more concrete and reliable information on this subject.

Keywords: Smoking, alcohol use, food addiction, applied psychology.

ÖZ

Amaç: Madde bağımlılığı önemli bir sorundur. Dünya genelinde beslenme alışkanlıklarının değişmesi ve hazır gıdalara olan ilginin artması da önemli bir konulardandır. Bu çalışmanın amacı, gençlerde nikotin bağımlılığı düzeyi ve alkol kullanımı ile yeme bağımlılığı arasındaki ilişkiyi araştırmaktır. Ayrıca bu ilişkide dürtüselliğin aracı değişken rolünü değerlendirmek amaçlanmıştır.

Gereç ve Yöntem: Çalışma 533 öğrenci ile gerçekleştirilmiştir. Veriler anket formu, Fagerström Nikotin Bağımlılığı Testi (FTND), Alkol Kullanım Bozuklukları Tanıma Testi (AUDIT-C), UPPS Dürtüsel Davranış Ölçeği ve Yale Yeme Bağımlılığı Ölçeği kullanılarak çevrimiçi olarak toplanmıştır. Verileri analiz etmek için korelasyon, MANCOVA ve aracılı regresyon analizi (Hayes, Model 4) kullanılmıştır.

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Bulgular: *Çalışmaya %79 kadın ve %21 erkek katılımcı dahil edilmiştir. Katılımcılar arasında sigara ve alkol tüketimi yaygınlığı sırasıyla %21,2 ve %15,8'dir. Şiddetli düzeyde yeme bağımlılığı prevalansı %5,4 olarak hesaplanmıştır. Bu çalışmanın bulguları, cinsiyetin alkol tüketimi ve nikotin bağımlılığının derecesi üzerinde önemli bir etkisi olduğunu ve bu etkilerin dürtüsel davranış özelliklerine bağlı olarak değiştiğini göstermektedir. Aracı regresyon analizinin sonuçları da nikotin bağımlılığının derecesinin dürtüsel davranışlara bağlı olarak yeme bağımlılığı üzerinde önemli bir etkiye sahip olduğunu, ancak bu etkinin alkol kullanımı için anlamlı olmadığını göstermektedir.*

Sonuç: *Çalışmamızın bulguları, yasal madde kullanımı ve yeme bağımlılığının farklı düzeylerinin gençler arasında yaygın olduğunu göstermektedir. Ayrıca, sonuçlarımız nikotin bağımlılığının derecesi ile dürtüsel davranışlara atfedilebilecek yeme bağımlılığı arasında önemli bir korelasyon olduğunu göstermektedir. Bu konuda daha somut ve güvenilir bilgiler elde etmek için farklı tekniklerin kullanımını da içeren daha fazla araştırma yapılması gerekmektedir.*

Anahtar Sözcükler: *Sigara kullanımı, alkol kullanımı, yeme bağımlılığı, uygulamalı psikoloji.*

INTRODUCTION

Substance abuse is a major global problem. The issue of substance use and mental health problems represents a significant challenge for individuals, families, communities, and, ultimately, societies. When the topic of substance use arises, the initial question that is typically posed is whether the substance in question is legal or illegal. This question remains a crucial aspect in the context of addressing substance use problems. It can be reasonably assumed that governments will continue to devote greater resources to the eradication of illicit substances. The inability to regulate the production and distribution of illicit substances, including cannabis, MDMA, heroin, amphetamines, methamphetamines, and synthetic cannabis, may result in unforeseen consequences on human health and behavior (1). The focus is on treatment and rehabilitation after use, which is a challenging process for both those receiving and providing treatment. Furthermore, only a small proportion of individuals with alcohol or drug use disorders receive treatment despite the well-documented adverse effects of addiction on physical, psychological, and quality of life (1).

A review of prevalence data can facilitate an understanding of this problem. In 2020, 58.7% (162.5 million people) of people aged 12 years and older are reported to have used tobacco, alcohol, or illicit substances regularly in the past month (2). In the United States, tobacco, alcohol, and cannabis are the substances most commonly used by adolescents and adults (1). The 2024 EUDA (European Union Drugs Agency) report indicates that the lifetime alcohol use rate among young adults aged 15-34 in Türkiye is 22.1% (male: 34.3%, female: 10.7%), while the lifetime tobacco use rate is 47% (male: 61.9%, female: 33.2%). In the same report, the rates of alcohol and tobacco use in the previous year were reported as 14.9 percent and 33.2 percent, respectively (3).

Another significant issue is that addiction is a concept that encompasses a wide range of behaviors. One such behavior is food addiction, which is a type of behavioral addiction that has been specifically defined in the literature. This definition involves examining individuals' eating habits. A critical examination of the underlying causes of this phenomenon reveals that the process of economic progress and globalization has profoundly shaped the dietary patterns and preferences of individuals. In particular, there has been a notable shift away from home-cooked meals towards the pervasive consumption of highly processed convenience foods, which are often referred to as "junk food." This shift has occurred particularly since the 20th century (4, 5). Ultra-processed foods are defined as foods and beverages that undergo a series of mechanical and chemical processes, including heating, freezing, frying, compression, irradiation, and microwave baking (6). These foods are typically composed of extracted food components, exhibiting minimal presence of natural and intact food substances. Ultra-processed foods encompass a range of products, including hydrogenated oils and fats, modified starches, hydrolyzed proteins, and ground or extruded offal "mix" or meat scraps. Furthermore, they contain flavorings, colorings, and other additives that are used to impart a 'hyper-flavor' (7, 8).

Some researchers have identified a high addictive potential in certain foods (9). As in substance addiction, the concept of craving also emerges as a key issue in this context. The issue

of food cravings has been the subject of considerable debate, with binge eating being linked to food craving for some time (10). It has been demonstrated that individuals who experience cravings tend to engage in more frequent snacking, adhere to more restrictive diets (11), and exhibit higher levels of body mass index (12). Moreover, the concept of food addiction has been discussed for some time (13). It has been proposed that obese individuals are predisposed to food addiction (14, 15) and that there may be links between reward regions in the brain and binge eating (15). Despite the substantial overlap between obesity and addictive eating behaviors, it is not possible to account for all cases of obesity. For instance, it is acknowledged that some individuals with a normal weight exhibit addictive eating behaviors (16).

In the context of the aforementioned information, the term "food addiction" is used to describe another eating behavior profile that exhibits a substance-like addiction to highly processed foods (e.g., pizza, potato chips, pastries) (17). Given the neurobiological and behavioral parallels between substance addiction and overeating behavior, it is unsurprising that scientific interest in eating addiction is on the rise (18,19). In particular, interest in the concept of food addiction has been driven by the findings of brain imaging studies that have demonstrated the presence of dopamine activity in brain regions associated with the reward system, as observed in substance addiction (20, 21). Furthermore, the Yale Food Addiction Scale (YFAS), based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), is a widely used instrument for diagnosing this condition. As with substance use disorders, the diagnostic process for food addiction includes criteria such as continued use despite negative consequences and repeated unsuccessful attempts to reduce use (17). There is a substantial body of research utilizing the YFAS scale (22–24).

The use of legal or illicit substances at an earlier age is considered a risk factor for the development of substance use disorders (25). Although the evidence bases for eating addiction is not as extensive as that for substance use disorders, it is widely acknowledged that addictive behaviors typically emerge at an earlier age. Consequently, this study is of significant importance within the field of addiction literature, both in terms of its subject matter and the fact

that it was conducted on a young population. The aim of this study is to investigate the relationship between the degree of nicotine dependence and alcohol consumption and food addiction in young people. It also aimed to assess the role of impulsivity as a mediating variable in this relationship.

This study hypothesizes that higher levels of nicotine dependence and alcohol consumption are associated with increased food addiction in young people and that impulsivity mediates this relationship. This suggests that impulsive individuals may be more susceptible to both substance use and food addiction.

MATERIALS and METHODS

Design: The study was conducted using a cross-sectional design. The approval of the Ethics Committee (Decision No. 2023/1-39 dated March 17, 2023) was obtained. Prior to the commencement of the study, informed consent forms were obtained from all participants. No identifying information (such as name and surname) was obtained from the participants. The responses were analyzed and evaluated anonymously and collectively.

Sample: The study sample consists of students of Sivas Cumhuriyet University with an average age of 21 years. The non-probability sampling method (snowball sampling) was used. Five hundred eighty students participated in the research, and the number of valid questionnaires was calculated as 533. Forty-seven students were not included in the statistical analyses because they did not answer the questions on the scales and some socio-demographic information, and the analyses were carried out on the data of 533 students.

Data collection: The data collection was conducted online using Google Forms, a free software. The data collection period was defined as spanning from 31 March 2023 to 19 December 2023. The process was conducted utilizing the following scales and questionnaires.

Questionnaire Form: It is a form prepared by the researcher and includes information on age, sex, class, weight, height, waist circumference and health status. The participants' body weight in kilograms (kg), height in centimeters (cm), and waist circumference were measured using the personal declaration method. From these measurements, the participants' body mass index (BMI) was calculated separately by dividing their

body weight (in kilograms) by the square of their height (in meters). The ranges of values were categorized according to the World Health Organization classification for adults (26): **Weak** (low weight): <18.50, **Normal**: 18.50-24.99, **Overweight**: 25.00-29.99, **Obese**: ≥30.

Fagerström Test for Nicotine Dependence (FTND): The scale was developed by Heatherton and colleagues (27). In 2004, Uysal et al. conducted a Turkish validity and reliability study and adapted a scale to assess risk and measure the degree of physical dependence on nicotine (28). Suppose the total score obtained from the test is less than or equal to 4. In that case, it means mild nicotine dependence, 5 means moderate nicotine dependence, and greater than or equal to 6 means severe nicotine dependence.

Alcohol Use Disorders Identification Test-Short Form (AUDIT-C): This scale (AUDIT) is a scale developed by the World Health Organisation to measure alcohol-related problems in the past 12 months. It is a 10-item scale that measures drinking habits, alcohol consumption, and alcohol-related problems. There is also a short form of the scale (AUDIT-C) that includes only the first three questions about alcohol use. The short form is most commonly used in screening for hazardous alcohol use (29). The scale enables the evaluation of risky alcohol use levels according to the obtained scores. The corresponding points are as follows: 0 to 4; low risk, 5 to 7; increasing risk, 8 to 10; higher risk and 11 to 12; possible dependence. The validity and reliability of the revised Turkish version was conducted by Saatçioğlu et al. The Cronbach alpha coefficient of internal consistency was 0.85 (30).

UPPS Impulsive Behaviour Scale: This scale, which includes a total of 45 questions, was developed by Whiteside and Lynam in 2001 to measure impulsivity (31). The validity and reliability study of the Turkish version of this scale was conducted by Yargıç et al. (2011), and it was reported to be a good tool for screening the traits related to impulsivity. The scale consists of 4 sub-dimensions: Urgency, Premeditation, Perseverance, and Sensation-Seeking (32).

Yale Food Addiction Scale (YFAS): This scale was developed by Gearhardt and colleagues in 2009 and is a 27-item scale that measures food addiction similarly to substance addiction symptoms (33). The scale has two scoring methods. At least one of the symptoms must be present to meet the criteria for food addiction, and three or more of the symptoms must be present in the past 12 months to be diagnosed with food addiction, in addition to clinical worsening. In our country, the validity and reliability study of the scale was first carried out by Bayraktar et al. in 2012, and the internal consistency coefficient was found to be 0.93 (34). Furthermore, a validity study was conducted by Büyüktuncer et al. in 2019, according to the DSM-V (35).

Data Analysis: The data was analyzed using SPSS v.25. Correlation, MANCOVA, and Mediated Regression Analysis were used to analyze. The statistical significance was accepted as 0.05 ($p < .05$).

Mediated regression analysis was conducted to examine whether or not impulsive behavior has a mediating role in the relationship between the degree of nicotine dependence or alcohol use and food addiction. The indirect effects macro of Preacher and Hayes (36) was used in the analyses. Bootstrapping, a computationally efficient method of resampling the dataset and estimating the indirect effect in each resampled dataset, was considered. Preacher and Hayes (36) defined mediation processes with only one mediating variable as simple mediation.

RESULTS

Participants' descriptive findings

Table-1 presents a descriptive overview of the participants. Most participants were female (79%), while the remaining 21% were male. Most participants were enrolled in social fields of study, including social work, psychology, political science, and Turkish language and literature (66.2%). The remaining 33.8% were enrolled in fields such as dentistry, medicine, computer science, electrical engineering, and architecture. The majority of the participants were in their first year of study.

A total of 4.9% of participants had been diagnosed with a physical illness, while 3.9% had been diagnosed with a psychiatric illness. The physical conditions reported included allergic rhinitis, asthma, diabetes, hepatitis B, anemia, migraine, reflux, rheumatism, thyroid, and pancreatitis. Psychiatric disorders were reported as anxiety disorder, depressive disorder, body dysmorphic disorder, bulimia nervosa, attention deficit hyperactivity disorder, epilepsy, obsessive-compulsive disorder, conversion disorder, generalized anxiety disorder, and social phobia. Additionally, 12.4% of participants reported recent regular use of a medicine with a doctor's recommendation, while 2.3% reported recent regular use of a medicine without a doctor's recommendation (Table-1).

Table-1. Descriptives Statistics.

Variables		n	%
Sex	Female	421	79
	Male	112	21
Department	Social science	353	66.2
	Natural science	180	33.8
Grade	1st grade	213	40
	2st grade	122	22.9
	3st grade	71	13.3
	4st grade	105	19.7
	postgraduate	22	4.1
Physical illness	Yes	26	4.9
	No	507	95.1
Psychiatric disease	Yes	21	3.9
	No	512	96.1
Use of medicines with doctor's advice	Yes	66	12.4
	No	467	87.6
Use of medicines without doctor's advice	Yes	12	2.3
	No	521	97.7

Descriptive Statistical Findings on Participants' Smoking, Drinking, and Food Addiction

Figures-1 and 2 illustrate the prevalence of smoking and alcohol consumption among the study participants. The data indicates that 21.2% of the participants engage in smoking habits, while 15.8% engage in alcohol consumption.

■ **yes** ■ **no** ■ **give up**
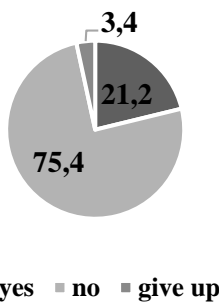
Figure-1. Cigarette use (%).

The final scoring using the YFAS scale revealed that 5.4% of the participants met the diagnostic criteria for severe food addiction, 1.1% for moderate food addiction, and 0.4% for mild food addiction. Additionally, 27% of the participants were assessed as not having a food addiction because they did not meet the clinical criteria, although they exhibited some symptoms of a food addiction (see Figure-3).

■ **yes** ■ **no** ■ **give up**
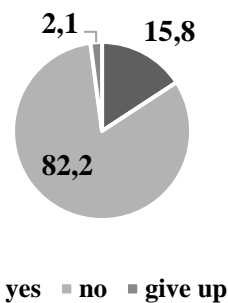
Figure-2. Alcohol use (%).

Findings on Correlation Analysis

Table-2 presents descriptive statistics and Pearson correlation coefficient values. Age, BMI, and alcohol consumption exhibited a significant positive correlation. The subscales of premeditation and perseverance demonstrated a negative relationship with food addiction, whereas impulsivity and sensation-seeking exhibited a positive and significant relationship. Additionally, there was a significant positive relationship between food addiction and degree of nicotine dependence. Alcohol use exhibited a significant negative relationship with premeditation and perseverance subscales. Furthermore, there is a significant positive correlation between alcohol consumption and the degree of nicotine dependence.
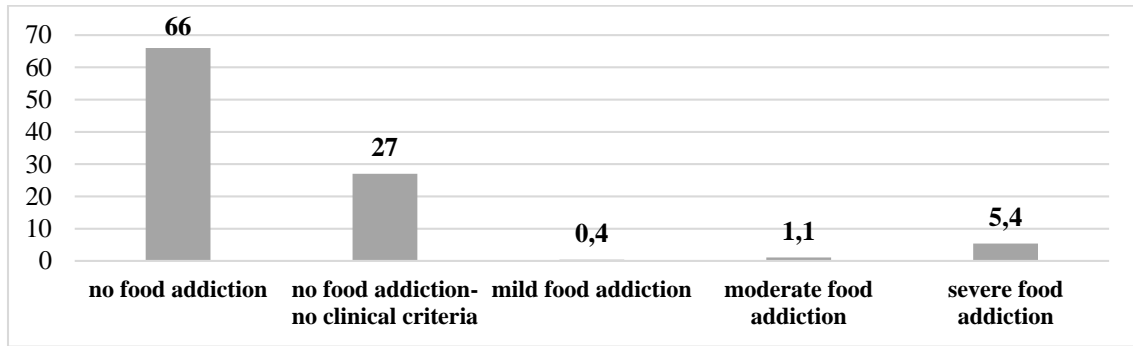
Figure-3. Rates of participants' food addiction diagnosis (%)

Table-2. Correlations between Variables.

Variables	1	2	3	4	5	6	7	8	9	10
1. Age	-									
2. Sex	-.138**	-								
3. BMI	.113**	-.077	-							
4. YFAS	-.052	.073	.032	-						
5. UPPS-premeditation	.049	-.034	-.010	-.137**	-					
6. UPPS-urgency	-.078	.044	-.024	.345**	-.205**	-				
7. UPPS-sensation seeking	-.051	-.164**	-.032	.163**	.090*	.268**	-			
8. UPPS-perseverance	.065	.001	.021	-.086*	.522**	-.257**	.164**	-		
9. FTND	.155	-.326**	-.018	.241**	-.009	-.043	-.008	-.002	-	
10. AUDIT-C	.194*	-.317**	-.004	.082	-.260**	-.049	-.046	-.245**	.304**	-
M	21.74	0.79	22.71	1.83	35.56	25.91	34.69	29.22	2.80	2.94
SD	4.576	0.41	8.04	2.76	5.09	6.92	7.53	5.12	2.52	2.32

Note. * $p < .05$; ** $p < .01$; **Sex:** 0: Male 1: Female, **BMI:** Body Mass Index, **YFAS:** Yale Food Addiction Scale, **UPPS:** Impulsive Behavior Scale, **FTND:** Fagerström Test for Nicotine Dependence, **AUDIT-C:** Alcohol Use Disorder Identification Test-Short form

Figures-4 and 5 illustrate the distribution of degree of nicotine dependence and alcohol consumption by sex. Furthermore, multivariate analysis of covariance (MANCOVA) was employed to ascertain whether there was a statistically significant difference between female and male scores on the AUDIT-C and FTND, controlling for participants' scores on the UPPS impulsive behavior. Consequently, the dependent variables were the degree of nicotine dependence and alcohol consumption, the independent variable was sex, and the covariate was impulsive behavior (see Table-3). The MANCOVA analysis revealed a statistically significant correlation between the degree of nicotine dependence ($F(1, 74) = 4.547$, $p < .05$, $\eta_p^2 = .06$) and alcohol use ($F(1, 74) = 25.427$, $p < .001$, $\eta_p^2 = .26$) among the youth. The male participants exhibited a higher degree of nicotine dependence ($M = 4.18$, $SD = 2.78$) and alcohol use ($M = 4.03$, $SD = 2.91$) than the female participants ($M = 2.50$, $SD = 2.36$; $M = 2.50$, $SD = 1.76$).

Findings on Degree of Nicotine Dependence and Alcohol Consumption By Sex
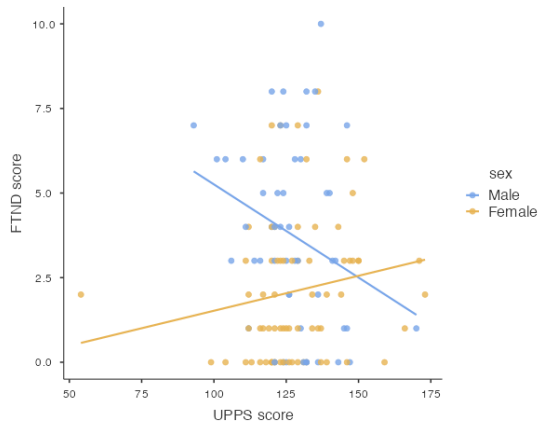
Figure-4. Scatter plot for FTND scores
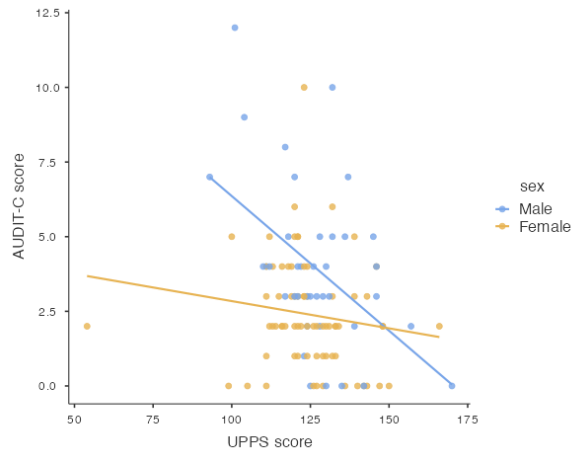
Figure-5. Scatter plot for AUDIT-C scores

Table-3. Findings on MANCOVA Analysis

	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	*p*	Partial Eta Squared (η_p^2)
Corrected model	FTND	83.762[a]	3	27.921	4.452	.006	.153
	AUDIT-C	111.734[b]	3	37.245	7.986	.000	.245
Intercept	FTND	28.516	1	28.516	4.547	.036	.058
	AUDIT-C	118.581	1	118.581	25.427	.000	.256
Sex	FTND	39.681	1	39.681	6.327	.014	.079
	AUDIT-C	35.721	1	35.721	7.660	.007	.094
UPPS	FTND	3.246	1	3.246	.518	.474	.007
	AUDIT-C	55.942	1	55.942	11.996	.001	.139
Sex * UPPS	FTND	29.723	1	29.723	4.739	.033	.060
	AUDIT-C	26.785	1	26.785	5.743	.019	.072
Error	FTND	464.084	74	6.271			
	AUDIT-C	345.099	74	4.664			

a. R Squared = .153 (Adjusted R Squared = .119)

b. R Squared = .245 (Adjusted R Squared = .214)

Table-4. Direct and Indirect Effects of Cigarette Use on Food Addiction.

	Unstand.	SE	*t*	*p*	LLCI	ULCI
Total Effect	0.3216	0.0953	3.3748	**0.01***	0.1329	0.5102
Direct Effect (*c'*-path)	0.3237	0.0946	3.4201	**0.0009***	0.1262	0.4603
Covariates						
Sex	1.0563	0.4825	2.1893	**0.0305***	0.1010	2.0117
BMI	0.0120	0.0181	0.6654	0.5071	-4.6987	-1.4673
Indirect Effect (a*b-path)	**Unstand.**	**BootSE**			**BootLLCI**	**BootULCI**
	0.0021	0.0135	-	-	0.0307	0.0263

Notes. *p<.05

Cigarette use (independent), impulsive behavior (mediator), food addiction (dependent)

cov= c("sex", "BMI"), **LLCI:** Lower limit confidence interval, **ULCI**: Upper limit confidence interval **Unstand.:** Unstandardized regression coefficient, **SE:** Standard error.
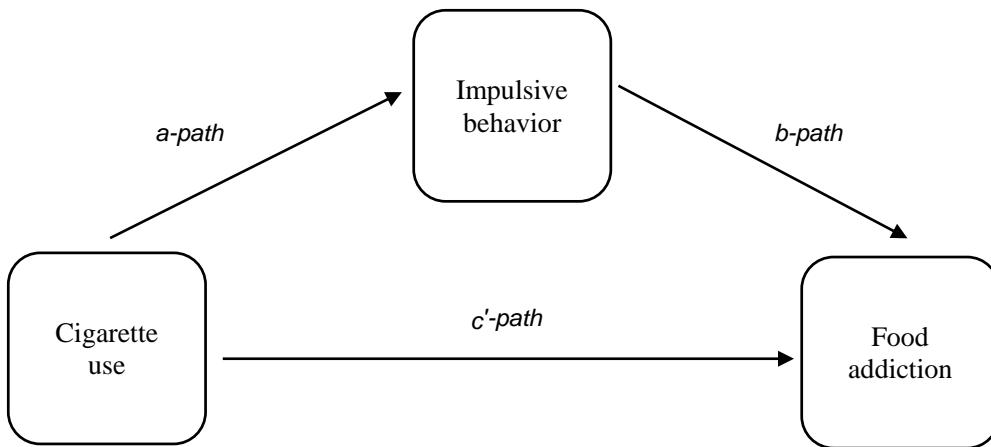
Figure-6. The mediating role of impulsive behavior on food addiciton in cigarette use (***a*b:***indirect effect, ***c'-******path:***direct effect)
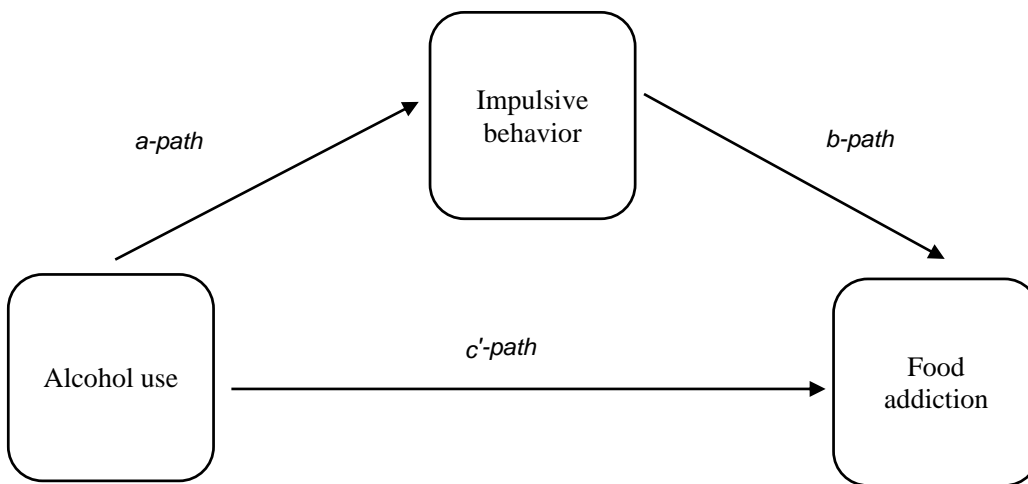
Figure-7. The mediating role of impulsive behavior on food addiction in alcohol use (***a*b:*** indirect effect, ***c'-path:*** direct effect)

Table-5. Direct and Indirect Effects of Alcohol Use on Food Addiction.

	Unstand.	SE	*t*	*p*	LLCI	ULCI
Total Effect	0.1459	0.1334	1.0938	0.2765	-0.1186	0.4104
Direct Effect (*c'*-path)	0.1895	0.1392	1.3611	0.1764	-0.0866	0.4656
Covariates						
Sex	0.6987	0.6522	1.0713	0.2865	-0.5945	1.9919
BMI	0.0100	0.0225	0.4446	0.6575	-0.0346	0.0546
Indirect Effect (a*b-path)	**Unstand.**	**BootSE**			**BootLLCI**	**BootULCI**
	-0.0436	0.0408	-	-	-0.1216	0.0440

Notes. *p<.05

Alcohol use *(independent),* ***impulsive behavior*** *(mediator),* ***food addiction*** *(dependent)*

***cov= c("sex", "BMI")* ,** **LLCI:** Lower limit confidence interval, **ULCI**: Upper limit confidence interval
Unstand.: Unstandardized regression coefficient, **SE:** Standard error.

Findings on Mediated Regression Analysis

The variables are presented in Figures-6 and 7 in accordance with Model 4, developed by Hayes (37). The dependent variable is food addiction, which is represented by the total score on the YFAS, which encompasses 11 criteria as outlined in the DSM-5. The independent variable is the total score on the FTND for the degree of nicotine dependence and the AUDIT-C for alcohol. The mediating variable is impulsive behavior, as indicated by the UPPS scores. As illustrated in Figures 5 and 6, the impact of nicotine dependence and alcohol consumption on the UPPS impulsive behavior score (a-path), the influence of the UPPS impulsive behavior score on nicotine dependence and alcohol use (b-path), and the effect of nicotine dependence and alcohol on food addiction (c′-path) were analyzed. Sex and BMI variables were also included in the analysis as covariates.

The degree of nicotine dependence, sex, and BMI variables did not have a statistically significant effect on UPPS impulsive behavior scores ($F_{3.121}$=0.0995, R^2=0.0025, p>.05). Therefore, the model (a-path) is not significant. The degree of nicotine ($β$=0.0953, p<.05) and sex ($β$=0.4854, p<.05) were found to have a significant effect on the YFAS scores. Consequently, the model (c′-path) was found to be significant ($F_{3.121}$=4.3686, R^2=0.0977, p<.01). Furthermore, the effect of the mediator and other variables on the YFAS scores was found to be significant ($F_{3.121}$=3.9862, R^2=0.1173, p<.01). The degree of nicotine dependence ($β$=0.0946, p<.001) and sex ($β$=0.4825, p<.05) were found to have a statistically significant effect on food addiction scores. The effect results regarding the mediating role of UPPS impulsive behavior are also presented in Table-4. Accordingly, the indirect effect (a*b-path) is significant, as evidenced by the bootstrap confidence interval, which does not include zero (y=.0021, SE=.0135, 95%CI [.0307, .0263]).

The results of the analysis of the direct and indirect effects of alcohol consumption on food addiction are presented in Table-5. No statistically significant difference was identified ($F_{4.105}$=0.5866, R^2=0.0163, p>.05).

DISCUSSION

The objective of this study was to examine the degree of nicotine dependence and alcohol consumption, as well as food addiction, in young people. Additionally, the study aimed to investigate the relationship between the degree of nicotine dependence and alcohol consumption of young people and food addiction and to assess whether the level of impulsive behavior plays a mediating role in this relationship. The findings of this study indicate that sex has a significant effect on alcohol consumption and degree of nicotine dependence, with these effects varying depending on impulsive behavior characteristics. The results of the mediated regression analysis also demonstrate that the degree of nicotine dependence has a significant effect on food addiction depending on impulsive behaviors, although this effect is not significant for alcohol use.

Tobacco use represents one of the most significant public health concerns globally. The World Health Organization (2022) reports that tobacco is the leading cause of preventable mortality, killing more than 8 million people annually. Of these deaths, over 7 million are attributed to direct tobacco use, while an additional 1.2 million are attributable to second-hand smoke exposure (38). The use of tobacco in the form of cigarettes or in various other forms results in the development of an addiction pattern, which is dependent on the duration and quantity of use. It is well-established that nicotine is the primary factor in the formation of this pattern (39). The present study indicates that the prevalence of smoking and alcohol consumption among the participants was 21.2% and 15.8%, respectively.

A considerable number of studies have been conducted on the issue of smoking among university students. For instance, a study conducted among undergraduate students in Greece reported a tobacco use rate of 35% (40). Another study conducted in Türkiye revealed that 23.4% of university students exhibited mild nicotine dependence, 2.8% exhibited moderate dependence, and 3.4% exhibited severe dependence. The same study calculated that the rate of risky alcohol consumption was 2.5%, risk-free alcohol consumption was 23.4%, and alcohol dependence was 2% (41).

Ethyl alcohol is the most commonly abused psychostimulant worldwide, following caffeine and tobacco. (39). The World Health Organization (WHO) has determined that the average value of pure alcohol consumption per

capita per year is 12.33 for France, 12.91 for Germany, 11.19 for Russia, 9.87 for the United States and that this value for our country is calculated at 1.4 liters for 2018 (42). A study conducted in Italy revealed that 19% of university students exhibited risky alcohol use (44). In a recent study in Türkiye, Atlam et al. (45) calculated the rate of risky alcohol use as 12% in a sample that included different segments of society (including university students). Although these data and other similar findings (including our own) were obtained through self-reporting, it can be said that the rates are remarkable.

It is well-established that sex plays a significant role in the consumption of tobacco and alcohol. Our study revealed that female and male young people exhibited distinct patterns of nicotine dependence and alcohol use, which were dependent on impulsive behavior. A study conducted on university students in our country revealed that the frequency of smoking and alcohol use was higher in males than in females, a finding that aligns with our own (46). These and similar findings suggest that males and young people may be at an elevated risk for legal substance use. In addition to substance use, the dietary habits of young people also warrant investigation. The consumption of food and drink outside the home has become a popular and alternative way of eating. Research has demonstrated that 2.5 billion people worldwide consume what is commonly referred to as "street food" (47). A report covering the years 2015-2018 in the US indicated that the rate of fast food consumption among children and adolescents on a given day was 36.3% (49). A further report published in the United States covering the years 2013-2016 indicates that 36.6% of adults consume fast food on a given day. Men consume fast food at a higher rate than women, with 37.9% of men consuming fast food on a given day compared to 35.4% of women (48).

In our country, there is a great deal of interest in the eating behavior of individuals and, consequently, weight gain. This has led to a plethora of studies being conducted in the literature. In particular, these studies focus on obesity. A review of the general statistics (TÜİK, 2019) reveals that, based on body mass index calculations, the prevalence of obesity among individuals aged 15 years and above in Turkey was 19.6% in 2016, with this figure increasing to 21.1% in 2019. In terms of gender, it has been demonstrated that there is a greater prevalence of obesity among females than males (female; 24.8%, male; 17.3%). It has been reported that obesity and overweight have become a global epidemic in both adults and children (15,50). One of the factors thought to be contributing to this epidemic is the easy accessibility of excessively fatty, salty, or sugary foods. However, it has been suggested that there are other factors influencing this epidemic (51).

The diagnostic process for food addiction, as with substance use disorders, includes criteria such as continued use despite negative consequences and repeated unsuccessful attempts to reduce consumption (17). In our study, food addiction was calculated using the YFAS scale (v.2.0). It was found that 5.4% of the adolescents met the diagnostic criteria for severe food addiction. A considerable body of research utilizing the YFAS scale is available in the literature (22–24). The findings of these studies indicate that the group with the highest consumption of processed foods exhibits more problematic eating behaviors than the group with the lowest consumption of processed foods (e.g., vegetables, fruit, protein).

A study conducted in Poland among individuals aged 18 to 82 years revealed that individuals in the younger age group and those with a higher body mass index (BMI) exhibited a greater preference for unhealthy foods, including crisps, salty snacks, fast food, and sugary fizzy drinks (52). A study conducted with university students in Istanbul reported a food addiction rate of 21.7% (n=65) and identified a significant relationship between food addiction and the level of impulsivity (53). In a further study conducted among university students, it was reported that 3.4% of the students were in the high-risk group in terms of food addiction (54). Given that impulsivity is a factor thought to be associated with alcohol/substance use (55, 56) and eating behavior (57), this study also investigated the participants' levels of impulsivity.

It has been proposed that there are parallels between drug addiction and food addiction, with changes and activation observed in certain brain regions. In this context, neurobiological evidence is particularly pertinent. Consequently, research examining the interrelationships between substance abuse issues and food addiction at various levels is crucial. For instance, a study conducted on individuals aged 14-21 (2017) revealed a significant positive correlation between smoking, alcohol consumption, substance use, and food addiction (58). In the

present study, we employed a mediation analysis approach, utilizing Hayes' Model 4, to examine the relationship between nicotine dependence and alcohol consumption and their respective effects on impulsive behavior. Our findings revealed that the direct and indirect effects of nicotine dependence on food addiction were statistically significant, whereas the direct and indirect effects of alcohol consumption on food addiction were not. In particular, our results indicate that the cultural characteristics of the sample studied may also be an effective variable. The fact that this relationship, which is clear for the degree of nicotine dependence, is not found for alcohol consumption may also be explained by the generally accepted patterns of alcohol consumption in the province of Sivas.

A significant finding of our study is that certain psychological characteristics of individuals were considered. In our study, the level of impulsive behavior in young people was found to be associated with legal substance use and food addiction. Appropriate statistical models were used to analyze the data. Previous studies (59, 60) have also highlighted the importance of impulsivity in eating behavior. These studies have suggested that individuals with high levels of impulsivity may have a lower ability to control their eating behavior than those with lower levels of impulsivity. However, further research is needed to confirm this association.

Limitations

The most significant limitation of our research is the potential for social desirability. Although this effect has been the subject of debate, the use of questionnaires and scales to obtain information from individuals can provide accurate and reliable data to some extent. Furthermore, the fact that the sample was selected using non-probabilistic methods may have influenced participants' motivation to participate in the research, with some individuals being more likely to volunteer than others. For instance, individuals with higher levels of smoking and/or alcohol consumption may have chosen not to participate in this research. Consequently, in subsequent studies where university students are selected as the target sample group, these limitations can be mitigated by employing more systematic sampling methods. In summary, it should be noted that sampling was not possible in this study, and therefore, the findings cannot be generalized to the entire population.

Furthermore, it is important to acknowledge that most of our research sample is female, which may influence the interpretation of the findings.

CONCLUSION

The findings of our study indicate that legal substance use and food addiction are prevalent among young people. Furthermore, our results demonstrate a significant correlation between the degree of nicotine dependence and food addiction, which can be attributed to impulsive behaviors. The abuse of substances or drugs is regarded as a social, security, physical, and mental health problem. There is a wealth of evidence indicating that the use of drugs at an early age can result in a high-risk clinical and psychosocial profile in the future (61). It is, therefore, necessary to identify other factors that may increase or encourage substance use behaviors among young people and to plan preventive research on these factors. At times, eating behavior and substance use can be seen as behaviors that trigger each other and/or occur together. Further research, including the use of different techniques, is required in order to obtain more concrete and reliable information on this subject.

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Assessment of serum bisphenol a levels in autoimmune thyroiditis: a case-control study

Otoimmün tiroidit'te serum bisfenol a düzeylerinin değerlendirilmesi: bir vaka-kontrol çalışması

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ABSTRACT

Aim: The precise mechanism underlying autoimmune thyroiditis is still unclear. The female preponderance suggests the role of estrogens in autoimmunity, and there is strong evidence about this role. Bisphenol A (BPA), a widely used endocrine disruptor, may have estrogenic effects and affect autoimmunity through estrogenic and non-estrogenic mechanisms. We aimed to determine the relationship between serum BPA levels and thyroid autoimmunity.

Materials and Methods: We performed a cross-sectional case-control study involving 143 autoimmune thyroiditis patients and 95 age-matched healthy controls. Serum BPA levels were measured using ELISA method.

Results: BPA levels were not significantly different between autoimmune thyroiditis and control groups. The percentage of antibody-positive patients, thyroid function, thyroid antibody levels, or BMI were not different between BPA quartiles. Also, there was no correlation between BPA levels and thyroid autoantibody levels.

Conclusion: Serum BPA levels were not significantly different in autoimmune thyroiditis. Also, we could not show any relationship between BPA, thyroid function tests, and thyroid antibody levels.

Keywords: Autoimmune thyroiditis, Bisphenol A, Endocrine disruptors

ÖZ

Amaç: Otoimmün tiroiditin altında yatan kesin mekanizma hala belirsizdir. Kadınların baskın olması, östrojenlerin otoimmünitedeki rolünü düşündürmektedir ve bu rol hakkında güçlü kanıtlar bulunmaktadır. Yaygın olarak kullanılan bir endokrin bozucu olan Bisfenol A (BPA), östrojenik etkilere sahip olabilir ve östrojenik ve östrojenik olmayan mekanizmalar aracılığıyla otoimmüniteyi etkileyebilir. Serum BPA düzeyleri ile tiroid otoimmünitesi arasındaki ilişkiyi belirlemeyi amaçladık.

Gereç ve Yöntem: 143 otoimmün tiroidit hastası ve 95 yaş eşleştirilmiş sağlıklı kontrol içeren kesitsel bir olgu-kontrol çalışması gerçekleştirdik. Serum BPA düzeyleri ELISA yöntemi kullanılarak ölçüldü.

Bulgular: BPA düzeyleri otoimmün tiroidit ve kontrol grupları arasında anlamlı ölçüde farklı değildi. Antikor pozitif hastaların yüzdesi, tiroid fonksiyonu, tiroid antikor düzeyleri veya BKİ açısından BPA dörtlükleri arasında fark saptanmadı. Ayrıca, BPA düzeyleri ile tiroid oto antikor düzeyleri arasında bir korelasyon yoktu.

Sonuç: Serum BPA düzeyleri otoimmün tiroiditte önemli ölçüde farklı değildi. Ayrıca, BPA, tiroid fonksiyon testleri ve tiroid antikor düzeyleri arasında herhangi bir ilişki gösteremedik.

Anahtar Sözcükler: Otoimmün tiroidit, bisfenol A, endokrin bozucular.

INTRODUCTION

Autoimmune thyroid disease is the most prevalent cause of hypothyroidism in iodine-sufficient areas. It is characterized by the presence of thyroid autoantibodies, mainly anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg). The precise mechanism underlying autoimmune thyroid disease is unclear. Both genetic and environmental factors are implicated in pathogenesis. Autoimmune thyroid disorders demonstrate a female preponderance, suggesting the role of estrogens in autoimmunity. Immune cells express estrogen receptors. There is compelling evidence of the relationship between estrogen and autoimmunity via diverse and complex modulatory and stimulating roles (1). This cascade of complex interaction of hormones and environmental factors in genetically susceptible individuals leads to autoimmune disorders, including autoimmune thyroiditis (2).

Bisphenol A (BPA) is widely used in plastics (3). Its widespread use results in high levels of exposure in humans and animals (4). It can bind to estrogen receptors and exert estrogenic or anti-estrogenic effects (5). Enhancement of the immune-stimulating effects of the estrogens. T cell aggregation, effects on prolactin levels and cytochrome P450, and promoting reactive oxygen species formation are the other components of BPA induced autoimmunity(6).

Given the higher prevalence of autoimmune thyroid disease in women, the potential role of BPA's estrogenic effects in its pathogenesis is a compelling area of study. Our aim was to assess serum BPA levels in autoimmune thyroiditis and control subjects, with the potential to reveal intriguing implications for this relationship.

MATERIALS and METHODS

Ethics statement

The study was rigorously approved by the local ethics committee of Ege University Faculty of Medicine with approval number 13-11/13 at 04/12/2013. All the participants provided oral and written informed consent, and we ensured that all study steps were in strict alignment with the Declaration of Helsinki Principles revised in 2008, instilling confidence in the study's integrity.

Subjects and study design

This is a cross-sectional study including 143 patients with autoimmune thyroiditis and 95 age-matched thyroid autoantibody-negative healthy controls. Autoimmune thyroiditis was diagnosed in patients with anti-TPO and/or anti-Tg antibody positivity and sonographic findings of thyroiditis (i.e., hypo-echogenicity and heterogeneity of the parenchyma). The patients under levothyroxine replacement treatment were included as long as their thyroid hormones were in the euthyroid range. Subjects negative for anti-thyroid antibodies and normal sonographic appearance of the thyroid were recruited as controls. Subjects with overt hypo/hyperthyroidism, ALT/AST levels over three times the upper limit of normal, estimated glomerular filtration rate under 60 ml/min/1.73 m^2, pregnancy, oral contraceptive use, acute systemic illness, and active malignancy were excluded. A detailed medical history and thorough physical examination were performed on all participants. Height (centimeters) and weight (kilograms) were measured. We calculated body mass index (BMI) by dividing weight (kg) by square meter of height (m^2). Circulating levels of TSH, Free Triiodothyronine (FT3), Free thyroxine (FT4), anti-TPO, and anti-Tg were measured using immunochemical methods (Beckman Coulter DXI 800, USA)

The venous blood of both the patient and control groups was drawn following an overnight fasting. The serum was separated and stored at -80°C. Using a competitive ELISA method, Serum BPA levels were determined using a commercial kit (IBL Co., Ltd., Gunma, Japan). Spectrophotometric measurement was performed at a wavelength of 450 nm using the Thermo Scientific Multiskan GO model ELISA reader (Finland). The absorbance curves were used to determine BPA concentrations. The results were expressed in ng/mL.

Statistical analysis

IBM SPSS version 25.0 (Chicago, IL, USA) was used for statistical analysis. Descriptive statistics of continuous variables were reported as median (interquartile range-IQR) and mean ± standard deviation (SD), frequencies and percentages were provided for categorical variables. For comparison of two continuous variables Mann-Whitney *U* or Student's *t-test* was used while for more than two variables, one-way ANOVA or Kruskall-Wallis test was employed. Depending on distribution, Spearman's rank or Pearson's correlation coefficient was used for correlations. We performed logistic regression analysis to investigate the relationship between autoimmunity status and BPA. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 238 age and BMI matched subjects were included in the study (26 Male/ 212 Female). One hundred forty-three (60.1%) of the patients were antibody positive while 95 (39.9%) were controls. The groups were not different in terms of thyroid hormone levels. BPA levels were not significantly different between autoimmune thyroiditis and control groups (p=0.998) (Table-1).

When the groups were compared by their levothyroxine replacement status, there was no significant difference in terms of BPA and thyroid function tests. Patients were grouped by their BMI (<25, 25-30, and >30 kg/m2), and there was no significant difference in BPA levels (data not shown).

Only a minority of the patients were male (10.1%). There were no significant differences in BPA levels between male and female groups (p=0.086). Likewise, thyroid function tests and thyroid antibody levels were similar according to gender.

No significant differences were detected in terms of thyroid function tests, thyroid autoantibody levels, age, or BMI between BPA quartiles (Table-2). Also, the ratio of antibody-positive subjects was not different between BPA quartiles (p=0,87).

There was no correlation between the levels of BPA and anti-Tg, anti-TPO, FT3, FT4, TSH, age, and BMI in autoimmune thyroiditis and control groups (Table-3).

Logistic regression analysis with a full model including age, BMI, BPA, TSH, FT4, and FT3 as independent variables and autoimmunity presence as dependent variable, the final model was significant for only TSH with an OR of 1.2 (%95 CI 1.01-1.42 p=0.04).

Table-1. Comparison of the demographic and laboratory characteristics of the subjects.

Variables	Autoimmune thyroiditis n=143	Controls n=95	P
Sex, F/M	14/129	12/83	0.529
Age, years	42.57 ± 11.86	44.53 ± 13.26	0.666
BMI, kg/m²	27.61 ± 4.97	27.23 ± 5	0.512
TSH(mU/L)	2.00 (2.4)	1.48 (1.82)	0.073
fT4(ng/dL)	0.86 (0.23)	0.87 (0.19)	0.943
fT3(ng/L)	2.92 (0.48)	2.93 (0.54)	0.183
Anti-T(IU/mL)	4.7 (75.9)	0.20 (0.95)	<0.001*
Anti-M(IU/mL)	255.3 (617.9)	0.70 (1.1)	<0.001*
BPA(ng/mL)	1.13 (62.74)	0.99 (65.81)	0.998

Table-2. Comparison of groups according to BPA Quartiles.

	p	Q1	Q2	Q3	Q4
BPA	<0.001	0.37(0.09)	0.84 (0.19)	1.85 (18.43)	85.6 (20.69)
Age	0.964	43.3 ± 10.91	42.67 ±12.18	44.69 ±12.88	42.78 ±14.07
BMI	0.767	27.63 ±5.84	27.88 ±4.72	26.96± 4.75	27.27± 4.58
TSH	0.511	1.87 (2.26)	1.78 (2.5)	1.46 (1.74)	1.91 (2.97)
FT4	0.63	0.82 (0.21)	0.87 (0.23)	0.88 (0.23)	0.87 (0.18)
FT3	0.91	2.97 (0.44)	2.92 (0.49)	2.97 (0.59)	2.9 (0.5)
Anti M	0.63	17.3 (273.7)	19.4 (265.5)	12.4 (297.2)	40.4 (642.8)
Anti T	0.99	0.5 (4.8)	1.4 (26.8)	1 (18.75)	1.4 (26.5)

Table-3. Evaluation of the relation between BPA and the other demographic and laboratory variables.

	Control		Autoimmune thyroiditis	
	r	p	r	p
BMI	-0.099	0.354	0.071	0.438
TSH	-0.092	0.373	0.071	0.402
fT4	0.149	0.150	-0.050	0.550
fT3	-0.139	0.183	-0.016	0.847
Anti-T	-0.187	0.071	0.071	0.404
Anti-M	0.128	0.290	0.103	0.223

DISCUSSION

The potential effects of BPA on autoimmunity, either through estrogenic or direct effects, have drawn attention to possible pathogenetic effects on autoimmune thyroiditis. However, the current literature has revealed inconsistent results. We couldn't demonstrate any significant difference between autoimmune thyroiditis and control groups as regards serum BPA levels. Also, we failed to show any association between serum BPA levels and antibody titters or antibody positivity. There was no difference in BPA, thyroid function tests and thyroid antibodies in terms of gender.

Chailurkit et al were the first to evaluate BPA in autoimmune thyroiditis. They found that increasing BPA quartiles were accompanied by an increasing proportion of antibody-positive patients. They also found that the BPA quartile, age, and gender were independent determinants of anti-TPO antibody presence (7). Nevertheless, Choi et al. could not reveal any association between thyroid antibodies and urinary bisphenol A (8). Recently, in line with the aforementioned results, Yuan et al. failed to demonstrate any association between urinary BPA and thyroid autoantibodies in women of childbearing age (9).

Conflicting results may arise for various reasons, such as the study population, the detection method for BPA, and the stability and half-life of BPA. Different detection methods may yield different results. Also, the stability of phenolic compounds varies according to the type of sample, such as urine or blood, and storage method (4). ELISA method is less sensitive than liquid chromatography-mass spectrophotometry (LC-MS) technique. (3, 10) Because of their relatively short half-life, serum BPA levels may be less reliable compared to urinary BPA for reflecting daily BPA exposure. Somehow, all of

our study participants had detectable BPA serum levels which indicates the widespread exposure to BPA in the community.

The urinary BPA levels have been found to be 42 times higher than the serum levels. Also, the timing of the urine or blood sampling is important in relation to meals and the BPA content of the meal. Albeit, serum BPA may be more accurate in reflecting tissue concentrations, so it should not be entirely overlooked (11). However, there is still controversy surrounding the clinically significant levels of BPA. Some studies suggest that levels in the nanomolar range are enough to exert its effects (12), (13)

Apart from thyroid autoimmunity, BPA affects the thyroid in other aspects as well. Kitamura et al stated that BPA acts as a thyroid hormone agonist (14) on the contrary, others showed antagonistic effects on thyroid hormone receptors (TR) (15, 16) Some in vitro studies suggest that BPA causes thyroid disruption at the translational level by upregulating or down-regulating the genes involved in thyroid hormone synthesis or their transcription factors (17).

A recent meta-analysis showed a positive correlation between FT4 levels and BPA levels in females (18). On the other hand, some other studies failed to indicate any association between BPA and thyroid function (19, 20). In our study, we could not define any differences in thyroid hormone levels between autoimmune thyroiditis and control groups. Also, thyroid hormones were not significantly different among BPA quartiles. There was no correlation between BPA levels and TSH, FT4, and FT3 levels.

A recent study examined the relationship between serum and urinary BPA and analogs and thyroid volume and function. They failed to show any relationship between thyroid volume and function and serum and urine levels of

bisphenols except bisphenol C (BPC) (21). Choi et al. demonstrated that in patients with thyroid autoantibody positivity, the risk of thyroid nodules increased with increasing urinary BPA levels (8) . In our study, we did not have access to thyroid volume data of all patients, but the nodule prevalence was not different between BPA quartiles (data not shown).

Epidemiological studies to evaluate the effects of BPA on thyroid function are limited for several reasons. BPA exposure begins in utero and continues for a lifetime. BPA exposure during the early stages of life may have a transgenerational effect, predisposing the subsequent generations to the risk of BPA-related disease. Since autoimmunity is a lifelong process, it is hard to build a cause-effect relationship between BPA and thyroid autoimmunity. Another obstacle to EDC studies is their lipophilic nature, leading them to accumulate on tissues, which eventually results in leakage from deposits and longstanding toxicity.

Main Points

Although we could not define any relationship between serum BPA levels and autoimmunity, it is hard to draw firm conclusions with EDC studies for several reasons. One of which is the multiple endocrine disruptor exposure in a time. Secondly, they exert their effects for an extended period of time due to their lipophilic nature and possible epigenetic effects. Hence epidemiological and prospective studies are hard to perform.

CONCLUSION

In conclusion, our study did not show any difference in BPA serum levels between autoimmune thyroiditis and control groups. Moreover, we did not determine any correlation between BPA and thyroid autoantibodies and thyroid function tests. Future studies are needed to determine the effects of endocrine disruptors on the thyroid, including novel biomarkers to detect tissue effects and studies including various endocrine disruptors to ascertain possible synergistic effects on autoimmunity or thyroid disruption.

Conflict of interest: The authors declare that they have no conflict of interest.

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Cilde uygulanan ağrılı elektrik uyarısıyla üst ekstremite kaslarında oluşan eşzamanlı refleks yanıtların elektromiyografi ile incelenmesi

Examination of simultaneous reflex responses in the upper extremity muscles by recording electromyography with painful electrical stimulation on the skin

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ÖZ

Amaç: Bu araştırmada, sağlıklı bireylerde ağrılı elektriksel uyarımın bilateral *m. brachioradialis* kasındaki refleks yanıtını klasik analiz ve frekans analiz yöntemlerini birlikte kullanarak incelemek amaçlanmıştır.

Gereç ve Yöntem: Dokuz sağlıklı katılımcının dominant el sırtına ağrılı elektrik uyaranı verilerek tek motor ünite ve yüzeyel elektromiyografi kayıtları alındı. Elektrik uyarısı 0,2 ms süreli kare puls şeklinde, 1-2 saniye aralıklarla rastgele uygulandı. Uyarıdan 400 ms öncesi ve 500 ms sonrasındaki kısmın ortalaması alındı. Kayıtlarda 16 motor ünite seçilerek analizi yapıldı. Yüzeyel elektromiyografi, peristimulus time histogram ve peristimulus frekansgram analizlerinin kümülatif toplam grafiklerinden refleks latans ve süre bilgileri elde edildi.

Bulgular: Dominant kolda motor ünitelerin hepsinde kütanöz sessiz periyot gözlemlendi. Peristimulus frekansgram'da motor ünitelerde gözlenen inhibisyon süresi peristimulus time histograma göre 94,8 ms daha uzun olduğu görüldü (p<0,05). Histogram ve yüzeyel elektromiyografide eksitasyon görülürken, frekansgramda gözlenmedi. Dominant taraftaki kasta inhibisyon gözlemlenirken diğerinde görülmedi.

Sonuçlar: Peristimulus frekansgram yöntemi motor nöronda oluşan postsinaptik potansiyeli doğru bir şekilde gösterir. Araştırmamızda frekansgram yöntemiyle inhibisyonun uzun bulunması medulla spinaliste inhibitör devrelerin uzun süreli inhibisyon oluşturduğunu desteklemiştir. Peristimulus time histogramda görülen eksitasyon peristimulus frekansgram analizinde görülmedi. Refleks incelemelerinde klasik yöntemlere ek olarak frekansgram yönteminin de kullanılması uygun olacaktır.

Anahtar Sözcükler: Kutanöz sessiz periyot, Peristimulus time histogram, Peristimulus frekansgram.

ABSTRACT

Aim: *This study aims to investigate the reflex response in bilateral m. brachioradialis to painful electrical stimuli in healthy individuals using classical and frequency analysis methods.*

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Materials and Methods: *Records of the single motor unit and surface electromyography were obtained by administering painful electrical stimulation to the dorsal surface of the dominant hand of nine healthy participants. The electrical stimulus, delivered in the form of a 0,2 ms square pulse, was randomly applied at intervals of 1-2 seconds. Averages are computed throughout 400 ms before and 500 ms after the stimulus. Analyses were conducted by selecting 16 suitable motor units from among the recordings. Reflex latency and duration were obtained from the cumulative sum graphs of surface electromyography, peristimulus time histogram, and peristimulus frequencygram analyses.*

Results: *A cutaneous silent period was observed in all motor units of the dominant arm. According to the peristimulus frequencygram approach, the inhibition time observed in motor units was found to be 94.8 ms longer than that found by the peristimulus time histogram approach ($p<0,05$). Excitation was observed in both the histogram and surface electromyography, but not in the frequencygram. Inhibition was observed in the muscle on the dominant side, but not on the other side.*

Conclusion: *The peristimulus frequencygram method has been shown in the literature to sample the postsynaptic potential developed on a motor nöron. In our study, the finding of long inhibition with the frequencygram method has supported that inhibitory circuits in the medulla spinalis create long-term inhibition. The excitation observed in the peristimulus time histogram was not seen in the peristimulus frequencygram analysis. We recommend the use of the frequencygram method in addition to classical methods in reflex examinations.*

Keywords: *Cutaneous silent period, peristimulus time histogram, peristimulus frequencygram.*

GİRİŞ

Periferik sinirlerin elektriksel uyarımı ile elde edilen kas yanıtları sinir iletiminin değerlendirilmesi ve reflekslerin elde edilmesi amacıyla rutin bir elektrodiagnostik teknik olarak geniş çapta kullanılmaktadır (1). Ağrılı bir kutanöz uyaranın ekstremiteye uygulanması durumunda, ekstremite motor nöronları, distal kaslardan proksimal kaslara doğru giderek azalan bir şekilde inhibe olur. Bu inhibitör refleks, geri çekme refleksinin bir bileşeni olarak kabul edilir ve "kutanöz sessiz periyot" (KSP) olarak adlandırılır (2, 3). Bu refleks yanıt, ciltteki ağrı reseptörlerinin uyarılması sonucunda ortaya çıkar ve ekstremiteleri zararlı uyarandan koruyan refleks mekanizmalarının inhibisyon kısmını oluşturur (4). Klinikte ve deneysel araştırmalarda KSP; ince miyelinli, nispeten yavaş ileten, küçük çaplı Aδ liflerinin ve bunların efferent motor yollarıyla spinal oligosegmental nöronal bağlantılarının incelenmesi için kullanılmaktadır.

Diyabetik polinöropati, üst motor nöron hastalığı, huzursuz bacak sendromu gibi birçok patolojik durumlarda periferik ve merkezi yolakların iletimi araştırmalarında sessiz periyot kullanılmıştır (5-7). Ayrıca, KSP sağlıklı ve nörolojik hastalığı olan bireylerde elektriksel, lazer ve mekanik ağrılı uyarım gibi yöntemlerle birçok araştırmada incelenmiştir (7-13).

İnsan motor nöronlarından elektriksel aktivite kaydı almak invaziv olduğundan, belirli bir afferent veya kortikal yolun uyarıldığı ve bu uyarının etkilediği motor nöronların yanıtlarının kaydedildiği yöntemler kullanılmaktadır (2, 14, 15). Duyusal bir sinir lifinin uyarılmasına yanıt olarak kaslarda gelişen refleks yanıtlar, yüzeyel elektromiyografi (SEMG) ve kas içinden tek motor ünite EMG'si (single motor unit EMG, SMU-EMG) ile incelenmektedir. Motor ünite aksiyon potansiyeli; motor nöronun kas sinir kavşağı yoluyla oluşturduğu kas lifi depolarizasyonunun intramüsküler kaydedilen potansiyelidir. Kaydedilen kas refleks cevaplarının analizinde SEMG kaydından uyaran etrafında ortalama alma ve kas içi kaydından ise uyaran etrafında zaman histogramı (peristimulus time histogram-PSTH) yaygın olarak kullanılmaktadır (8, 16). Peristimulus time histogram (PSTH), uyarılan duyusal bir nöronun motor ünite üzerindeki etkisiyle meydana gelen potansiyel değişikliklerinin zamanla dağılımını gösteren bir grafiktir. Bu yöntem, sinaptik potansiyellerin tahmin edilmesinde kullanılır. Tek bir motor ünitenin uyarı öncesi veya sonrasındaki aksiyon potansiyel sayısı histogramda ilgili zaman aralığa eklenir ve bu motor nöronunun uyarılmaya verdiği yanıtın zamanlamasını

incelememize olanak sağlar. Ancak, son zamanlarda SEMG ve PSTH yöntemlerinin sayım ve senkronizasyon hataları nedeniyle refleks sürelerini doğru göstermediği belirtilmektedir. Refleks cevabın oluşturduğu uyarılmış net sinaptik potansiyelin genliği ve şekli hakkında yanıltıcı sonuçlara yol açtığı öne sürülmektedir (8, 13, 16-18). Bu klasik analiz yöntemlerinin yanı sıra, yakın dönemde frekans analizi kullanılarak peristimulus frekansgram (PSF) yöntemi geliştirilmiştir. Bu yöntem, bir motor ünitenin uyaran öncesi ve sonrası anlık frekanslarının zamanla değişimini göstermektedir. Frekans analiz yönteminin temel dayanağı, bir motor nörona giren akım ile deşarj frekansı arasında doğrusal bir ilişki olduğu gerçeğidir (19, 20). Bu deşarj frekansı, motor nöronun uyarılma seviyesini doğrudan yansıtır ve dolayısıyla motor nöron membran potansiyelini gösterir. Peristimulus frekansgram, tek motor ünitelerin anlık deşarj hızlarını uyarının etrafında zaman ekseninde gösterir (17, 21, 22). Rat beyin dilimi hipoglossal motor nöronlarında net giriş akımının zamanlamasını PSF yöntemi klasik yöntemlere kıyasla daha doğru bir şekilde belirlediğini gösterilmiştir (17, 18). Bu yönteme göre motor ünite aksiyon potansiyeli frekans değeri, motor nöron membranının o anki uyarılma düzeyini yansıtır. Bir motor nörona in vitro bir akım enjekte edildiğinde, motor nöron belirli bir frekansta boşalır. Motor nöron boşalım frekansındaki artış eksitasyonu, azalma ise inhibisyonu temsil ederken, bunları tanımlamada PSF'nin daha doğru bilgiler sağladığı bildirilmiştir (16, 17, 22). PSF analizi için en az 120 uyaran verilmesi gerekmektedir. Bu da kayıt süresinin biraz daha uzamasına neden olmaktadır (8). Refleks yanıtın genliği, latansı ve süresini belirlemek için SEMG, PSTH ve PSF grafiklerinde anlamlı ancak zor fark edilebilen değişiklikleri belirlemek için kümülatif toplam (CUSUM) grafikleri daha kullanışlı olduğu bildirilmektedir (9, 17, 23).

Bu çalışmada, sağlıklı bireylerde el cildine uygulanan ağrılı elektriksel uyarımla kaslarda oluşan refleks cevapta görülen inhibisyon (kutanöz sessiz periyot –KSP), *m. brachioradialis*'te araştırıldı. Motor nöronun gerçek sinaptik ilişkisinin mekanizmasını ve profilini doğru bir şekilde ortaya çıkartmak amacıyla klasik analiz yöntemleri yanı sıra PSF analiz yöntemi de kullanılmıştır. Çalışmamızın hipotezleri şu şekilde ortaya konmuştur: Ağrılı cilt uyarımıyla önkol kaslarında farklı analiz yöntemleriyle kontrlateral tarafta sessiz periyot

görülür. İnhibisyon süresi SEMG ve PSTH-CUSUM ile hesaplanandan daha uzundur. PSTH-CUSUM grafiğinde eksitasyon olarak gözlenen potansiyel gerçekte inhibisyonun devamıdır. Ağrı reseptörleri ile motor sinir arasındaki bağlantılar frekansgram ile daha doğru bir şekilde belirlenir. Her iki üst ekstremitede *m. brachioradialis*'lerden eşzamanlı olarak SEMG ve SMU-EMG kayıtları alınan ve bu kayıtları PSF analiz yöntemiyle inceleyen bir çalışmaya literatürde rastlanmamıştır.

GEREÇ ve YÖNTEM

Bu deneysel araştırma, İzmir Katip Çelebi Üniversitesi Klinik Araştırmalar İlaç Dışı Uygulamalar Etik Kurulu'nun 15.12.2022 tarihli ve 71 karar No'lu onayı ile gerçekleştirilmiştir. Araştırma, 2023 yılının Şubat-Aralık ayları arasında, İzmir Katip Çelebi Üniversitesi Tıp Fakültesi Biyofizik Anabilim Dalı laboratuvarında yürütülmüştür.

Katılımcılar

Bu çalışma, herhangi bir nörolojik veya kas hastalığı olmayan, psikoaktif ilaç kullanmayan, 1 kadın ve 8 erkek olmak üzere toplam 9 sağlıklı bireyden elde edilen 16 motor ünitenin analiziyle gerçekleştirilmiştir. Katılımcılar, deneyden 12 saat önce analjezik ilaç ve 72 saat önce antikoagülan ilaç kullanmamaları, deneyden 6 saat önce ise kahve içmemeleri konusunda uyarıldılar ve yaş ortalaması 38,25±9,2 idi. Çalışmanın amacı ve yöntemi hakkında katılımcılara bilgi verilmiş ve yazılı onamları alınmıştır.

Kayıt

Yüzeyel EMG kayıtlarında, her bir kas için bir çift tek kullanımlık, kendiliğinden yapışan Ag-AgCl yüzeyel EMG kayıt elektrotları kullanılmıştır. Bireyin omzu nötr pozisyonda ve dirseği 90 derece fleksiyonda iken, ön kolu araştırmacı tarafından sabitlendi. Bu pozisyonda kişinin ön kolunu fleksiyona getirmesi istenerek *brachioradialis* kası belirlendi (24). Kas konumunun belirlenmesi sonrası kasın en geniş kısmı hizasındaki cilt alkolle silindi ve cilt zımparasıyla hafifçe zımparalandı, yüzeyel elektrotlar kas üzerine 2 cm aralıklarla yerleştirildi. Elektrotlar ve cilt arasındaki empedansın 5 kΩ'nun altında olmasına dikkat edildi. Dudağa takılan bir "lip clip" elektrot toprak elektrodu olarak kullanıldı. Tek motor ünite EMG (SMU-EMG) kayıtlarında, kas aktivitesi bir çift bipolar intramüsküler elektrot kullanılarak

kaydedildi. İntramüsküler elektrotlar 100 μm çapında, 30 μm teflon kaplı gümüş tellerin (Ag3T, Leico Industries, NJ, ABD) teflon tabakası telin ucundan 1 mm kadar sıyrıldı (bir motor ünitenin aktivitesini kaydedebilmesi için) ve 25G iğne içine yerleştirildi ve uç kısmı kıvrılarak sterilize edildi. Tel elektrotlar iki yüzeyel elektrodun ortasından 25G iğne ile kas içine yerleştirilerek iğne geri çekildi ve teller kasın içinde bırakıldı. Kayıt öncesi bireylere, kaslarını birkaç kez kasmaları istendi (5, 8, 13).

Elektrot hazırlıkları tamamlandıktan sonra katılımcılar, omuzları nötr pozisyonda ve dirsekleri 90 derece fleksiyonda tutacak şekilde üretilmiş ahşap bir düzeneği bulunan rahat bir koltuğa oturdular. El bileklerinden kol düzeneğe sabitlendi (Şekil-1).

Biyopotansiyeller yükselteçte (1902 Amplifier, Cambridge Electronic Design Limited, Cambridge, England) büyütüldü. Elde edilen tüm veriler, analog dijital çevirici cihazıyla (1401 ADC, Cambridge Electronic Design Limited, Cambridge, England), Spike 2 yazılımı (Cambridge Electronic Design Limited, Cambridge, England) kullanılarak bilgisayara kaydedildi.

Maksimum istemli kas kasılması (MVC) düzeyini belirlemek için katılımcıların *m. brachioradialis*'leri maksimum seviyede 10 saniye süreyle kasmaları istendi ve 15 saniye aralık verilerek üç kez tekrarlandı. Elde edilen kayıtlar doğrultularak ortalama genlikleri belirlendi. Tümünün ortalaması alınarak MVC genliği elde edildi (25, 26).

Daha sonra bireylerin kas reaksiyon zamanı ölçüldü. Bunun için, algı eşiğinin üç katı kadar şiddette, 5-10 saniye aralığında rastgele gelen ortalama 50 elektrik uyarısı verildi ve katılımcıdan uyarı hissedildiğinde kasını hafifçe kasması istendi (27, 28). Elde edilen SEMG sinyalleri doğrultulup, ortalamaları alınarak reaksiyon zamanı belirlendi.

Eşzamanlı sağ ve sol *m. brachioradialis*'lerden SEMG ve SMU-SEMG kaydı alındı. Gönüllüden gündelik işlerinde kullandıkları kol sorularak dominant ekstremite belirlendi. Katılımcılara ellerini orta pronasyonda tutmaları ve *m. brachioradialis*'i aktive edebilmek için kollarını dirsekten hafif fleksiyona getirmeleri istendi. Kişinin kasını motor ünite uyarılma frekansı yaklaşık 10 Hz olacak şekilde, tek motor ünite kayıtları deney boyunca ayrı bir monitörde gösterilerek görsel geri bildirim sağlandı.

Gönüllüden alınan ilk kayıttan sonra uyaran şiddetinin yeterli olup olmadığını belirlemek için, ikinci bir kayda geçmeden SEMG ortalaması alınıp, kasta inhibisyon görüldüğü değerlendirilerek diğer kayıtlara devam edildi. Motor ünitenin kaybolması ve yeni motor ünitelerin kısa süreliğine ortaya çıkması durumunda kayıt analiz için kullanılmadı.
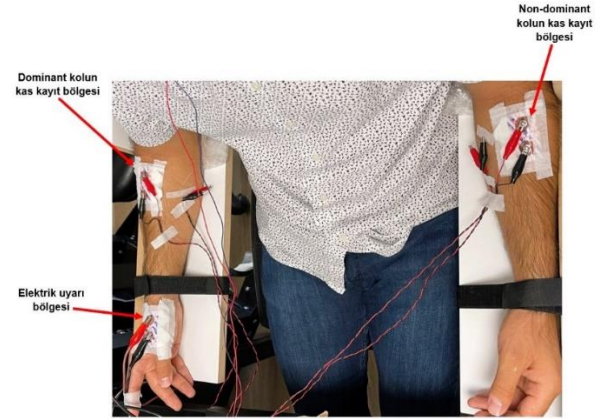
Şekil-1. Kayıt sırasında elektrot bağlantıları.

Uyarı Prosedürü

Brachioradialis kasının innervasyonu C6 spinal kökten gerçekleştiğinden; algı ve ağrı eşiğini belirlemek için elektrik uyarıları C6 dermatomunun dominant elin sırt kısmına uygulandı. Uyaranlar cilt hazırlandıktan sonra, kendinden yapışan bir çift Ag-AgCl elektrodu kullanarak, sabit akım stimülatörüyle (Digitimer Constant Current DS7A, Digitimer Limited, Hertfordshire, England) verildi. Kayıtlara başlamadan önce gönüllülerin algı eşiği, elektrik uyarısı beş kez azaltılıp arttırılarak belirlendi. Beş uyarıdan üçünü ancak hissettiği uyarı seviyesi bireyin algı eşiği olarak belirlendi.

Bu çalışmada ağrı seviyesini belirlemek için, 0-10 arası derecelendirilmiş (0: ağrı yok, 10: yüksek şiddette ağrı) subjektif bir ağrı değerlendirme aracı olan görsel analog ölçeği (Visual Analog Scale-VAS) kullanıldı. Ağrı seviyesini belirlemek amacıyla, algı eşiğinin katları olacak şekilde elektrik uyarı şiddeti kademeli bir şekilde arttırılıp, katılımcılardan VAS kullanarak geri bildirim alındı. Ağrı seviyesi VAS değerinin 4-6 seviyelerine karşılık gelecek şekilde miliamper (mA) olarak belirlendi (9). Kayıt boyunca el sırtına 0,2 ms süren kare puls şeklinde, 1-2 saniye aralığında rastgele gelen, en az 300 ağrılı elektrik uyarısı uygulandı (2, 29, 30).

Analiz

Yüzeyel EMG kayıtlarındaki sinyaller 1000 kat yükseltildi, 20-500 Hz aralığında band geçiren bir

filtre uygulandı ve 2000 Hz frekans hızı ile örneklendi (27). Hem SEMG hem de SMU-EMG kayıtlarında 50 Hz çentik filtre kullanıldı. Ham EMG verilerin tam dalga doğrultma işlemi uygulandıktan sonra, uyarıdan 400 ms önce ile 500 ms sonrasındaki kısmın ortalaması spike 2 yazılımı ile alındı. Ortalama alma işleminde uyarı artefaktı ortalamayı etkilediğinden veriler Excel dosyasına aktarıldı ve uyarıdan 4 ms sonrasına kadar olan verilerin yerine uyaran öncesi SEMG değerlerinin ortalaması yazılarak artefakt giderildi (31).

Tek motor ünite EMG kayıtları 1000 kat yükseltildi, 20000 Hz örneklendi ve 200-10000 Hz aralığında band geçiren filtre uygulandı (5, 11). Bipolar intramüsküler elektrotlardan gelen tek motor ünite potansiyellerinin belirlenmesi için, kayıttan sonra (off-line) Spike 2 yazılımında bulunan "microprocessor-based waveform analysis" seçeneği kullanıldı. Bu yöntem kayıt boyunca takibini yaptığımız motor ünitelerin doğru bir şekilde analizinin yapılmasını sağlamaktadır. Seçilen motor ünite aktiviteleri PSTH, PSF ve bunların CUSUM grafiklerini oluşturmak için kullanıldı.

Yüzeyel ve tek motor ünite kayıtlarının ortalaması alındıktan sonra, bu verilerin CUSUM grafikleri oluşturuldu. Kümülatif toplam grafiğinin oluşturulması şu şekilde yapılmıştır: Elektrik uyarımı etrafında oluşturulan grafiklerden uyaran öncesi dönemde maksimum pozitif ve maksimum negatif sapmalar belirlendi. Bu iki değerden en büyük olanı, simetrik bir 'hata aralığı' olarak tanımlandı. Hata aralığını aşanlar refleks olarak değerlendirildi. Uyarı sonrasında CUSUM grafiği üzerinde aşağıya doğru gözlemlenen sapmalar hata aralığının sınırlarını aştığında inhibisyon, hata aralığındaki sınırı yukarı doğru aşan sapmalar da eksitasyon olarak kabul edilmiştir (9, 22, 31). Kümülatif toplam grafiğinde, uyarının verildiği an ile hata aralığının sınırlarını aştığı ilk anlamlı CUSUM sapmasının belirlendiği dönem arasındaki zaman dilimi, refleks latansı olarak tanımlandı. Kümülatif toplam grafiğinin yukarı doğru kıvrıldığı an refleksin sonlanma zamanı kabul edildi. Refleks süresi ise refleksin bitişi ve başlangıcı arasındaki zaman farkı olarak belirlendi (8, 27).

İstatistiksel Analiz

Araştırmada elde edilen verilerin istatistiksel değerlendirilmesi IBM SPSS v.25 bilgisayar paket programı kullanılarak gerçekleştirildi. Sürekli değişkenlerin normal dağılıma uygunluğu Shapiro-Wilk testi ile değerlendirildi. Reflekslerin analizinde kullanılan yöntemlerde, latans ve süre değerleri arasında anlamlı bir fark olup olmadığı, normal dağılıma uymadığı için parametrik olmayan testlerden wilcoxon işaretli sıralar testi ile değerlendirildi. İstatistiksel analizlerde parametrik olmayan testlerin kullanılması sebebiyle, refleks cevapların ortalama latansları, bitişleri ve süreleri medyan (çeyrekler aralığı, IQR) şeklinde ifade edilmiştir. Tüm istatistiksel analizlerde p değeri 0,05, güç 0,80 ve etki büyüklüğü 0,90 olarak belirlendi. $p < 0,05$ ise istatistiksel olarak anlamlı kabul edilmiştir.

BULGULAR

Toplamda 16 gönüllüden kayıt alınmakla birlikte, motor üniteleri seçilebilen dokuz katılımcının (1 kadın, 8 erkek) kayıtları kullanıldı. Kayıt hazırlıkları dışında katılımcılardan alınan her EMG kaydı ortalama 15 dakika sürdü. Gönüllülerden en az iki kayıt alınarak bunlardan 16 motor ünite elde edildi. Katılımcıların algı eşiği ortalaması 1,79±0,46 mA (ortalama±SD) idi. Katılımcılarda ağrı duyumu algı eşiğinin ortalama 31±13,26 katı kadar şiddette oluştu. Elektrik uyarısı şiddeti ise ortalama 54,1±24,6 mA seviyesinde uygulandı. Katılımcılarda uygulanan elektrik uyarı şiddeti, VAS 4-6 aralığında olup, ortalama VAS değeri 4,9 olarak hesaplandı. Analizi yapılan kayıtların ortalama SEMG genliği 0,246±0,081 mV olarak ölçüldü. Bu değer, gönüllülerin maksimum istemli kas kasılması (MVC) ortalamasının %12,58±10,4'ü kadardı. Bireylerin dominat kol *brachioradialis* kasının reaksiyon zamanı 136±13,7 ms olarak belirlendi. Yüzeyel EMG'de KSP latansı 60,5 (10,7) (median, IQR) ms, süresi ise 57 (32,2) ms, PSTH analizinde ise KSP latansı 53,7 (16,1) ms, süresi 64 (40) ms bulundu. Frekansgram ile KSP süresi 158,8 (92,3) ms ve bitiş zamanı 212,8 (100,3) ms olarak hesaplandı. Tablo-1'de uyaran uygulanan kolun *m. brachioradialis*'e ait SEMG, PSTH ve PSF-CUSUM analiz sonuçları sunulmuştur. Kastaki inhibisyon nedeniyle frekans bilgisi olmadığından frekansgram analizinde latans belirlenememektedir. Bu nedenle PSTH latans değerleri PSF analizi için de latans olarak kabul edilmiştir (8, 11, 32). Yüzeyel EMG ve PSTH analiz yöntemlerinde KSP'yi takiben bir eksitatör refleks ve sonrasında bunu takip eden ikinci bir inhibisyon görüldü. Ancak PSF analizlerinde KSP sonrası eksitasyon yanıtı gözlenmedi, bunun yerine devam eden uzun süreli bir inhibisyon gözlendi.
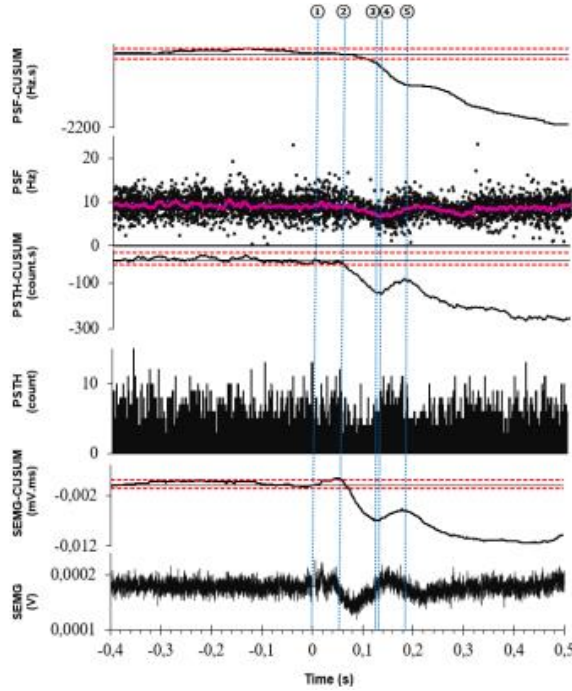
Şekil-2. Bir bireye ait EMG kaydının uyaran etrafında ortalama SEMG, PSTH, PSF ve CUSUM grafikleri.

Ağrılı elektrik uyarısı sonrası bir bireye ait SEMG ve SMU-EMG kayıtlarından elde edilen sonuçlar üç farklı analiz yöntemi ve bunların CUSUM grafikleri ile gösterilmiştir. Şekilde 1 ile 5 arasındaki dikey çizgiler sırayla; (1) sıfır noktasındaki uyarma anı; (2) PSTH-CUSUM ile belirlenen KSP'nin başlangıcı; (3) PSTH-CUSUM ile belirtilen KSP'nin bitişi; (4) PSTH-CUSUM ile belirlenen eksitasyon başlangıcı; (5) PSF-CUSUM ile belirtilen KSP'nin bitişini göstermektedir. PSF-CUSUM grafiği karşılaştırılmalı incelendiğinde KSP'nin daha uzun olduğu ve eksitasyonun olmadığı izlenmektedir. CUSUM grafiklerindeki kırmızı yatay çizgiler ise, CUSUM hata aralığını göstermektedir. PSF kaydındaki ortalama deşarj hızı, kalın çizgi ile PSF üzerinde gösterilmiştir.

Analiz yöntemlerinin istatistiksel ikili karşılaştırmalarında PSTH ile ölçülen KSP latansı, SEMG'den 6,8 ms daha kısa bulundu ($P<0,05$). Ancak iki analiz arasında KSP bitiş ve süreleri arasında anlamlı farklılık görülmedi. Yüzeyel EMG eksitasyon süresi PSTH ile belirlenen süreden anlamlı olarak daha uzun bulundu ($P<0,05$). Kütanöz sessiz periyot bitiş ve süreleri arasında klasik analiz yöntemleri (SEMG, PSTH) ile PSF yöntemini kıyasladığımızda anlamlı bir farklılık bulundu ($p<0,05$). Frekansgram analiz yönteminde elde edilen refleks süresi ve bitişi, SEMG ve PSTH yöntemlerinden anlamlı bir şekilde daha uzun olduğu gözlemlendi ($p<0,001$). Bu sonuçlar Tablo-2'de gösterilmiştir. Şekil-2'de ise bir gönüllüye ait aynı zaman ekseninde SEMG, PSTH, PSF ve CUSUM grafiklerinde süre farklılıkları izlenmektedir.

Tablo-1. SEMG, PSTH ve PSF CUSUM Analiz Sonuçları.

| | SEMG | PSTH | PSF |
	Medyan (IQR)	Medyan (IQR)	Medyan (IQR)
KSP Latansı	60,5 (10,7)	53,7 (16,1)	53,7 (16,1) [†]
KSP Bitişi	120 (21,3)	118 (34,8)	212,8 (100,3)
KSP Süresi	57 (32,2)	64 (40)	158,8 (92,3)
Eksitasyon Latansı	122,3 (22)	129 (23)	‡
Eksitasyon Bitişi	178 (22)	173 (35)	‡
Eksitasyon Süresi	55,7 (21)	39 (17,6)	‡

[†] *PSF de latansını belirleyecek frekans olmadığından PSTH latansları girilmiştir.*

‡ *Eksitasyon görülmedi.*

Değerler ms cinsindendir.

Tablo-2. Kutanöz Sessiz Periyot ve Eksitasyon Refleks Yanıtlarında SEMG, PSTH ve PSF Analiz Yöntemlerinin İkili Karşılaştırılmaları

	SEMG ve PSTH p*	SEMG ve PSF p*	PSTH ve PSF p*
KSP Latans	0,002	†	†
KSP Bitiş	0,717	0,001	0,000
KSP Süre	0,078	0,000	0,000
Eksitasyon Latans	0,785	‡	‡
Eksitasyon Bitiş	0,209	‡	‡
Eksitasyon Süre	0,005	‡	‡

* Wilcoxon işaretli sıralar testi
† PSF deki latans PSTH'dan alındığından değer girilmemiştir.
‡ Eksitasyon görülmedi.

Tablo-3. Literatürde ve bu araştırmada üst ekstremite kaslarında elde edilen kütanöz sessiz periyot sonuçları karşılaştırılması.

Araştırmalar	Kayıt yeri	Uyarı yeri ve tipi	Analiz yöntemi	SEMG		PSTH		PSF
				Latans	Süre	Latans	Süre	Süre
Kofler ve Poustka (2005)	Tenar kaslar	2. el parmağı, elektrik uyarı (ipsilateral latans)	SEMG	41±4	85,9±18,8			
Kumru ve ark. (2009)	Tenar kaslar	2. el parmağı, elektrik uyarı (ön uyaransız uyarım)	SEMG	44,9±3	79,2±5,7			
Kofler (2003)	FDI kası	2. el parmağı, elektrik uyarı	SEMG	46±5	81±18			
Inghilleri ve ark. (2002)	FDI kası	5. el parmağı, elektrik uyarı	SEMG	69,4±4,34	46,7±6,79			
Kahya ve ark. (2010)	FDI kası	El sırtı C8 dermatom, elektrik uyarı	SEMG, PSTH, PSF	63,7±2,8	74±6,6	58 ±2,8	71,6±8,1	124,5±11,7
Çalışmamızın sonuçları Değerler median(IQR)	Brachioradia lis kası	El sırtı C6 dermatom, elektrik uyarı	SEMG, PSTH, PSF	60,5(10,7)	57(32,2)	53,7(16,1)	64(40)	158,8(92,3)

Değerler ortalama±standart sapma, ms.

TARTIŞMA

Bu çalışmada, sağlıklı bireylerde dominant el sırtına uygulanan ağrılı elektriksel uyarımla her iki *brachioradialis* kasında ortaya çıkan KSP'yi klasik analiz yöntemleri (SEMG, PSTH) ve motor ünitelerin çalışma frekansına dayalı olan PSF analiz yöntemi kullanarak incelemek amaçlandı. Distal üst ekstremite kaslarında frekansgram analiz yöntemi ile KSP incelenmiş (8, 9), ancak sağ ve sol *brachioradialis* kaslarında KSP'nin PSF ile incelendiği çalışmaya rastlanmamıştır.

Klinikte KSP kolay uygulanabilir ve non-invaziv olması nedeniyle yaygın olarak SEMG ile incelenmektedir. Yüzeyel elektromiyografi ile motor nöronların toplam sinyalleri ölçülebilir. Ancak, diğer kaslardan gelen gürültüyü en azana indirmek ve farklı boyutlardaki motor ünitelerini belirlemek için tek motor ünite elektromiyografi (SMU-EMG) daha kullanışlıdır. Bu yöntem, motor ünitelerin değişikliklerini daha iyi bir çözünürlükle belirlemeye yardımcı olabilir. Bu nedenle, klinik uygulamalarda SEMG ve SMU kayıtlarının birlikte kullanılması, merkezi sinir sistemi işlevlerini daha iyi anlamamıza katkı sağlayabilir (5, 8). Kayıtlarımızı SEMG ve SMU-EMG ile elde ettik. Refleks yanıtların değerlendirilmesinde klasik analiz yöntemlerinin (SEMG, PSTH) hatalara yol açtığı ve PSF analiz yöntemi daha doğru sonuçlar verdiği bildirilmektedir (9, 11, 16, 17, 33). Kayıtlarımızın analizinde uyaran etrafında ortalamasını alıp SEMG, PSTH ve PSF ve bunların CUSUM grafiklerini oluşturduk.

Araştırmacılar KSP çalışmalarında aynı uyarım yeri ve kastan alınan kayıtların değerlendirilmesinde aynı yöntemleri kullanmalarına rağmen farklılıklar görülmektedir. Örneğin, Kofler ve Poustka (34) ve Kumru ve ark. (21) tenar kaslarda, SEMG ile latansı sırasıyla 41±4 ve 44,9±3 ms; süreyi 85,9±18,8 ve 79,2±5,7 ms bulmuşlardır. Bu farklılıklar yöntemlerde standardizasyon ihtiyacını ve refleksin farklı yöntemlerle araştırılmasının gerekliliğini ortaya koymaktadır.

Frekansgramda elde ettiğimiz inhibisyon bitişi 212,8 (100,3) ms dir. *Brachioradialis* kasında reaksiyon zamanı olarak elde ettiğimiz 136±13,7 ms değerini dikkate aldığımızda inhibisyon süresi içinde kaldığı görülmektedir. Literatür incelendiğinde, refleks yanıtların değerlendirilmesinde reaksiyon zamanı içerisinde kalan kısmının refleks olarak kabul edilmemesi yönünde bilgiler bulunmaktadır (8, 9, 27). Reaksiyon zamanından sonra PSF'de inhibisyonun görüldüğünden inhibisyonun oldukça etkin olduğu düşünülebilir. Bu konunun açığa kavuşturulabilmesi için yeni araştırmalar gerekmektedir.

PSTH ve PSF'den Elde Edilen Bulgularının Karşılaştırılması

Peristimulus time histogram uyarıdan sonra belirli bir zamanda motor ünite aksiyon potansiyellerinin ortaya çıkma sayısına dayanmaktadır. Uyaranı takiben motor nöronun ağrılı uyaran nedeniyle inhibisyonundan sonra düzenli bir hızda, senkronize bir şekilde deşarj olması nedeniyle sayıya dayalı olan PSTH bunu bir eksitasyon olarak tanımlamaktadır (18, 31). Ancak, bu senkronize deşarjların frekansı, motor ünite boşalımlarının anlık frekans değerini gösteren PSF yöntemiyle incelendiğinde; düşük frekanslı olduğu, dolayısıyla motor nöronun halen inhibisyon etkisinde bulunduğu bildirilmektedir (9, 13, 16, 17, 22, 35). Türker ve Powers'in (17) yaptığı beyin dilimleri incelemeleri çalışmasında, düzenli olarak deşarj olan motor nöronlarda, PSTH ve SEMG yöntemleriyle elde edilen sonuçların sayım ve senkronizasyondan kaynaklı hatalar içerdiği vurgulamıştır. Ancak SEMG ve PSTH yöntemleri daha kolay uygulandığından araştırmalarda ve klinikte sıkça kullanılmaktadır. Öte yandan, frekansa dayalı yöntem, uyarıdan sonra herhangi bir zamanda aksiyon potansiyellerinin sayısından etkilenmeyen, yalnızca aksiyon potansiyellerin anlık boşalma hızındaki değişiklikleri gösterdiğinden bu tür hataları içermemektedir (16, 17). İnhibe edilmiş motor ünitelerin deşarj oranı önemli ölçüde azalır, bu nedenle PSF yöntemi PSTH'da inhibisyondan sonra görülen eksitasyonun gerçek bir eksitasyon olmadığı, inhibitör refleksin bir devamı olduğunu gösterir (11, 16-18, 33). Araştırmamızda bu literatürle uyumlu olarak PSTH'da eksitasyonun gözlendiği aynı zaman aralığında PSF'de düşük frekansta motor nöron deşarjları olduğu görüldü.

Frekansgram yönteminde inhibisyonun başlangıcını (latans) belirlemek için aksiyon potansiyelleri olmadığından refleksin başlangıcına ilişkin yeterli bir bilgi sağlayamamaktadır (11, 13, 35). Frekansgram yönteminin kullanıldığı çalışmalarda SEMG ve PSTH yöntemlerinin reflekslerin varlığı ve latanslarını belirlemekte kullanıldığı görülmektedir (17, 22, 31). Bu yüzden araştırmamızda PSF latansı için PSTH ile elde edilen değeri kullandık. Literatürde SEMG, PSTH ve PSF için CUSUM grafiklerinin kullanımı önerilmektedir (9, 17, 22, 23). Biz de ilgili analiz yöntemlerinin CUSUM grafiklerini kullandık.

Kutanöz sessiz periyodun araştırıldığı çalışmalarda el kaslarında SEMG latans ve süresi sırasıyla ms olarak bir çalışmada 44,9±3, 79,2±5,7, diğer bir çalışmada 41±4, 85,9±18,8, farklı bir araştırmada ise 69,4±4,34, 46,7±6,79 olarak belirlenmiştir (21, 34, 36). Literatürdeki bu sonuçlar Tablo-3'te topluca gösterilmiştir. Çalışmamızda ise, *brachioradialis* kası SEMG'de KSP latansı 60,5 (10,7) (median, IQR) ms, süresi ise 57 (32,2) ms bulundu. Bu sonuçlar literatürde elde edilen sonuçlarla uyumludur. Daha önceki bir araştırmamızda *First Dorsal Interosseous* (FDI) kasında sessiz periyodun PSTH analiziyle elde edilen latans ve süre sonuçları sırasıyla 58 ±2,8, 71,6±8,1 ms bulunmuştur (9). Bu çalışmamızda latansı 53,7 (16,1) ms, süresi 64 (40) ms olarak benzer bulundu.

Literatürde PSF ile ilgili üst ekstremitede çok fazla çalışma bulunmamakla birlikte yapılan bir çalışmada (9) PSF'de refleks süresi 124,5±11,7 ms olarak bulunmuştur (Tablo-3). Araştırmamızda KSP süresi 158,8 (92,3) ms olarak ölçüldü (Tablo-1).

Araştırmamızda PSF incelemesinde eksitasyon görülmemekle birlikte PSTH'da her inhibisyondan sonra bir eksitasyon gözlenmiştir. Frekansgram refleks süresi PSTH yöntemiyle belirlenen refleks süresinden 94,8 ms daha uzun bulunmuştur (p<0,001). Daha önce tartışıldığı gibi PSF'de görülen refleks süresinin uzunluğu inhibisyonun devamı olduğundan, PSF refleks süresi PSTH'dan daha uzun ortaya çıkmaktadır. Refleksin bitiş zamanı PSF'de 212,8 (100,3), PSTH'da 118 (34,8) ms olarak belirlendi (p<0,001). Daha önceki çalışmamızda refleks bitişi PSF'de 184,7±13,3, PSTH'da 129,6±9,2 ms bulunmuştur (9). Bahsi geçen çalışmada olduğu gibi bu çalışmamız da refleksin bir eksitasyon

olmadan uzun süreli bir inhibisyon oluşturduğu düşüncesini desteklemiştir (Tablo-2).

Kütanöz Sessiz Periyodu Takiben Oluşan Eksitasyon Refleksi

Bazı araştırmacılar, KSP sonrası görülen eksitatör refleks yanıtın transkortikal uzun döngü reflekslerin karışması sonucu ortaya çıktığını bildirmişler (10, 37, 38). Kumru ve arkadaşları (21), ön uyaran uygulayarak KSP'yi takip eden eksitasyon yanıtını ve bu yanıtın irkilme refleksiyle ilişkisini değerlendirmişler. Çalışma sonucunda ön uyarılı stimülasyonunu takiben eksitasyon fazının ortalama amplitüdünde bir azalma gözlemlendiğini, ancak KSP parametrelerinde herhangi bir değişiklik olmadığını bildirmişler. Bu sonuçlarla, araştırmacılar eksitatör fazın somatosensoriyel irkilme refleksinin bir komponenti olduğunu belirtmektedirler. Ayrıca, bazı araştırmacılar eksitasyon refleksinin, motor ünitelerin inhibisyon sonrası yeniden senkronizasyonu ile ilişkili olduğunu ileri sürmüşlerdir (9, 34, 39, 40). Ancak, KSP'nin PSF ile incelendiği araştırmalarda bu yanıtın gerçek bir eksitatör refleks yanıt olmadığı ve aslında inhibisyonun devamı olduğu söylenmektedir (8, 13, 16, 17, 27).

Çalışmamızda PSTH analizinde eksitasyonun latansı 129 (23), bitişi 173 (35) ve süresi 39 (17,6) ms olarak belirlendi. Diğer bir araştırmamızda ise, FDI kasında PSTH ile belirlenen eksitasyon latansı 133,6± 8,8, bitişi 185,2± 10,2 ve süresi 51,7± 4,2 ms olarak benzer bir şekilde bulunmuştur (9). Ancak PSF analizinde inhibisyon bitiş zamanının 212,8 (100,3) ms olduğu dikkate alındığında, PSTH ile eksitasyon başlangıcı olan 129 (23) ms'de halen inhibisyonun devam ettiği görülmektedir (Şekil-2).

İki Ekstremite Kasından Eş Zamanlı Kaydedilen EMG Sonuçları

Kofler ve Poustka (34) bilateral ve ünilateral tenar kaslarında KSP'yi araştırmışlardır. Bu çalışmada sağlıklı katılımcıların SEMG sonuçlarında; dominant elde ikinci parmağın elektrik uyarısıyla aynı taraftaki tenar kaslarda KSP gözlenirken, diğer elde tenar kaslarda KSP gözlenmemiştir. Bu sonuçlarla KSP'yi ileten nöral devrenin üst ekstremitelerde tek taraflı etkili olduğunu düşüncesini bildirmişlerdir. Araştırmamızda bahsi geçen çalışmayla uyumlu olarak; dominant tarafa yapılan elektriksel uyarımın dokuz katılımcıda aynı taraftaki kaslarında KSP oluşturduğu gözlenirken diğer ekstremitede KSP görülmedi.

Kas Kasılma Düzeyinin KSP Üzerinde Etkileri

Belli bir düzeyde kasılan kasta inhibitör bir refleks görülebilmektedir. Kasma düzeyi ile ilişkili olarak araştırmacıların bir kısmı kas kasılma düzeyinin artmasının KSP'nin latansını ve süresini kısaltabileceğini belirtmişlerdir (14, 25). Diğer bir kısmı ise, maksimum kas kasılmasının %10'u ila 60'ı aralığında olmasının KSP parametrelerini etkilemediğini bildirmişlerdir (26, 41). Ayrıca bir araştırma %5 MVC'nin altında ve %25 MVC ile kasılan kasta benzer KSP'un oluştuğunu gözlemlemiştir (34). Araştırmamızda katılımcıların kaslarını maksimum istemli kasılma (MVC) düzeyinin ortalama %12,58±10,4'ü kadar kasmalarını geri bildirim vererek sağladık. Tek motor ünite kayıtlarında düşük kas kasılma düzeyi kullanılması, fazla sayıda motor ünitenin aktive edilmemesini sağlayarak analizi kolaylaştırmaktadır (8, 9).

Bu araştırmanın kısıtlılıkları şunlardı: İki ekstremiteden eş zamanlı kayıt yapılması gerekliliği, katılımcıların her iki ekstremitede *brachioradialis* kaslarını aynı düzeyde kasmaları ve her iki kolda tek motor ünite aksiyon potansiyellerini deney sonuna kadar devam ettirmelerindeki zorluk, uzun süreli kayıt alma gerekliliği nedeniyle takip edilen motor ünitenin kaybolmasıydı. Geleceğe yönelik yapılacak çalışmaların klinik faydalarının daha fazla olması için; KSP'nin refleks parametrelerinin eldesinde standardizasyonun geliştirilmesi, farklı analiz yöntemlerinin birlikte kullanılması, KSP'nin etkilendiği farklı hasta gruplarında ayrıntılı çalışmaların yapılması ve KSP oluşumunda supraspinal etkileşimlerin ortaya konmasının gerekli olduğunu düşünmekteyiz.

SONUÇ

Bu çalışmada motor ünite frekansını gösteren PSF yöntemiyle inhibisyon süresinin SEMG ve PSTH'a göre uzun olduğu gözlenmiştir. Bu sonuç medulla spinaliste inihibitör devrelerin bilinenden daha fazla sinaptik bağlantılar içerdiğini veya inhibitör yolakların daha uzun süreli etkisini sürdürdüğünü düşündürmektedir. Ayrıca PSF'de PSTH'da görülenin aksine eksitasyonun görülmemesi de önemli bir farklılıktır. Kontraleteral kasta tek bir katılımcı dışında diğer katılımcılarda refleks görülmemesi üst ekstremitede KSP'un spinal segmentte karşı yarıya etkin bir şekilde dağılmadığı düşüncesini desteklemektedir. Bunun daha net bir şekilde ortaya konması daha fazla katılımcıyla, farklı

uyarı tipleri ve farklı şiddetlerde ipsilateral ve kontralateral EMG kayıtları alınarak mümkün olabilir. SEMG, PSTH ve PSF grafiklerinde refleks cevabının genliğini ve latansını belirlemek için anlamlı ancak zor fark edilebilen değişiklikleri tespit etmek için CUSUM grafikleri daha kullanışlıdır. Refleks araştırmalarında aynı metodlarla farklı sonuçların görülmesinin engellenmesi; analiz yöntemlerinde standardizasyonu ve yeni yöntemlerin geliştirilmesi ile mümkün olacağı düşünülmektedir. Farklı analiz yöntemlerinin kullanılması ile refleks yanıtların daha doğru bir şekilde belirlenmesini ve nöral devrelerin doğru tanımlanmasını sağlayabilir. Bu nedenle, refleks latansları ve sürelerini belirlemek için klasik ve frekansgram analiz yöntemlerinin birlikte kullanılması önerilmektedir.

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The effects of separation-individuation characteristics in adolescents with anorexia nervosa

Anoreksia nervoza tanılı ergenlerde ayrılma-bireyleşme özelliklerinin etkileri

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ABSTRACT

Aim: This study aimed to investigate the relationship between separation-individuation characteristics, body mass index (BMI), and quality of life in adolescents diagnosed with anorexia nervosa (AN).

Materials and Methods: The study included a sample of 21 cases who were presented at the

Ege University Child and Adolescent Psychiatry Unit and received a diagnosis of anorexia nervosa. The study utilized the Sociodemographic Data Form, the Separation-Individuation Test of Adolescence (SITA), and the Children's Quality of Life Inventory (PedsQL).

Results: Negative correlations were found for the scores of rejection expectancy and need denial from the separation-individuation subscales with peer enmeshment and quality of life functionality ($r = -0.67$; $p = 0.002$; $r = -0.61$; $p = 0.007$). A positive correlation was found between the practicing-mirroring scores of the cases and the affect-related quality of life functionality ($r = 0.55$; $p = 0.018$).

Conclusion: The study suggests that difficulties in separation-individuation among adolescents with anorexia nervosa are linked to a decline in quality of life. Recognizing and addressing separation-individuation challenges, which are believed to contribute to the development of anorexia nervosa and its psychodynamic etiology, is crucial for improving quality of life and ensuring effective treatment.

Keywords: Separation-individuation, anorexia nervosa, adolescent psychiatry.

ÖZ

Amaç: *Bu çalışmanın amacı yeme bozukluğu tanılı ergenlerdeki ayrılma-bireyleşme özellikleri ile, beden kitle indeksleri (VKİ) ve yaşam kaliteleri arasındaki ilişkiyi saptayabilmektir.*

Gereç ve Yöntem: Ege Üniversitesi Tıp Fakültesi Çocuk ve Ergen Ruh Sağlığı Hastalıkları birimine başvuran ve anoreksiya nervoza tanısı alan 21 olgu araştırma örneklemini oluşturmaktadır. Çalışmada Sosyodemografik Veri Formu, Adölesan Ayrılma Bireyleşme Testi [AABT], Çocuklar İçin Yaşam Kalitesi Ölçeği (ÇİYKÖ) formları kullanılmıştır.

Bulgular: Olguların ayrılma bireyleşme alt ölçeklerinden reddedilme beklentisi ve ihtiyacı inkâr etme skorlarıyla; yaşıtlarıyla ilişkileri ile ilgili yaşam kalitesi işlevselliği arasında negatif yönde korelasyon saptanmıştır (r=-0.67; r=-0.61; p=0.002; p=0.007). Olguların pratik yapma-aynalama skorları ile duygulanımla ilişkili yaşam kalitesi işlevselliği arasında ise pozitif yönde bir korelasyon saptanmıştır (r=0.55; p=0.018).

Sonuç: Bu çalışmada AN tanılı ergenlerdeki ayrılma bireyleşme güçlüklerinin yaşam kalitesinde düşüş ile ilişkili olduğu bulunmuştur. AN gelişiminde, psikodinamik etiyolojide yeri olduğu düşünülen ayrılma-bireyleşme ile ilgili güçlüklerin fark edilip desteklenmesi, yaşam kalitesinin artırılması ve etkin tedavi açısından göz ardı edilmemesi gereken bir konudur.

Anahtar Sözcükler: Ayrılma bireyleşme, anoreksiya nervoza, ergen psikiyatri.

INTRODUCTION

Anorexia nervosa (AN) is an eating disorder that progresses with problems related to weight loss and malnutrition and has a higher mortality rate and suicide risk compared to the general population (1, 2). It is thought to be more common than its prevalence suggests, since the disease has a wide spectrum and includes subclinical forms that do not fully meet the diagnostic criteria (3, 4). Weight loss is often due to food restriction, but weight loss can also occur due to energy expenditure (such as exercise) or consumption of metabolism-boosting products (5). This disorder usually occurs in adolescence or pre-adolescence, and physical and psychiatric comorbidities are frequently observed (6, 7). In this disorder, patients overestimate their beliefs about their eating attitudes (1).

Anorexia nervosa is a disorder with multiple causes in which many genetic, neurobiological, psychological, developmental, and sociocultural factors play roles in its development (8). Opinions about the psychodynamic etiology of the disorder focus on the separation-individuation period. According to Mahler's theory, in the separation-individuation period covering the first three years of life, the human offspring who is physically separated from his mother but "born without being adjusted to the outside world" also separates from their mother spiritually and begins to perceive themselves as a separate individual (9). A healthy separation, which is expected to mature in this period, has the most critical importance for the development of individuation and sense of identity (10). After this period, which Mahler defined as the separation-individuation process in the first three years, Blos defined adolescence as the second separation-individuation period (11). According to Blos, in the second separation-individuation period, the adolescent should say goodbye to internalized parental designs, find affection objects outside the family, and adapt to the adult world (11). In the psychodynamic literature, the symptoms of anorexia nervosa are stated to develop in response to the intense anxiety that occurs with the repeated experience of the separation-individuation period (12). It was suggested that the pressure to be independent during adolescence activates difficulties with separation and individuation that originated during feeding from infancy and are associated with eating disorders (13).

Quality of life is the subjective sum of physical, psychological, and social functionality and well-being sub-dimensions, as well as the general health of the individual (14). In anorexia nervosa, which affects many systems in the body, there is no effect on the quality of life in the early period. However, the quality of life decreases with the increase in somatic symptoms in the later period (15). In various studies on the subject, there was a decrease in the quality of life in AN (16, 17). Quality of life was used as a criterion for AN in the 1990s (18).

This study aimed to investigate the relationship between separation-individuation characteristics, BMI, and quality of life in adolescents diagnosed with anorexia nervosa. Our hypothesis was that separation-individuation characteristics would affect body mass index and quality of life in AN patient. While there are many studies on the deterioration of quality of life in anorexia nervosa, the effect of separation-individuation characteristics on quality of life in adolescents with anorexia nervosa forms a gap in the literature. We hope that our study will contribute to filling this gap in literature.

MATERIALS and METHODS

Our study included 20 female and 1 male case aged 12-18 years, who applied to XXXX Child Psychiatry Outpatient Clinic with eating and weight loss problems and were diagnosed with anorexia nervosa through psychiatric interviews. Written informed consent was obtained from all patients, and ethics committee approval was obtained from the ethics committee of XXXX (Decision no: 19-8.1T/54). PedsQL (18, 23), SITA (19, 20), and the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime Version (K-SADS-PL) (21, 22) were used for the evaluation of the cases, and their diagnoses were first made by experienced resident physicians and then finalized by the responsible faculty members. Comorbid diagnoses were evaluated with the same method. The inclusion criteria for patients in this study were as follows: they had to meet the DSM-5 diagnostic criteria for anorexia nervosa, be between the ages of 12 and 17, and voluntarily agree to participate in the study. Cases with clinically below-normal mental capacity and cases with autism and bipolar disorder were excluded from the study. All patients were evaluated by a pediatrician. Exclusion was planned for those with serious physical illnesses or conditions that could lead to neurological deficits. However, no patients were excluded from the study for these reasons. The study was initially planned with 34 patients. Unfortunately, 6 patients did not attend follow-up appointments, 2 patients declined to complete the forms, 3 patients could not obtain parental consent, 1 patient was excluded due to cognitive limitations, and 1 patient was excluded due to comorbid bipolar disorder. Ultimately, the study was completed with 21 patients.

After case selection, a sociodemographic data form was given to all cases, in which general information such as age and family characteristics was questioned. The cases were weighed, and care was taken to ensure that the weighing process was carried out in the early hours of the day. The body mass index (BMI) of the patients was calculated and recorded.

Tools

Schedule for Affective Disorders and Schizophrenia for School Aged Children, Present and Lifetime Version, K-SADS- PL (21, 22)

K-SADS-PL is used to detect current and lifelong psychopathologies in children and adolescents. It is a semi-structured interview form. The form consists of three parts. In the first part, demographic characteristics of the child, general health status, previous psychiatric admissions, general information such as treatment, family and peer relationships, and school information are questioned. The second part includes screening questions and evaluation criteria that examine specific psychiatric symptoms. If there are positive symptoms as a result of the screening interview, the additional symptom list is questioned in order to better evaluate that psychopathology.

Children's Quality of Life Inventory (PedsQL) (18)

The scale developed by Varni et al. aims to measure general social functioning and quality of life in the 2-18 age group. The scale measures physical, emotional, social, and academic functioning. In the section on physical functionality, there are eight items; in the section on emotional functionality, there are five items; and in the section on social functionality, there are five items. In the section concerning school-related issues, there are three items for the 2-4 age group and five items for other age groups.

Separation-Individuation Test of Adolescence (SITA) (19)

Developed by Levine and colleagues in 1986, the SITA includes a series of behavioral expressions related to relationships with parents, teachers, and peers. It is a Likert-type scale consisting of 85 items. The subscales of the SITA have been identified as separation anxiety, engulfment anxiety, peer enmeshment, teacher enmeshment, practicing-mirroring, need denial, rejection expectancy, and nurturance seeking.

Sociodemographic Data Form

This form is a brief information sheet containing questions about the child's age, family characteristics, accompanying illnesses, the presence of psychiatric disorders in the family, and similar inquiries.

Statistical Analysis

Data was analyzed using appropriate statistical methods. Analyses were performed using the SPSS 25.0 (Statistical Package for Social Sciences) software package. Before statistical analysis, Shapiro-Wilk and Kolmogorov-Smirnov tests were used to evaluate whether numerical variables were normally distributed. Variable correlation was evaluated with Pearson correlation in the case of normal distribution and Spearman correlation for non-normal distribution.

The independent samples t-test was used to compare paired groups. A power analysis was conducted for the statistical analysis of the research. The required sample size to achieve 80% power (α = 0.05) for large effect size (r = 0.5) was 34. In our study, this sample size was not achieved, and the observed statistical power for 21 patients is 58.7%, which is one of the limitations of the study.

RESULTS

The ages of the subjects included in the study ranged from 12 to 18 years, and the mean age was calculated as 15.1. While 95.2% (n=20) of the cases included in our study were female, 4.8% (n=1) were male. Other information about sociodemographic data and comorbidity rates is shown in Table-1.

When Table 1 is examined, 85.7% (n=18) of the 21 cases with anorexia nervosa (AN) had at least one comorbid psychiatric diagnosis. In our sample, the most common comorbidity was major depression (MDD) in 71.4% (n=15), followed by ADHD in 42.9% (n=9), generalized anxiety disorder (GAD) in 28.6% (n=6), and obsessive-compulsive disorder (OCD) in 23.8% (n=5). When the 18 cases with comorbid psychiatric diagnoses and the 3 cases without comorbidity were compared in terms of SITA scores, no statistically significant difference was observed in any of the subscales (p>0.05). In addition, when the cases were divided into two groups—those with and without MDD—AN cases with MDD comorbidity had higher separation anxiety and engulfment anxiety scores, and lower practice and mirroring scores than AN cases without MDD (p: 0.04; 0.00; 0.00). The scores for the cases diagnosed with AN, with and without major depressive disorder, are shown in Table-2.

The cases in our sample were evaluated in terms of the relationship between SITA scores and PedsQL scores. A negative correlation was found between rejection expectancy and need denial scores from the SITA subscales, and quality of life functionality related to their relationships with peers (r=-0.67; r=-0.61; p=0.002; p=0.007). With further analysis, the relationship between rejection expectancy and quality of life functionality related to relationships with peers was significant, but the relationship between need denial and quality of life functionality related to relationships with peers was not significant (p=0.002; p=0.102; r²=0.506; r²=0.122). A positive correlation was found between the practice-mirroring scores of the cases and affect-related quality of life functionality (r=0.55; p=0.018), and further regression analysis found this relationship to be significant (p=0.023, r²=0.466). In addition, when the relationship between SITA scores and BMI percentiles was evaluated, a positive correlation was found between practice-mirroring scores and BMI percentiles (r=0.54; p=0.03) (Table-3). Regression analysis was performed to evaluate the relationship between practice-mirroring scores and BMI, and a significant relationship was found (p=0.016, r²=0.349).

Table-1. Sociodemographic Findings and Comorbidity.

Cases of Anorexia Nervosa (n=21)	YES n (%)	NO n (%)
Psychopathology in the Family	7 (33.3)	14 (66.6)
Eating Disorder in Mother	1 (4.7)	20 (96.3)
Eating Disorder in Father	0 (0)	21 (100)
Eating Disorder in Siblings	4 (30.7)	9 (69.3)
Additional Psychiatric Diagnosis	n (%)	
Presence of comorbidity	18(85.7)	
Major Depression	15 (71.4)	
Attention Deficit Hyperactivity Disorder (ADHD)	9 (42.9)	
Generalized Anxiety Disorder (GAD)	6 (28.6)	
Obsessive Compulsive Disorder (OCD)	5 (23.8)	

Table -2. Evaluation of the scores for cases with and without MDD comorbidity from the SITA scale

		N	X	t	P	
Separation Anxiety	MDD + MDD -	15 6	25.60 16.66	2.113	**0.04**	
Engulfment Anxiety	MDD + MDD -	15 6	22.66 13.66	4.608	**0.00**	
Peer Enmeshment	MDD + MDD -	15 6	29.00 29.66	-0.186	0.85	
Teacher Enmeshment	MDD + MDD -	15 6	18.93 19.00	-0.35	0.97	
Practicing-Mirroring	MDD + MDD -	15 6	26.46 35.60	-4.520	**0.00**	
Need Denial	MDD + MDD -	15 6	12.33 13.50	-0.615	0.54	
Rejection Expectancy	MDD + MDD -	15 6	26.53 25.50	0.375	0.71	
Nurturance Seeking	MDD + MDD -	15 6	25.06 22.66	0.925	0.36	

MDD: MAJOR DEPRESSIVE DISORDER (+): yes; (-):no

Table-3. Analysis of the Relationships Between Separation Individuation Test Results of the Cases with BMI Percentiles and PedsQL Scores.

	BMI Percentiles		PedsQL Affect Scores		PedsQL Others Scores	
	P value (χ2)	correlation coefficient (r value)	P value (χ2)	correlation coefficient (r value)	P value (χ2)	correlation coefficient (r value)
Separation Anxiety	0.61	0.13	0.38	-0.21	0.33	-0.24
Engulfment Anxiety	0.80	0.06	0.90	-0.03	0.91	-0.02
Peer Enmeshment	0.07	0.45	0.61	0.12	0.22	0.30
Teacher Enmeshment	0.79	0.06	0.59	0.13	0.37	0.22
Practicing-Mirroring	**0.03**	**0.54**	**0.01**	**0.55**	**0.03**	**0.51**
Need Denial	0.31	-0.26	0.24	-0.29	**0.00**	**-0.61**
Rejection Expectancy	0.40	-0.22	**0.01**	**-0.55**	**0.00**	**-0.67**
Nurturance Seeking	0.92	0.02	0.93	0.02	0.71	-0.09

BMI: Body Mass Index, PedsQL: Children's Quality of Life Inventory

DISCUSSION

This study examined the effects of separation-individuation characteristics on the body mass index and quality of life of adolescent patients with AN. According to our findings, among the cases diagnosed with AN, the quality of life was better in those with better practice and mirroring skills, which are positive separation-individuation characteristics. On the contrary, subjects who had negative separation-individuation characteristics, such as rejection expectancy and need denial, had lower quality of life. Additionally, the body mass index percentiles of the cases with AN who had better practice and mirroring skills were also higher. Moreover, it seems that patients with anorexia nervosa accompanied by depression experience greater difficulties in separation and individuation.

Separation and individuation skills are expected to be acquired during adolescence. Self and object images are expected to form during this period (19). In this period, which is full of crises

that need to be resolved for healthy mental development, difficulties related to separation and individuation were associated with depression, eating disorders, delinquency, and substance use (20). Similar to our study, in a study conducted using the SITA scale and investigating the relationship between separation-individuation characteristics and depression, the depression scores of young people with separation-individuation difficulties were higher (24). According to studies, parents of depressed adolescents have more controlling parental attitudes, and separation from parents who cannot leave their children creates difficulties for adolescents (25). The relationship between eating pathologies and depression has been known for a long time. A recent review and meta-analysis evaluated 30 longitudinal studies examining the relationship between eating pathologies and depression. According to the results of the study, eating pathologies increase the risk of developing depression, and depression increases the risk of developing eating pathologies (26). In our sample, a significant comorbidity rate of major depression (MDD) was observed, with 71.4% of participants exhibiting this condition, which aligns with findings reported in the existing literature. Furthermore, individuals with anorexia nervosa (AN) who also presented with MDD demonstrated elevated scores on the separation anxiety and engulfment anxiety subscales of the SITA test. These subscales are indicative of the challenges faced in the separation-individuation process, reflecting difficulties in establishing autonomy and personal identity. Conversely, these patients scored lower on the practice-mirroring subscale, a measure that typically serves as a positive indicator of successful separation and individuation.

Based on these findings, it appears that the difficulties in separation and individuation among individuals with anorexia nervosa are significantly associated with the comorbidity of depression. The presence of depressive symptoms may exacerbate the challenges related to separation-individuation, suggesting that the psychological complexities inherent in AN are compounded by co-occurring depressive disorders. Consequently, addressing and treating the accompanying depressive symptoms could prove beneficial in facilitating healthier separation-individuation processes among adolescents with anorexia nervosa. This highlights the importance of an integrated treatment approach that simultaneously targets both eating disorder symptoms and comorbid depressive conditions to enhance overall psychological well-being.

Quality of life, a multifaceted concept closely linked to an individual's overall well-being, has been extensively studied within the context of eating disorders. A recent review analyzed 12 cross-sectional studies and 5 cohort studies, revealing a significant association between dietary habits and quality of life among children (27). Additionally, a review published in 2019 concluded that eating disorders in children and adolescents were correlated with a diminished quality of life (28).

While numerous studies in literature highlight a decline in quality of life among individuals diagnosed with anorexia nervosa, there remains a notable gap in research specifically exploring the relationship between separation-individuation characteristics and quality of life in this population. Understanding how these developmental processes interact with the experiences of individuals suffering from anorexia nervosa could provide valuable insights into their overall well-being and inform targeted interventions aimed at improving their quality of life. Further investigation in this area is essential to fill this research void and enhance our comprehension of the complex dynamics at play in eating disorders.

CONCLUSION

Deaths from anorexia nervosa mostly occur due to suicide and medical complications of AN (29). The longer the disease persists, the higher the risk of medical complications and death. After diagnosis, treatment should commence promptly, aiming for remission. Given the diverse causes of AN, it is recommended to employ multiple treatment methods, including weight restoration and other interventions, particularly psychoeducation. Efforts should also focus on supporting adolescents in terms of separation and individuation. Strengthening the adolescent in this area during AN treatment can enhance treatment outcomes and improve the patient's quality of life.

LIMITATIONS

There are several limitations in this study. The first limitation is the small sample size of the participants. To obtain more accurate statistical

results, the study should be repeated with larger samples. The second limitation of our study was the absence of a control group. We recommend that further studies compare anorexia nervosa groups with control groups consisting of healthy adolescents. Another limitation is the restricted number of male patients. There is a need for further research involving larger sample groups that include a greater number of male patients.

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The perioperative outcomes of gastric cancer surgery ın octogenarians

Yaşlı hastalarda mide kanseri cerrahisinin perioperatif sonuçlarının değerlendirilmesi

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ABSTRACT

Aim: Gastric cancer is the fifth most common cancer in the world and the third most common cause of cancer-related deaths. Its incidence is also increasing in elderly patients. A patient-based, multidisciplinary treatment approach is required in the evaluation of elderly patients. Surgical resection is the curative method in the treatment of gastric cancer. We aimed to evaluate the early surgical outcomes, mortality and morbidity of patients aged 80 years and older who were operated for gastric cancer.

Materials and Methods: Patients aged 80 years and older who underwent surgery for gastric adenocarcinoma between January 2015 and June 2022 were retrospectively analyzed. Demographic, clinical, and histopathological parameters and postoperative outcomes were evaluated.

Results: Twenty-three patients aged 80 years and older were included in the study. The mean age was 81.95 ± 3.11 (Range 80-96) years. 8 were female and 15 were male. 5 patients were ASA I, 11 patients were ASA II, and 7 patients were ASA III. Three patients received neoadjuvant chemotherapy. Total gastrectomy was performed in 11 patients, distal gastrectomy in 5 patients, and proximal gastrectomy in 7 patients. The mean number of lymph nodes removed was 19.87±12.61. The mean hospital stay length was 9.34 ± 3.45 days. The mortality rate in the first ninety days of our study was 8.69% and morbidity was 13,04%.

Conclusion: Octogenarians are a special patient group. The treatment plan should be individualized. If possible, all medically fit patients should undergo curative surgery.

Keywords: Octogenarian, gastric cancer, gastric surgery, perioperative outcomes.

ÖZ

Amaç: Mide kanseri dünyada en sık görülen beşinci kanserdir. Toplumun yaşlanmasıyla beraber ileri yaş grubunda da mide kanseri görülebilmektedir. Çalışmamızın amacı 80 yaş ve üstü hastalarda mide kanseri cerrahisinin erken dönem sonuçlarının değerlendirilmesidir.

Gereç ve Yöntem: Ocak 2015 - Haziran 2022 tarihleri arasında mide kanseri nedeniyle opere edilen 80 yaş ve üstü hastalar; klinik, histopatolojik ve erken dönem perioperatif sonuçlar retrospektif olarak değerlendirildi.

Bulgular: Çalışmaya toplam 23 hasta dahil edildi. Hastaların yaş ortalaması 81.9 idi. Hastaların sekizi kadın 15'i erkekti. Üç hastaya neoadjuvan sistemik tedavi uygulanmış idi. On bir hastaya total gastrektomi, beş hastaya distal gastrektomi, yedi hastaya proksimal gastrektomi uygulandı. İlk 90 günde iki hastada (%8,69) eksitus, üç hastada (%13,04) morbidite gelişti.

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Sonuç: *Yaşlı hasta grubu özel bir hasta grubudur. Tedavi süreci planlanırken hasta bazlı karar verilmelidir. Mümkünse medikal olarak fit hasta grubuna cerrahi rezeksiyon uygulanmalıdır.*
Anahtar Sözcükler: *Yaşlı hasta, mide kanseri, mide kanser cerrahisi.*

INTRODUCTION

With the increase in the elderly population, the need for oncologic surgical intervention is increasing.(1) Advanced age is known to increase surgical mortality and morbidity. Elderly patients require individualized treatment modalities according to their comorbidities and physical performance status. People over 65 years of age have a life expectancy of over 20 years, while those over 80 years of age have a life expectancy of approximately 9 years. In the United States of America (USA), the population over 65 years of age is expected to account for 21% of the total population by 2040, while in Western Europe, the population over 60 years of age is expected to increase to 37% by 2050.(2, 3) As the population ages, the number of surgeries performed on elderly patients has been increasing over the years. While 1.5 million patients over the age of 75 underwent surgery in the UK in 2006-2007, this number increased to 2.5 million in 2015-2016. In the US, 12% of the population is over 65 years of age, while 40% of hospital admissions and 48% of hospitalizations are in patients aged 65 and over (1).

Although major abdominal surgical procedures are becoming more common with optimized multidisciplinary approaches, advances in surgical techniques and improved perioperative care for the elderly, advanced age remains a risk factor. Comorbidities such as cardiovascular disorders, respiratory diseases and renal failure, advanced disease at the time of diagnosis, sarcopenia, limited physical capacity and higher incidence of chemotherapy toxicity are factors that complicate treatment in elderly patients.(2–5) Gastric cancer is the fifth most common cancer in the world and the third most common cause of cancer-related deaths. Its incidence is also increasing in elderly patients. A patient-based, multidisciplinary treatment approach is required in the evaluation of elderly patients. Surgical resection is the curative method in the treatment of gastric cancer. In the literature, we have limited data on surgical outcomes in elderly patients. According to some surgical traditions, advanced age is a contraindication for surgical treatment of gastric cancer (6, 7). In our study, we aimed to evaluate the early surgical outcomes, mortality and morbidity of patients aged 80 years and older who were operated for gastric cancer.

MATERIALS and METHODS

The study was initiated after ethics committee approval number 23-11T/31 (Date: 02.11.2023) was obtained from Ege University Medical Research Ethics Committee.

Patients who underwent surgery for gastric cancer between 2015 and 2022 were evaluated retrospectively. Patients aged 80 years and older were included in the study. Patients with pathology other than gastric adenocarcinoma, surgeries performed for emergency cases such as bleeding and perforation, and patients under 80 years of age were excluded. Patients aged 80 years and older who were operated under elective conditions with a pre-diagnosis of gastric cancer were included in the study. Data was transferred from the prepared case report forms to the database created in Excel file. Age, gender, ASA-PS Score (8) (American Society of Anesthesiologists, Physical Status Classification System), comorbidities, tumor location and surgical procedure, pathology results (TNM staging according to AJCC 8th edition was used (9)), perioperative morbidity and mortality data in the first ninety days, and re-admissions to the hospital within ninety days were analyzed by descriptive statistical methods. Morbidity and mortality data were presented using the Clavien-Dindo Classification (10).

RESULTS

23 patients, eight females and 15 males, were included in the study. The mean age of the patients was 81.95 (± 3.11) years (min: 80, max: 96). Five patients (21%) were ASA-PS 1, 11 patients (47%) were ASA-PS 2, and seven patients (30%) were ASA-PS 3. Comorbidities of the patients are shown in Table-1.

Three locally advanced patients received neoadjuvant systemic treatment. When tumor localization was evaluated, 7 patients had tumor localization in the proximal stomach and 16 patients had tumor localization in the distal and corpus junction. 11 patients underwent total

gastrectomy, 7 patients underwent proximal gastrectomy, and 5 patients underwent distal gastrectomy. The mean number of excised lymph nodes was 19.87 ± 12.61 and the mean number of metastatic lymph nodes was 9.22 ± 9.82. The pathologic examination results of the patients are shown in Table-2.

The mean hospital stay length was 9.34 ± 3.45 days. One patient who underwent total gastrectomy developed anastomotic leakage, which was managed by endoscopic stenting, however the patient died on postoperative day 23 due to sepsis. In a patient who underwent proximal gastrectomy, sudden cardiac arrest developed on postoperative day 3 and the patient died. The mortality rate in the first ninety days of our study was 8.69%.

Aspiration pneumonia developed in one patient and the patient was discharged on postoperative day 15 with medical treatment. Three patients were readmitted to the hospital due to nausea and vomiting. One patient's complaints resolved with intravenous fluid therapy. Two patients underwent an upper GI endoscopy. One patient had stenosis at the anastomosis line, balloon dilatation was performed. There was no pathology observed in the other patient. Clinical complaints were resolved with IV hydration. The patient was discharged on the 5th day of follow-up.

Table-1. Comorbidities.

Comorbidity	Number
Coronary Arterial Disease	6
Hypertension	12
Diabetes Mellitus	5
Cerebrovascular Disease	1
Hearth failure	3
Chronic Kidney Failure	1
Chronic Obstructive Lung Disease	1
Parkinson Disease	1

Table-2. Pathological Features.

T			%
	1	2	8,69
	2	3	13,04
	3	4	17,39
	4	14	60,86
N			
	0	4	17,39
	1	3	13,04
	2	5	21,73
	3	11	47,82
Subtype			
	Tubular	9	39,13
	Mucinous	2	8,69
	Poorly Cohesive	8	34,78
	Mixed	4	17,39
Lymphovascular Invasion		15	65,21
Perineural Invasion		11	47,82

Table-3. Morbidity and Mortality within 90 days.

Patient No	Clavien – Dindo Classification	
1	5	Sudden Cardiac Arrest PO 3rd
2	5	Anastomotic leakage, managed with endoscopic stent, mortality was occurred due to sepsis, on PO 23th day
3	2	Aspiration Pneumoniae – managed with IV Antibiotics, discharged on PO 15th day
4	2	Readmission with nausea and vomiting, managed with IV fluid therapy
5	3	Readmission with nausea and vomiting, upper GI endoscopy revealed no pathology, managed with IV fluid therapy
6	3	Readmission with nausea and vomiting, upper GI endoscopy revealed anastomotic stenosis, managed with balloon dilatation.

DISCUSSION

There is no clear definition for the term "elderly patient" in literature. While the definition of "geriatric patient" can be used for patients over 65 or 70 years of age, the definition of "octogenarian" refers to patients 80 years of age and older. We preferred to include octogenarians in this study. Although it is known that age increases surgical mortality and morbidity, patients in the same age group do not carry the same surgical risk. Patients have different comorbidities and physical performance status. Different assessment models have been developed for these (11).

Gastric cancer prevalence increases in elderly patients (12). Some studies suggest that older patients may not have the same chance of curative treatment compared to younger patients due to the general belief that older patients have a worse prognosis regardless of surgery (13, 14). Due to advanced age and comorbidities, perioperative complications and mortality rates are more common in elderly patients than in younger patients; however, with the advancement of surgical and anesthesia techniques and improvements in postoperative patient care, perioperative morbidity and mortality have decreased and surgical outcomes of elderly patients have improved (15).

Advanced age and high ASA score are associated with mortality and morbidity. Perioperative mortality rates in elderly patients have been reported between 0-10% in the literature; in our study, the perioperative mortality rate was 8.6% (16, 17). Fujiwara et al. compared the mortality and morbidity of patients aged 80 years and older with those aged 79 years and younger; mortality rate was 4.3% vs. 0.9% and respiratory complication rates were 6% vs.

2.1%.(18) In contrast to this study, Konishi et al. reported 0 mortality and 30% morbidity in a series of 134 cases in patients aged 85 years and older, and suggested that curative surgery with D2 lymphadenectomy should be performed even in elderly patients (19). Schendel et al. reported 10% mortality in a series of 130 cases aged 57 years and older; however, they showed that disease-free survival was longer with surgery alone (20). Fujisaki et al. argued that laparoscopic gastrectomy can also be performed in patients aged 75 years and older (21).

Gagniere et al. published a meta-analysis of 13 research articles published between 2008 and 2017, including a total of 2544 patients, investigating the outcomes of CRS and HIPEC in elderly patients. It was found that advanced age did not affect 30-day postoperative morbidity, anastomotic leakage rate, reoperation, hospital readmission and length of hospital stay, but increased 90-day postoperative mortality. In subgroup examinations, it was found that the rate of Grade 3 and higher complications according to the Clavien-Dindo Classification increased above the age of 70 years. Although the authors did not set a clear age limit in the study, they emphasized that morbidity increased above the age of 70 years (22).

The immune system and respiratory function decline in elderly patients, which may lead to an increase in complications such as pneumonia and sepsis (23, 24). One of the reasons for the weak immune response of elderly patients is vitamin and mineral deficiency (25). Gastric cancer causes cachexia, vitamin and mineral deficiency, which can lead to a reduced immune response. Elderly patients and patients with gastric cancer should be evaluated as a multidisciplinary team in terms of nutritional

status - necessary nutritional support should be provided (26).

The most important limitation of our study is its retrospective nature. Since elderly patients are not included in randomized controlled trials, there is no strong evidence to confirm the efficacy of any treatment in this age group. When high-quality evidence from randomized trials is not available, we can benefit from methodologically well-planned retrospective studies. However, it is obvious that the elderly patients included in retrospective studies are selected patients and do not reflect the general elderly patient population. Therefore, unintentional bias in this article and in the literature is inevitable.

CONCLUSION

Octogenarians are a special patient group. This study showed that gastric cancer surgery can safely performed in octogenarians. The treatment plan should be individualized. If possible, all medically fit patients should undergo curative surgery.

Conflict of interest: Statement and Authorship Contributions

All the authors are in agreement with the content of the manuscript.

All the authors have no conflict of interest.

The manuscript has not been published previously and is not under consideration.

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Psikiyatrik hastalığı olan bireylerde COVID-19 salgını ve aşısına yönelik tutumlarının incelenmesi

Investigation of attitudes towards the COVID-19 outbreak and vaccine in individuals with mental health diseases

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ÖZ

Amaç: Aşılama, pandemi sürecinde enfeksiyonun önlenmesi ve kontrol edilmesi için kilit bir stratejidir; bu nedenle toplumun özel bir kısmı olan psikiyatrik tanılı hastalarda COVID-19 aşısına yönelik tutumlar ve aşıya karar verme sürecini etkileyen faktörleri araştırmayı amaçladık.

Gereç ve Yöntem: Psikiyatri poliklinik başvuruları bulunan çalışmaya alınma, dışlanma kriterlerini karşılayan depresif bozukluk, anksiyete bozukluğu, obsesif kompulsif bozukluk, bipolar bozukluk, psikotik bozukluk, kişilik bozukluğu tanıları olan 200 hastaya sosyodemografik veri toplama formu, COVID-19 hastalık algısı ölçeği, COVID-19'un nedenleri algısı ölçeği, COVID-19'un kontrolü algısı ölçeği, COVID-19'dan kaçınma tutumları ölçeği ve COVID-19 aşısına yönelik tutumlar ölçeği uygulanmıştır.

Bulgular: Hastaların %77'si (n=154) COVID-19 ile ilgili gelişmeleri takip ettiği, %40'ının (n=80) COVID-19 geçirdiği, %53,5'inin (n=107) ailesinde COVID-19 geçiren bir kişinin olduğu, %22,5'inin (n=45) yakınlarından birinin COVID-19' dan dolayı hayatını kaybettiği saptanmıştır. Hastaların %93,5'i (n=187) hastalıktan korunduğunu, %78,5'inin (n=157) hastalıktan korkulması gerektiğini, %18,5'inin (n=37) çevrelerindeki insanların kurallara uyduğunu belirtmektedir. Hastaların %88'inin (n=176) en az bir doz COVID-19 aşısı olduğu saptanmıştır. COVID-19 ile ilgili gelişmeleri takip edenlerde ve COVID-19'dan korkanlarda aşılanma oranı daha yüksek bulunmuştur (p=0.008, p=0.042). Aşılanma durumunun sosyodemografik özelliklerle ilişkisine bakıldığında yaş, cinsiyet, medeni durum, eğitim düzeyi ve çalışma durumuna göre farklılık göstermediği saptanmıştır (p>0.05). Aşılanma durumunun psikiyatrik tanılara göre farklılık gösterdiği saptanmıştır (p=0.04). Bipolar bozukluk ve psikotik bozukluk tanılı hastalarda aşılanmama oranının daha yüksek olduğu saptanmıştır.

Sonuç: Çalışmamız, psikiyatrik hastalığı olan bireylerin COVID-19 salgını ve aşısına yönelik tutumlarının daha iyi anlaşılmasına katkı sağlamaktadır. Bipolar bozukluk ve psikotik bozukluk tanılı hastalarda aşılanmama oranının daha yüksek olduğu saptanmış, ayrıca COVID-19 ile ilgili gelişmeleri takip eden ve hastalıktan korkan bireylerde aşılanma oranının daha yüksek olduğu görülmüştür. Bu bulgular, psikiyatrik hastalığı olan bireylere yönelik daha etkili destek ve müdahale stratejilerinin geliştirilmesine yardımcı olabilir.

Anahtar Sözcükler: Aşı, COVID-19, psikiyatrik hastalıklar.

ABSTRACT

Aim: *Investigation of attitudes towards the COVID-19 vaccine and factors affecting vaccine decision-making in patients with psychiatric diagnoses.*

Summary: Investigation of attitudes towards the COVID-19 vaccine and factors affecting vaccine decision-making in patients with psychiatric diagnoses. Vaccination is a crucial strategy for preventing and controlling infection during the pandemic.

Material and Methods: *A form for collecting sociodemographic data, as well as perception scales for COVID-19 disease, its causes, control, and overall perception, were administered to 200 patients who met the inclusion and exclusion criteria at the psychiatric outpatient clinic. Additionally, the scales for attitudes towards disease avoidance and the COVID-19 vaccine were also administered.*

Results: *It was determined that 77% (n=154) of the patients followed the developments regarding COVID-19, 40% (n=80) had COVID-19, 53.5% (n=107) had someone in their family who had COVID-19, and 22.5% (n=45) had a relative who lost their life due to COVID-19. 93.5% (n=187) of the patients stated that they were protected from the disease, 78.5% (n=157) stated that the disease should be feared, and 18.5% (n=37) stated that the people around them followed the rules. It was determined that 88% (n=176) of the patients had at least one dose of COVID-19 vaccine. The vaccination rate was higher in those who followed the developments regarding COVID-19 and those who were afraid of COVID-19 (p=0.008, p=0.042). When the relationship between vaccination status and sociodemographic characteristics was examined, it was found that there was no difference according to age, gender, marital status, education level and employment status (p>0.05). It was found that vaccination status differed according to psychiatric diagnoses (p=0.04). It was found that the rate of not being vaccinated was higher in patients diagnosed with bipolar disorder and psychotic disorder.*

Conclusion: *Our study contributes to a better understanding of the attitudes of individuals with psychiatric illnesses towards the COVID-19 pandemic and the vaccine. It was found that the rate of non-vaccination was higher in patients diagnosed with bipolar disorder and psychotic disorder, and it was also observed that the vaccination rate was higher in individuals who followed developments regarding COVID-19 and were afraid of the disease. These findings may help develop more effective support and intervention strategies for individuals with psychiatric illnesses.*

Keywords: *COVID-19, mental health diseases, vaccine.*

GİRİŞ

Koronavirüs hastalığı 2019 (COVID-19), son derece hızlı bir şekilde küresel ölçekte pandemik bir boyuta ulaşmış ve özellikle mevcut sağlık zorlukları ve sorunlarla ilişkili olarak eşi benzeri görülmemiş bir küresel krize sebep olmuştur. Psikiyatrik tanısı olan, özellikle ağır ruhsal hastalık tanısı konmuş hastalar, COVID-19 enfeksiyonuna karşı savunmasız ve yüksek risk grubunu oluşturur. Araştırmalar, bu popülasyonda yüksek enfeksiyon oranları ve kötü prognozun yanı sıra yüksek ölüm oranlarını da gözlemlemiştir (1-3).

Aşılama, pandemi sürecinde enfeksiyonun önlenmesi ve kontrol edilmesi için kilit bir stratejidir; bu nedenle COVID-19 aşısına yönelik tutumlar ve aşıya karar verme sürecini etkileyen faktörler üzerine araştırmalar yapılmaktadır (4-7). Ruhsal bozukluğu olan hastaların aşıya yönelik tutumlarını araştıran çalışmalarda bu hasta popülasyonunda aşılama oranlarının genel popülasyona göre daha düşük olduğu bildirilmiştir (6,8). Genel nüfusu kapsayan çalışmalar, aşı tereddüdünün, COVID-19 aşısına olan güven eksikliği ve komplo teorilerine duyulan inançla ilişkili olduğunu bildirmiştir (9). Aşılar, salgının kontrol altına alınması ve toplum bağışıklığı oluşturulması için hayati öneme sahiptir. Ancak, psikiyatrik hastalığı olan bireylerin COVID-19 aşısına yönelik tutumları, çeşitli faktörlerden etkilenebilir ve bu durum da aşılama programlarının başarısını gölgeleyebilir. Bugüne kadar psikiyatrik hastalığı olan bireylerin COVID-19 salgını ve aşılamaya yönelik tutumları yeterince araştırılmamıştır.

Bu çalışmanın amacı, psikiyatrik hastalığı olan bireylerin COVID-19 salgını ve aşısına yönelik tutumlarını daha derinlemesine incelemektir. Bu bağlamda, bireylerin algıları, bilgi düzeyleri, endişeleri, güven seviyeleri ve aşılama niyetleri gibi faktörler üzerinde durulacaktır. Ayrıca, psikiyatrik hastalığı olan bireylerin aşılama programlarına katılımını artırmak için etkili stratejilerin belirlenmesi amacıyla, bu tutumların nedenleri ve etkileyen faktörler de analiz edilecektir. Bu çalışma, psikiyatrik hastalığı olan bireylerin COVID-19 salgını ve aşısına yönelik tutumlarını anlamak için bir temel oluşturmayı ve aşıya erişim ve kabulü artırmak için politika ve

müdahaleler geliştirmesine yardımcı olabilir. Bu çalışmada, COVID-19 salgını sürecinde psikiyatrik hastalığa sahip bireylerin aşıya yönelik tutumlarını incelemeye yönelik olarak aşağıdaki hipotezler test edilmiştir

H1: Psikiyatrik tanılı hastalarda aşılanma durumu tanılara göre farklılık göstermektedir.

H2: COVID-19 ile ilgili gelişmeleri takip eden hastalarda aşılanma oranları daha yüksektir.

H3: COVID-19'dan korkanlarda aşılanma oranı daha yüksektir.

H4: Eğitim düzeyi yüksek olanlarda aşılanma oranı daha yüksektir.

YÖNTEM

Çalışmaya 01.03.2022-01.06.2022 tarihleri arasında Rize Devlet Hastanesine ayaktan psikiyatri poliklinik başvuruları bulunan çalışmaya alınma, dışlanma kriterlerini karşılayan 200 hasta dahil edildi. Çalışmaya 18-65 yaş arası, okur yazar, iletişim kurmaya engelli hali olmayan, çalışmaya katılmaya istekli, mental retarde, demans hastalığı olmayan polikliniğe ardışık sıra ile başvuran şizofreni, bipolar bozukluk, depresif bozukluk, anksiyete bozuklukları, yeme ve beslenme bozuklukları, kişilik bozuklukları, alkol-madde kullanım bozukluğu tanılı hastalar çalışmaya dahil edilmiştir. Tüm olgular çalışma hakkında bilgilendirilerek yazılı onamları alınmıştır. Çalışmadan dışlanma ölçütleri; 18-65 yaş aralığı dışında olma, görüşme yapmaya ya da ölçeğin uygulanmasına engel bir durumun bulunması, çalışmaya katılmayı kabul etmemek olarak belirlendi. Çalışmaya alınma ve dışlanma kriterlerine göre, toplamda 24 hasta çalışmadan dışlanmıştır. Dışlanma nedenleri arasında 18-65 yaş aralığında olmama, mental retardasyon veya demans gibi iletişim kurmayı engelleyen durumlar ve çalışmaya katılmayı reddetme yer almaktadır. Bu hastalar veri toplama sürecine dahil edilmemiştir.

Uygulama

Rize Devlet Hastanesi Psikiyatri Polikliniğine ardışık sıra ile başvuran hastalara çalışmanın amacı ve yöntemi hakkında bilgi verilmesi ardından aydınlatılmış onam formunu doldurarak çalışmaya katılmayı kabul eden, çalışmaya alınma ölçütlerini karşılayan hastalarla yüz yüze görüşülüp Sosyodemografik Veri Toplama Formu (sosyodemografik özellikler ve klinik özellikleri), COVID-19 Hastalık Algısı Ölçeği, COVID-19'un Nedenleri Algısı Ölçeği, COVID-19'un Kontrolü

Algısı Ölçeği, COVID-19'dan Kaçınma Tutumları Ölçeği ve COVID-19 Aşısına Yönelik Tutumlar Ölçeği uygulanmıştır.

Veri Toplama Araçları

Sosyodemografik Veri Toplama Formu: Hastaların sosyodemografik özellikleri, hastalığın klinik özellikleri gibi araştırmanın bağımsız değişkenleri konusunda bilgi edinmek amacıyla, literatür bilgilerinden yararlanılarak, çalışmacılar tarafından hazırlanmış olan bir anket formu kullanılacaktır.

COVID-19 Hastalık Algısı Ölçeği: Ölçeğin geçerlilik ve güvenilirlik çalışmasını Geniş ve arkadaşları gerçekleştirmiştir. Bu ölçek, yedi maddeden oluşmaktadır ve beşli likert ölçeği kullanılarak "Tehlikelilik" ve "Bulaştırıcılık" olmak üzere iki alt boyuttan oluşmaktadır. Katılımcılar, ifadeleri "Kesinlikle katılmıyorum (1)", "Katılmıyorum (2)", "Kararsızım (3)", "Katılıyorum (4)", "Kesinlikle katılıyorum (5)" şeklinde değerlendirmektedirler. İlk alt boyut olan "Tehlikelilik", COVID-19'un yarattığı tehlike algıları ve inançlarını içermektedir. İkinci alt boyut olan "Bulaştırıcılık" ise hastalığın bulaşıcılığına ilişkin algıları içermektedir. Ölçeğin "Tehlikelilik" alt boyutundaki bazı maddeler, ters olarak kodlanmıştır. Alt boyuttaki madde puanlarının toplanmasıyla elde edilen toplam puan, o alt boyuttaki madde sayısına bölünerek 1 ile 5 arasında bir değer elde edilmektedir. Yüksek puanlar, "Tehlikelilik" alt boyutunda hastalıkla ilgili tehlike algısının yüksek olduğunu, "Bulaştırıcılık" alt boyutunda ise virüsün bulaşıcı olduğu algısını göstermektedir. Ölçeğin toplam Cronbach alfa katsayısı 0.74; "Tehlikelilik" alt boyutunun Cronbach alfa katsayısı 0.64 ve "Bulaştırıcılık" alt boyutunun Cronbach alfa katsayısı 0,75 olarak bulunmuştur. (10).

COVID-19'un Nedenleri Algısı Ölçeği: Geniş ve ekibinin yaptığı bir araştırmada, ölçeğin geçerlilik ve güvenilirlik çalışması yapılmıştır. Bu ölçek, beşli Likert ölçeği kullanılarak 14 maddeden oluşmakta ve üç alt boyuta ayrılmıştır. Ölçeğin ilk alt boyutu "Komplo" olarak adlandırılmıştır ve medyada sıkça dile getirilen komplo inançlarını (biyolojik savaş, aşı satma çabaları vb.) içermektedir. İkinci bileşen olan "Çevre", COVID-19 salgınının olası nedenlerini sosyal ve fiziksel çevre açısından ele almaktadır. Bu bileşen, sağlıksız beslenme, küresel ısınma, doğal kaynakların kirlenmesi gibi faktörlere değinmektedir. Son bileşen olan "İnanç", COVID-19'un nedenine dair dini ve ruhsal açıklamaları

içermektedir. Bu inançlar arasında salgının kaderle ilişkilendirilmesi veya Tanrı'nın bir gazabı olarak algılanması gibi düşünceler bulunmaktadır. Ölçekte ters maddeler bulunmamaktadır. Alt boyuttaki madde puanlarının toplanmasıyla elde edilen toplam puan, o alt boyuttaki madde sayısına bölünerek 1 ile 5 arasında bir değer elde edilir. Bu değerin yüksek olması, ilgili alt boyuttaki algının yüksek olduğunu göstermektedir. Toplam ölçek için Cronbach alfa katsayısı (10).

COVID-19'un Kontrolü Algısı Ölçeği: Geniş ve diğer araştırmacılar tarafından yürütülen bir çalışmada, ölçeğin geçerlilik ve güvenirlik analizi gerçekleştirilmiştir. Bu ölçek, beşli Likert ölçeği kullanılarak 12 maddeden oluşmakta ve üç alt boyuta ayrılmıştır. Ölçeğin ilk alt boyutu "Makro Kontrol" olarak adlandırılmıştır ve kurumsal, ulusal veya küresel düzeyde alınan tedbirlerin etkililiği ile ilgili inançları içermektedir. İkinci alt boyut "Kişisel Kontrol" olarak adlandırılmıştır ve kişisel önlemlerin hastalığa yakalanma riskini azaltmadaki etkililiği ile ilgilidir. Son alt boyut "Kontrol Edilebilirlik" olarak adlandırılmıştır ve hastalığın kontrol edilebilir olduğu inancını değerlendirmektedir. Bu alt boyuttaki maddeler ters olarak puanlanmaktadır. Alt boyuttaki madde puanlarının toplanmasıyla elde edilen toplam puan, o alt boyuttaki madde sayısına bölünerek 1 ile 5 arasında bir değer elde edilmektedir. Yüksek puanlar, ilgili alt boyuttaki algının yüksek olduğunu göstermektedir. Toplam ölçek için Cronbach alfa katsayısı 0,79; "Makro Kontrol" alt boyutunun Cronbach alfa katsayısı 0,83, "Kişisel Kontrol" alt boyutunun Cronbach alfa katsayısı 0.80 ve "Kontrol Edilebilirlik" alt boyutunun Cronbach alfa katsayısı 0,78 olarak bulunmuştur (10).

COVID-19'dan Kaçınma Tutumları Ölçeği: Geniş ve diğer araştırmacıların yaptığı bir çalışmada, ölçeğin geçerlik ve güvenirlik analizi yapılmıştır. Bu ölçek, beşli Likert ölçeği kullanılarak 10 maddeden oluşmakta ve bilişsel kaçınma ile davranışsal kaçınma olmak üzere iki alt boyuta ayrılmıştır. Bilişsel kaçınma, belirli düşünceleri veya durumları zihinsel olarak reddetme eğilimini değerlendirirken, davranışsal kaçınma belirli davranışları yapmaktan kaçınma eğilimini değerlendirir. Ölçekte ters maddeler bulunmamaktadır. Alt boyuttaki madde puanlarının toplanmasıyla elde edilen toplam puan, o alt boyuttaki madde sayısına bölünerek 1 ile 5 arasında bir değer elde edilmektedir. Yüksek puanlar, ilgili alt boyuttaki kaçınmanın yüksek olduğunu göstermektedir. Toplam ölçek için

Cronbach alfa katsayısı 0.88; bilişsel kaçınma alt boyutunun Cronbach alfa katsayısı 0,92 ve davranışsal kaçınma alt boyutunun Cronbach alfa katsayısı 0,97 olarak bulunmuştur. Bu sonuçlar, ölçeğin güvenirliğinin yüksek olduğunu göstermektedir (10).

COVID-19 Aşısına Yönelik Tutumlar Ölçeği: Geniş ve arkadaşlarının gerçekleştirdiği bir çalışmada ölçeğin geçerlik ve güvenirlik analizi yapılmıştır. Bu ölçek, 9 maddeden oluşmakta ve "Olumlu Tutum" ve "Olumsuz Tutum" olmak üzere iki alt boyuta ayrılmıştır. Olumlu tutum alt boyutu, aşıya yönelik olumlu düşünceleri içermektedir. Öte yandan, olumsuz tutum alt boyutu aşının olumsuz yönlerini değerlendirmektedir. Ölçekte 5'li Likert ölçeği kullanılmış olup, olumsuz tutum alt boyutu maddeleri ters olarak puanlanmıştır. Alt boyuttaki madde puanlarının toplanması ve madde sayısına bölünmesiyle 1-5 arasında bir değer elde edilmektedir. Yüksek puanlar, ilgili alt boyuttaki tutumun güçlü olduğunu göstermektedir. Toplam ölçek için Cronbach alfa katsayısı 0,80; olumlu tutum alt boyutu için Cronbach alfa katsayısı 0,96 ve olumsuz tutum alt boyutu için Cronbach alfa katsayısı 0,78 olarak bulunmuştur. Bu çalışmada toplam ölçek için Cronbach alfa katsayısı 0.81; olumlu tutum alt boyutu için 0.94 ve olumsuz tutum alt boyutu için 0,82 olarak hesaplanmıştır. Bu sonuçlar, ölçeğin güvenirliğinin kabul edilebilir düzeyde olduğunu göstermektedir (10).

Çalışmanın etik kurul onayı Recep Tayyip Erdoğan Üniversitesi Girişimsel olmayan Klinik Araştırmalar Etik Kurulu, 24.03.2022 tarih 2022/91 sayılı kararı ile alınmış olup çalışma için kurum izni de alınmıştır. Bu çalışmada yapılan tüm uygulamalar, kurumsal ve/veya ulusal araştırma komitesinin etik standartlarına ve 1964 Helsinki Bildirgesi'ne ve daha sonraki düzeltmelerine veya karşılaştırılabilir etik standartlara uygun yapılmıştır.

İstatistiksel analiz

İstatistiksel analizlerde Windows SPSS 26.0 programı (Statistical Package for the Social Sciences Inc.) kullanıldı. Tanımlayıcı istatistikler ve yaş gibi sürekli değişkenler ortalama ± standart sapma, cinsiyet gibi kategorik değişkenler ise frekans ve yüzde olarak verildi. Kategorik verilerin analizinde Ki-kare testi, bağımsız grupların ortalamalarının karşılaştırılmasında bağımsız gruplar t-testi, sürekli verilerin birbiriyle olan ilişkisinde Pearson

korelasyon analizi, kategorik verilerin regresyon analizinde Binary lojistik regresyon analizi kullanıldı. İstatistiksel anlamlılık düzeyi tüm değerler için p<0,05 olarak kabul edildi.

BULGULAR

Katılımcıların yaş ortalaması 39.61±12,81 yıl, %70'i (n=140) kadın, %60'ı (n=120) evli, %68'i (n=136) çalışıyor, %54,5'nin (n=109) eğitim düzeyi lise ve üzeriydi. Hastaların %31,5'i (n=63) depresif bozukluk, %36,5'i (n=73) anksiyete bozukluğu, %9'u (n=18) obsesif kompulsif bozukluk, %8,5'i (n=17) bipolar bozukluk, %9,5'i (n=19) psikotik bozukluk, %3'ü (n=6) kişilik bozukluğu tanısına sahipti (Tablo-1).

Hastaların %77'si (n=154) COVID-19 ile ilgili gelişmeleri takip ettiği, %40'ının (n=80) COVID-19 geçirdiği, %53,5'inin (n=107) ailesinde COVID-19 geçiren bir kişinin olduğu, %22,5'inin (n=45) yakınlarından birinin COVID-19' dan dolayı hayatını kaybettiği saptanmıştır. Hastaların %93,5'i (n=187) hastalıktan korunduğunu, %78,5'inin (n=157) hastalıktan korkulması gerektiğini, %18,5'inin (n=37) çevrelerindeki insanların kurallara uyduğunu belirtmektedir. Hastaların %88'inin (n=176) en az bir doz COVID-19 aşısı olduğu saptanmıştır. COVID-19 ile ilgili gelişmeleri takip edenlerde ve COVID-19'dan korkanlarda aşılanma oranı daha yüksek bulunmuştur (p=0.008, p=0.042) (Tablo-2). Hastaların aşılanma dozlarına bakıldığında %84'ü (n=168) iki doz, %37'si (n=74) üç doz, %3,5'i (n=7) dört doz ve %1'i beş doz aşı olmuştur (Şekil-1).
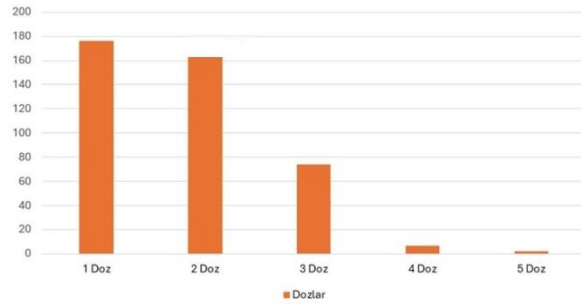
Şekil-1. Hastaların aşılanma dozları.

Aşılanma durumunun sosyodemografik özelliklerle ilişkisine bakıldığında yaş, cinsiyet, medeni durum, eğitim düzeyi ve çalışma durumuna göre farklılık göstermediği saptanmıştır (p>0.05). Aşılanma durumunun psikiyatrik tanılara göre farklılık gösterdiği saptanmıştır (p=0.04). Bipolar bozukluk ve

psikotik bozukluk tanılı hastalarda aşılanmama oranının daha yüksek olduğu saptanmıştır (p=0.04) (Tablo-3).

Uygulanan ölçeklerin aşılanma durumuna göre karşılaştırılması sonucunda COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik (p=0.001), COVID-19 Nedenler Algısı Ölçeğinin Çevre (p=0.02), COVID-19 Kontrol Algısı Ölçeğinin Makro (p=0.05), COVID-19 Aşısına Yönelik Tutumlar Ölçeğinin Olumlu Tutum (p=0.001) ve Olumsuz Tutum (p=0.001) alt boyutu puanları açısından anlamlı farklılık vardı (Tablo-4).

Uygulanan ölçeklerin birbiriyle olan ilişkisine bakıldığında COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik alt boyutu ile COVID-19 Kontrol Algısı Ölçeğinin Makro alt boyutu (r=-0.194, p<0.01) ve Kişisel alt boyutu (r=-0.156, p<0.05) arasında negatif yönde bir ilişki varken COVID-19 Aşısına Yönelik Tutumlar Ölçeğinin Olumlu Tutum (r=0.173, p<0.05) ve Olumsuz Tutum (r=0.257, p<0.01) arasında pozitif yönde bir ilişki saptanmıştır. COVID-19 Aşısına Yönelik Tutumlar Ölçeğinin Olumlu Tutum alt boyutu ile COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik alt boyutu (r=0.173, p<0.05) COVID-19 Hastalık Algısı Ölçeğinin Bulaştırıcılık alt boyutu (r=0.222, p<0.01), COVID-19 Kontrol Algısı Ölçeğinin Makro alt boyutu (r=0.144, p<0.05), COVID-19 Aşısına Yönelik Tutumlar Ölçeğinin Olumsuz Tutum alt boyutu (r=0.601, p<0.01) arasında pozitif yönde bir ilişki varken, COVID-19 Nedenler Algısı Ölçeğinin Komplo alt boyutu (r=-0.202, p<0.01) arasında negatif yönde bir ilişki saptanmıştır. COVID-19 Aşısına Yönelik Tutumlar Ölçeğinin Olumsuz Tutum alt boyutu ile COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik alt boyutu (r=0.257, p<0.01) ile pozitif yönde bir ilişki varken COVID-19 Nedenler Algısı Ölçeğinin Komplo alt boyutu (r=-0.277, p<0.01) ile negatif yönde bir ilişki vardı (Tablo-5).

Psikiyatrik tanılı hastaların aşı olma durumunu etkileyen faktörlerin tespit edilmesi amacıyla iki durumlu (Binary) lojistik regresyon analizi yapılmıştır. Bunun için öncelikle literatüre dayalı olarak ağır ruhsal hastalık varlığı, COVID-19 ile ilgili gelişmeleri takip etme, COVID-19 korkusu, COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik alt boyutu ve COVID-19 Aşısına Yönelik Tutumlar Ölçeği Olumlu ve Olumsuz Tutumlar alt ölçeği bağımsız değişkenler olarak belirlenmiştir. Yapılan analiz sonucunda kurulan modelin Hosmer ve Lemeshow Uyum İyiliği Testi sonucu beklendiği gibi p>0,05 olarak bulunmuş (p=0.654)

ve modelin iyi uyum gösterdiğini ortaya koymuştur. Ayrıca kurulan model bağımlı değişkenin %55,1'ini açıklamıştır (Nagelkerke R^2 =0.551). COVID-19 Aşısına Yönelik Tutumlar Ölçeği Olumlu Tutum ve COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik alt boyutu puanlarının aşılanma durumu üzerine anlamlı bir etkisi bulunmaktadır (p=0.000) (Tablo-6).

Tablo-1. Katılımcıların sosyodemografik ve klinik özellikleri.

Yaş (Ort ± SS)		39,61 ± 12,81	
		n	**%**
Cinsiyet	Kadın	140	70
	Erkek	60	30
Medeni durum	Bekar	80	40
	Evli	120	60
Eğitim düzeyi	Okuryazar Değil	8	4
	İlköğretim	58	29
	Ortaöğretim	25	12.5
	Lise	57	28.5
	Üniversite	52	26
Çalışma durumu	Çalışıyor	136	68
	Çalışmıyor	64	32
Psikiyatrik tanılar	Depresif bozukluk	63	31.5
	Anksiyete bozukluğu	73	36.5
	Obsesif kompulsif bozukluk	18	9
	Bipolar duygudurum bozukluğu	17	8.5
	Psikotik bozukluk	19	9.5
	Kişilik bozukluğu	6	3
	Madde kullanım bozukluğu	1	0.5
	Uyum bozukluğu	3	1.5

Ort: Ortalama, SS: Standart Sapma

Tablo-2. Aşılanma durumunun COVID-19 özellikleri ile ilişkisi.

	COVID-19 aşılanan 176 (%88)	COVID-19 aşılanmayan 24 (%12)	X^2	p
COVID-19 ile ilgili gelişmeleri takip ediyor musun?	Evet:141 (%80.1) Hayır:35 (%19.9)	Evet:13 (%54.2) Hayır:11 (%45.8)	8.029	0.008*
COVID-19 geçirdin mi?	Evet:68 (%38.6) Hayır:108 (%61.4)	Evet:12 (%50) Hayır:12 (%50)	1.136	0.375
Aile üyelerin COVID-19 geçirdi mi?	Evet:90 (%51.1) Hayır:86 (%48.9)	Evet:17 (%70.8) Hayır:7 (%29.2)	3.294	0.083
COVID-19' dan dolayı yakınlarından biri hayatını kaybetti mi?	Evet:40 (%22.7) Hayır:136 (%77.3)	Evet:5 (%20.8) Hayır:19 (%79.2)	0.43	1
COVID-19' den korunuyor musun?	Evet:165 (%93.8) Hayır:11 (%6.2)	Evet:22 (%91.7) Hayır:2 (%8.3)	0.151	0.658
COVID-19' dan korkuyor musun?	Evet:142 (%80.7) Hayır:34 (%19.3)	Evet:15 ((%62.5) Hayır:9 (%37.5)	4.137	0.042*
COVID-19 kurallarına çevrendeki insanlar uyuyor mu?	Evet:30 (%17) Hayır:146 (%83)	Evet:7 (%29.2) Hayır:17 (%70.8)	2.058	0.164

*Pearson ki-kare istatistiksel analiz; p<0.05

Tablo-3. Aşılanma durumunun sosyodemografik ve klinik özellikler ile ilişkisi.

		COVID-19 aşılanan n (%)	COVID-19 aşılanmayan n (%)	t/X²	p
Yaş (Ort ± SS)		39.63 ± 12.87	39.41 ± 12.66	-0.07	0.93
Cinsiyet	Kadın	125 (89.3)	15 (10.7)	0.73	0.47
	Erkek	51 (85)	30 (15)		
Medeni durum	Bekar	68 (85)	12 (15)	0.28	0.37
	Evli	108 (90)	12 (10)		
Eğitim düzeyi	Okuryazar Değil	7 (87.5)	1 (12.5)	5.03	0.28
	İlköğretim	53 (91.4)	5 (8.6)		
	Ortaöğretim	24 (96)	1 (4)		
	Lise	46 (80.7)	11 (19.3)		
	Üniversite	46 (88.5)	6 (11.5)		
Çalışma durumu	Çalışıyor	56 (87.5)	8 (12.5)	0.02	1
	Çalışmıyor	120 (88.2)	16 (11.8)		
Psikiyatrik tanılar	Depresif bozukluk	59 (93.7)	4 (6.3)	14.54	0.04*
	Anksiyete bozukluğu	67 (91.8)	6 (8.2)		
	Obsesif kompulsif bozukluk	15 (83.3)	3 (16.7)		
	Bipolar duygudurum bozukluğu	13 (76.5)	4 (23.5)		
	Psikotik bozukluk	13 (68.4)	6 (31.6)		
	Kişilik bozukluğu	6 (100)	0 (0)		
	Madde kullanım bozukluğu	1 (100)	0 (0)		
	Uyum bozukluğu	2 (66.7)	1 (33.3)		

*Pearson ki-kare istatistiksel analiz; p<0.05

Tablo-4. Aşılanma durumunun uygulanan ölçekler ile ilişkisi

		COVID-19 aşılanan Ort ± ss	COVID-19 aşılanmayan Ort ± ss	t	p
COVID-19 hastalık algısı ölçeği	Tehlikelilik	3.05 ± 0.74	2.36 ± 0.71	-4.3	0.001*
	Bulaştırıcılık	4.23 ± 0.85	3.88 ± 0.79	-1.88	0.06
COVID-19 nedenler algısı ölçeği	Komplo	2.7 ± 1.06	3.11 ± 1.23	1.74	0.08
	Çevre	2.62 ± 0.83	3.02 ± 0.81	2.2	0.02*
	İnanç	2.66 ± 1.09	2.7 ± 1.12	0.18	085
COVID-19 kontrol algısı ölçeği	Makro	3.12 ± 1.02	3.55 ± 1.03	1.95	0.05*
	Kişisel	3.24 ± 081	3.42 ± 0.75	1	0.31
COVID-19'dan kaçınma tutumları ölçeği	Bilişsel kaçınma	1.85 ± 0.87	1.91 ± 0.79	0.28	0.78
	Davranışsal kaçınma	2.49 ± 0.79	2.47 ± 0.83	-0.08	0.93
COVID-19 aşısına yönelik tutumlar ölçeği	Olumlu tutum	4.05 ± 0.89	2.29 ± 0.92	-9.04	0.001*
	Olumsuz tutum	3.75 ± 0.9	2.64 ± 0.69	-5.76	0.001*

Ort: Ortalama, SS: Standart Sapma *Bağımsız Gruplar t Testi, p<0.05

Tablo-5. Uygulanan ölçekler arasındaki ilişkisi

	1	2	3	4	5	6	7	8	9	10	11
Tehlikelilik (1)	1	-.0081	-0.09	0.073	-0.099	-.194**	-0.156*	0.003	0.04	0.173*	0.257**
Bulaştırıcılık (2)	-0.081	1	-0.095	-0.033	-0.009	.038	0.067	0.070	0.076	0.222**	0.121
Komplo (3)	-0.09	-0.095	1	0.189**	0.206**	-.070	0.046	-0.002	0.11	-0.202**	-0.277**
Çevre (4)	0.073	-0.033	0.189**	1	0.369**	-.048	0.212**	0.266**	0.305**	-0.117	-0.015
İnanç (5)	-0.099	-0.009	0.206**	0.369**	1	.188**	0.223**	0.301**	0.184**	0.029	-0.049
Makro (6)	-0.194**	0.038	-0.07	-0.048	0.188**	1	0.422**	0.054	-0.045	0.144*	0.055
Kişisel (7)	-0.156*	0.067	0.046	0.212**	0.223**	.422**	1	0.087	0.007	0.132	0.081
Bilişsel kaçınma (8)	0.040	0.076	0.11	0.305**	0.184**	-.045	0.007	0.735**	1	-0.045	-0.029
Davranışsal kaçınma (9)	0.003	0.07	-0.002	0.266**	0.301**	.054	0.087	1	0.735**	0.081	0.031
Olumlu tutum (10)	0.173*	0.222**	-0.202**	-0.117	0.029	.144*	0.132	0.081	-0.045	1	0.601**
Olumsuz tutum (11)	0.257**	0.121	-0.277**	-0.015	-0.049	.055	0.081	0.031	-0.029	0.601**	1

*Pearson korelasyon analizi, $p<0.05$, **$p<0.001$

Tablo-6. Aşılanma durumu üzerine değişkenlerin etkisinin lojistik regresyon analizi

	B	S. Hata	Wald	Sd	p	Odds Oranı	%95 Güven Aralığı	
							Min.	Maks.
Ağır ruhsal hastalık	-0.403	0.667	0.365	1	0.546	0.668	0.181	2.471
COVID-19 gelişmeleri takip	0.582	0.634	0.843	1	0.358	1.790	0.517	6.200
COVID-19 korkusu	-0.124	0.709	0.031	1	0.861	0.884	0.220	3.543
Tehlikelilik	1.121	0.423	7.035	1	0.008	3.069	1.340	7.030
Olumlu tutum	1.407	0.339	17.200	1	0.000	4.082	2.100	7.935
Olumsuz tutum	0.432	0.427	1.022	1	0.312	1.540	0.667	3.557
(Sabit)	-7.088	1.710	17.182	1	0.000	0.001		

*Bağımlı Değişken: Aşılanma Durumu Hosmer ve Lemeshow Uyum İyiliği $p=0.654$. Nagelkerke $R^2=0.551$

TARTIŞMA

Çalışmamız psikiyatri hastalarında COVID-19 salgını ve aşısına yönelik tutumları değerlendiren kısıtlı sayıdaki çalışmadan biridir. Önceki çalışmalar psikiyatrik tanısı olanlarda aşılanma oranının daha düşük olduğunu belirtmektedir bu nedenle COVID-19 salgını sırasında psikiyatrik tanının aşılama oranları ve tutumlara etkisi araştırılmıştır (6, 8, 11). Bireyin COVID-19 enfeksiyonu ve tedavisine yönelik yaklaşımları, kültürel ve sosyal çevresinin, algıları, tutumları, inançları ve geçmiş deneyimlerinden etkilenmektedir (10). Bizim çalışmamızda psikiyatrik hastalarda aşı olma durumunun sosyodemografik verilerle bir ilişkisi olmadığı saptanmıştır. Çalışmamızla benzer şekilde Kılıç ve arkadaşlarının yapmış olduğu çalışmada da aşı olma durumunun yaş, cinsiyet, eğitim durumu

ve bir işte çalışıyor olma durumuyla ilişkili olmadığı saptanmıştır (12). Çalışmamızda hastaların %88'i aşı olmuştu. Ülkemizde genel toplumda aşılanma oranı %93,3'tür (13). Benzer şekilde Çin'de yapılan bir araştırma, depresyon ve anksiyete tanısı alan hastalarda COVID-19 aşısının kabul edilme oranının %96,2 olduğu saptanmıştır (6). Ülkemizde ağır ruhsal hastalığı olan bireylerde yapılan çalışmada da aşılanma oranının %83,7 olduğu belirtilmektedir (12). Çalışmamızda aşılanma oranının psikiyatrik tanılara göre farklılık gösterdiği saptanmıştır. Özellikle ağır ruhsal hastalık olarak belirtebilecek psikotik bozukluk ve bipolar bozukluk hasta grubunda aşılanmama oranı daha yüksek bulunmuştur. Çalışmamızdaki bulguları destekleyecek şekilde ruhsal hastalığı veya şizofreni hastalarında COVID-19 aşılama oranlarının genel popülasyona göre daha düşük olduğunu bildirmiştir (1, 14, 15). Kapsamlı bir kohort çalışmasında ayrıca ruhsal bozukluğu olan hastalarda aşılama oranlarının (%60,4) genel popülasyona (%74,9) göre anlamlı derecede düşük olduğu ve bu oranın psikotik bozukluğu olan hastalarda (%56,9) daha da düşük olduğu rapor edilmiştir (11). Bazı çalışmalar psikotik bozukluk tanısı alan hastalarda aşılama oranlarının daha düşük olduğunu göstermiştir (14). Ülkemizde ağır ruhsal bozukluğu olan hastalarda yapılan başka bir çalışmada ise aşılanma oranının %90'a yakın olduğu ve psikiyatrik tanılara göre aşılanma oranlarında anlamlı bir farklılık olmadığı saptanmıştır (16). Ağır ruhsal bozukluğu olan hastalarda COVID-19 aşısının önündeki yaygın engeller arasında aşının yan etkileri ve etkinliğine ilişkin endişeler, aşılara duyulan güvensizlik ve yanlış bilgilendirme yer alabilmektedir (17). Özellikle şizofreni hastalarında tedavi kararı verme kapasitesinin azalması, aşı kullanımını olumsuz etkileyebilmektedir (18).

Çalışmamızda COVID-19 ile ilgili gelişmeleri takip edenlerde aşılanma oranlarının daha yüksek olduğu saptanmıştır. Sosyal ağların etkileri de aşı kaygılarının azaltılmasında veya aşılmasında rol oynayabilir. Ruhsal bozukluğu olan bireylerin COVID-19 aşısı hakkında yeterli bilgiye sahip olmama veya bilgilendirme kampanyalarından yeterince faydalanamama olasılığı vardır. Bu durum, aşıya olan güveni azaltabilir ve aşılanma oranlarını düşürebilir (19). Güvenilir bilgi kaynaklarına erişimi iyileştiren müdahaleler yoluyla bu aşılanma engellerin aşılması, bu popülasyonda aşılama oranlarının artmasına

yardımcı olabilir (19, 20). Çalışmamızda, aile üyelerinden birinin COVID-19 geçirmiş olduğu bireylerde aşılanma oranlarının daha yüksek olduğu tespit edilmiştir. Bu durum COVID-19 ile ilgili bilgi ve farkındalık düzeyinin artmış olmasıyla ilgili olabilir. COVID-19 ile ilgili bilgi ve farkındalık düzeyinin aşılanma üzerindeki etkisi oldukça önemlidir. Daha yüksek bilgi ve farkındalık düzeyine sahip bireylerin genellikle aşıya daha olumlu bir yaklaşım sergilediği gözlemlenmektedir. Bu durum, aşının etkinliği, güvenliği ve faydaları hakkında doğru bilgilere sahip olmanın, bireylerin aşılanma kararı verme sürecini etkilediğini göstermektedir (21). COVID-19 aşıları ve enfeksiyonu hakkında hedefe yönelik, özel bilgiler sağlamanın ebeveynlerin çocukları aşılama konusundaki niyetini artırdığı ve aşı tereddütlerini azalttığı gösterilmiştir (22). Literatürdeki çalışmalar, aile bireylerinin veya arkadaşlarının COVID-19'dan etkilendiğini tanıyan bireylerin aşı oranlarının daha yüksek olabileceğini öne sürüyor. Araştırmalar, bir kişinin COVID-19 hastalığına yakalandığını veya bu hastalıktan öldüğünü bilmenin, en az tek bir COVID-19 aşısı dozu alma olasılığının daha yüksek olmasıyla ilişkili olduğunu buldu (23). Ayrıca, COVID-19 nedeniyle sağlık sorunu yaşayan birini tanıyan bireylerin aşı olma olasılığı daha yüksek saptanmıştır (24). Bu bulgular, kişinin sosyal çevresinde hastalıkla ilgili kişisel deneyiminin aşılama kararlarını etkileyebileceğini ve aşı alımını artırabileceğini göstermektedir. COVID-19'dan korkulması gerektiğine inanan bireylerde aşılanma oranlarının daha yüksek olduğu gözlemlenmiştir. COVID-19 korkusu ile aşılama oranları arasındaki ilişki çok yönlüdür. COVİD-19 korkusunun aşı isteğini öngördüğü, artan korkunun ise aşı olma olasılığının arttığı görüldü (25). Kanser hastaları gibi belirli popülasyonlarda, daha yüksek düzeydeki COVİD-19 fobisi, COVID-19 aşılarına olan istekliliğin artmasıyla ilişkilendirilmiştir (26). Psikiyatrik hastalıkları olan kişiler genellikle toplumun en savunmasız gruplarından birini oluştururlar. Bu hastalar, aşılama kararı alırken çeşitli endişelerle karşılaşabilirler. Bunlar arasında aşının güvenilirliği, yan etkileri, yaygın yanlış bilgiler, korku ve güvensizlik gibi faktörler yer alabilir. Yapılan bir pilot müdahale çalışması sonucunda psikiyatrik tedavi sağlayıcıları, ağır ruhsal hastalığı olan hastalarda aşılama oranlarını önemli ölçüde artırabildiği saptanmıştır. Tedavi sağlayıcıları hastalarıyla güvenilir bir ilişki içinde oldukları için, aşıya ilişkin endişeleri ele

almak ve doğru bilgi sağlamak için önemli bir konumdadırlar. Bu, hastaların aşıya yönelik endişelerini azaltabilir ve aşılama oranlarını artırabilir (27).

Çalışmamızda COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik alt boyutu puanları yüksek olanlarda aşılanma oranı daha yüksek saptanmıştır. COVID-19 Hastalık Algısı Ölçeğinin Tehlikelilik alt boyutu puanları yüksek olan bireylerde aşılanma oranlarının daha yüksek olduğu bulgusu, aşı kabulünün kişinin hastalığın ciddiyeti konusundaki algısıyla ilişkili olduğunu gösterebilir. Yani, hastalığın tehlikeli olduğunu düşünen bireyler, kendilerini ve çevrelerini korumak için aşıyı daha fazla kabul edebilirler (25). Bu bulgu, sağlık uzmanlarının ve kampanya yürütücülerinin aşı kabulünü artırmak için doğru bilgi ve farkındalık oluşturmanın önemini vurgular. Tehlikelilik algısını artırmak, insanların COVID-19'un ciddiyetini daha iyi anlamalarına ve buna göre aşıyı kabul etmelerine yardımcı olabilir (28). Bu nedenle, COVID-19'a ilişkin algıların, aşılanma oranlarını belirlemede önemli bir rol oynadığına dair bu tür bulgular, aşı kampanyalarının ve sağlık iletişiminin nasıl şekillendirileceği konusunda fikir verici olabilir. Hastalığın ciddiyeti ve aşının etkili olduğuna dair doğru bilginin vurgulanması, aşı kabulünü artırabilir ve sonuçta toplum sağlığını korumaya yardımcı olabilir.

Çalışmamızda COVID-19 Aşısına Yönelik Tutumlar Ölçeğinin Olumlu ve Olumsuz Tutum alt boyutu puanları yüksek olanlarda aşılanma oranı daha yüksek saptanmıştır. COVID-19 Aşısına Yönelik Tutum Ölçeğinin Olumlu Tutum alt ölçeğinde daha yüksek puan alan bireylerin aşı olma olasılıkları daha yüksektir. Bu ilişki, yaşam memnuniyeti, COVID-19 aşısı bilgisi ve bilime güven gibi çeşitli faktörlerin, bireyin aşıya yönelik tutumunu ve dolayısıyla aşılama davranışını etkileyebileceğini gösteren bulgularla desteklenmektedir (29). Ülkemizde yapılan bir başka araştırmada ise aşı tereddüdü ve direnci olan bireylerin aşılara olan güvenlerinin düşük, komplo teorilerine olan inançlarının ise yüksek olduğu ve bunun da aşılama oranlarını olumsuz etkilediği vurgulandı (30). COVID-19 aşısına karşı tereddüdü olanlarda, aşı tereddüdü ve aşı karşıtlığı ölçeklerinde daha yüksek puanlar saptanmıştır (31). Ebeveynlerin çocukluk çağı aşılarına yönelik tutumlarının, COVID-19 aşılarına yönelik tutumlarla ilişkili olduğu, tereddüt eden ebeveynlerin ise COVID-19 aşısına karşı daha az olumlu tutum sergiledikleri görüldü (32). Son olarak, gençler arasında aşı tereddüdünün azaltılmasında doğru ve yeterli bilgilendirmenin önemli olduğu ve aşılamayı destekleyecek olumlu sosyal davranış ve değerlerin önerildiği saptandı (33).

Çalışmamızın öne çıkan özellikleri literatürdeki çalışmaların çoğunlukla ağır ruhsal hastalıklar gibi spesifik ruhsal bozukluğu olan hastalarla yürütülmüşken çalışmamızda tüm ruhsal hastalık türleri dahil edilmiştir. Ayrıca çalışmamız hastalarla bire bir yüz yüze şekilde COVID-19 algısını ve aşıya yönelik tutumlarına ilişkin niteliksel ve derinlemesine görüşmelerin sağlanmış olmasıdır. Çalışmamızın bazı sınırlılıkları ise araştırmanın kesitsel tasarımı, genel popülasyona genellenemez oluşu, araştırmanın tek merkezde yapılmış olması ve zamanlamasıdır.

SONUÇ

Sonuç olarak çalışmamız literatürde bu alanda yürütülmüş kısıtlı sayıda ve bazı yönleriyle ülkemizdeki ilk çalışmadır. Çalışmamız, psikiyatrik hastalığı olan bireylerin COVID-19 salgını ve aşısına yönelik tutumlarının daha iyi anlaşılması, bu popülasyona yönelik etkili destek ve müdahale stratejilerinin geliştirilmesine olanak tanır. Ayrıca, psikiyatrik hastalığı olan bireylerin sağlıkla ilgili kararlarını etkileyen faktörleri anlamak için daha geniş kapsamlı bir araştırma ve müdahale çağrısında bulunmaktadır.

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Evaluation of the accuracy of estimated fetal weight in pregnancies complicated by premature prelabour rupture of membranes (PPROM)

Doğum öncesi erken membran rüptürü (PPROM) ile komplike gebeliklerde tahmini fetal ağırlığın doğruluğunun değerlendirilmesi

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ABSTRACT

Aim: Premature prelabour rupture of membranes (PPROM) can affect ultrasound measurements of the fetus and therefore, estimated fetal weight (EFW) as there is a reduced amount of amniotic fluid. The aim of this study was to test the accuracy of EFW calculated with the Hadlock IV method in pregnancies complicated by PPROM by comparisons with birthweight.

Materials and Methods: A retrospective screening was made of the data of patients admitted with a diagnosis of early membrane rupture to Kayseri Training and Research Hospital between May 2018 and June 2020. Singleton pregnancies between 23^{+0}-36^{+6} weeks with estimated fetal weight (EFW) measured by ultrasonography within 2 weeks before delivery was included in the study. The accuracy of the EFW was determined by the absolute percent difference between BW and EFW (abs[EFW-BWW]/BW100).

Results: A total of 137 patients were included in the study according to the sample size analysis. The mean difference between birthweight and EFW was 7,04% (0,04-44,5). The difference (min-max) between EFW and BW was 10,93% (0,11-34,6) when BMI was ≥30 kg/m2 and 9,61% (0,79-34,6) in the presence of anhydramnios. And these were statistically significant, p=0,001 and p=0,007 respectively. However, the results for oligohydramnios, primiparity and breech presentation were not statistically significant (p>0,05).

Conclusion: In patients with PPROM, EFW measured by the Hadlock IV method can be estimated with a margin of error of 7,04%. This difference increases in obese women and anhydramnios and prediction becomes difficult.

Keywords: Premature prelabour rupture of membranes, Hadlock's formula, ultrasonography, estimated fetal weight.

Note: This study was presented as an oral presentation at the Izmir Democracy University 4th International Congress, which took place between 9-11 November 2022.

ÖZ

Amaç: *Doğum öncesi erken membran rüptürü (PPROM), fetüsün ultrason ölçümlerini ve dolayısıyla amniyotik sıvı miktarında azalma nedeniyle tahmini fetal ağırlığı (EFW) etkileyebilir. Bu çalışmanın amacı PPROM ile komplike olan gebeliklerde Hadlock IV yöntemi ile hesaplanan EFW'nin doğruluğunu doğum ağırlığı ile karşılaştırarak test etmektir.*

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Gereç ve Yöntem: *Mayıs 2018- Haziran 2020 tarihleri arasında Kayseri Eğitim ve Araştırma Hastanesi'ne erken membran rüptürü tanısıyla başvuran hastaların verilerinin retrospektif taraması yapıldı. 23^{+0}-36^{+6} hafta arası tekil gebelikler ile tahmini fetal ağırlık (EFW) doğumdan önceki 2 hafta içinde ultrasonografi ile ölçülenler çalışmaya dahil edildi. EFW'nin doğruluğu, doğum ağırlığı (BW) ile EFW (abs[EFW-BWW]/BW100) arasındaki mutlak yüzde farkla belirlendi.*

Bulgular: *Örneklem büyüklüğü analizine göre toplam 137 hasta çalışmaya dahil edildi. Doğum ağırlığı ile EFW arasındaki ortalama fark %7,04 (0,04-44,5) idi. EFW ve BW arasındaki fark (min-maks) BMI ≥30 kg/m2 olduğunda %10,93 (0,11-34,6), anhidramnios varlığında %9,61 (0,79-34,6) idi. Bunlar istatistiksel olarak anlamlıydı; sırasıyla p=0,001 ve p=0,007. Ancak oligohidramniyoz, primiparite ve makat prezentasyon sonuçları istatistiksel olarak anlamlı değildi (p>0,05).*

Sonuç: *PPROM'lu hastalarda, Hadlock IV yöntemiyle ölçülen EFW, %7,04'lük bir hata payı ile tahmin edilebilmektedir. Obez kadınlarda ve anhidroamniyozda bu fark artmakta ve tahmin zorlaşmaktadır.*

Anahtar Sözcükler: *Doğum öncesi erken membran rüptürü, Hadlock formülü, ultrasonografi, tahmini fetal ağırlık.*

Not: *Bu çalışma, 9-11 Kasım 2022 tarihleri arasında gerçekleşen İzmir Demokrasi Üniversitesi 4. Uluslararası Kongresi'nde sözlü sunum olarak sunulmuştur.*

INTRODUCTION

Premature prelabour rupture of membranes (PPROM) is the opening of the amniotic membrane before the 37th gestational week and the loss of amniotic fluid though this opening (1, 2). Although seen in only 3% pregnancies, PPROM accounts for 20% of all perinatal mortality. It has been reported to be a complication of 140,000 pregnancies per year in the USA (1-3). Although the etiology of PPROM is multifactorial, the most important factor is subclinical infection. PPROM involves risks associated with primarily chorioamnionitis, cord prolapse, detached placenta, and anhydramnios, which require a prolonged hospital stay and antibiotic treatment (4).

With a reduced amount of amniotic fluid measured on fetal ultrasound, PPROM can affect the estimated fetal weight (EFW). In the measurement of EFW, there are several factors with an effect, such as maternal weight, skin structure, fetal position and the amount of amniotic fluid (5, 6). The Hadlock et al method is the most commonly used method in the calculation of EFW (7). There are very few studies in literature that have evaluated the accuracy of the Hadlock method in EFW measurement in cases with PPROM by comparison with the actual birthweight (8, 9).

The aim of this study was to compare the EFW with the actual birthweight in pregnancies complicated by PPROM to determine the accuracy of EFW calculated with the Hadlock IV method in such cases.

MATERIAL and METHOD

A retrospective screening was made of the data of patients with a diagnosis of early membrane rupture admitted to the Kayseri Training and Research Hospital between May 2018 and August 2020. Approval of the study was granted by the Hospital Ethics Committee (130/2020) and all procedures were in compliance with the Helsinki Declaration. Written informed consent for participation in the study was obtained from all the study subjects.

This study was presented as an oral presentation at the Izmir Democracy University 4th International Congress, which took place between 9-11 November 2022. A total of 137 subjects were included in the study to provide 90% power in a 95% confidence interval according to the sample calculation made using G-Power 3.1 program (8).

The patients included in the study were those with a singleton pregnancy with EFW value measured as 23^{+0}-36^{+6} weeks on ultrasonography within 2 weeks before birth. Patients were excluded if they had fetal anomalies, multiple pregnancy or no EFW value measured within 2 weeks before birth. The diagnosis of early membrane rupture was made from the combination of patient history, physical examination, biochemical, microscopic and ultrasonographic findings.

The data related to maternal age, height, weight, BMI, ethnicity, gestational age, hypertension, diabetes, and chorioamnionitis were retrieved from the hospital data records system.

Ultrasonographic measurements were taken on a ClearVue 550 (Phillips, Holland) ultrasonography device using a C5-2 probe (2D convex 3-5 Mhz). The deepest pocket and amniotic fluid index were recorded in the ultrasound measurements. EFW was calculated using the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) measurements in the Hadlock IV formula of Log10 BW = 1.3596 + 0.0064(HC) + 0.0424(AC) + 0.174(FL) + 0.00061(BPD)(AC) - 0.00386(AC)(FL). All the measurements were taken by a single physician experienced in obstetric ultrasonography (ANA).

Oligohydramnios was defined as amniotic fluid index (AFI) <5th percentile for gestational age, or AFI <5cm and/or the deepest pocket <2cm. Anhydramnios was defined as the deepest pocket <1cm (3). We did not find any polyhydramnios cases with fetal ultrasonography measurements in the last two weeks, who developed premature membrane rupture and delivered at our center. Therefore, polyhydramnios cases, another cause of PROM, are not included in our sample size.

Preeclampsia was defined as blood pressure of 140 mm Hg systolic or higher or 90 mm Hg diastolic or higher occurring after 20 weeks of gestation in a woman with previously normal blood pressure and protein excretion of 0.3 g or higher in a 24-hour urine sample (10).

The diagnosis of Gestational Diabetes Mellitus (GDM) was defined as a blood glucose level of 200 mg or more in the 50-gram oral glucose tolerance test (OGTT) performed between the 24th and 28th weeks of pregnancy if diabetes is diagnosed in the first trimester or early second trimester with the standard diagnostic criteria of a hemoglobin A1C (HbA1C) of 6.5% or greater, a fasting plasma glucose of 126 mg/dL or greater, or a 2-hour glucose of 200 mg/dL or greater on a 75-g oral glucose tolerance test, it is considered pregestational diabetes (11).

As a result of the retrospective screening, a total of 1352 patients who developed membrane rupture between 23+0-36+6 weeks of gestation between May 2018-August 2020 were identified. It was determined that 567 of these patients did not give birth in this hospital. Of the 785 patients who gave birth in our hospital, 258 had multiple pregnancies and 148 had various degrees of fetal

anomalies. It was observed that the remaining 242 patients did not have fetal biometry measurements within two weeks before birth. The remaining 137 patients were included in the study.

The BMI threshold value of 30 kg/m2 was determined based on the WHO obesity classification. This value was taken as basis because BMI> 30 kg/m2 and above is considered obesity (12).

This accuracy was calculated as a percentage by subtracting BW from EFW and dividing this value by BW, and the formula abs[EFW-BW] / BW x 100 was used.

The diagnosis in each case was made by the attending physician or the treatment team and not by a stated protocol.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS 25.0 software. Categorical variables were stated as number (n) and percentage (%) and continuous variables as mean ± standard deviation (SD), or where appropriate, as median, minimum and maximum values. The McNemar test statistic was used in comparisons of categorical variables. In the comparison of antenatal and postnatal continuous measurements, variables conforming to normal distribution were compared using the independent groups t-test and those not showing normal distribution with the Wilcoxon test. A value of p<0,05 was accepted as statistically significant in all the tests.

RESULTS

The demographic data and clinical characteristics of all the patients are shown in Table-1. The mean difference between EFW and birthweight was 7.04% (0.04-44.5). The difference (min-max) between EFW and BW was 10.93% (0.11-34.6) when BMI was ≥30 kg/m2 and 9.61% (0.79-34.6) in the presence of anhydramnios. And these were statistically significant, p=0,001 and p=0,007 respectively. However, the results for oligohydramnios, primiparity and breech presentation were not statistically significant (p>0,05) (Table-2). This value was found to be 3.3% in cases where ultrasonography measurements were made within ≤7 days.

Table-1. Demographic data and characteristics of patients.

	n	Values
Age (year) (n) (mean±SD)	137	26,1±6,1
Height (cm) (n) (mean±SD)	137	165,2±6,0
Weight (kg) (n) (mean±SD)	137	75,9±10,6
BMI (kg/m2) (n) (mean±SD)	137	27,8±3,5
Gravida (n) [median (min-max)]	137	2(0-5)
Parity (n) [median (min-max)]	137	2(0-5)
APGAR 1. min. (n) (mean±SD)	137	6,9±1,7
APGAR 5. min. (n) (mean±SD)	137	8,4±1,5
Gestational week at PPROM (week) (n) (mean±SD)	137	31,2±2,4
Birth week (week) (n) (mean±SD)	137	32,5±2,5
EFW (n) (mean±SD)	137	1860,2±511,9
Birth weight (gram) (n) (mean±SD)	137	1997,1±515,7
Birth weight difference percentage [median (min-max)]	137	7.04 (0.04-44.5)
Nullipara (n, (%))	54	39,4
BMI≥30 kg/m2 (n, (%))	55	40,1
Type II DM (n, (%))	13	9,5
GDM (n, (%))	4	2,9
Preeclampsia (n, (%))	13	9,5
Oligohydramnios (n, (%))	22	16,1
Anhydramnios (n, (%))	26	19,0
Chorioamnionitis (n, (%))	6	4,4
Cesarean rate (n, (%))	43	31,4
Breech presentation (n, (%))	35	25,5
Ultrasonography date ≤7 days (n, (%))	69	50,4
Fetal sex (n, (%))		
Female	66	48,2
Male	71	51,8
Turkish (n, (%))	107	78,1
Syrian (n, (%))	30	21,9

BMI: Body mass index, PPROM: premature prelabour rupture of membranes, EFW: Estimated fetal weight, GDM: Gestational diabetes mellitus.

Table-2. Evaluation of the margin of error in birth weight according to various factors.

Birth weight difference %		P
BMI<30 (n=82)	**BMI≥30 (n=55)**	
5.01 (0.04-44.5)	10.93 (0.11-34.6)	**0,001***
Nulliparous (n=54)	**Parous (n=83)**	
7.30 (0.11-27.12)	6.31 (0.04-44.5)	0.798*
Non-oligohydramnios (n=115)	**Oligohydramnios (n=22)**	
6.90 (0.04-44.5)	7,64 (1,1-29,7)	0.464*
Non-anhydramnios (n=111)	**Anhydramnios (n=26)**	
5.56 (0.04-44.5)	9.61 (0.79-34,6)	**0.007***
Cephalic presentation (n=102)	**Breech presentation (n=35)**	
6.15 (0.04-44.5)	7.57 (0.79-34,6)	0.134*

* Mann Whitney U Test

DISCUSSION

In this study, we investigated the accuracy of EFW measured with the Hadlock IV method according to birth weight in pregnancies complicated by PROM. We found that the margin of error increased statistically significantly in the presence of obesity, but the increase was not statistically significant in the presence of oligohydramnios, anhydramnios, nulliparity and breech presentation.

Accurate calculation of the estimated fetal weight is extremely important in determining the baby's survival risk, especially in premature births, and in determining the need for neonatal intensive care (NICU). Many formulas have been used so far to determine the estimated fetal weight. Guralp et al. found an 8.7±7.5% margin of error in EFW measured by the Hadlock I method in preterm fetuses (13). Sheeana et al., in their study on 1220 fetuses, claimed that Hadlock 1 was more accurate than Hadlock 2 and reported a margin of error of 3.19% (14).

In a study conducted in France, 578 singleton pregnancies between 22-34 weeks were included and ultrasonography was performed within 2 days before birth. The Hadlock formula was compared with the INTERGROWTH formula, and it was reported that the Hadlock formula gave more accurate results (15).

EMR is one of the important causes of prematurity. Additionally, it may cause negative events such as chorioamnionitis, placenta abruption, and cord prolapse (1-4).

Aviram et al. state that 21 formulas, including Hadlock IV, give similar results in pregnancies complicated by PROM, and although the Ott formula gives the best performance, the difference can be neglected (16). Warshafsky et al. also investigated the accuracy of the Hadlock IV method in patients with PROM and showed that Hadlock IV provided an accurate EFW in PROM patients, regardless of fetal gender or amniotic fluid levels (17).

Esin et al., in their study with 234 PROM patients, found that the total median % error difference between EFW and birth weight was 11.7% of the birth weight and that the Hadlock formula used performed better with an overall median error difference of 10.5% (18). We used the Hadlock IV method in our study and concluded that the difference in birth weight was 7.04%. This

supports the studies on the accuracy of the method.

However, when the amount of amniotic fluid and other factors come into play, the results are contradictory. Duncan et al. It is stated that the accuracy of the hadlock formula in patients with PROM is affected by nulliparity, anhydramnios and the mother's BMI (8).

In another study, no effect of oligohydramnios on EFW accuracy was found (19). Karahanoğlu et al. (6), in their study with 1069 term isolated oligohydramnios and 182 term isolated polyhydramnios cases, show that EFW gives accurate results, although there is a possibility of overestimation for both polyhydramnios and oligohydramnios. Blitz et al. state that the amount of amniotic fluid affects the measurement, but BMI does not have such an effect (20).

In our study, the difference increased statistically significantly if the BMI was 30 kg/m2 and above, while the difference increased in anhydramnios, oligohydramnios, nulliparity and breech presentation, but the results were not statistically significant.

In terms of the accuracy of the estimated fetal weight, when the ultrasonography is performed it is also important. Duncan et al. stated that the margin of error decreased significantly in ultrasonography performed 7 days or less after birth. In our study, 50.4% of the patients had ultrasound performed within the last 7 days and the margin of error in these patients was found to be 3.3%. This value is statistically significant (p<0,001).

As a result, in pregnancies complicated by PPROM, the estimated fetal weight is 7.04% inaccurate compared to the birth weight. While there is a statistically significant increase in this rate in patients with BMI≥30 kg/m2, a non-statistically significant increase is observed in oligohydramnios, anhydramnios, nulliparity and breech presentation.

CONCLUSION

As a result, the most trusted and preferred method in the world for predicting birth weight is still the Hadlock IV method. It is observed that this method has a very low margin of error in the presence of PROM. However, in order to further reduce the margin of error in predicting birth weight, it is of great importance to prevent obesity during pregnancy and to record

measurements within 7 days before birth, and more extensive studies are needed to minimize the margin of error in EFW, especially in obese women.

Declaration of interest statement

The authors certify that they have NO affiliations with or involvement any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Conflict of interest: The authors declare that they have no conflict of interest.

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Evaluation of the association of ligamentum mucosum with anterior knee pain and the effect of ligamentum mucosum excision due to femoral attachment on arthroscopic surgery time

Ligamentum mukozumun diz önü ağrısı ile ilişkisi ve ligamentum mukozumun femoral tutunma yerinden eksizyonunun artroskopik cerrahi süresine etkisi

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ABSTRACT

Aim: The main purpose of the study was to determine the duration of excisions made from the femoral attachment of the ligamentum mucosum during knee arthroscopy and the duration of excision from the portion attached to the retropatellar fat pad and evaluate which anatomic area excision shortens the surgical time. The second purpose of this study was to determine whether the excision of ligamentum mucosum has an effect on anterior knee pain with age.

Materials and methods: 64 patients were included in this retrospective study. Patients were assigned into two groups based on the ligamentum mucosum excision technique, and ligamentum mucosum excision times were calculated. Ligamentum mucosum excision was performed from the femoral attachment site in 34 and retropatellar fat pad attachment in 30 patients. Patients were divided into two groups younger or older than 40 years of age, regardless of the excision technique. All patients were evaluated with Kujala scores.

Results: Mean excision time of ligamentum mucosum was found to be 17.4 ±3.4 s in group excision from the femoral intercondylar node and the mean excision time was found to be 49 ±10.7 s from retropatellar fat pad attachment region. The mean Kujala score in the >40-year-old group was 84.8 ±6.4, and in the <40-year-old group was 94 ±5.5.

Conclusion: Excision made from the intercondylar node can shorten the surgical time relative to that of the excision made from the retropatellar fat pad. Clinical results of patients <40 years of age were better than those of patients aged >40 years of age.

Keywords: Anterior knee pain, knee, ligamentum mucosum.

ÖZ

Amaç: *Çalışmanın ilk amacı, diz artroskopisi sırasında ligamentum mukozasının femoral yapışma yerinden yapılan eksizyonların süresini ve retropatellar yağ yastıkçığına bağlı kısımdan yapılan eksizyon süresini belirlemek ve hangi anatomik alan eksizyonunun ameliyat süresini kısalttığını belirlemektir. Bu çalışmanın ikinci amacı, yaşla birlikte diz önü ağrısı üzerine ligamentum mukozum eksizyonunun etkisi olup olmadığını belirlemektir.*

Gereç ve Yöntem: *Bu retrospektif çalışmaya 64 hasta dahil edildi. Hastalar ligamentum mukozum eksizyon tekniğine göre iki gruba ayrıldı. Ligamentum mukozum eksizyon süreleri hesaplandı. Hastaların 34'ünde femoral yapışma bölgesinden ligamentum mukozom eksizyonu ve 30'unda retropatellar yağ yastıkçığı yapışma bölgesinden eksizyon yapıldı. Hastalar eksizyon tekniğinden bağımsız olarak 40 yaş altı veya 40 yaş üstü olmak üzere iki gruba ayrıldı. Tüm hastalar Kujala skorları ile değerlendirildi.*

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Bulgular: *Femoral interkondiler alandan yapılan ligamentum mukozum eksizyonunun ortalama süresi 17,4 ±3,4 sn ve retropatellar bölgeden yapılan ortalama eksizyon süresi 49 ±10,7 sn olarak bulundu. >40 yaş grubunda ortalama Kujala skoru 84,8 ±6,4, <40 yaş grubunda ise 94 ±5,5 olarak tespit edildi.*

Sonuç: *İnterkondiler alandan yapılan eksizyon, retropatellar yağ yastıkçığından yapılan eksizyona göre cerrahi süreyi kısaltabilir. 40 yaş <hastaların klinik sonuçları >40 yaş hastalara göre daha iyidir.*

Anahtar Sözcükler: *Diz, ligamentum mukozum, ön diz ağrısı.*

INTRODUCTION

The ligamentum mucosum (LM) is the remnant of the membrane that divides the knee embryonically (1). Histological examinations of the LM revealed nerve endings at the attachment point of the retropatellar fat pad that played a role in the kinematics of the knee extensor mechanism (2).

LM was excised before arthroscopic visualization of the lateral compartment during standard knee arthroscopy. After visualization of the lateral femoral condyle, arthroscopic visualization of the lateral compartment was performed. An important factor that prolongs the surgical time during knee arthroscopy is the location of the LM anterior to the anterior cruciate ligament. LM is a ligamentous structure that originates from the synovial layer of the knee joint. It starts from the intercondylar area of the femur and ends at the center of the intrapatellar fat pad (3). LM has been detected in the knee at the rate of 79.2%, and its isolation from the anterior cruciate ligament occurs at the rate of 92.7% (4). Another cadaveric study revealed that the LM was detected in the knee at the rate of 83% (5).

In knee arthroscopy, after examining the medial and lateral gutters of the patellofemoral joint in knee extension, the knee was flexed to 90 degrees. If the evaluation of the medial tibiofemoral joint and the medial meniscus indicated an LM, excision of the LM was performed from the medial to the lateral end, usually with a shaver, until the lateral femoral condyle entered the imaged area. When the knee was flexed by 30–45 degrees, the femoral attachment of the LM was very well visualized. In this situation, where the LM was the loosest at this knee flexion angle (6).

Paciniani corpuscles are located inside the intrapatellar fat pad; these nerve endings provide pressure perception. It helps the proprioceptive property of the infrapatellar fat pad (7). It is believed that the retropatellar fat pad is effective in the flexion and extension of the knee with the menisci. Moreover, it has been stated that

fibrosis in the retropatellar fat pad after knee surgeries may limit knee movements, especially in extension (5). The LM restricts the movement of the retropatellar fat pad in the anterior direction (8). After excision of the LM, the retropatellar fat pad moves anteriorly due to the increase in the pressure with the intra-articular fluid and does not prevent the arthroscopic view, which reduces the need for retropatellar fat pad resection (9).

The main purpose of the study was to determine the duration of excisions made from the femoral attachment of the LM during knee arthroscopy and the duration of excision from the portion attached to the retropatellar fat pad and evaluate which anatomic area excision shortens the surgical time. The second aim of this study was to determine whether the excision of ligamentum mucosum has an effect on anterior knee pain with age.

The hypothesis of this study is to determine whether ligamentum mucosal excision sites shorten the surgical time and to determine the relationship between ligamentum mucosum and anterior knee pain.

MATERIALS and METHODS

Study design

The research was approved by the institutional review board (IRB) of the authors and conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all patients who participated in this study.

In this study, patients who underwent knee arthroscopy for various intra-knee pathologies at our clinic during 2018–2020 were retrospectively evaluated. Patients aged 18–60 years, with Kujala scores in their follow-up files and a follow-up period of at least 12 months were included in the study (10). Patients aged <18 years and >60 years, with a follow-up period of <12 months, and without Kujala scores in their follow-up files were not included in the study. Patients who had a meniscal tear, anterior cruciate ligament tear, and osteochondral lesions were not included in the study. In all, 286 knee arthroscopies were

performed in our clinic during these 3 years; the examination of these patients' files showed that LM was detected during the arthroscopic surgeries of 182 patients. Among these 182 patients, 64 patients with chronic anterior knee pain did not show meniscal tear, anterior cruciate ligament tear, or osteochondral lesion, but their LM was excised. The records of these 64 patients during arthroscopy were reviewed, and the patients were assigned into two groups based on the ligamentum mucous excision technique used, and the LM excision times were calculated. It was determined that the LM excision was performed from the femoral attachment site in 34 of the 64 patients and the retropatellar fat pad attachment in 30 patients. LM excision times were determined and calculated as the time between the initiation of debridement with a shaver or a punch to the appearance of the lateral femoral condyle.

Surgical technique

Arthroscopic LM excision methods

All surgeries were performed under a tourniquet after cefazolin sodium prophylaxis 1 hour before induction of anesthesia. During the surgery, while the patients were still lying in the supine position, a silicone riser pad was placed under both their knees and the knees were left free to remain in 90° flexion. After diagnostic arthroscopy was completed using the standard anterolateral portal, the anteromedial working portal. The LM was detected and excised from the femoral attachment or the attachment of the retropatellar fat pad.

Excision from the femoral attachment

After detecting the LM, the knee was flexed at 30°–45° and the LM was excised with a shaver from the femoral attachment area. (Figure-1-2)
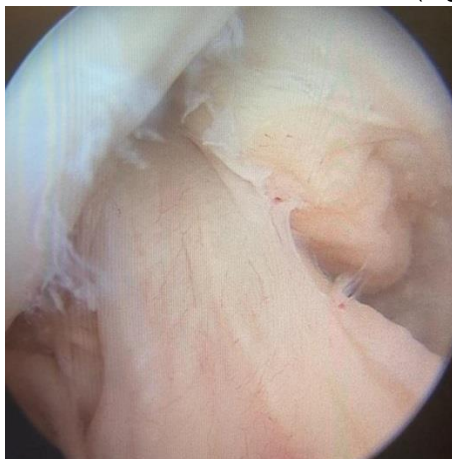
Figure-1. Arthroscopic view of the ligamentum mucosum in the intercondylar area
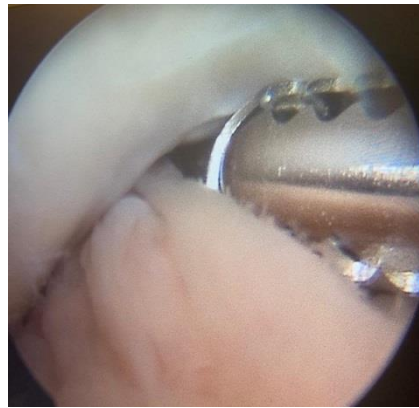
Figure-2. Appearance of anterior cruciate ligament after arthroscopic ligamentum mucous excision

Excision from the retropatellar fat pad attachment

After detecting the medial femoral condyle, the LM was excised with a shaver, starting from the medial side of the attachment of the LM to the retropatellar fat pad to the lateral side at 90° knee flexion.

Evaluation of patients

The age, follow-up period, and Kujala scores in the medical files of the patients included in this study were determined. The LM excision times and excision techniques were determined from the arthroscopic records of the patients, and the patients were accordingly divided into two groups. To evaluate the efficacy of age on clinical outcomes, since the age of 40 is the beginning of the middle age group, the patients were divided into two groups younger or older than 40 years of age, regardless of the excision technique used.

Statistical analysis

The suitability of the data for normal distribution was tested; since they were not normally distributed, the non-parametric Mann–Whitney U-test was applied to compare the numerical variables, and the Chi-square-Fisher Exact test was used for categorical data. A p-value of <0.05 at the 95% confidence interval was considered to indicate statistical significance.

RESULTS

In this study, 64 patients met the criteria of the study and were followed up. Of these, 34 patients were men and 30 were women. The mean age of these patients was 39 ±12.2 years. The mean follow-up period of the patients was 18.6 ±3.2 months. The mean Kujala scores at the last

follow-up of the patients were determined to be 90.2 ±7.4. The mean time to excision of the LM was 32.2 ±17.6 s.

The mean age of the patients in the group who underwent excision of the LM from the femoral intercondylar node was 38.3 ±12.2 years. In this group, the follow-up period was determined to be 18.3 ±3 months, and the mean Kujala score was 91.2 ±7.2. The mean excision time of LM was found to be 17.4 ±3.4 s in this group. There were 34 patients in this group.

The mean age of the patients was 39.8 ±12.4 years in the group that underwent LM excision from the retropatellar fat pad attachment region. The follow-up period was determined to be 19 ±3.4 months. The mean Kujala score was 89.2 ±7.6. In this group, the excision time of the LM was determined as 49 ±10.7 s. There were 30 patients in this group. (Table-1)

Statistical comparison of the results of both groups demonstrated a statistically significant difference between the Kujala scores (p≤0.05). No statistically significant difference was detected

between the follow-up periods and the age values of the patients. A statistically significant difference was detected between the surgical times (p ≤ 0.05). Excisions of the LM from the femoral intercondylar region were performed in a shorter time than the excision of the LM from the retropatellar fat pad attachments.

When the clinical results were evaluated by dividing the patients into 2 groups of patients aged <40 years and those aged >40 years, independent of the LM excision technique used. It was determined that there were 38 patients under the age of 40 years and 26 patients above the age of 40 years. The mean Kujala score in the >40-year-old group was 84.8 ±6.4, and the mean Kujala score in the <40-year-old group was 94 ±5.5. As a result of the statistical evaluation by age, a statistically significant difference was noted between the Kujala questions of the groups under 40 and those over the age of 40 years (p ≤ 0.05). The Kujala scores of patients <40 years of age were better than those of patients >40 years of age (Table-2).

Table-1. Demographic data and the clinical results of the study groups according to ligamentum mucosum excision site.

	Number of patients	Mean age (year)	Follow-up time (month)	Mean Kujala score	Mean surgical excision time (s)
Group with excision from the intercondylar node	34	38,3 ±12,2	18,3 ±3	91,2 ±7,2	17,4 ±3,4
Group excision from retropatellar fed pad	30	39,8 ±12,4	19 ±3,4	89,2 ±7,6	49 ±10,7
P value	p≥0,05	p≥0,05	p≥0,05	p≥0,05	p≤0,05

Table-2. Clinical results of the groups according to age.

	Number of patients	Mean Kujala scores
Patients under age 40 years	38	94±5,5
Patients over age 40 years	26	84,8 ±6,4
P value	p≤0,05	p≤0,05

DISCUSSION

The main findings of this study are; Excisions of the LM from the femoral intercondylar region were performed in a shorter time than the excision of the LM from the retropatellar fat pad attachments and LM excision is effective in reducing anterior knee pain in patients under 40 years of age.

It has been suggested that nociceptive nerve endings of the infrapatellar pad are associated

with anterior knee pain. Some studies have suggested that LM may cause anterior knee pain. LM and retropatellar fat pad act in combination as enthesis organs (5). When there is tension in the LM and retropatellar fat pad, pain may develop with the nociceptive nerve pathway, leading to anterior knee pain (11). Although it is believed that anterior knee pain and scores may be lower due to damage to the proprioceptive cells in the retropatellar fat pad in LM excisions made from the retropatellar fat pad, no statistically significant

difference was detected between the clinical outcomes of the two groups.

LM starting from the intercondular node of the femur and ending in the superior proximal of the retropatellar fat pad was classified as abnormal LM. It was found that patients with this type of LM had greater osteochondropathy in the superior intercondular node of the femur and patellofemoral sulcus (12). Moreover, the clinical results of 11 out of 12 patients who underwent LM excision with the complaint of isolated anterior knee pain were found to be good (3). Furthermore, Kujala scores and clinical results in the last control of our patients who underwent LM excision were evaluated as good.

There are no clinical studies on isolated infrapatellar fat pad excision yet. Some studies have been conducted during anterior cruciate ligament reconstructions. In the examination of patients who underwent anterior cruciate ligament reconstruction and underwent opening of the anterior portal, patellar tendinitis was detected in 72% of patients; secondary look arthroscopy was performed in 5.4% of the patients, fibrosis was detected in the infrapatellar fat pad, and LM hypertrophy was detected in 12.5% of the patients. It is stated that it causes fibrosis and hypertrophy in the anterior compartment (13). In another study, it was found that partial resection of the infrapatellar fat pad in patients who underwent anterior cruciate ligament reconstruction did not affect the knee scores and clinical outcomes of the patients (14). As a result of the studies performed during anterior cruciate ligament reconstructions, excision of the retropatellar fat pad was not very effective on the clinical outcomes. In our study, no statistically significant difference was detected between the clinical outcomes of the group in whom the retropatellar fat pad of the LM excision was performed and the group performed by the intercondylar node. In the group where excision was made by the retropatellar fat pad; partial excision occurred in the retropatellar fat pad. The absence of a statistically significant difference between the clinical outcomes between the groups suggests that the excision of the retropatellar fat pad did not affect the clinical outcomes.

There is no study in the literature on the relationship between LM excision and age. According to the results of our study, a statistically significant difference exists between the clinical results after excision of the LM in patients <40 years of age and those after LM excisions in patients >40 years of age. This finding suggests that age is an important factor in anterior knee pain, except for LM excision. We believe that this relationship can be better determined by conducting prospective randomized studies on this subject.

The middle lobe of the infrapatellar fat pad is connected to the intercondylar node with the LM. In this way, when the knee is extended, it aligns with the intercondylar node (15). The volume of the infrapatellar fat pad decreases with a decrease in the intra-articular knee volume in terminal flexion and extension (16). The LM, which attaches to the middle lobe of the infrapatellar fat pad, acts as a ligament that pulls the infrapatellar fat pad from the top when the knee is extended (15). It was determined that the tension of the LM increased when the knee reached the forward flexion degree and that the tension was the least at the intermediate flexion degrees (6). Moreover, the best visualization of the LM with arthroscopy in the intercondylar node excisions of the LM was the intermediate flexion degrees of the knee, and it was compatible with the biomechanical and anatomical features described in the literature. Excisions of the LM from the femoral intercondylar region can be performed in a shorter time compared to excision from the retropatellar fat pad attachments.

CONCLUSION

Excision made from the intercondylar node can shorten the surgical time relative to that of the excision made from the retropatellar fat pad. Clinical results of the patients <40 years of age were better than those of patients aged >40 years of age.

Conflict of interest: The authors declare that they have no conflict of interest.

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Hepatoselüler kanserli hastalarda lokal bölgesel tedaviler ve sorafenib kombinasyonun etkinlik ve güvenilirliği: tek merkez deneyimi

Efficacy and safety of combined locoregional therapy and sorafenib in hepatocellular carcinoma: single-center experience

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ÖZ

Amaç: Hepatoselüler karsinom, dünya genelinde önemli bir sağlık sorunu olan bir karaciğer kanseridir. Çoğu hasta unrezektabl evrede tanı alır. Lokal bölgesel tedaviler ve sorafenib gibi sistemik tedaviler, Hepatoselüler kanser tedavisinde kullanılan önemli yöntemlerdir. Bu çalışmada, Lokal bölgesel tedaviler ve sorafenib kombinasyonunun Hepatoselüler kanser hastalarındaki etkinliği ve güvenilirliği retrospektif olarak araştırılmıştır.

Gereç ve Yöntem: 2018-2023 yılları arasında Medipol Mega Üniversite Hastanesi'nde takip edilen 20 Hepatoselüler kanser tanılı hasta çalışmaya dahil edildi. Hastaların tümüne Lokal bölgesel tedaviler ve sorafenib tedavisi uygulandı. Progresyonsuz sağkalım ve genel sağkalım süreleri, yan etkiler ve prognostik faktörler değerlendirildi.

Bulgular: Kombinasyon tedavi sonrası objektif yanıt oranı %90, hastalık kontrol oranı %95 olarak bulundu. Ortanca progresyonsuz sağkalım 11,1 ay, ortanca genel sağkalım 18,6 ay olarak saptandı. Çok değişkenli analizde, progresyonsuz sağkalım için lokal bölgesel tedavi tipi ve nakil varlığı, genel sağkalım için ise ECOG performans durumu bağımsız prognostik faktörler olarak belirlendi. En sık görülen yan etki dermatolojik yan etkiler oldu.

Sonuç: Çalışmamız, Hepatoselüler Kanser hastalarında Lokal bölgesel tedaviler ve sorafenib kombinasyonunun güvenli ve etkili olabileceğini göstermektedir. Bu kombinasyon, progresyonsuz sağkalım ve genel sağkalım sürelerini uzatabilir. Ancak, bu konuda daha büyük, prospektif çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Genel sağkalım, Hepatoselüler karsinom, lokal bölgesel tedavi, sorafenib.

ABSTRACT

Aim: Hepatocellular carcinoma represents a significant global health concern, with a substantial proportion of patients presenting at an unresectable stage. Locoregional therapies and systemic agents such as sorafenib have emerged as pivotal modalities in the management of Hepatocellular carcinoma. This retrospective study aimed to evaluate the efficacy and safety of combining Locoregional therapies with sorafenib in patients diagnosed with Hepatocellular carcinoma.

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Materials and Methods: *A cohort of 20 Hepatocellular carcinoma patients followed at Medipol Mega University Hospital between 2018 and 2023 was retrospectively analyzed. All patients underwent Locoregional therapies in conjunction with sorafenib treatment. Progression-free survival and overall survival rates, adverse events, and prognostic factors were assessed.*

Results: *Upon receiving combination therapy, an objective response rate of 90% and a disease control rate of 95% were observed. Median progression-free survival was determined to be 11,1 months, while median overall survival was 18,6 months. Multivariate analysis revealed that Locoregional therapies type and transplantation status were independent prognostic indicators for progression-free survival, whereas ECOG performance status was an independent prognostic factor for overall survival. Dermatological adverse events were the most frequently reported.*

Conclusion: *Our findings suggest that the concurrent administration of Locoregional therapies and sorafenib may offer a safe and effective therapeutic strategy for patients with Hepatocellular carcinoma. This combined approach has the potential to prolong progression-free survival and overall survival. However, to definitively establish the clinical benefits of this regimen, larger-scale, prospective trials are warranted.*

Keywords: *Hepatocellular carcinoma, overall survival, locoregional therapy, sorafenib.*

GİRİŞ

Hepatoselüler Karsinom (HSK) dünyada en sık görülen beşinci kanserdir. Hastaların çoğu anrezektabl evrede tanı almaktadır (1). Bu evredeki hastalarda lokal bölgesel tedavi (LBT) seçenekleri olarak, Trans arteriyel kemo-embolizasyon (TAKE), Trans arteriyel radyo-embolizasyon (TARE), Radyofrekans ablasyon (RFA), Mikrodalga ablasyon (MDA) gibi LBT'ler kullanılmaktadır. Lokal ablasyon stratejileri genellikle uygun konumlarda üç cm'ye kadar olan küçük tümörleri tedavi etmek için kullanılır. Üç cm'den büyük tümörlere genellikle arteriyel embolizasyon işlemleri yapılmaktadır (2, 3).

TAKE, ablasyon veya transplant ile tedavi edilemeyen ileri ve anrezektabl HSK hastaları için sağkalım avantajına sahip LBT yöntemlerinden biridir. Portal ven trombozu olmayan hastalarda uygun bir yaklaşımdır (4). TARE, mikrosferler yttrium-90 (Y-90) ile empreyne edilir ve tümörün kan dolaşımı yoluyla yönlendirilir. HSK'nın arteriyel hipervaskülaritesi, bu partiküllerin yüksek oranda tümöral alımına izin vererek, iskemiye neden olmadan yüksek radyasyon dozları kullanabilme avantajı sağlar. Yan etki bakımdan TAKE'ye göre daha tolerabl bir yöntemdir. Transplantasyona köprülemede "down staging" yaparak katkı sağlamanın yanında yüksek radyasyon ile radyasyon hepatektomisi de yapılabilmektedir (5-7). Ancak, LBT'ye rağmen nüks riski yüksektir. Karşılanmamış bir medikal ihtiyaç mevcuttur. LBT sonrası gelişen iskemi ve tümör mikro çevresinde meydana gelen değişiklikler nedeniyle vasküler endotelyal büyüme faktörü (VEGF) vb. moleküllerin arttığı bazı çalışmalarda gösterilmiştir. Preklinik modellerde, LBT ile anti-anjiyojenik ajanların kombinasyonunun tümör hacminde ve damar yoğunluğunda azalma, tek başına LBT'ye kıyasla sağkalımları uzattığı gözlemlenmiştir (8-10).

Sorafenib bir anti-VEGF inhibitörü multi-kinaz inhibitörüdür. Sorafenib tedavisinin plasebo ile kıyaslandığı SHARP çalışmasında, ileri evre HSK hastalarında hem progresyonsuz sağkalım (PSK) hem genel sağkalım (GS) faydası gösterildi. Sorafenib, dünya genelinde en yaygın kullanılan ve en çok çalışılan sistemik tedavilerden biridir (11, 12). Çalışmamızda, merkezimizde takip ve tedavi edilen HSK tanılı hastalardaki LBT ve sorafenib tedavisinin kombine kullanımda PSK ve GS üzerine olan etkilerinin analiz edilmesi amaçlanmıştır.

GEREÇ ve YÖNTEM

2018-2023 yılları arası Medipol Mega Üniversite Hastanesi'nde takipli başlangıçta opere edilemeyen 18 yaş üstü, histolojik kanıtlanmış HSK tanılı 20 hasta çalışmaya dahil edildi. Tek merkezli retrospektif bir gözlem çalışması olarak tasarlandı. Tedavi sonrası nakil olabilen hastalar da dahil edildi. Sorafenib tedavisi 2x400mg dozunda LBT'den yedi gün önce başlandı ve LBT'den sonra tolerasyona göre dört ile yedi gün sonra başlandı. Hastalarda LBT ve sorafenib tedavilerinin kombinasyonun etkinliği ve sağkalımlar üzerine etkinliği değerlendirildi. Medipol Üniversitesi etik kurulundan onay alındı. Etik kurul sayı: E-10840098-202.3.02-5683, Karar No: 870 olarak belirlenmiştir. Kategorik değişkenler, hasta sayısı ve her bir kategorideki hasta yüzdesi belirlendi ve tedavi grupları arasındaki istatistiksel farkları test etmek için Ki-

Kare veya Fisher testi kullanıldı. PSK ve GS oranları Kaplan-Meier yöntemiyle hesaplandı ve log-rank testi PSK ve GS oranlarında gruplar arasında muhtemel farklılıkları karşılaştırmak için kullanıldı. Cox regresyon modeli kullanılarak sağkalım ile değişkenler arasındaki tek değişkenli ve çok değişkenli ilişki araştırıldı. %95 güven aralığında (95% CI) hazard oranları hesaplandı. Tüm testlerde p değeri için 0,05 istatistiksel anlamlılık sınırı olarak kabul edildi. Analizler SPSS 22 versiyonu kullanılarak yapıldı.

BULGULAR

Hastaların ortalama yaşı 59 (37-78) olup, %70'i Child A, %65'i ise Barcelona Clinic Liver Cancer (BCLC) evre B idi. Hastaların çoğunda (%60) majör vasküler invazyon varken (MVI), %70 hastada tümör boyutu 5-10 cm idi. LBT olarak, %65 hastada TARE, %30 hastada kombine tedavi uygulanırken, altı hastaya (%30) LBT ve sorafenib sonrası karaciğer nakli yapılabilmişti (Tablo-1).

LBT ve sorafenib sonrası yanıtlara bakıldığında; beş hastada tam yanıt (%25), 13 hastada parsiyel yanıt (%65) ve bir hastada (%5) stabil hastalık elde edilmişi. Objektif yanıt oranı (OYO) %90 iken, hastalık kontrol oranı (HKO) %95 idi. Tedavi ile AFP düşüşü istatiksel olarak anlamlı idi (p=0,002) (Tablo-2).

Tablo-1. Hasta Bazal Özellikleri.

Hasta Sayısı(n)	20
Cinsiyet (%)	Kadın %25, Erkek 75
HBV, HCV, Diğer (%)	HBV veya HCV pozitifliği %85
Yaş (medyan, n)	59
ECOG-PS	ECOG-PS 0 %70, ECOG-PS 1 %30
Child-Pugh Sınıflaması	Chilld A %70, Child B %30
BCLC Evresi	Evre A %5, Evre B %66, Evre C %25, Evre D %5
LN Tutulumu (%)	%45
Majör Vasküler İnvazyon (%)	%60
Minör Vasküler İnvazyon (%)	%65
Tümör Çapı(cm)	≤5 cm %5, 5-10 cm %70, ≥ 10 cm %25
TAKE (%)	%5
TARE (%)	%65
Kombine	%30
Tablo-1 devamı	
Lokal Bölgesel Tedavi sayısı (ortalama)	2 (1-5)
Karaciğer Nakli (%)	%30

*HBV: Hepatit B Virüsü, HCV: Hepatit C Virüsü

†ECOG-PS: Eastern Cooperative Oncology Group, Performans Statütüsü

‡BCLC: Barcelona Clinic Liver Cancer

§ TAKE: Transarteryel Kemoembolizasyon

¶ TARE: Transarteryel Radyoembolizasyon

Tablo-2. Yanıt Oranları

Tam Yanıt (%)	%25
Kısmi Yanıt (%)	%65
Stabil Hastalık (%)	%5
Progresif Hastalık (%)	%5
Objektif Yanıt Oranı (%)	%90
Hastalık Kontrol Oranı (%)	%95
Tedavi Öncesi AFP (ortalama, IU/L)	585(2-105840)
Tedavi Sonrası AFP (ortalama, IU/L)	24(2-69000)
§ AFP: Alfa-fetoprotein	

Tablo-3. Sorafenib İlişkili Yan Etkiler

Tüm Yan etkiler (%)	%60
Dermatolojik (Derece, %)	Derece-1 %16,7 Derece-2 %75 Derece-3 %8,3
Gastrointestinal (Derece, %)	Derece-1 %66,7 Derece-2 %33,3
Yan etki nedeniyle tedavi kesilmesi (%)	%10
Ortanca Sorafenib Süresi (ay)	7,6(4-35,6)

Ortanca PSK süresi 11,1 ay (%95 CI 7,7-14,5) ve ortanca GS 18,6 aydı (%95 CI 9,2-28,1). (Şekil-1 ve 2). Tek değişkenli analizde, PSK için, MVI varlığı (p=0,04), lenf nodu (LN) varlığı (p=0,01), BCLC evresi (p=0,013), tümör çapı (p=0,033), ECOG PS (p=0,012) ve tedavi sonrası AFP düşüşü anlamlı prognostik faktörlerdi. Diğer taraftan, GS için LN varlığı (p=0,028), ECOG PS (p=0,003) ve tedavi sonrası AFP (p=0,012) tek değişkenli analizde anlamlı prognostik faktörler olarak saptandı. Çok değişkenli analizde ise, PSK için LBT tipi (p=0,019, HR: 0,10, %95 CI 0,08-0,65) ve nakil varlığı (p=0,016, HR: 1,34, %95 CI 0,99-5,67), GS için ise yalnızca ECOG PS (p=0,048, HR: 12,8, %95 CI 1,02-21,3) bağımsız prognostik faktörler olarak bulundu. Ortanca sorafenib kullanım süresi: 7,6 aydı (4-35,6). En sık görülen yan etki dermatolojik yan etki olup, derece üç (%8,3), derece iki (%75) ve derece bir (%16,7) gözlendi. Hastaların %66'sında derece bir gastrointestinal yan etki gözlendi (Tablo-3).
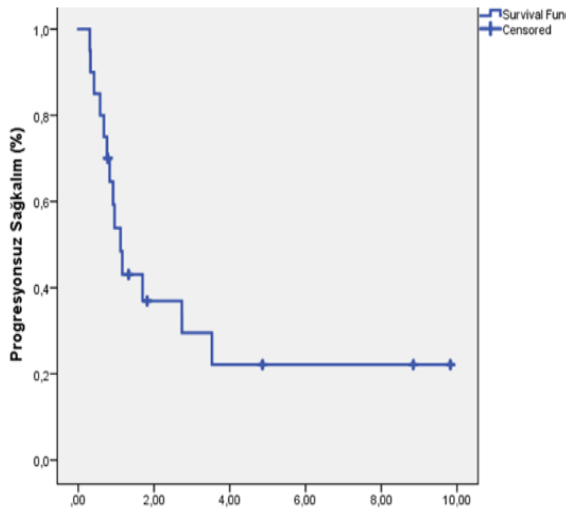
Şekil-1. Progresyonsuz sağkalım.
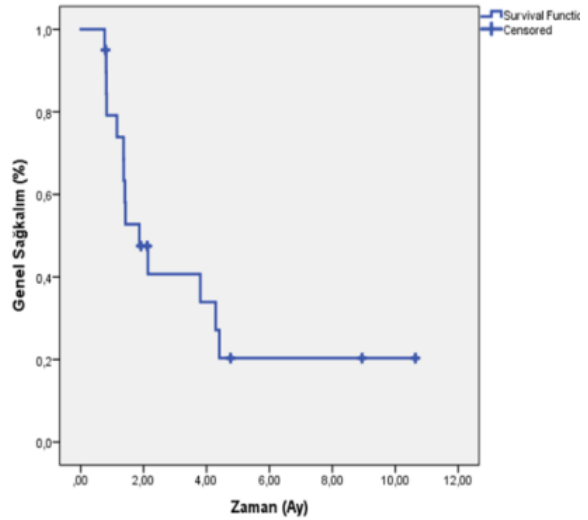
Şekil-2. Genel sağkalım.

TARTIŞMA

Sorofenib ve LBT kombinasyonunun hem sıralı hem de eş zamanlı uygulamanın güvenli ve uygulanabilir olduğu gösterilen bir dizi tek kollu faz I ve II çalışmalarda değerlendirilmiştir. Sorafenib eş zamanlı LBT uygulanan hastalarda VEGF artışı, sorafenibin potansiyel VEGF baskılamasını sağlayarak tümör progresyonu önleyebilir. Nitekim LBT sonrasında VEGF düzeyinin arttığı, LBT'den sonra sorafenib başlanması ile VEGF düzeyinde düşüş olduğu faz I ve faz II çalışmalarda gösterildi (13-15).

Faz II START çalışmasının Asyalı hastalarda yapılan ara analizi, TAKE ve sorafenib eş zamanlı verildiğinde olumlu sonuçlar vermiştir. Kombinasyon tedavisinde HKO %91,2, OYO %52,4, 18. ayda %90'ın üzerinde GS oranı elde edildi (16).

Koreli bir Faz II çalışmasında PSK süresinin sadece yedi ay olduğunu göstermiştir. Bu çalışmada altı aylık PSK oranı %52 ve ortalama GS süresi 20,8 ay olarak bulunmuştur (17).

Faz III Asya çalışması, TAKE'ye yanıt veren hastalarda sorafenib kullanımının PSK süresini önemli ölçüde uzatmadığını ortaya koymuştur

(sorafenib + TAKE: 5,4 ay -e karşı TAKE: 3,7 ay). Bunun nedeni, TAKE'den sonra sorafenib verilmesine kadar geçen uzun zamandır. Çalışmada hastaların %60'ında randomizasyondan önce dokuz haftadan fazla tedavi gecikmesi yaşanmıştır (18).

Bizim çalışmamızda sorafenib ve LBT kombinasyon tedavisinde OYO %90, HKO %95, ortanca PSK süresi 11,1 ay (%95 CI 7,7-14,5) ve ortanca GS süresi 18,6 aydı (%95 CI 9,2-28,1). Ortanca sorafenib kullanım süresi 7,6 ay ve yan etki nedeniyle sorafenib kesilmesi %10 olarak gözlendi. Bu bulgular faz-II START çalışması ve Faz-II Kore çalışması ile uyumlu idi.

SONUÇ

BCLC evre-B ve evre-C HSK hastaları günümüzde TAKE, TARE veya kombine kullanımı (TAKE+TARE) ile tedavi edilmektedir. Erken veya orta evrelerde, lokal tedaviler ile tedavi edilen HSK hastalarında, sistemik bir tedavinin eklenmesi, neoanjiyojenezi azaltarak nüks süresini ve muhtemelen sağkalımı uzatabilir (19, 20).

Lokal tedaviler ve moleküler hedefli ilaçların birleştirilmesi, HSK tedavisinde yeni bir yaklaşım olup, hala açık kalmış birçok soru bulunmaktadır. Hedefli tedavilerin kullanım zamanı ve sayısı, lokal tedavi döngü sayısı ve uygulama sıklığı açığa kavuşturulamayan konular olarak görülmektedir. HSK tanılı hastalarda LBT'lere sorafenib'in eklenmesinin yararlı olabileceğini düşünmekteyiz. Bu tarz hastalar multidisipliner tümör konseylerinde tartışılmalı ve kombinasyon tedavilerinin potansiyel etkisi göz önünde bulundurulmalıdır. Bu konuda daha fazla hasta içeren faz-III klinik çalışmalara ihtiyaç vardır.

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The cytotoxic effects of bio-transformed propolis on healthy and colon cancer cell lines

Biyotransforme propolisin sağlıklı ve kolon kanseri hücre hatları üzerindeki sitotoksik etkileri

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ABSTRACT

Aim: Propolis has a lot of properties related to human health: antioxidant, antimicrobial, antiseptic, antibacterial, anti-inflammatory, antimutagenic, and immunomodulating. Its possible effects on different cancers are among the priorities of these properties. In this study, the comparative cytotoxic effects of bio-transformed propolis samples on colon cancer cells and healthy colon epithelium cells were investigated.

Materials and Methods: *Lactobacillus plantarum* strains used in this study are based on their well-documented ability to metabolize phenolic compounds during fermentation, effectively modifying their structure and activity. These strains exhibit cinnamoyl esterase activity, which is critical for reducing allergenic compounds such as DMEA and CAPE in propolis. Additionally, *L. plantarum* is widely utilized in biotransformation processes due to its safety, adaptability, and efficiency in enhancing the bioactive properties of natural products, making it an ideal candidate for optimizing the cytotoxic potential of propolis against colon cancer cells.

Results: The highest reduction was determined for ferulic acid, and the lowest reduction was obtained by using ethanol as a solvent using ultrasound treatments in the presence of water at optimal conditions (300 W/40 kHz). It was found that propolis samples showed dose-dependent cytotoxic effects on the colon cancer cell line (HCT-116) and healthy colon epithelium cell line (CCD-841 CoN) at 24, 48, and 72 hours.

Conclusion: Our results show that the method of propolis extraction, and the type of biotransformation reaction are very important in terms of effect on the cytotoxicity of colon cancer cells. Consequently, in this study, the demonstration of the bio-transformed propolis to have the ability to destroy cancerous cells without causing severe damage to healthy cells reveals that it can have the potential that can be used in cancer treatment.

Keywords: Colon cancer, propolis, biotransformation, cytotoxicity, polyphenol.

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ÖZ

Amaç: *Propolisin insan sağlığıyla ilgili birçok özelliği vardır: antioksidan, antimikrobiyal, antiseptik, antibakteriyel, anti inflamatuar, antimutajenik ve immün modülatör. Farklı kanserler üzerindeki olası etkileri bu özelliklerin öncelikleri arasındadır. Bu nedenle, bu çalışmada biyotransforme edilmiş propolis örneklerinin kolon kanseri hücreleri ve sağlıklı kolon epitel hücreleri üzerindeki karşılaştırmalı sitotoksik etkileri araştırılmıştır.*

Gereç ve Yöntem: *Farklı çözücüler (etanol; polietilen glikol - PEG; su) ve farklı ultrason işlemleri (300 W/40 kHz (5, 10 ve 15 dakika) kullanılarak yapılan ekstraksiyon prosedürüne başlamadan önce, propolis örneklerinin biyotransformasyonu üç farklı L. plantarum suşu (ISLG-2, ATCC-8014 ve Visbyvac) ile çeşitli konsantrasyonlarda (%1,5; %2,5; %3,5) gerçekleştirildi. Örneklerin fenolik profili sıvı kromatografisi-kütle spektrometrisi/kütle spektrometrisi (LCMS/MS) ile analiz edildi. Propolisin HCT-116 ve CCD-841 CoN hücreleri üzerindeki sitotoksik etkileri WST-8 yöntemi ile belirlendi.*

Bulgular: *En yüksek indirgeme ferulik asit için belirlenirken, en düşük indirgeme optimum koşullarda (300 W/40 kHz) su varlığında ultrasonik işlemler kullanılarak çözücü olarak etanol kullanılarak elde edildi. Propolis örneklerinin 24, 48 ve 72. saatlerde kolon kanseri hücre hattı (HCT-116) ve sağlıklı kolon epitel hücre hattı (CCD-841 CoN) üzerinde doza bağlı sitotoksik etkiler gösterdiği bulundu.*

Sonuç: *Propolis ekstraksiyon yönteminin ve biyotransformasyon reaksiyon tipinin kolon kanseri hücrelerinin sitotoksisitesi üzerindeki etki açısından çok önemlidir. Bu çalışmada biyotransforme edilmiş propolisin Sağlıklı hücrelere ciddi zarar vermeden kanserli hücreleri yok edebilen bu yöntemin kanser tedavisinde de kullanılabilecek potansiyele sahip olduğu ortaya çıkarılmıştır.*

Anahtar Sözcükler: *Kolon kanseri, propolis, biyotransformasyon, sitotoksisite, polifenol.*

INTRODUCTION

Colorectal cancer is the third most common cancer worldwide (6.1% of total cases) and ranks fourth among cancer-related deaths (9.2% of total cases) (1,2). It has been thought that its incidence can increase by 60% till 2030 (3). Increased age population, negative modern dietary habits, smoking, low physical exercise, and obesity have been pointed out to be among the causes of this increase in patients with colorectal cancers in developed countries. Nowadays, surgical, radiotherapy (rectal), neoadjuvant, and palliative chemotherapy methods have been used in the treatment of primary and metastatic colorectal cancer. However, these treatment options are known to have limited effects on treatment rates and survival (4). Not getting enough responses from late diagnosis, and chemotherapeutic drugs constitutes an important obstacle in the treatment of the disease (3).

Many drugs are isolated from natural products (3). Propolis is a complex natural product, enriched in terms of beeswax and bee secretions, and is collected from plant buds, and exudates by honey bees (5). The content of propolis varies depending on its geographical origin, and plant sources (6,7). Propolis, containing polyphenols, terpenes, and flavonoids, is a bioactive compound that has anti-inflammatory, antimicrobial, antioxidant, and antiviral activities (8).

The potential beneficial effects of propolis on human health are closely related to the polyphenol compounds in its ingredients. These compounds can be extracted with different solutions, such as water, ethanol, and polyethylene glycol (6). Propolis, which has been used for centuries due to its beneficial properties, nowadays commercially offered in various forms, such as capsules, creams, powders and mouthwash solutions (5,6). Although propolis possesses antioxidant, antimicrobial, antiseptic, antibacterial, anti-inflammatory, antimutagenic, and anti-tumorigenic effects (9–18), its utilization is limited since it causes various allergic reactions.

Approximately 10% of individuals are sensitive to propolis, primarily due to allergenic compounds such as caffeic acid esters, 1,1-dimethylallyl caffeic acid ester (DMEA), benzyl caffeates, geranyl caffeate, and related cinnamic acid derivatives (19). Among these, DMEA and its isomers constitute the majority of propolis content, accounting for 87% of its composition, with 63% of these isomers linked to DMEA. Another allergenic compound, caffeic acid phenethyl ester (CAPE), demonstrates allergenic properties similar to those of DMEA (20).

It has been suggested that a less allergenic form of propolis could be developed through biotransformation processes utilizing lactic acid bacteria (21). Previous studies have highlighted that certain strains of *Lactobacillus helveticus*

and *Lactobacillus plantarum* with cinnamoyl esterase activity are capable of reducing the allergenic compounds in propolis (Patent No: TR2015 16914B, dated 2018/07/23). Specifically, using *L. plantarum* for the extraction and biotransformation of propolis effectively decreases the levels of allergenic molecules such as DMEA and CAPE (6, 22).

Changes in phenolic compounds during the biotransformation are directly related to selected microorganisms and strains. Lactobacillus plantarum is the most preferred type of lactic acid bacteria, as it takes place in the metabolism of phenolic compounds during the fermentation of various plant materials (23). In our previous studies, it was shown that *L. lantarum* strains reduce the amount of allergen molecules in propolis.

In our study, *Lactobacillus plantarum* strains were selected to reduce the allergenic effects of propolis. These strains exhibit unique enzymatic activities, particularly on phenolic compounds, which are critical for their effectiveness. Through cinnamoyl esterase activity and related mechanisms, *L. plantarum* effectively reduces allergenic compounds such as DMEA and CAPE in propolis. These distinctive features make it an ideal candidate for enhancing both the safety and therapeutic potential of propolis.

Existing research on propolis presents notable limitations, particularly in addressing its allergenic effects. While many studies focus on the bioactive properties of propolis, there is a lack of innovative biotechnological approaches aimed at mitigating allergenic components (6, 21). Additionally, the variability of phenolic compounds in propolis, influenced by geographical and botanical origins, hinders standardization and therapeutic application (19). This variability often results in inconsistent efficacy and safety profiles in its clinical use.

Our approach is innovative in addressing these gaps by employing *L. plantarum* strains with specific enzymatic activities to bio-transform propolis. This process not only reduces allergenic compounds but also optimizes the antitumor properties of propolis, particularly in colon cancer cells. By focusing on reducing allergenicity and enhancing therapeutic efficacy, this study provides a novel strategy for improving the safety and clinical potential of propolis in cancer treatment and beyond.

One of the aims of this study is to improve the methods which will be used to increase the cytotoxic effect of propolis on colon cancer lines, besides investigating the cytotoxic effects of bio-transformed propolis on colon cancer and healthy cell lines.

In the present study, the cytotoxic effects of the propolis extracts obtained on both colon cancer cells and healthy colon epithelium cells by processing biotransformation with various *L. plantarum* strains were investigated.

In the present study, the cytotoxic effects of the propolis extracts, obtained by the biotransformation using various L. plantarum strains, were investigated on both colon cancer cells and healthy colon epithelium cells.

MATERIALS and METHODS

The Preparation of The Propolis Samples and The Process of The Biotechnological Transformation

Propolis samples were obtained from the Sarkikaraagac district of Isparta city (Coordinates: Latitude: 388040 45.9800 N and Longitude: 318210 59.0000 E) in the Mediterranean region of Turkey in July. Raw propolis samples were milled by conventional machines. Particle size was determined as 35 mesh (0.5 mm) by applying the sieve analysis method to the milled samples.

The biotransformation was carried out by using *L. plantarum* strains (ISLG-2, ATCC-8014, and Visbyvac) at different concentrations (1.5%, 2.5%, and 3.5%). The propolis samples (w/v:1/1) were treated with different solutions (ethanol: 10%; poly-ethylene glycol PEG: 40%; water) and the ultrasonication treatment was applied at 300 W/40 kHz (5, 10, and 15 minutes) using water.

The incubation was performed at 30˚C for 24-72 hours under anaerobic conditions. After biotransformation, the obtained bioproducts were treated with 70 mL of ethyl acetate and incubated at room temperature for 10 minutes. The phenolic fraction extraction was followed by centrifugation of the mixture at 1500 × 3 rpm for 5 minutes. The solid residues were separated using ethyl acetate. The obtained solid extracts were dried and dissolved in 100 mL methanol. After centrifugation at 4000 × g for 1 minute, the supernatants were diluted in appropriate conditions for analyses.

Determination of Phenolic Content of The Propolis Extracts (LCMS/MS Analysis)

In this study, the analyses were realized using liquid chromatography coupled to tandem mass

spectrometry (LC-MS/MS). 1 mg/mL stock solution of each molecule was prepared in acetonitrile and methanol. Chemical stock solution of 1 mg/mL (caffeic acid, caffeic acid phenethyl ester, 1,1-dimethyl allyl ester caffeic acid, benzyl ester caffeic acid, ferulic acid, salicylic acid, genticic acid, catechin, chlorogenic acid, vanicic acid, and ethyl ferlate) was prepared in acetonitrile, and 1 mg/mL chemical stock solution (gallic acid, synergic acid, catechol, kaempferol, epicatechin, quercetin, myricetin, beta carotene, routine, maleic acid, 4-hydroxybenzoic acid, trans cinnamic acid, protocatechuic acid, p-coumaric acid, ellagic acid, cyanidine, narenin, pelargonin, and deiphinin) was prepared in methanol. These stock standard solutions were diluted to the working concentrations of 1-1000 pg/mL (diluted with a water/acetonitrile (50/50%). The mixture containing 0.1% formic acid up to the working concentrations of 1-10000 ng/mL except salicylic acid), and these solutions were used to obtain calibration curves for each chemical.

Quantitative analysis was performed by using a Waters® ACQUITY™ TQD tandem quadruple UPLC-MS/MS system consisting of an ACQUITY Ultra Performance™ liquid chromatography system, an ACQUITY XEVO TQD, and multiple reaction monitoring (MRM) (Waters mode, Milford, MA) in electrospray ionization (ESI). This UPLC-MS/MS system was controlled with MassLynx™ 4.1 software.

Chromatographic analysis was performed using the Waters Acquity™ UPLC I-Class system. The essential separation was achieved using the Waters Analytical Acquity UPLC BEH C18 columns (2.1 mm x 50 mm, 1.7 um, Waters, Milford, MA, ABD). Mobile phase A (0.2% formic acid [v/v] in water) and mobile phase B (0.1% formic acid in ACN) were operated with a gradient elution at 0.4 mL/min as follows: %75 A (0-0.5 min.), %75 A → %2 A (0.5-2.1 min.), %2 A → %75 A (2.1-2.7 min.), %75 A (2.7-4.0 min.). The column temperature was adjusted to 60°C, and the autosampler temperature was kept at 10°C. The capillary voltage, the source temperature, the thaw temperature, the con gas flow, the thaw gas flow, and the ion energy were set to 3730 V, 150°C, 40 L/s, 600 L/s, and 0.5 V, respectively, for MRM data collection.

Cell Culture
HCT-116 human colon cancer and CCD-841 CoN human normal colon epithelial cell lines were provided from the American Type Culture Collection (ATCC). The cells were grown in McCoy's 5A (HCT-116) and Eagle's Minimum Essential Medium EMEM (CCD-841 CoN) media

containing 2 mM L-glutamine, 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin and incubated in the incubator in a moist environment containing 5% CO_2 at 37 °C.

Cytotoxicity Experiments
WST-8 (water-soluble tetrazolium salt) assay was used for determination of cell viability and cytotoxicity studies. For this aim, HCT-116 and CCD-841 CoN cells ($2x10^4$) were seeded into 96-well plates at a density of $8x10^3$ cells per well, respectively. After 24 hours, different concentrations of the transformed and non-transformed propolis extracts (25–1000 µg/mL) were separately added to the cells. The wells into which no substances were added were considered as controls. 10 µl of WST-8 solution (CCK-8, Sigma-Aldrich, USA) was added to each well at the end of 24-, 48-, and 72-hour cycles. The optical density (OD) of each well was determined by absorbance at 450 nm wavelength (reference wavelength: 620 nm) using a microplate reader (Thermo-Scientific, Multiskan FC, Finland) for periods of 1-4 hours.

The percentage of cytotoxicity value was determined by using the absorbance data obtained from these experiments and the following formula: **% cytotoxicity = 100 − {[O.D. (experimental value) / O.D. (control value)] × 100}**

Statistical Analysis
The experimental results were indicated as the mean ± standard error of the mean (SD). For analyzing the data, a two-way ANOVA, and posthoc test were used as Bonferroni test. The p-values less than or equal to 0.05 are considered statistically significant.

The graphical and statistical analysis were done using the Graphpad v8 (GraphPad Software, Inc., Avenida de la Playa La Jolla, USA) program. IC_{50} values were calculated using CalcuSyn v2 (Biosoft) software. The experiments were studied in 3 replicates.

RESULTS
Effects of biotransformation on the phenolic compounds and the allergen molecules
The evaluation of allergic molecules in the bio-transformed propolis samples was performed after preliminary studies, including the determination and classification of important phenolic compounds.

The phenolic compounds were determined in the samples subjected to biotransformation by different *L. plantarum* strains (ISLG-2, ATCC-

8014, and Visbyvac), used at different concentrations (1.5%, 2.5%, and 3.5%), prior to the extraction procedure by using different solvents (ethanol, polyethylene glycol – PEG, water) and different ultrasound treatments (300 W/40 kHz) for 5, 10, and 15 minutes. It has been known that the ultrasound treatment enhances the extraction yield.

The conditions and design of testing model related to ultrasound treatments were optimized by using the other food matrix (unpublished).

Considering the importance of the reduction of allergenic molecules (DMAE-1,1-dimethyl allyl ester caffeic acid, CAPE-caffeic acid phenethyl ester, FA-ferulic acid, and TSA-trans cinnamic acid), the best prominent effects of biotransformation are summarized (14 design combinations) in Table-1.

As can be seen in Table-1, in all cases except the extraction done only with water treatment, the biotransformation process for the used *L. plantarum* strains, inoculum concentration, and the extraction treatment (ethanol, PEG, electrical treatment conditions) caused the reduction of allergenic molecules.

Comparing to the percentage of reduction effectiveness, the order from the highest to the lowest values could be ordered as followed: ferulic acid > DMAE > CAPE > trans-cinnamic acid.

The highest reduction was determined for ferulic acid using ultrasound treatments in the presence of water at optimal conditions (300 W/40 kHz). Increasing the retention time (5, 10, 15 minutes)

leads to a lower reduction ratio of this compound. The best performance of *L. plantarum* was achieved with strain L2 with inoculum value of 1.5%. It is not required to increase the inoculum value up to 3.5% in order to reach the required reduction of this compound.

Considering the CAPE compound, the lowest reduction was obtained by using ethanol as a solvent during the extraction and ultrasound treatment with conditions of 300 W/40 kHz/10 and 15 minutes retention time. During biotransformation, the best results were obtained using *L. plantarum* L2 with inoculum concentration of 2.5%, followed by *L. plantarum* L1 with inoculum concentration of 2.5%.

The values related to DMAE reduction were found to be in a similar manner with ferulic acid. The highest reduction of this compound was obtained by applying ultrasound at conditions of 300 W/40 kHz/5 and 15 minutes retention time in addition to using the different solvent extraction procedures prior to the biotransformation.

Furthermore, in terms of the culture used, the best results and hence the best performance rate were provided with L. plantarum L2 with a 1.5% vaccination rate and with L. plantarum L1 with a 2.5% vaccination rate.

It was observed that trans-cinnamic acid reduction values are also related to the applied ultrasound treatment (300 W/40 kHz) with 5- and 10-minute retention times before the biotransformation.

Table-1. Quantities of allergen molecules were compared for all transformed samples with their non-transformed samples (the solvents of the samples are the same). The arrow sign indicates that the amount has increased, while the descending arrow indicates that the amount is decreasing. CAPE: caffeic acid phenethyl ester DMAE: DMAE caffeic acid

Propolis	DMAE	CAPE	Ferulic acid	Trans cinnamic acid
Water (Non-transformed sample)	250	170	530	30.63
Water, L2 2.5 Inoculum rate (%)	546 ↑	448 ↑	2270 ↑	464.6 ↑
Water, L3 3.5 Inoculum rate (%)	990 ↑	1501 ↑	1551 ↑	87.41 ↑
Ethanol (Non-transformed sample)	898	879	1363	94
Ethanol, L2 2.5 Inoculum rate (%)	308 ↓	272 ↓	2452 ↑	37 ↓
PEG (Non-transformed sample)	456	398	2112	48.3
PEG, L2 3.5 Inoculum rate (%)	84 ↓	78 ↓	422 ↓	41 ↓
Water, 40 kHz/5 min (Non-transformed)	1921	1510	4225	327
Water, 40 kHz/5 min, L2 1.5 Inoculum rate (%)	838 ↓	1120 ↓	2327 ↓	16.3 ↓
Water, 40 kHz/10 min (Non-transformed)	1181	963	3275	127
Water, 40 kHz/10 min, L1 2.5 Inoculum rate (%)	625 ↓	516 ↓	1871 ↓	24 ↓
Water, 40 kHz/10 min L3 3.5 Inoculum rate (%)	1004 ↓	773 ↓	1498 ↓	51 ↓
Water, 40 kHz/15 min (Non-transformed)	975	796	3355	133
Water, 40 kHz/15 min L2 2.5 Inoculum rate (%)	261 ↓	238 ↓	1565 ↓	143 ↑

Table-2. Samples with the most and least major polyphenolic ingredients (Specimens in which 12 major polyphenolic contents of more than 50 ng/ml were detected in propolis samples and the most and least were stated).

Polyphenols	The Most Detected Sample	The Least Detected Sample
1. Kaempferol	Ethanol, L2 2.5 Inoculum rate (%)- 2113 ng/ml	PEG, L2 3.5 Inoculum rate (%) - 175 ng/ml
2. Vanillic acid	Ethanol, L2 2.5 Inoculum rate (%)- 723 ng/ml	Water, L3 3.5 Inoculum rate (%) - 73 ng/ml
3. Myricetin	Ethanol, L2 2.5 Inoculum rate (%)- 921 ng/ml	Water, 40 kHz/10 min, L1 2.5 Inoculum rate (%) - 18 ng/ml
4. Ellagic acid	Ethanol, L2 2.5 Inoculum rate (%)- 156 ng/ml	PEG (non-transformed sample) - 25 ng/ml
5. Epicatechin	Ethanol, L2 2.5 Inoculum rate (%)- 88 ng/ml	Water, 40 kHz/5 min, L2 1.5 Inoculum rate (%) - 1 ng/ml
6. Naringenin	Water, 40 kHz/5 min (non-transformed) - 369 ng/ml	PEG, L2 3.5 Inoculum rate (%) - 16 ng/ml
7. Pelargonin	Water, 40 kHz/5 min (non-transformed)- 119 ng/ml	Water, 40 kHz/15 min L2 2.5 Inoculum rate (%) - 13 ng/ml
8. Quercetin	Water, 40 kHz/5 min (non-transformed) - 393 ng/ml	Water, L3 3.5 Inoculum rate (%) - 81 ng/ml
9. P coumaric acid	Water, 40 kHz/5 min (non-transformed) - 506 ng/ml	Water (non-transformed sample) - 89 ng/ml
10. 4 hydroxybenzoic acid	Water, L2 2.5 Inoculum rate (%) - 69 ng/ml	Water, 40 kHz/10 min, L1 2.5 Inoculum rate (%) - 7 ng/ml
11. Caffeic acid	Water, L2 2.5 Inoculum rate (%) - 1029 ng/ml	PEG, L2 3.5 Inoculum rate (%) - 224 ng/ml
12. Chlorogenic acid	Water, 40 kHz/5 min, L2 1.5 Inoculum rate (%) - 259 ng/ml	Water, 40 kHz/15 min L2 2.5 Inoculum rate (%) - 23 ng/ml

Table-3. IC_{50} values of Propolis samples on HCT-116 cells at the 24th, the 48th and the 72nd hours.

Propolis (solvents)	Inoculum rate (%)	Ultrasound application	IC_{50} values (µg / mL)		
			24h	48h	72h
Ethanol + Phosphate buffer	No	No	1567	---	---
Ethanol + Phosphate buffer	L2 (2.5%)	No	---	---	---
PEG + Phosphate buffer	No	No	1345	1187	---
PEG + Phosphate buffer	L2 (3.5%)	No	1345	682.9	779.8
Water	No	No	1255	957	890.84
Water	L2 (2.5%)	No	1340	799.6	678.3
Water	L3 (3.5%)	No	1487	1563	1489
Water	No	40 kHz/5 min	1075	1046	596.77
Water	L2 (1.5%)	40 kHz/5 min	1012.7	841.5	772.5
Water	No	40 kHz/10 min	---	---	---
Water	L1 (2.5%)	40 kHz/10 min	1081	1486	1547
Water	L3 (3.5%)	40 kHz/10 min	>2000	1642	>2000
Water	No	40 kHz/15 min	525.7	395.8	552.3
Water	L2 (2.5%)	40 kHz/15 min	817.9	553.8	722.5

*L1: ISLG-2, **L2:** ATCC 8014, **L3:** Visbyvac strains of *L. plantarum.*

In both non-transformed and transformed samples of propolis, we determined that 12 phenolic compounds were above 50 ng/ml in quantity, except for allergen molecules. These 12 polyphenols are: vanilic acid, paracoumaric acid, quercetin, kaempferol, caffeic acid, myricetin, epicatechin, ellagic acid, chlorogenic acid, 4-hydroxy benzoic acid, pelargonine and naringenin (4 polyphenols were found below 50 ng/ml; these were rutin, salicylic acid, gentisic

acid, and protocatechuic acid). The concentration of these compounds varies between each other, depending on the type of extraction used and the biotransformation conditions.

Table-2 gives the list of those having the most, the least, and the highest increase and the highest decrease in percent according to the percentages of the main polyphenolic components the samples contain.

Comparative Cytotoxic Effects of Propolis Samples on Colon Cancer and Healthy Colon Epithelium Cells

It was found that propolis samples showed dose-dependent cytotoxic effects on the colon cancer cell line (HCT-116) and healthy colon epithelium cell line (CCD-841 CoN) at 24, 48, and 72 hours. The results are shown in the following Figures (1-5) in a comparative manner. IC_{50} values of the propolis samples for colon cancer cells (HCT-116) are given in Table-3.

The propolis sample, dissolved in 20% ethyl alcohol, has shown 6.91, 2.93, 1.62, and 2.45-fold higher cytotoxic effects on colon cancer cells compared to healthy colon cells at the concentrations of 100 µg/mL, 500 µg/mL, 1000 µg/mL, and 1500 µg/mL at the 24th hour, respectively (Fig. 1). No such effect was observed at the 48th and 72nd hours.
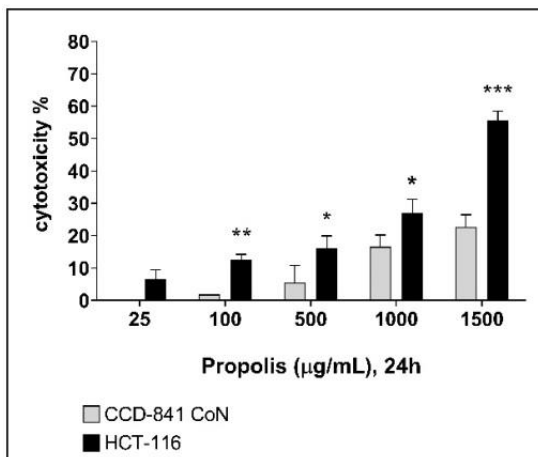
Figure-1. The cytotoxic effects of the propolis samples, dissolved in 20% ethyl alcohol, on colon cancer and the healthy colon epithelium cells at the 24th hour (*p<0.05, **p<0.01, ***p<0.001).

Compared to healthy colon cells, the sample of propolis, dissolved in 50% PEG, showed 7.46- and 3.23-fold higher cytotoxic effects at the concentrations of 500 µg/mL and 1000 µg/mL at the 24th hour, respectively; higher cytotoxic

effects became 2.76- and 2.29-fold at the concentrations of 1000 µg/mL and 1500 µg/mL at the 48th hour, respectively, and 1.94-fold at the concentrations of 1500 µg/mL at the 72nd hour on colon cancer cells (Fig. 2a, 2b and 2c).
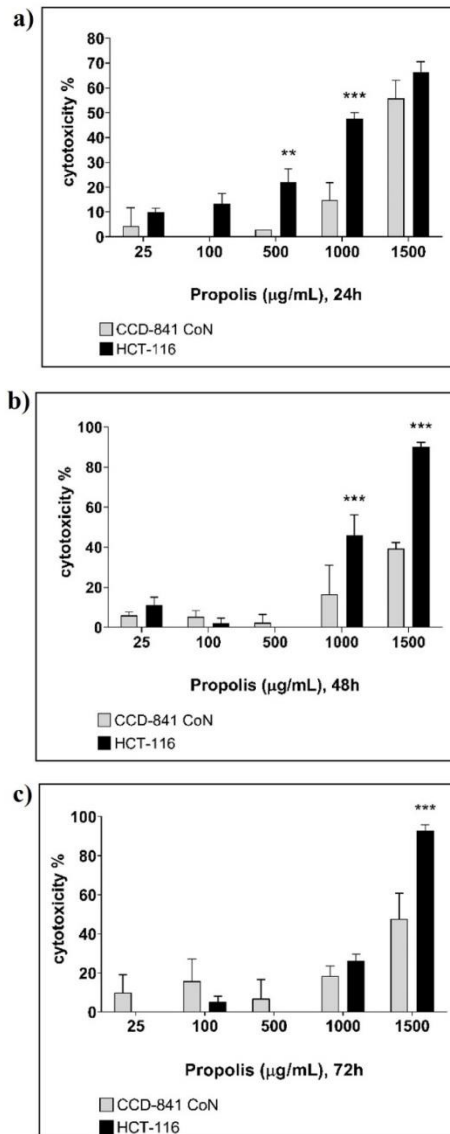
Figure-2a, 2b, 2c: The cytotoxic effects of the propolis sample, dissolved in 50% PEG, on colon cancer, and healthy colon epithelium cells at the 24th (2a), the 48th (2b), and the 72nd (2c) hours (*p<0.01, ***p<0.001).

The sample, dissolved in water, on which ultrasound was applied at 40 kHz for 10 min and inoculated with 2.5% L. plantarum ISLG-2 strain, did not show a cytotoxic effect on CCD-841 CoN cells at the 24th hour and the 48th hour, but the significant cytotoxic effect was observed on HCT-116 cancer cells (Figure-3a, 3b).
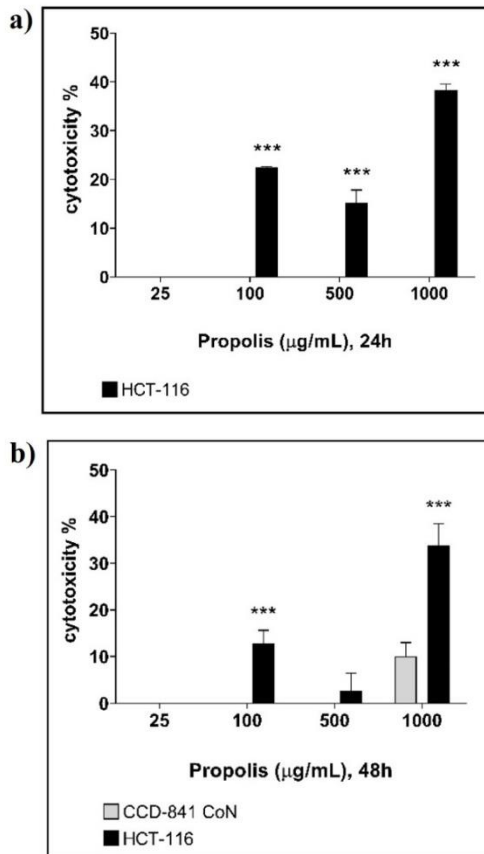
Figure-3a, 3b. The cytotoxic effect of the sample, dissolved in water, on which 40 kHz ultrasound was applied for 10 min, and which was inoculated with *L. plantarum* ISLG-2 strain of 2.5%, on colon cancer, and healthy colon epithelium cells at the 24th hour (3a), and the 48th hour (3b) (***$p<0.001$).

Figure-4a, and 4b: Cytotoxic effects of the propolis sample, dissolved in water, treated with 40 kHz ultrasound for 5 min, and which was inoculated with 1.5% *L. plantarum* ATCC® 8014 strain, both on colon cancer and healthy colon epithelium cells at the 24th (4a) and the 48th hour (4b) (*$p<0.05$, ***$p<0.001$).

Compared to the healthy colon cells, the sample dissolved in water, on which 40 kHz ultrasound was applied for 5 min, and which was inoculated with 1.5% L. plantarum ATCC® 8014 strain, has shown 4.7 and 1.81 times higher cytotoxic effects on colon cancer cells at the concentrations of 500 and 1000 µg/mL at the 24th hour, respectively (Figure-4a).

The sample, treated as above, has shown 2.63 and 1.96 times higher cytotoxic effects, respectively, on colon cancer cells at the concentrations of 500 and 1000 µg/mL at the 48th hour compared to the healthy colon cells (Figure-4b). Such an effect was not observed at the 72nd hour.

The sample, dissolved in water, on which 40 kHz ultrasound was applied for 15 min, and which was inoculated with 2.5% L. plantarum ATCC® 8014 strain, has shown 4.32 and 2.83 times higher cytotoxic effects at the concentrations of 100 µg/mL, and 500 µg/mL on colon cancer cells compared to the healthy colon cells at the 24th hour, respectively.

While it has shown cytotoxic effects on HCT-116 colon cancer cells at the ratios of 35%, 31.2%, and 39.6%, respectively, at the concentrations of 25 µg/mL, 100 µg/mL, and 500 µg/mL at the 48th hour, it did not show the cytotoxic effects on CCD-841 CoN healthy colon cells (Figure-5a, 5b).
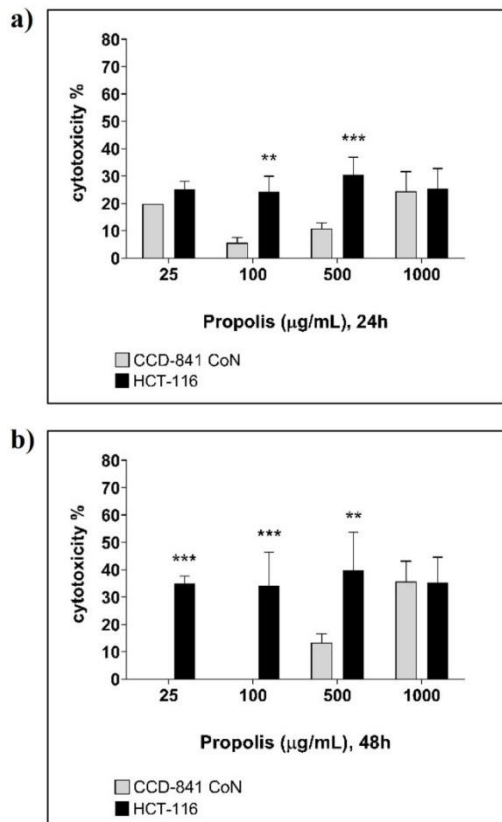
Figure-5a, 5b: Cytotoxic effects of the propolis sample, dissolved in water, treated with 40 kHz ultrasound for 15 min, and which was inoculated with 2.5% *L. plantarum* ATCC® 8014 strain, on colon cancer, and healthy colon epithelium cells at the 24th (5a), and 48th (5b) hours (*$p<0.01$, ***$p<0.001$).

DISCUSSION

According to the results we obtained from the cytotoxicity studies, the sample dissolved in water, sonicated at 40 kHz for 5 min, and inoculated with 1.5% *L. plantarum* ATCC 8014 and the sample dissolved in water, sonicated at 40 kHz for 15 min, and inoculated with 2.5% *L. plantarum* ATCC 8014 strains have shown more cytotoxic effect on the colon cancer cells than the healthy colon cell line. Similarly, the sample dissolved in ethanol and the sample dissolved in PEG were also found to have higher cytotoxic effects in the colon cancer cells than in the healthy colon cell lines.

The samples dissolved in water, treated by ultrasound at 40 kHz for 10 min, and inoculated with 2.5% *L. plantarum* ATCC 8014 strain have shown cytotoxic effects on the colon cancer cells, but they have not shown a cytotoxic effect on the healthy colon cell line.

The same amount of propolis samples used in these experiments caused significantly more cancer cell line death when compared to that of the healthy colon cell line.

The other remaining normal and the transformed samples showed more cytotoxic effects in the healthy cells compared to the cancerous cell lines.

The extract of propolis in ethanol has been used in the study of Masashi Ishihara et al. and its effects on $CaCO_2$, HCT116, HT29, and SW480 colon cancer cell lines have been compared with the effect on healthy colon epithelial cell lines. While the propolis sample at the same concentration prevents the development of colon cancer cell lines, it does not cause a significant change in the healthy cell line (24). Calhella et al. have investigated the cytotoxic effect of ethanolic extract of Portuguese propolis on MCF7-breast adenocarcinoma, NCI-H460-non-small cell lung carcinoma, HCT15-colon carcinoma, HeLa-cervical carcinoma, HepG2-hepatocellular carcinoma, and non-tumor primary cells (PLP2). While the samples that were used at the same concentrations have shown cytotoxic effects on all cell lines (cancer and healthy), a lower IC_{50} value was obtained in the cancer cell lines than that of the healthy cell line (25). In our study, we found that the extract of propolis in ethanol has a higher cytotoxic effect in the cancer cell line than in the healthy cell line.

The findings of our study align with and extend upon previous research on the cytotoxic effects of propolis in cancer cell lines. For example, Ishihara et al. and Calhella et al. demonstrated that ethanolic extracts of propolis exhibit significant cytotoxic effects on colon cancer cells, often attributed to the high solubility and polyphenolic content of ethanol-based preparations (24,25). However, our results highlight the added value of biotransformation via Lactobacillus plantarum inoculation and ultrasonic treatment. Specifically, biotransformation increased the selectivity of propolis by enhancing its cytotoxic effects against colon cancer cells while reducing its impact on healthy colon epithelial cells, addressing a critical limitation in previous studies (26).

Since propolis is easily soluble in ethanol, the cytotoxicity values of propolis ethanol extracts on the cancerous, and the healthy cell lines were found to be closer each other with respect to that of its extract in water. Propolis dissolves in water

at a ratio of 1% at most, but it dissolves in ethanol up to 20%. In this study, it was shown the polyphenolic content of the ethanolic extract of propolis to be much higher. Some studies done on the same cancer cell lines may yield different results. The reason for this result is that the content of propolis collected from different geographies is different from each other. Even propolis samples collected from various regions of the same country can have different effects on the same cell lines.

In a study made with propolis provided from Greece, it has been observed that while propolis samples have shown cytotoxic effects on the fibrocarcinoma cell line, it had no effect on the fibroblast cell line (27). Tubi-bee propolis was used in another study associated with the same cell lines; it has been determined that the propolis samples show cytotoxic effects on both cancerous and healthy cell lines (28). There are two main reasons for obtaining different results from the studies associated with the same cell lines: 1. There are nearly 300 active components in the content of propolis, and these components can be extracted at different ratios from different solvents. 2. The polyphenolic content in propolis samples collected from the different regions differs in terms of both quantity and variety.

The content analysis of propolis collected from the different regions of Turkey, and purchased from Azerbaijan were carried out in a study by our team.

The main polyphenols in the propolis of each region were found to be less or more in comparison with the propolis collected from the other regions. Although all propolis samples are qualitatively similar in terms of polyphenols in their contents, they can be quantitatively separated from each other (29). In a review article, it has been stated that the region where propolis is collected and climate are the main factors affecting the content of propolis. There are more subgroups of polyphenols specifically in different regions and climatic conditions. There are more polyphenol subgroups in different regions and specifically in different climatic zones. For example, propolis collected from the mild climate zone, where Turkey is also located, contains flavonoids more than those collected from other parts of the world. This result is related to the characteristics of vegetation of the region itself (30). In a study comparing the content and biological activities of propolis

collected from different regions of Hungary, it has been shown that propolis has different content and different biological activities (31). According to this and our study results, it can be pointed out that propolis samples collected from different regions may have different contents and different biological activities.

Another factor that can also affect is the choice of solvent, and extraction method. In most of the studies made on biological activity up to now, only ethanolic extracts of propolis have been used. There are very few studies in which different solvents and extraction methods are tried.

In one of these studies, total polyphenol contents of propolis extracts and antioxidant capacity of propolis have been compared using different extraction methods. This research has made possible the extraction of propolis with different solvents and techniques, especially for people with alcohol intolerance (32).

Since three different solvents, various sonication, and extraction methods were tried in our study, and the samples have different concentrations, it can be accepted to be obtained different results from the studies related to the cancerous and healthy cell lines.

In a similar study, the biological activity results of propolis extract made in polyethylene glycol and those made in ethanol have been compared. It has been found that the extract in polyethylene glycol is at least as effective as the extract in ethanol (33).

In our study, the sample extract made in polyethylene glycol showed more cytotoxic effects than the sample extraction in ethanol.

Transformation affects the polyphenolic content of propolis. To the best of our knowledge, a study that investigates the effects of transformed propolis (i.e., modified propolis in terms of polyphenolic content) on cancer cell lines has not been made. Therefore, in this study, the different extraction methods that affect polyphenol concentration were also investigated in order to compare the effect of the transformed propolis on the cancerous cells.

In a study, the biological activities of propolis samples have been compared using different solvents. It has been found the propolis sample extracted in ethanol to be more effective, and shown the reason for this effect to be the higher total polyphenolic content of propolis extracted in ethanol (34).

One study reported that induction of apoptosis, cell cycle arrest in the G2/M phase, epithelial-mesenchymal transition (EMT)-related markers, and phosphoinositide 3-kinase/protein kinase B signaling pathways are among the mechanisms of action of kaempferol (35). In a study made on human hepatic cancer cells, it has been shown kaempferol to induce autophagy through AMPK, and AKT signaling molecules, and to cause G2/M arrest through downregulation of CDK1/cyclin B (36). In a study made on SW480 colon cancer cells, it has been shown kaempferol to up-regulate TRAIL receptors, thereby inducing apoptosis (37). In a previous study, we found that kaempferol reduced the expression of BIRC7 (livin) and cIAP-2, which are from the family of apoptosis inhibitory proteins. In addition, we have shown that kaempferol causes a decrease in the expression of HSP70 protein, which is known to promote cancer cell invasiveness in prostate cancer. In our same study, we have also found that kaempferol reduced the amount of Clusterin, which is thought to have a critical role in colon cancer progression. The downregulation of Claspin, which is involved in the regulation of the cell cycle by interacting with Chk1, results in decreasing cell viability. In our previous study, we have determined that the level of Claspin decreased significantly with the application of kaempferol to HCT-116 colon cancer cells (38).

In this study, we found that propolis sample dissolved in water, ultrasonicated at 40 kHz for 5 min, and inoculated with *L. plantarum* strain ATCC 8014 (1.5%) had higher cytotoxic effects in colon cancer cells than healthy colon cells. We have seen that both the amount of allergen molecules such as, ferulic acid, DMAE, and CAPE, decreased significantly and the number of molecules such as, kaempferol, chlorogenic, vanillic, and salicylic acid, increased significantly compared to the non-ultrasonicated control. The increase in kaempferol and other phenolic substances in this sample may be the cause of the higher cytotoxic effect on cancer cells.

It has been shown that myricetin induces apoptosis of HCT-15 human colon cancer cells, and may be useful in the development of therapeutic agents for human colon cancer (39). The human flap endonuclease 1 (hFEN1) enzyme is accepted to represent an important target in the DNA damage response system for anticancer drug development. In a study made with this enzyme, it has been shown that myricetin inhibits HFEN1 in the HT-29 colon

cancer cell line, and it will be able to be used as a new agent in cancer treatment (40). In a study, it has been stated that M10, which is a new myricetin derivative, alleviate endoplasmic reticulum stress and prevents ulcerative colitis, and colorectal tumor, and myricetin is an effective compound in preventing colon cancer together with using it in colon cancer treatment (41). In our study, the propolis sample dissolved in water, ultrasonicated at 40 kHz for 15 min, and inoculated with *L. plantarum* ATCC 8014 strain (2.5%) showed greater cytotoxic effect in colon cancer cells than healthy colon cells. In this sample, it was found that while the amount of allergen molecules decreased, at the same time, the amount of myricetin both was more than that of the non-transformed control and 3 times greater than that of the non-ultrasonicated control. These increments explain why this sample is more effective in colon cancer cells than healthy cells.

Anti-proliferative effects of chlorogenic acid have also been demonstrated in the studies made on HT-29 colon cancer cell line (42,43). In a study made by Ekbatan et al., it has been shown that chlorogenic acid inhibits cell proliferation by causing cell cycle arrest at the S-phase on Caco-2 colon cancer cells. In the same study, they found that apoptosis was induced by activation of caspase-3 by chlorogenic acid in colon cancer cells (26). It has been reported that chlorogenic acid was capable of arresting cell cycle at G0/G1 phase and inducing apoptosis in HL-60 acute promyelocytic leukemia cells (44). In another study, it has been found chlorogenic acid to cause the cell cycle arrest in the S-phase of HCT-116, and HT-29 human colon cancer cell lines, and inhibit cell viability through ERK inactivation (45). In an *in vivo* study made with chlorogenic acid, it has been reported to be determined antitumoral activity in murine models (46). In the studies made on the cancer and healthy cell lines, it has been found that chlorogenic acid has cytotoxic activity on colon cancer cell lines (DLD-1), endometrium cancer cells (ECC-1), and renal cell carcinoma cells (A498), whereas it has no toxic effect on human embryonic kidney cells (HEK293) (47,48). In a study made on renal cell carcinoma cells, chlorogenic acid has been shown to inhibit the PI3K/Akt/mTOR path by down-regulating the ratio of p-Akt/Akt, and p-mTOR/mTOR (48). In the present study, the propolis sample dissolved in water, ultrasonicated at 40 kHz for 10 min, and

inoculated with *L. plantarum* ISLG-2 strain (2.5%) exhibited cytotoxic effect on colon cancer cells, whereas it did not show such an effect on healthy colon epithelial cells. A 2-fold increase in the amount of chlorogenic acid compared to the non-transformed control can be the cause of this. Also, the amount of ferulic acid, DMAE, and CAPE allergen molecules in this sample decreased as a result of the transformation.

Our analysis revealed a significant reduction in allergenic compounds such as ferulic acid, CAPE, and DMAE, accompanied by increased levels of kaempferol, chlorogenic acid, and myricetin. These changes are probably responsible for what was observed in the transformed samples.

As a result, in our study, it has been shown that the extracts of propolis in ethanol and polyethylene glycol have significantly more cytotoxic effects on the colon cancer cell line compared to the healthy cell line. Kaempferol, a flavonoid well-documented for its anti-cancer properties, induces apoptosis through TRAIL receptor upregulation, downregulation of anti-apoptotic proteins (e.g., BIRC7, cIAP-2), and suppression of cell survival pathways such as PI3K/Akt (35). Chlorogenic acid further supports this activity by inducing cell activating caspase-3 in cancer cells, as observed in studies on HT-29 and Caco-2 colon cancer cell lines. Similarly, myricetin has been shown to inhibit DNA repair enzymes, such as hFEN1, and to alleviaum stress, both of which are critical for cancer cell survival (26).

The significant enrichment of these compounds in our bio-transformed samples explains their enhanced efficacy, and our results provide additional evidence that the regional origin and extraction method significantly influence the biological activity of propolis.

It has been determined that some propolis samples dissolved in water, ultrasonicated, and transformed have also higher cytotoxic effects on colon cancer cells than healthy colon cells or have not shown cytotoxic effects on healthy cells while they have shown cytotoxic effects on cancer cell line.

In different solvents, polyphenols are transferred into extracts of propolis in different ratios; this situation determines the cytotoxic effect of propolis. The cause of having different cytotoxic effects of propolis collected from different regions on the same cell lines depends on the number of polyphenols in their content. Therefore, it is of great importance to determine the number of phenolic substances in its ingredient when the anticancer effects of propolis are investigated.

Determining the best solvent that may transfer polyphenol, which is sensitive to specific cell line, into extracts of propolis will also make treatments more specific and effective. These studies will guide propolis to be used safer, and more original in the studies that will be made in the future.

CONCLUSION

In light of the promising findings from our study, the potential clinical applications of bio-transformed propolis are highly encouraging. The enhanced selectivity and cytotoxicity of bio-transformed propolis, particularly through the modification of polyphenolic content by Lactobacillus plantarum inoculation and ultrasonic treatment, opens new avenues for its use as a targeted therapeutic agent for colon cancer. These modifications have not only improved the efficacy of propolis against cancer cells but also reduced its impact on healthy cells, addressing a critical challenge in cancer therapy. The ability to manipulate the phenolic composition of propolis through biotransformation can lead to more personalized and effective treatment strategies. Moreover, due to their reduced allergenic compounds and the selective cytotoxic effects, it could be suggested that bio-transformed propolis could be developed as a safer, and natural adjunct in cancer treatment. Focusing future research in clinical settings, focusing on optimizing extraction methods, identifying specific cell-line targeting phenolic compounds, and ensuring bioavailability, will be crucial in utilizing the full potential of bio-transformed propolis for cancer therapy.

Author Contribution Statement

E.Y.S. designed and directed the project. H.K.Y. carried out the propolis sample's

biotransformation process. B.D. and H.M. carried out the extraction of the propolis samples and content analysis with liquid chromatography/mass spectrometry. L.M.O.C. and N.S.G. carried out the cell culture and cytotoxicity. All authors participated in the analysis of the data. N.S.G., L.M.O.C. and H.M. wrote the article. All authors discussed the results and contributed to the final manuscript.

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Single-center and reverse evaluation of febrile neutropenic attacks of patients followed with acute myeloid leukemia diagnosis

Akut myeloid lösemi tanısı ile takip edilen hastaların febril nötropenik ataklarının tek merkezli ve geriye dönük değerlendirilmesi

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ABSTRACT

Aim: Since fever may be the only symptom in patients with hematological malignancy, fever in neutropenic patients is considered to be related to infection until proven otherwise. The standard approach is the rapid evaluation of febrile neutropenic (FEN) episodes and initiation of appropriate antibiotic treatment. In this study, it was aimed to examine the duration of FEN, infection categories, symptoms and examination findings, isolated microorganisms and mortality rates of the patients we followed up in our clinic due to acute myeloid leukemia.

Materials and Methods*:* We examined 155 adult patients with AML who were treated at the Adult Hematology Clinic. In our study, we retrospectively evaluated 337 chemotherapy-related FEN episodes in terms of infection categories, offending pathogenic microorganisms and mortality rates.

Results: 43.92% (n=148) of the patients had pathological examination findings accompanying the fever. FEN attacks in 249 patients had clinically and/or microbiologically proven foci of infection. The most frequent infection site was the lungs (41.5%). The most frequently isolated microorganisms were found to be *coagulase negative staphylococcus* (37.30%) and E.coli (36.21%). 32.94% of the patients (n=111) were recorded as smokers. When the relationship between smoking and lung infection was investigated, it was determined that the incidence of lung infection was higher in smokers (p= 0.007). In 91 (27%) of those who had a FEN attack in our hospital resulted in death. It was the lung infection that mostly correlated with the mortality (40.71%).

Conclusion: Each clinic can determine empirical antibiotic treatment policies by investigating infectious agents. It can manage the FEN episode process more accurately by providing the necessary infection control measures.

Keywords: Febrile neutropenic attacks, acute myeloid leukemia, neutropenia.

ÖZ

Amaç: Hematolojik maligniteye sahip hastalarda tek semptom ateş olabildiği için nötropenik hastalarda ateş aksi ispat edilene kadar enfeksiyon ile ilişkili kabul edilir. Febril nötropenik (FEN) ataklarının acil olarak değerlendirilip uygun antibiyotik tedavisinin hızlıca başlanması standart yaklaşımdır. Bu çalışmada akut myeloid lösemi (AML) nedeniyle kliniğimizde takip ettiğimiz hastaların FEN süreleri, enfeksiyon kategorileri, semptom ve muayene bulguları, izole edilen mikroorganizmalar ve mortalite oranlarının incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Araştırmamıza erişkin hematoloji kliniğinde yatarak tedavi gören 155 erişkin AML tanılı hasta alındı. Kemoterapiye bağlı gelişen 337 FEN atağı; enfeksiyon kategorileri, izole edilen patojen mikroorganizmalar, mortalite oranları açısından geriye dönük olarak değerlendirildi.

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Bulgular: *Hastaların %43,92'sinde (n= 148) ateşe eşlik eden patolojik muayene bulgusuna rastlanmıştır. 249 hastadaki FEN ataklarında klinik ve/veya mikrobiyolojik olarak kanıtlanmış enfeksiyon odakları vardı. En sık saptanan enfeksiyon odağının akciğer olduğu görülmüştür (%41,5). İzole edilen mikroorganizmaların en sık %37,30 ile koagülaz negatif stafilokok (KNS) ve %36,21 ile E. coli olduğu görülmüştür. Hastaların %32,94'ü (n=111) sigara kullandığı kaydedilmiştir. Sigara kullanma ile akciğer enfeksiyonu arasındaki ilişki incelendiğinde, sigara içenlerde akciğer enfeksiyonu görülme sıklığının fazla olduğu belirlendi(p=0,007). FEN ataklarının %27'sinin (n= 91) mortal seyrettiği görülmüştür. Mortaliteyle en fazla ilişkili olan %40,71 ile akciğer enfeksiyonuydu.*

Sonuç: *Her klinik, enfeksiyon etkenlerini araştırarak, ampirik antibiyotik tedavi politikalarını belirleyebilir. Gerekli enfeksiyon kontrol önlemlerini sağlayarak FEN epizod sürecini daha doğru yönetebilir.*

Anahtar Sözcükler: *Febril nötropenik atak, akut myeloid lösemi, nötropeni.*

INTRODUCTION

While it is aimed to increase the survival time with intensive chemotherapy protocols applied in acute myeloid leukemia (AML) patients, hospital stays due to the disease and chemotherapy are prolonged and bone marrow toxicity may develop. These patients are at increased risk of developing serious infections, especially in the neutropenic period. Patients receiving chemotherapy or having neutropenia may not have the expected classical symptoms and signs of infection due to decreased inflammatory response. Since fever may be the only symptom in these patients, it should be considered to be related to infection until proven otherwise and urgent intervention should be performed. Rapid evaluation of the patient and initiation of empirical antibiotic therapy as soon as possible is of vital importance. The primary disease of the patient, the chemotherapy protocol he/she received, the factors detected in the previous febrile neutropenia (FEN) attack, the level and duration of neutropenia are important in the selection of empirical antibiotics. Additionally, the causative profile of each clinic is also important in the selection of empirical antibiotics. For this purpose, FEN periods, infection categories, symptoms and examination findings, isolated microorganisms and mortality rates of the patients we followed up in our clinic for AML were all examined.

MATERIALS and METHODS

This study included 155 adult AML patients who were hospitalized in the Adult Hematology Clinic of Ege University Medical Faculty Hospital between January 2012 and January 2016. AML was diagnosed by clinical, complete blood count, peripheral smear, bone marrow aspiration and/or bone marrow biopsy, histochemical staining, flow cytometry and/or cytogenetic evaluation according to French-American-British and World Health Organization criteria. 337 FEN attacks that developed during the hospitalization of these patients were retrospectively examined. A single measurement of 38.3°C and above or a temperature measurement of 38.0–38.2°C for 1 hour without any environmental factors was considered fever. Neutropenia was considered when the neutrophil level was below 500/mm³ or when the neutrophil level was between 500–1000/mm³ and was expected to fall below 500/mm³ within 24–48 hours. Blood culture positivity was defined as the growth of a known pathogenic microorganism in at least one blood culture. It was determined that blood cultures were studied using an automated system (BACTEC). Patient data were obtained from the electronic file system of Ege University Faculty of Medicine Hospital. Age, gender, diagnosis, additional diseases, smoking habit, presence of urinary catheter and central venous catheter, major symptoms and findings accompanying fever, detected primary infection focus, abnormal radiological findings, culture results, and survival status were reviewed and recorded from the patient files. Local ethics committee approval was obtained before starting the study.

SPSS.23.0 statistical package program was used. Measurement variables are presented either mean ± standard deviation (SD) or categorical variables as numbers and percentages (%). Intergroup comparison of qualitative variables was done with chi-square analysis, and performed quantitative variables was done with Mann-Whitney U test. P < 0.05 was considered statistically significant. The ethics committee approval was received for this study.

RESULTS

In our study, 337 FEN attacks developed in 155 adult patients who were hospitalized in the Ege University Medical Faculty Hospital were examined. While 36.73% (n=57) of the patients were women, 63.22% (n= 98) were men. The mean age was 51.56 (SD±14.82). While the average number of FEN attacks for men was 2.98, it was 2.93 for women. No statistically significant difference was found between genders and FEN attack numbers (p=0.867).

Culture (kx) positivity was detected in 54.90% (n=185) of FEN attacks. Of the patients with positive cultures, peripheral blood cultures were detected in 57.30% (n=106), catheter-catheter tip positivity in 45.41% (n=84), and urine positivity in 26.49% (n=49). During the 337 FEN period, blood kx positivity was found to be 31.4% (n = 106).

Nothing was isolated in 45.10% of the patients. 48.66% of the isolates were bacteria, 2.67% were fungi and 3.57% were both bacteria and fungi. 62.16% of the isolated bacteria were gram negative, 54.05% were gram positive and 1.08% were anaerobic. It was also observed that both gram positive and gram-negative bacteria were isolated in the same patient. (Table-1).

While 32.94% (n= 111) of the patients were smoking, 67.06% (n= 226) were non-smokers. When the relationship between the presence of lung infection according to smoking status was investigated, 144 of the non-smoking patients did not have lung infection, while lung infection was observed in 82 patients. While lung infection was seen in 47.74% of smokers, it was seen in 25.66% of non-smokers. According to the analysis, it was found that the frequency of lung infections was significantly higher in smokers (p= 0.007). In our hospital, 27% (n= 91) of FEN attacks resulted in exitus (ex). While looking at the relationship between the detected area of infection and mortality, mostly the patients with pulmonary infection died (40.71%), then patients with gastrointestinal (GIS) (32.30%), genitourinary (GUS) (26%), and lastly the catheter infections (5.30%). In some patients, more than one focus of infection was detected in the same febrile neutropenia attack. The results regarding the distribution of infection foci and relationship between area and mortality are shown in Table-2 and Table-3.

Table-1. Agents isolated.

Agent Isolated	N (number)	Percent (%)
CNS	69	37.30
E. coli	67	36.21
Enterococci	32	17.30
Klebsiella	23	12.43
Acinetobacter	16	8.65
ESBL+ E. coli	12	6.49
Pseudomonas	11	5.95
VRE	10	5.40
Candida	7	3.78
Enterobacter	7	3.78
Stenotrophomonas	7	3.78
Aspergillus	6	3.24
Aeromonas	4	2.16
Streptococci	4	2.16
CRE	4	2.16
Proteus	3	1.62
Staphylococcus aureus	**3**	1.62
Corynebacterium	2	1.08

**Abbreviations*: CNS: Coagulase-negative staphylococci, ESBL: Extended spectrum beta lactamases, VRE: Vancomycin resistant enterococcus spp., CRE: Carbapenem resistant erobacteriaceae.*

Table-2. Distribution of infection foci.

		N (number)	Percent (%)
Detected Focus of Infection	**Present**	249	73.89
	Absent	88	26.11
Lung Infection		140	56.22
Catheter Infection		82	32.93
Gastrointestinal System Infection		65	26.10
Genitourinary Tract Infection		50	20.08
Soft Tissue Infection		28	11.24
Sinus Infection		13	5.22

Table-3. The relationship between infection area and mortality.

Infection Groups	Mortality		
	Ex: N (number)	Ex: Percent (%)	Lives: N(number)
Lung infection	57	40.71	83
Catheter Infection	21	25.30	62
Gastrointestinal System Infection	21	32.30	44
Genitourinary Tract Infection	13	26	37
Soft Tissue Infection	7	25	21
Sinus Infection	3	23.07	10

DISCUSSION

In neutropenic attacks, the only sign of infection may sometimes be bacteremia, and identifying the causative agents is crucial for managing the patient. Bacteremia rates ranging from 10-25% to 45.9% have been reported in various studies (1, 2). In our study, the rate of bacteremia was found to be 31.4%.

The causative agents of febrile neutropenic attacks shifted from gram-negative bacteria to gram-positive bacteria before the 2000s; however, there has been a resurgence of gram-negative bacteria today (3, 4). Currently, antibiotic-resistant gram-negative strains are emerging (3, 4). In our study, 62.16% of the causative agents of febrile neutropenic attacks were gram-negative and 54.05% were gram-positive bacteria. It was also observed that both gram positive and gram-negative agents were detected in the same patient. Coagulase-negative staphylococci (CNS) were the most common cause among gram-positives, while E. coli was the most common among gram-negatives.

Considering the distribution of infection foci, in a study by Gupta A. et al. examining 382 febrile episodes in 95 AML patients, lung infection was reported at 46%, gastrointestinal system infection at 33%, and soft tissue infection at 22% (5). In our study, clinical and/or microbiologically proven focus of infection was found in 73.89% (n= 249) of the patients. Lung infection was present in 56.22% (n=140), and catheter infection in 32.93% (n=82) of these patients. The frequency of lung infections in our clinic was found to be above the average stated in the literature (Table-2).

As is well-known, smoking is an important risk factor for developing chronic obstructive pulmonary disease (COPD) and can also worsen lung function in patients with lung infections. Studies on community-acquired pneumonia have shown that 72% of pneumonia cases requiring intensive care hospitalization had a history of smoking (6). Similarly, smoking has been identified as a risk factor for the need for intensive care in viral pneumonias (7). A review published in 2017 stated that cigarette exposure is a recognized risk factor for both acute and chronic respiratory diseases. In our study, the

frequency of lung infections was also found to be higher in smokers (p= 0.007).

In our study, we examined the relationship between the infection site and mortality. Accordingly, patients with pulmonary infections had the highest mortality rate at 40.71%, while gastrointestinal (GIS), genitourinary (GUS), and catheter-related infections had mortality rates of 32.3%, 26%, and 25.3%, respectively. When considering the distribution of infection sites in the literature, lung infections account for 46%, GIS infections for 33%, and soft tissue infections for 22% (5). In a study conducted in a hematology clinic, including 60 patients, it was observed that the most common reason for referral to the intensive care unit during a febrile neutropenic (FEN) episode was respiratory failure, with a rate of 38.5% (8). GIS infections also contribute significantly to mortality. Delphine et al. reported that acute abdomen developed in 171 (5.3%) of their 3222 cancer patients. Neutropenic enterocolitis was detected in 33% of patients who developed an acute abdomen, and 22% of these patients died (9).

While mortality rates in febrile neutropenic patients were 75% before the use of empirical antibiotic treatments, today, mortality has been reduced to 5-10% with advances in diagnosis, treatment options, and standardized approaches in FEN attacks (10). In our study, the mortality rate in febrile neutropenic patients was 27%, higher than the average reported in the literature.

Patients with the highest risk of developing neutropenia-associated infections are those with AML and those undergoing allogeneic bone marrow transplantation who are receiving high-dose chemotherapy and induction therapy. Although our results are generally consistent with the literature, the rates of lung infections, catheter-related infections, and mortality in our clinic were found to be higher than those reported in the literature (Table-3).

CONCLUSION

Multidrug-resistant organisms pose a major challenge in the management of neutropenic febrile patients with hematological malignancies, including AML. Future directions to improve outcomes require advances in biomarker research as well as innovative treatment approaches to facilitate diagnosis and disease monitoring (11). When FEN attacks of AML patients were examined retrospectively in our clinic, it was observed that the isolated microorganisms were gram-negative, and lung infections were the most common as the infection site. Today, mortality in FEN attacks is gradually decreasing; however, it is still an important cause. In conclusion; each clinic's determination of its own infectious agent profile may contribute to empirical antibiotic selection and better management of this process.

Conflict of interest: This study has not received any financial support from any institution, person or commercial organization. There is no conflict of interest.

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Protective effect of peroral capsaicin administration in ethanol-induced gastric ulcer model in rats

Sıçanlarda etanol kaynaklı gastrik ülser modelinde peroral kapsaisin uygulamasının koruyucu etkisi

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ABSTRACT

Aim: Capsaicin is a compound that possesses antioxidants and anti-inflammatory properties, and it is found in almost all bitter fruits. The objective of our study was to investigate the protective effect of capsaicin in an ethanolic ulcer model.

Materials and Methods: The male *Wistar albino* rats were divided into four groups, comprising a control group and three experimental groups (n=8). The control group (C) administered a single dose of distilled water subcutaneously. The Capsaicin (CAP) group was administered a single dose of 1 mg/kg CAP via gavage. The ulcer group administered a single dose of 1ml absolute alcohol via gavage, 30 minutes following the administration of a single dose of distilled water via gavage. The U+CAP group administered 1ml of absolute alcohol 30 minutes following the administration of 1 mg/kg CAP. Macroscopic and microscopic ulcer scores, as well as mucosal barrier integrity, were evaluated in the gastric tissues that had been removed. The levels of total oxidant status (TOS) and total antioxidant status (TAS) were determined in the tissue samples, and an oxidative stress index (OSI) was calculated. **Results:** In comparison to the U group, the macroscopic and microscopic mucosal lesions, TOS, OSI levels and IL-1, TNF-α, NFκB and Caspase 3 expressions were found to be decreased in the U+CAP group, while TAS levels were observed to be increased.

Conclusion: The results of the study demonstrated that capsaicin protects mucosal integrity through its antioxidant, anti-inflammatory and anti-apoptotic properties in the ethanol-induced gastric ulcer model.

Keywords: Ethanol, ulcer, capsaicin, caspase 3, NF-κB.

ÖZ

Amaç: *Kapsaisin, hemen tüm acı biberlerin yapısında bulunan anti-oksidan ve anti-inflamatuar özelliklere sahip bir bileşiktir. Çalışmamızda etanolik ülser modelinde kapsaisin'in koruyucu etkisi araştırılmıştır.*

Gereç ve Yöntem: *Erkek Wistar albino sıçanlar biri kontrol üçü deney grubu olmak üzere dört gruba ayrılmıştır (n=8). Kontrol (C) grubuna tek doz distile su subkutan uygulanmıştır. Kapsaisin (CAP) grubuna tek doz 1 mg/kg CAP gavaj yoluyla uygulanmıştır. Ülser (U) grubuna tek doz distile suyun gavaj yoluyla uygulanmasından 30 dakika sonra tek doz 1 ml mutlak alkol gavaj yoluyla uygulanmıştır. U+CAP grubuna 1 mg/kg CAP uygulanmasından 30 dakika sonra 1 ml mutlak alkol uygulanmıştır. Çıkarılan mide dokularında makroskobik ve mikroskobik ülser skorları ve mukozal bariyer bütünlüğü değerlendirilmiştir. Dokuda total oksidan status (TOS) ve total anti-oksidan status (TAS) seviyeleri ölçülmüş ve oksidatif stres indeksi (OSI) hesaplanmıştır.*

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Bulgular: *U grubuyla karşılaştırıldığında, makroskobik ve mikroskobik mukozal lezyonlar, TOS, OSI seviyeleri ve IL-1, TNF-α, NFκB ve Kaspaz 3 ekspresyonlarının U+CAP grubunda azaldığı, ancak TAS seviyesinin arttığı görülmüştür.*
Sonuç: *Çalışma, kapsaisin'in etanol kaynaklı gastrik ülser modelinde anti-oksidan, anti-inflamatuar ve anti-apoptotik özellikleriyle mukozal bütünlüğü koruduğunu göstermiştir.*
Anahtar Sözcükler: *Etanol, ülser, kapsaisin, kaspaz 3, NF-κB.*

INTRODUCTION

Peptic ulcer disease (PUD) represents a significant and pervasive public health concern, with an estimated incidence of approximately 1 in 1,000 cases per year in the general population across the globe (1). Histopathologically, PUD is defined as damage that reaches the submucosal layer in the stomach, resulting from the disruption of the balance between the factors that form the mucosal barrier, such as bicarbonate secretion, adequate mucosal blood flow, the regeneration capacity of epithelial cells, and the production of gastric acid and pepsin (2). Today, Helicobacter pylori infection is held primarily responsible for the etiology of peptic ulcer disease, but it is well known that factors such as nonsteroidal anti-inflammatory drug (NSAID) use, stress, smoking and alcohol are also causative agents of peptic ulcer (3). Ethanol has been frequently used to create ulcer models in animal studies because it disrupts the mucosal barrier by reducing mucosal microcirculation and triggers inflammation by increasing free oxygen radicals in the tissue (4, 5).

Capsaicin (CAP) is a compound that is present in almost all varieties of chili peppers and is responsible for the spicy aroma that is characteristic of these peppers (6). *In vivo* and *in vitro* studies have demonstrated that CAP and related but non-pungent capsinoids (capsiate, dihydrocapsiate, nordihydrocapsiate) possess the capacity to elicit a range of beneficial effects, including analgesic, antioxidant, anti-inflammatory and anti-carcinogenic properties (7). In a sepsis model created in rats, CAP administered subcutaneously at a dose of 1 mg/kg was shown to reduce plasma interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels and malondialdehyde (MDA) levels in liver, lung and heart tissues (8). Li et al. showed that CAP reduced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), TNF-α and nitric oxide (NO) levels in a lipopolysaccharide-induced inflammation model in BALB/c mice (9).

Although CAP has been shown to have a mucosal protective effect in NSAID, stress and ethanol-induced ulcer models by desensitizing afferent nerve fibers via transient receptor potential cation channel subfamily V member 1 (TRPV1) receptors, no studies were found demonstrating the efficacy of orally administered CAP in an ethanol-induced ulcer model (10, 11, 12).

The aim of our study was to demonstrate the efficacy of CAP in the ethanol-induced gastric ulcer model in *Wistar albino* rats through histopathological, biochemical and immunohistochemical methods.

MATERIALS and METHODS

Male *Wistar albino* rats (n=32, weighing 300-350 g each) from the Animal Care and Research Unit of Bülent Ecevit University (Zonguldak, Turkey) were used in the present study. The appropriate environmental conditions for the care of the animals (room temperature of 20 ± 1°C, humidity of 60 ± 10%, and a 12/12 hour light/dark cycle) were provided throughout the experiment. The subjects were permitted free access to food and water. The study was conducted in accordance with the guidelines set forth in the Guide for the Care and Use of Laboratory Animals, published by the US Public Health Service. Prior to the commencement of the study, approval was obtained from the Institutional Animal Ethics Committee of Bülent Ecevit University (Zonguldak, Turkey; 2020-06-27/02).

Experimental Design

A total of four groups were constituted, comprising one control group and three experimental groups, each with eight rats. The control group administered a single dose of CAP solvent (distilled water) subcutaneously. The CAP group administered a single dose of 1 mg/kg CAP via gavage. The ulcer (U) group administered a single dose of 1ml absolute alcohol via gavage, 30 minutes following the administration of a single dose of CAP solvent via gavage. The U+CAP group administered 1 ml of absolute alcohol for 30 minutes following the administration of 1 mg/kg CAP.

Macroscopic Ulcer Score

One hour after the administration of absolute alcohol, the animals were euthanized, and their stomach tissues were excised. The gastric tissues were incised along with the greater curvature and washed with isotonic NaCl solution. They were then flattened on paraffin plates to allow for the examination of the mucosal folds. The dimensions of the lesion area and the total stomach surface area were determined macroscopically by affixing transparent millimeter-scale paper templates to the stretched stomach surface. The macroscopic ulcer score was calculated using the following formula: lesion area/total stomach surface area x 100.

Histopathological Evaluations

Following the application of a 10% formalin fixative, paraffin blocks were obtained from stomach tissues that had undergone the requisite tissue processing steps and were embedded in paraffin. In order to determine the histological features of the stomach, 5µ thick sections were taken from the paraffin blocks and subjected to staining with either hematoxylin-eosin (H&E) and periodic acid Schiff (PAS) dyes. The stained tissues were imaged using the Axio Lab A1 microscope (Zeiss, Germany). Each 1 cm section was subdivided into three areas, and a microscopic ulcer score was calculated for each area based on the following criteria:

0: normal mucosa, 1: epithelial damage, 2: glandular disruption, vasocongestion or oedema of the upper parts of the mucosa, 3: vasocongestion or oedema extending to the middle parts of the mucosa, 4: mucosal damage involving the entire mucosa (13). The mean score for each area was deemed to represent the microscopic ulcer score for that particular section.

Evaluation of TAS, TOS and OSI in Tissue

Stomach tissue homogenates were prepared and total antioxidant status (TAS) and total oxidant status (TOS) measurements were performed in accordance with the protocol provided with the commercially purchased Rel Assay (Rel Assay Diagnostics kit, Mega Medicine, Gaziantep, Turkey) (14). Oxidative stress index was calculated with the formula TOS/TAS × 100 (15).

Immunohistochemical Evaluations

In gastric tissues, expressions of inflammatory cytokines interleukin1-β (IL-1β), TNF-α, caspase 3, a common pathway caspase in apoptotic cell death, and NF-κB, a transcription factor, were demonstrated by immunohistochemistry.

Stomach sections with a thickness of 5 microns were taken from paraffin-embedded samples. The sections were initially incubated with rabbit anti-NF-κB (1:200 dilution, Novus Biologicals, USA), anti-caspase-3 (1:200 dilution, Sigma-Aldrich, Germany), anti-TNF-α (1:200 dilution, Sigma-Aldrich, Germany) and anti-IL-1-β antibodies (1:100 dilution, Novus Biologicals, USA) at +4°C for 24 hours. Subsequently, immune complexes formed on tissues incubated with a biotinylated goat anti-rabbit secondary antibody (Thermo Fisher Scientific, USA) were detected by incubation with 3,3'-diaminobenzidine tetrachloride (Vector Laboratories, DAB Substrate Kit, Peroxidase (HRP), with Nickel, USA). Subsequently, a counterstain was applied using hematoxylin. The H-score was conducted on the sections using the Axio Lab A1 microscope (Zeiss, Germany) in accordance with the following criteria: 0: no staining, 1+: weak but detectable staining, 2+: medium or prominent staining, 3+: intense staining. The H-score value for each section was calculated by multiplying the percentage of stained cells for each density category by its density. The scoring was conducted under a light microscope at 40x objective magnification on 20 randomly selected fields on each section, with the mean scores employed for statistical analysis. H-score = $\sum i$ i xPi, i; density score, Pi; cell percentage (16).

Statistical Analysis

The data analysis was conducted using the Jamovi 2.3.21 package program. The immunohistochemical H-score index was calculated as the median (minimum-maximum). Firstly, the suitability of the data for normal distribution was evaluated using the Shapiro-Wilk test. Subsequently, one-way analysis of variance was employed to compare the normally distributed quantitative values. The Kruskal–Wallis test was employed for the comparison of normally distributed qualitative variables. A Mann-Whitney U test with Bonferroni correction was employed for the purpose of conducting pairwise comparisons. $p < 0.05$ was considered significant.

RESULTS

Effect of CAP on Ethanol-Induced Gastric Mucosal Injury

In Groups C and CAP, the gastric mucosa exhibited no macroscopic abnormalities. There

was no statistically significant difference between the ulcer scores observed at the macroscopic level in the C and CAP groups (p>0.05). The width of erosion areas and macroscopic ulcer scores in the gastric tissues of rats in the U+CAP group were significantly lower compared with those in the U group (p<0.01) (Figure-1 and Figure-2).
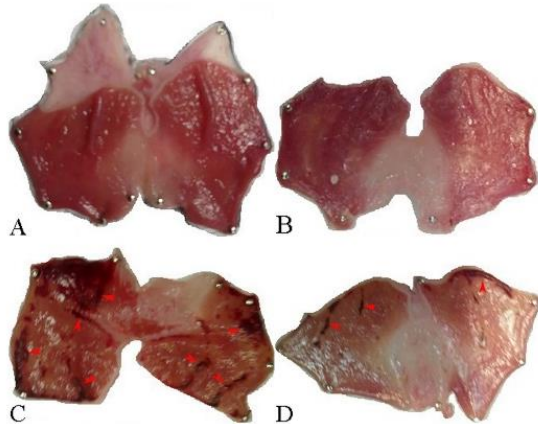
Figure-1. Macroscopic view of gastric lesions in all groups. No lesions were observed in the gastric mucosa of rats in the C (A) and CAP (B) groups. The rats in the U group (C) have extensive hemorrhagic lesions on the mucous membrane of the stomach. Rats in U+CAP group (D) have limited gastric mucosal hemorrhagic lesions. Red arrowheads indicate ulcerated areas.

Figure-2. *Significant when compared with C, CAP and U+CAP groups (P<0.01). **Significant when compared with C, CAP and U groups (p<0.01).

The gastric mucosa of the subjects belonging to the C and CAP groups showed a normal histological appearance microscopically, and there was no statistically significant difference between the microscopic ulcer scores of these two groups (p>0.05). The microscopic ulcer score of the C and CAP groups showed a statistically significant difference compared to both the U (p<0.01 for both) and U+CAP (p<0.01 for both) groups. The microscopic evaluation of H&E-stained sections of the stomach tissues of subjects belonging to the U group revealed denudation in both surface epithelium and glandular epithelium, as well as vasocongestion, erythrocyte extravasation, localized areas of mucosal necrosis and inflammatory cell infiltration. In contrast, both the lesion width and depth were significantly reduced in the U+CAP group (p<0.01). Furthermore, it was observed that the submucosal thickness was markedly increased in the U group, whereas the submucosal thickness was within the normal range in the U+CAP group (Figure-3 and Figure-4).
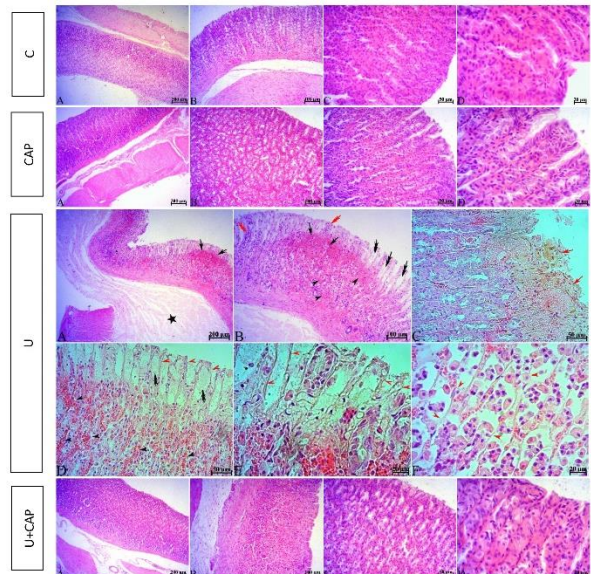
Figure-3. Histological evaluation of rat stomach mucous membranes in all groups. Epithelial desquamation (red arrowhead), mucosal hemorrhage (black arrow), vascular congestion (black arrowhead), inflammatory cell infiltration (red double arrow), mucosal necrosis (red arrow), oedema in lamina propria (black double arrow) and submucosa (black star). H&E staining. Scale bar; In C, CAP and U+CAP groups A; 200µm, B; 100 µm, C; 50 µm, D; 20 µm. In U group A; 200 µm, B; 100 µm, C, D; 50 µm, E, F; 20 µm
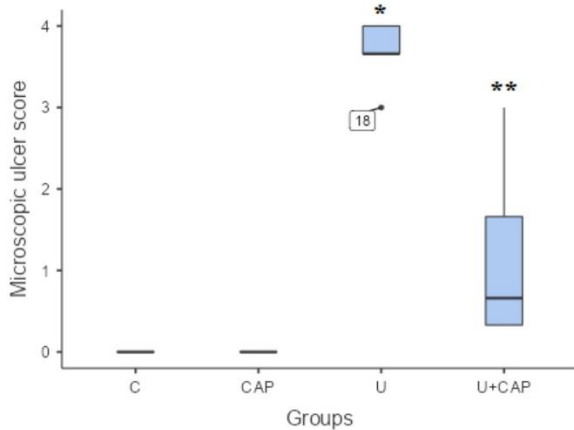
Figure-4. *Significant when compared with C, CAP and U+CAP groups (P<0.01). **Significant when compared with C, CAP and U groups (p<0.01).

The PAS staining method was applied for the purpose of evaluating the mucus barrier that covers the stomach surface. The intense magenta staining observed on the mucosal surface in the U+CAP group in comparison to the U group indicated that the glycoprotein mucus barrier was intact (Figure-5).
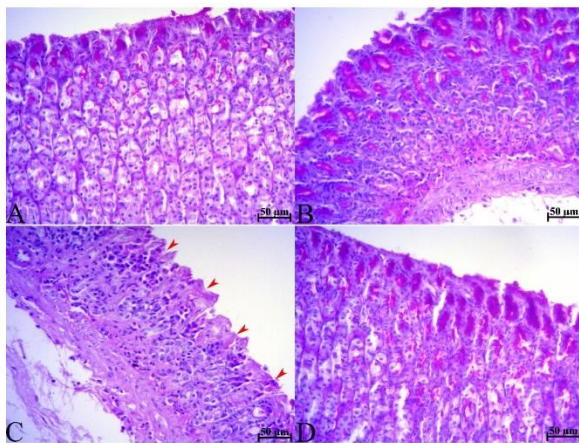
Figure-5. Histological assessment of the integrity of the mucus barrier in the gastric mucosa of rats in all groups. A: C group, B: CAP group, C: U group, D: U+CAP group. Disruption of the mucus barrier in group U, manifested by loss of magenta staining on the mucosal surface (red arrowhead). PAS staining. Scale bar; 50μm.

Effect of CAP on TAS, TOS and OSI Levels

A comparison of the TAS, TOS and OSI values in stomach tissue revealed no statistically significant difference between the C and CAP groups (p>0.05 for all). TOS value was higher in the U group compared to the C, CAP and U+CAP groups (p<0.01, p<0.01 and p<0.05, respectively). TAS value was lower in the U group compared to the C, CAP and U+CAP groups (p<0.01 for all). In the intergroup comparison in terms of OSI, a statistically significant difference was found between the U group and the C, CAP and U+CAP groups (p<0.01 for all) (Figure-6).
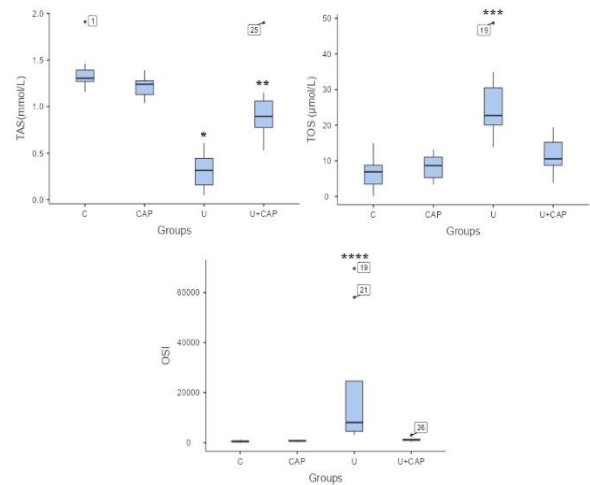
Figure-6. *Significant when compared with C, CAP and U+CAP groups (P<0.01). **Significant when compared with C and U groups (p<0.05 and p<0.01 respectively. ***Significant when compared with C, CAP and U+CAP groups (p<0.01, p<0.01 and p<0.05 respectively). ****Significant when compared with C, CAP and U+CAP groups (p<0.01 for all).
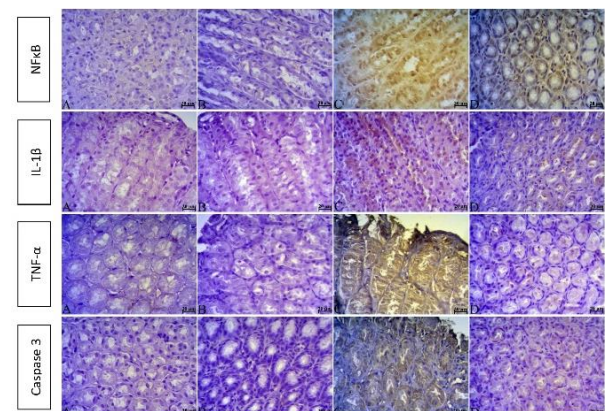
Figure-7. Results of immunohistochemical staining for IL-1, TNF-α, NF-κB and caspase 3 in stomach tissues obtained from subjects in all groups. Scale bar; 20 μm.

Effect of CAP on Immunohistochemical Staining Results

The expression of IL-1-β, TNF-α, caspase-3 and NF-κB in the mucosa was evaluated by immunohistochemical staining of gastric sections. Immunohistochemical staining of gastric tissues from groups C and CAP showed that all protein expressions were very weak. When the h-scores of these two groups were compared, no statistically significant difference was found (p>0.05). A statistically significant difference was found between the U group and the C, CAP and U+CAP groups for all protein expressions evaluated (p<0.01 for each) (Figure-7 and Figure-8).
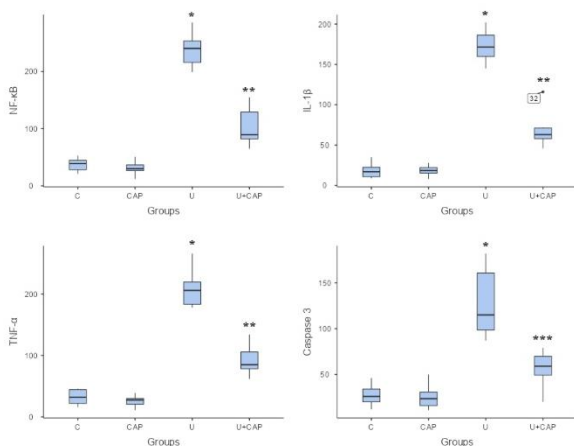
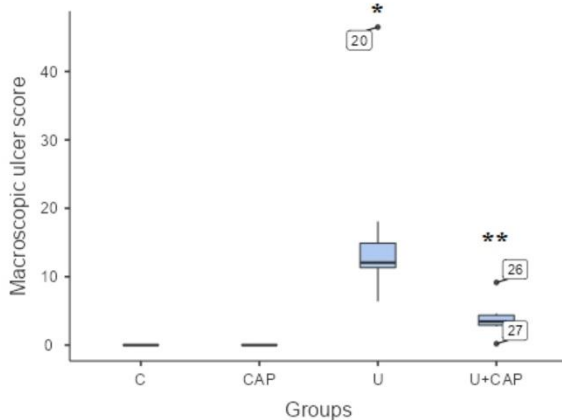**Figure-8.** *Significant when compared with C, CAP and U+CAP groups (p<0.01). **Significant when compared with C, CAP and U groups (p<0.01). ***Significant when compared with C, CAP and U groups (p<0.05, p<0.05 and p<0.01 respectively).

DISCUSSION

Our study is the first to show that oral administration of capsaicin has a protective effect on ethanol-induced gastric ulcers.

Alcohol consumption is a common cause of peptic ulcer. Ethanol has been demonstrated to exert a detrimental effect on the gastric mucosa through a number of mechanisms, including direct damage, increased gastric acidity, decreased mucosal prostaglandin levels, disruption of mucosal microcirculation, and the induction of oxidative stress and inflammation (17, 18). In experimental studies in which gastric ulcers were induced through the use of ethanol, a range of histopathological changes were frequently observed. These included epithelial necrosis, hemorrhage, mucosal and submucosal oedema, inflammatory cell infiltration and loss of the surface mucus layer. Additionally, large ulcer areas were commonly observed at the macroscopic level (19, 20). The results of our study showed that subjects in the ulcer group had macroscopic and histopathological features similar to findings described in the literature. However, it was observed that the macroscopic and microscopic ulcer scores in the stomach tissues of the subjects who were administered capsaicin in conjunction with ethanol exhibited a notable decline.

The alteration in the equilibrium between oxidants and antioxidants within the tissue, with a predominance of oxidants, is referred to as oxidative stress (21). The primary targets of oxidant agents are cellular lipids, proteins, and nucleic acids. The peroxidation of cellular lipids by oxidants gives rise to the formation of MDA. In addition, reactive oxygen species (ROS) have the potential to cause damage to nucleic acids, proteins and other macromolecules in the cell (22). Sarıyer et al. observed an increase in myeloperoxidase (MPO) and MDA levels in the gastric mucosa of *Sprague-Dawley* rats subjected to an ethanol-induced ulcer model. The administration of astaxanthin, an antioxidant, to the diet resulted in a notable reduction in MDA and MPO levels, accompanied by a decline in macroscopic and microscopic ulcer scores (23). Zhao et al. demonstrated that ethanol administration resulted in a reduction in superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and total antioxidant status (TAS) levels in the gastric mucosa of *Wistar albino* rats, concomitant with an increase in MDA levels. However, Dan-Shen-Yin treatment, an antioxidant phenolic compound mixture employed in traditional Chinese medicine, was observed to restore the antioxidant balance (24). The current study revealed a significant difference in TAS and TOS levels between subjects who were administered ethanol and those who were treated with capsaicin and ethanol. These findings are in accordance with those reported in the existing literature.

A significant consequence of oxidative stress at the cellular level is the induction of an inflammatory response, which itself represents a source of ROS production (25). The pivotal molecule that establishes the interconnection between ROS and inflammation is NF-κB, which serves as a principal regulator of proinflammatory genes. A variety of cytokine responses, including those associated with IL-1, IL-6 and TNF-α, are

primarily regulated by NF-κB (26). In experimental ulcer models induced by ethanol, in which Hsuan et al. used aibika flower flavonoid extract (27) and Fan et al. administered *Dendrobium officinale flos* water extract as therapeutic agents (28), they demonstrated that these antioxidant agents markedly reduced the elevated ROS levels in the tissue resulting from ethanol exposure, concurrently with a reduction in the expression of NF-kB and proinflammatory cytokines.

Apoptosis is a well-controlled and extremely specialized process of cell death in which a cell destroys itself and it is vital for cellular homeostasis that the process of apoptosis is properly regulated (29). Both ROS and inflammatory stimuli have been demonstrated to trigger apoptosis. At low levels of ROS, the cell can undergo DNA repair and survival through p53 activation. Conversely, at high levels of ROS, p53 directs the cell to the intrinsic apoptotic pathway by regulating the levels of pro-apoptotic (Bcl-2-associated X protein (Bax), BH3 interacting-domain death agonist (Bid), Apoptotic protease activating factor 1 (Apaf-1)) and anti-apoptotic [B cell lymphoma/leukemia type 2 (Bcl-2), B-cell lymphoma-extra-large (Bcl-X$_L$), surviving] proteins and activating caspase 9 (30). In addition to the NF-κB-mediated increase in proinflammatory cytokines during the inflammatory process, elevated levels of ROS inhibit phosphatases, resulting in enhanced phosphorylation of the Inhibitor of κB (IκB) (31). This, in turn, leads to its degradation, thereby facilitating NF-κB activation. Consequently, an augmented proinflammatory cytokine response, particularly TNF-α, instigates the extrinsic apoptotic pathway via by binding to tumor necrosis factor receptor 1 (TNF-R1) and activating caspase 8 (29). Irrespective of whether the process of apoptosis is initiated by intrinsic or extrinsic mechanisms, the activation of caspase 3 ultimately leads to cellular demise (32). Recent studies have demonstrated the involvement of apoptosis in the pathogenesis of ethanol-induced gastric ulceration. Alamoudi et al. observed an increase in ROS, NF-kB, proinflammatory cytokines and caspase 3 in ethanolic ulcers in rats. They also demonstrated that hesperidin caused a significant decrease in these parameters and a significant improvement in the histological appearance of the gastric mucosa, which they attributed to its antioxidant and anti-inflammatory effects (33). Similarly, Lin et al. demonstrated that galangin, which possesses antioxidant and anti-inflammatory properties, led to a reduction in the levels of MDA, NO, NF-κB, pro-inflammatory cytokines IL-1, IL-6, IL-8, TNF-α as well as caspase 3 in ethanolic ulcer models induced in BALB/c mice (34). Our study yielded similar results to those observed by other researchers, indicating a correlation between the decline in caspase 3 expression and the reduction in ROS, NF-kB, IL-1 and TNF-α levels in the gastric mucosa of subjects who were administered capsaicin.

CONCLUSION

The results of our study revealed that capsaicin exerts its protective effect on the gastric mucosa in the ethanol-induced ulcer model through its antioxidant, anti-inflammatory and anti-apoptotic properties.

Conflict of interest: There is no conflict of interest among the authors.

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The efficacy of multiparametric prostate MRI in making treatment decisions and predicting early recurrence in localized prostate cancer

Multiparametrik prostat MRI'nın lokalize prostat kanserinde tedavi seçimi ve erken nüks saptamaya etkisi

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ABSTRACT

Aim: This study investigates the potential role of multiparametric magnetic resonance imaging (mp-MRI) in localized prostate cancer, its impact on treatment decision-making and its predictability of the likelihood of cancer recurrence after treatment.

Material and Method: The relationships between ISUP grade groups, prostate volume, prostate-specific antigen (PSA), and free PSA values that determine the risk classification of 114 cases diagnosed with localized prostate cancer, mp-MRI findings, including index lesion diameter, apparent diffusion coefficient (ADC) value, capsule contact length, extracapsular extension, and presence of seminal vesicle invasion, and biochemical recurrence, were investigated.

Results: Of the 114 patients included in the study, 49 underwent radiotherapy and 61 underwent radical prostatectomy as curative treatments. Four patients were enrolled in an active surveillance protocol to delay potential side effects. PSA or local recurrence occurred in 13 (11.4%) patients during the follow-up period. There was a significant correlation between stable disease and absence of extracapsular invasion (*p=0.022*) and ISUP grade (*p=0.025*). There was also a significant correlation between index lesion diameter (*p=0.005*), capsule contact length (*p=0.015*), and recurrence. Additionally, the ADC value decreased as the ISUP grade and clinical stage increased (*p=0.001*).

Conclusion: This study's findings indicate that mp-MRI can be used for risk stratification and making risk-based treatment decisions in localized prostate cancer patients.

Keywords: Prostate cancer, multiparametric prostate MRI, risk factors, lesion diameter, capsule contact length.

ÖZ

Amaç: *Çalışmada amacımız, prostat kanseri tanısında, giderek kullanımı artan multiparametrik prostat MRG'nin lokalize hastalıkta tedavi kararlarındaki potansiyel rolünü ve tedavi sonrası erken dönemde nüksü belirlemede prognostik önemini araştırmaktır.*

Gereç ve Yöntem: *Lokalize prostat kanseri tanısı alan 114 olgunun risk sınıflamasını belirleyen ISUP derecesi, prostat spesifik antijen (PSA), serbest PSA, prostat volüm ile mp-MRI incelemede indeks lezyon çapı, görünür difüzyon katsayısı (ADC) değeri, kapsül temas uzunluğu, ekstrakapsular uzanım ve seminal vezikül invazyonu varlığının biyokimyasal nüks ile ilişkisi araştırılmıştır. Gruplar arasındaki radyolojik ve klinik özelliklerin dağılımındaki farklılıklar, ki-kare testi kullanılarak istatistiksel olarak değerlendirildi.*

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Bulgular: *Çalışmaya dahil edilen 114 hastanın 49'una radyoterapi, 61'ine radikal prostatektomi olmak üzere küratif tedavi uygulanmıştır. Dört hasta ise yan etkileri ertelemek amacıyla aktif izlem protokolüne alınmıştır. Takipte 13 (11,4%) hastada PSA veya lokal nüks meydana gelmiştir. Nüksüz hastalık ile ekstrakapsüler invazyon yokluğu(p=0,022) ve ISUP derecesi (p=0,025) arasında istatistik olarak anlamlı bir ilişki gözlenmiştir. İndeks lezyon çapı ölçümü (p=0,005) ve kapsül temas uzunluğu ölçümü(p=0,015) ile erken dönem nüks hastalık arasında istatiksel olarak anlamlı ilişki gözlenmiş olup, ISUP derecesi arttıkça ve klinik evre arttıkça ADC değeri düşmekteydi (p=0,001).*

Sonuç: *Bu çalışmanın bulguları, mp-MRI'nin lokalize prostat kanseri hastalarında risk sınıflandırması ve riske dayalı tedavi kararları vermek için kullanılabileceğini göstermektedir.*

Anahtar Sözcükler: *Prostat kanser, multiparametrik MRI, risk faktörleri, lezyon çapı, kapsül temas uzunluğu.*

INTRODUCTION

Prostate cancer tops the charts as the most common cancer affecting men globally (1). Most patients with prostate cancer are diagnosed in the localized disease stage. Therefore, this patient group is treated with local treatment options such as active surveillance, radiotherapy (RT), or radical prostatectomy (RP) based on their risk classification (2). When planning optimal treatment for men with prostate cancer, it is essential to avoid unnecessary overtreatment in the low-risk patient group and not to cause recurrence of the disease and treatment failures in men who choose active surveillance (3). Currently used risk stratification for prostate cancer relies on findings like PSA level, ISUP grade, and clinical stage from digital rectal exams. However, these findings can vary depending on the examining physician, highlighting the need for more accurate assessment of clinically significant lesions, disease extent at diagnosis, and future progression risk (4). For example, interobserver agreement for clinical staging is strikingly low, and ISUP grade of approximately one-third of patients are increased based on radical prostatectomy materials relative to biopsy specimens (5, 6). Therefore, new assessment methods are needed to improve the risk stratification of prostate cancer patients.

Many publications in the literature address risk assessments after radical prostatectomy (7). However, these reference standards may not apply to determining prognosis in patients undergoing RT (8, 9). In particular, the fact that ISUP's grade is histopathologically upgraded after radical prostatectomy reveals the necessity of additional criteria in the pre-RT evaluation.

MRI is gaining importance in prostate cancer imaging due to its reliability in detecting clinically significant cancers. This allows for better patient selection for biopsy and more precise targeting of lesions during the procedure. The spread of cancerous tissue within the prostate gland and its spread to surrounding tissues is better assessed by MRI. In addition, detailed information about the exact location and size of the cancerous tissue provides guidance in planning treatments such as surgery or radiotherapy.

Considering the foregoing, the objective of this study is to investigate the potential role of mp-MRI, which is increasingly used in the diagnosis of prostate cancer, in making treatment decisions in localized prostate cancer patients and its prognostic value in predicting early recurrence after treatment.

MATERIALS and METHODS

Study design and setting

Prior to conducting this retrospective study, informed consent was obtained from all participants. The study protocol was approved by the University's ethics committee (Approval Date: 24.05.2023, Approval No.424), ensuring adherence to the ethical principles outlined in the revised Declaration of Helsinki adopted by the World Medical Association General Assembly in Edinburgh in 2000.

Population and Sample

A total of 406 patients who underwent mp-MRI in our tertiary university hospital between 2015 and 2022 were identified from the SECTRA picture archiving and communication system (PACS) (Sectra Workstation IDS7; Sectra AB, Linköping, Sweden). These patients' biopsy and follow-up results were accessed from the hospital archive system (MIAMED, 1.0.1.3295). Consequently, 221 patients, who were determined to have been diagnosed with prostate cancer, constituted the study population. Of these patients, patients with

metastatic disease at the time of diagnosis (n: 11), no lesion finding on MRI at the time of diagnosis (n: 70), whose information could not be accessed as they were treated outside our hospital after having an MRI in our hospital (n: 12), and whose MRI scan quality was not optimal (n:14) due to reasons such as hip replacement artifact, motion artifact, etc. were excluded from the study. The remaining 114 patients constituted the study sample. These patients' demographic and clinical characteristics, including age, ISUP grade, tumor rate in biopsy materials, PSA and free PSA values, treatments received, and follow-up duration after treatment, were obtained from the patients' archive files and recorded.

MRI Analysis

All mp-MRI scans performed before the treatment were reviewed by an abdominal radiologist (A.K.) with 18 years of experience knowing the diagnosis of prostate cancer but blinded to the clinical and pathological results. The Prostate Imaging-Reporting and Data System (PI-RADS) score was determined according to the PI-RADS v2.1 guideline published by the European Society of Urogenital Radiology (ESUR) in 2019 with the latest revisions. Prostate volume was measured via axial and sagittal T2WI, the longest dimension of the index lesion via axial T2WI images and apparent diffusion coefficient (ADC) maps, and the index lesion's capsule contact length via axial T2WI images. In addition, all images were evaluated together, and the presence of accompanying lymphadenopathy, extra prostatic extension, and seminal vesicle invasion was assessed. The ADC value was determined by selecting the largest region of interest (ROI) of the targeted tissue in the ADC map containing the largest tumor section, but without approaching the tumor borders so that the signal would not interfere with the normal tissue.

Local staging of the tumor was performed using mp-MRI using the American Joint Committee on Cancer guidelines (10). Cases where no lesion could be detected on MRI but were histopathologically diagnosed with prostate cancer were excluded from the study.

MRI Protocol

All mp-MRI examinations were performed using a 3.0 Tesla Magnetic Resonance Imaging (3T-MRI) device (MAGNETOM Spectra; Siemens Healthcare, Erlangen, Germany) and a 16-channel phased array body coil (Siemens Healthcare, Erlangen, Germany). MRI sequences utilized were T1 weighted-imaging (T1WI) and T2WI sequences in the axial plane, high-resolution small field-of-view T2 (FOV T2) sequences in axial, sagittal, and coronal planes, diffusion-weighted imaging (DWI) (b 50, b800, b 1000 and b1400 sec/mm^2), ADC map and T1-weighted dynamic contrast-enhanced (T1W DCE) sequences with fat suppression in the axial plane (Table-1). ADC maps were automatically calculated by the software (Syngo via Siemens Medical Systems) using all available b-values integrated into least squares monoexponentially fitting. DCE sequences were taken at the thirtieth, sixtieth-, and ninetieth-seconds following administration of the contrast material.

Table-1. MRI sequence parameters.

Parameters	Axial TSE T2WI	Coronal TSE T2WI	Echo planar imaging DWI	Axial T1W DCE
TR (ms)	3720	4320	7700	5.12
TE (ms)	101	101	94	1.78
FOV (mm)	154x70	200x20	211x250	259x259
Matrix size	218x320	224x320	122x144	138x192
Slice thickness (mm)	3	3.5	5	3.5
b-values (s/mm^2)	-	-	b0, b400, b800, b1000, b1400	-
Flip angle	160	160	90	15

TR: Repetition time; TE: Echo time; FOV: Field of view; T2WI: T2-weighted imaging; DWI: Diffusion-weighted imaging; T1W:cT1 weighted-imaging; DCE: Dynamic contrast-enhanced.

Table-2. Demographic and clinical characteristics of the patients included in the study.

Variables (n=114)	
Age (years). Median (IQR)	65.4 ± 7.7
Prostate volume (cc)	50.9 ± 25.2
PSA (ng/mL)	8.86 (±7.33)
Free PSA	2.89 (±10.29)
PIRADS score. n (%)	
Stage 2	1 (0.9%)
Stage 3	18 (15.8%)
Stage 4	53 (46.5%)
Stage 5	42 (36.8%)
ISUP grade. n (%)	
Grade 1	70 (61.4%)
Grade 2	16 (14.0%)
Grade 3	14 (12.3%)
Grade 4	8 (7.0%)
Grade 5	6 (5.3%)
Clinical stage. n (%)	
T2 A	32 (28.1%)
T2 B	18 (15.8%)
T2 C	35 (30.7%)
T3 A	17 (14.9%)
T3B	12 (10.5%)
Pelvic lymphadenopathy. n (%)	**8 (7.0%)**
Extracapsular extension. n (%)	**31 (27.2%)**
Seminal vesicle invasion	**16 (14.0%)**
İndex lesion diameter (mm)	**14.18 (±8.25)**
ADC	**732.83 (±191.45)**
Capsule contact length (mm)	**11.43 (±12.23)**
Percent of biopsy cores	**11.65 (±14.52)**

PSA: Prostate-specific antigen; ADC: Apparent diffusion coefficient SD. Standard deviation, p value <0.05

Table-3. The relationships between stable or recurrent disease status and the categorical variables.

		Follow-up results				
		stable		recurrence		
		N	%	N	%	*p.
PIRADS	2	1	100.0%	0	0.0%	
	3	17	94.4%	1	5.6%	
	4	49	92.5%	4	7.5%	.267
	5	34	81.0%	8	19.0%	
Pelvic lymphadenopathy	yes	7	87.5%	1	12.5%	.919
	no	94	88.7%	12	11.3%	
Extracapsular extension	yes	24	77.4%	7	22.6%	**.022**
	no	77	92.8%	6	7.2%	
Seminal vesicle invasion	yes	12	75.0%	4	25.0%	.065
	no	89	90.8%	9	9.2%	
Gleason score	3+3=6	64	91.4%	6	8.6%	
	4+3=7	11	78.6%	3	21.4%	
	3+4=7	16	100.0%	0	0.0%	
	4+4=8	7	87.5%	1	12.5%	**.025**
	4+5=9	2	50.0%	2	50.0%	
	5+5=10	1	50.0%	1	50.0%	
Clinical stage	T2A	32	100.0%	0	0.0%	
	T2B	16	88.9%	2	11.1%	
	T2C	31	88.6%	4	11.4%	.106
	T3A	13	76.5%	4	23.5%	
	T3B	9	77.8%	3	22.2%	

*Significance level according to chi-square test results

Statistical Analysis

Statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, U.S., 2019), Jamovi (Version 2.3.28, The Jamovi Project, 2023), and JASP (Version 0.19.1, Jeffreys' Amazing Statistics Program, 2024). SPSS was utilized for basic statistical analyses, while Jamovi and JASP were employed for advanced statistical procedures, visualization, and ROC curve analyses with associated metrics. The sample size was determined to ensure at least 80% test power and 5% types-1 error rate for each variable analyzed. To determine the appropriate statistical tests, the normality of continuous variables was assessed using the Kolmogorov-Smirnov test for samples exceeding 50 and the Skewness-Kurtosis test. Based on these tests, parametric tests were employed due to the confirmation of normal distribution in the continuous variables. Descriptive statistics were generated for the collected data. Continuous variables are presented as mean ± standard deviation (SD), while categorical variables are represented by frequency (n) and percentage (%) values. Categorical variables were compared between groups using the independent samples t-test for two groups and one-way analysis of variance (ANOVA) for three or more groups. Following a significant ANOVA result, Duncan's multiple range test (DMRT) was used to identify specific groups with statistically different means. The relationship between continuous variables was assessed with Pearson correlation analysis. Chi-square tests were employed to evaluate associations between categorical variables. Receiver Operating Characteristic (ROC) curve

analyses were conducted to determine optimal cut-off values for imaging parameters. The Youden index (J = sensitivity + specificity - 1) was used to identify optimal cut-off points. Area Under the Curve (AUC) values were calculated with 95% confidence intervals, and DeLong's test was used to compare the diagnostic performance of different parameters. Sensitivity, specificity, positive and negative predictive values, and likelihood ratios were calculated for the identified cut-off points. A p-value of less than or equal to 0.05 was considered statistically significant.

RESULTS

Patients' Characteristics

The mean age of the patients included in the study was 65.4 ± 7.7 years, and the mean follow-up period was 31.7 ± 14.2 months. Patient's demographic and clinical characteristics are detailed in Table-2. Of the 110 patients who received curative treatment, 49 underwent radiotherapy and 61 underwent radical prostatectomy. Four additional patients were enrolled in an active surveillance protocol. Among patients receiving curative treatment, biochemical recurrence occurred in 13 patients (11.4%; 7 in the surgery group and 6 in the radiotherapy group). The active surveillance group was excluded from recurrence analysis due to different progression criteria.

The relationships between stable or recurrent disease status and the categorical variables are given in Table-3. Accordingly, there was a statistically significant relationship between stable disease status and the absence of extracapsular invasion. The majority (92.8%) of the cases lacking extracapsular invasion remained clinically stable (*p=0.022*). Similarly, there was a statistically significant correlation between the patients' ISUP grade and stable disease status (*p=0.025*). On the other hand, there was no statistically significant relationship between the variables other than those listed above and stable or recurrent disease status (p>0.05).

Mp-MRI Findings

The distribution of patients' mp-MRI findings by stable or recurrent disease status revealed a statistically significant difference between index lesion diameter and recurrent disease status (*p=0.005*). Accordingly, the index lesion size was significantly higher in patients with recurrent disease than in patients with stable disease (Table-4).

Similarly, there was a statistically significant difference between patients' capsule contact length in MRI and recurrent disease status(*p=0.015*). Accordingly, the capsule contact length was significantly higher in patients with recurrent disease than in those with stable disease (Table-4).

Additionally, it was determined that ADC value was significantly correlated with the ISUP grade and clinical stage. Accordingly, as the ISUP grade and the clinical stage increased, the ADC value decreased (*p=0.001*) (Table-5).

Furthermore, it was determined that the capsule contact length was significantly correlated with findings such as pelvic lymphadenopathy, extracapsular extension, and seminal vesicle invasion. Accordingly, patients with the said findings had significantly higher capsule contact lengths than others (*p=0.001*) (Table-6).

Table-4. Distribution of patients' mp-MRI findings and clinical findings by stable or recurrent disease status.

	Follow-up results		
	stable	recurrence	*p.
Index lesion diameter (mm)	13.41±7.57	20.15±10.97	.005
ADC	736.42±183.88	705.00±249.92	.580
Capsule contact length (mm)	10.44±10.62	19.15±19.92	.015
PSA	9.27±7.65	5.65±2.23	.093
Free PSA	3.09±10.92	1.38± 7.1	.574
Percent of biopsy cores	10.30±10.08	22.15±31.67	.004

Values are shown as mean ± standard deviation. ADC: Apparent diffusion coefficient; PSA: Prostate-specific antigen

*Significance levels according to independent samples t-test results.

Table-5. Analysis of correlations of ADC with Gleason score and clinical stage.

		ADC (**)	*p.
Gleason score	3+3=6	792.97±190.00 [a]	.001
	4+3=7	627.43±186.08 [b]	
	3+4=7	664.56±167.47 [b]	
	4+4=8	607.00±106.05 [b]	
	4+5=9	653.00±67.12 [b]	
	5+5=10	575.00±52.33 [c]	
Clinical stage	T2A	868.8±181.27 [a]	.001
	T2B	689.22±151.13 [a]	
	T2C	764.29±174.60 [a]	
	T3A	578.41±114.51 [b]	
	T3B	566.00±57.71 [b]	

* Values are shown as mean ± standard deviation. Significance levels according to one-way ANOVA test results.
a.b.c: Shows the difference between the groups (Tukey's post-hoc test)
** ADC: apparent diffusion coefficient

Table-6. Analysis of the correlations of capsule contact length with categorical variables

		Capsule contact length (mm)	p.
Lymphadenopathy	Yes	33.25±17.25	.001
	No	9.79±10.11	
Extracapsular extension	Yes	25.23±14.22	.001
	No	6.28±5.79	
Seminal vesicle invasion	Yes	30.63±13.50	.001
	No	8.30±8.69	

* Values are shown as mean ± standard deviation. Significance levels according to independent samples t-test results

ROC Curve Analysis

ROC curve analysis was performed to determine optimal cut-off values for both index lesion diameter and capsule contact length in predicting recurrence (Table-7, Figure-1). The index lesion diameter demonstrated fair diagnostic performance with an AUC of 0.712 (95% CI: 0.619-0.793, p=0.006), yielding an optimal cut-off value of >15 mm (sensitivity: 69.23%, specificity: 70.30%). Similarly, capsule contact length showed good diagnostic accuracy with a higher AUC of 0.778 (95% CI: 0.678-0.860, p=0.001), with an optimal cut-off value of >18 mm (sensitivity: 66.67%, specificity: 81.25%). The positive likelihood ratio was notably higher for capsule contact length (3.56, 95% CI: 1.86-6.81) compared to index lesion diameter (2.33, 95% CI: 1.46-3.73), suggesting stronger predictive value for recurrence. These findings indicate that both parameters, particularly capsule contact length, can serve as valuable imaging biomarkers for risk stratification in localized prostate cancer, with capsule contact length >18 mm associated with a significantly increased risk of recurrence.

Table-7. ROC curve analysis results for index lesion diameter and capsule contact length.

Parameter	Index Lesion Diameter	Capsule Contact Length
AUC (95% CI)	0.712 (0.619-0.793)	0.778 (0.678-0.860)
Cut-off value	>15 mm	>18 mm
Sensitivity	69.23%	66.67%
Specificity	70.30%	81.25%
Youden index	0.3953	0.4792
Recurrence rate	13 (11.40%)	9 (10.11%)
p-value	**0.006**	**0.001**

Note: Bold p-values indicate statistical significance (p≤0.05)
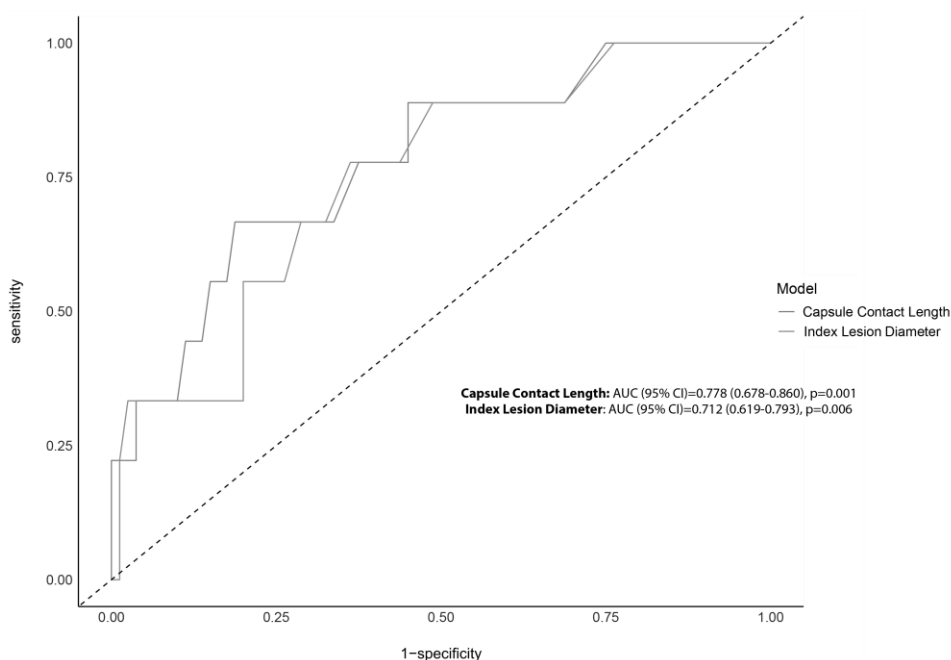
Figure-1. ROC analysis for index lesion diameter and capsular contact length. The optimal cut-off value for index lesion diameter was determined to be >15 mm (AUC: 0.712, 95% CI: 0.619-0.793, p=0.006), and for capsular contact length, it was >18 mm (AUC: 0.778, 95% CI: 0.678-0.860, p =0.001).

DISCUSSION

In this study, we evaluated the impact of mp-MRI findings on treatment decisions and prognosis in patients with localized prostate cancer. Our findings demonstrated significant associations between index lesion diameter (p=0.005) and capsule contact length (p=0.015) with biochemical recurrence. Moreover, we found that ADC values showed an inverse correlation with both Gleason score and clinical stage (p=0.001). The absence of extracapsular extension showed significant correlation with stable disease status (p=0.022). Additionally, patients' Gleason categories demonstrated significant association

with disease stability (p=0.025). These findings suggest that quantitative mp-MRI parameters can serve as important prognostic indicators in localized prostate cancer management.

Localized prostate cancer is characterized by the absence of identifiable regional lymph nodes or distant metastases. In this context, three primary treatment options are available for localized prostate cancer patients: active surveillance, surgery, and RT. Several cohort studies have identified a 0% to 6.1% risk of metastasis and death from prostate cancer in selected patients under active surveillance and thus concluded that active surveillance is beneficial in this patient group (11). Clinicians traditionally classify

prostate cancer diagnoses as low-, intermediate-, and high-risk based on a combination of factors: ISUP grade (tumor grade), prostate-specific antigen (PSA) level, and clinical stage determined by digital rectal examination. While the specific treatment options for prostate cancer may vary depending on individual factors, surgery and radiation therapy are commonly considered effective treatments for men with advanced-stage prostate cancer (11). One of the main objectives of using MRI is to accurately determine the tumor focus with the highest Gleason pattern and the T-stage of the tumor in order to make an accurate risk classification. Despite a smaller sample size compared to other relevant studies, this research identified a significant relationship between low ISUP grade and stable disease status, aligning with established literature. However, more importantly, it was found that as the ISUP grade and the clinical stage increase, the ADC value decreases in diffusion sequences. Taking samples from the tissue with a low ADC value while planning a biopsy allows for determining the highest Gleason score, which we think may increase the effectiveness of the treatment, especially in planning active surveillance or RT.

The other radiological parameter we found to be related to local recurrence was the index lesion size. Patients with recurrent disease had significantly larger index lesion sizes, supporting the established link between larger tumor volume in radical prostatectomy specimens and negative outcomes like recurrence, lymph node involvement, metastasis, and mortality (4). In the literature, lesions greater than 15 mm have been associated with a higher risk of extracapsular extension (ECE) and biochemical (PSA) recurrence (2,12,13). This criterion is used to differentiate PI-RADS 4 and 5 in the PI-RADS v.2 guidelines, and Gorovets et al (14). found a significant relationship between index lesion size and recurrence after stereotactic body radiotherapy (SBRT) in PI-RADS4 and PI-RADS 5 cases. Dahan et al (15). suggested that patients with a larger index lesion may benefit from an intraprostatic dose increase via external beam radiation therapy (EBRT) or an increase in brachytherapy. T stage has an important place in the risk stratification of prostate cancer patients in terms of selecting the optimal treatment in clinical practice. Yet, index lesion size, an mp-MRI parameter, is not included in the risk stratification. Some studies reported that tumor volume, another MRI parameter, has shown promising results over pathological tumor volume, with its

relatively high correlation and low interobserver variability. However, other studies had reported that tumor volume showed poor correlation, mainly when only T2WI was used. Therefore, it has been speculated that tumor volume measurement in T2WI and DWI sequences can give results close to tumor volume in pathological preparations (4).

Since T1 tumors were not visible on MRI, we did not include them in our study. Nonetheless, another critical prognostic MRI finding is simply whether a lesion is visible or not. As a reason, visible lesions have been reported to increase the risk of developing metastases and death from prostate cancer more than tenfold compared to invisible lesions (15).

Our study revealed a positive correlation between capsule contact length and recurrent disease, with patients experiencing recurrence having significantly longer contact lengths. In addition, we also found pelvic lymphadenopathy, ECE, and seminal vesicle invasion to be significantly higher in cases with high capsule contact length. It has been shown in the literature that the tumor-to-capsule contact length on MRI is a strong predictor of ECE, with good to excellent interobserver agreement (16). A major challenge in diagnosing extracapsular extension (ECE) of prostate cancer lies in the subjective visual assessment of MRI findings by radiologists, leading to potential variability between examiners with different levels of experience. Capsule contact length, however, presents a unique opportunity for objective evaluation. It is currently the only known measurable and reproducible determinant for ECE. Despite its promise, there is a lack of standardized methods for measuring tumor contact surface. Studies have employed different approaches, including straight and curvilinear measurements. According to Eurboonyan et al. (17), measuring the total length of tumor-capsule contact along the longitudinal axis (absolute tumor-capsule contact length) provides a more accurate estimate of extra prostatic extension (ECE) compared to the curvilinear measurement. In addition, they found that the capsule contact length values above the 15 mm cut-off value, which they measured in dynamic contrast series, were highly correlated with the presence of ECE (17). While the American College of Radiology (ACR) recommends a 10mm contact surface as an indicator of ECE in their PI-RADS v2 guidelines, several studies have proposed or utilized higher cut-off values, ranging from 11mm to 20mm (17).

Due to ECE, patients experience a higher risk of lymph node or bone metastasis, tumor

recurrence after treatments like radical prostatectomy or radiation therapy, often requiring adjuvant therapy (18). Hricaket al (19), described asymmetry of the neurovascular bundle, tumor occlusion of the neurovascular bundle, a swollen prostate contour, obliteration of an irregular or prickly capsular margin, and the rectal-prostatic angle, capsular retraction, tumor capsule contact surface greater than 1 cm, and findings indicating direct tumor spread and a tear in the capsule on T2-weighted imaging as imaging findings associated with EPE. However, there are discrepancies between the results of various mp-MRI prostate cancer staging studies, which feature a wide range of sensitivity (23%-90%) and specificity (30%-95%) (18).

In our study, the absence of extra prostatic invasion was associated with stable disease status, in line with the literature findings. However, the inability to distinguish focal or non-focal spread in EPE by imaging causes diagnostic and prognostic uncertainty. The definition of ECE is also unclear in pathology. Various clinical variables, including the surgical procedure, the pericapsular environment, and the lack of guidelines for a true capsular space, can complicate determining the presence or extent of ECE (20). However, pretreatment diagnosis of ECE, which cannot be detected even during the surgery, is essential in planning the surgery and RT. Then again, ECE is often microscopic and is, therefore, below the detection threshold of mp-MRI.

Beyond the conventional MRI findings, our study demonstrated that quantitative parameters derived from mp-MRI, specifically the index lesion diameter and capsule contact length, can serve as objective imaging biomarkers for risk stratification. While both parameters showed significant predictive value for recurrence, capsule contact length emerged as a particularly robust predictor with superior diagnostic accuracy. This finding aligns with previous studies suggesting that tumor-capsule contact measurements might better reflect the biological aggressiveness of prostate cancer compared to simple size measurements alone. The higher specificity of capsule contact length in predicting recurrence suggests its potential utility in identifying patients who might benefit from more aggressive treatment approaches. Notably, our identified cut-off values for both parameters demonstrated practical clinical utility, with capsule contact length showing particular promise in risk stratification. These quantitative thresholds could potentially complement existing risk assessment tools, although prospective validation in larger cohorts would be valuable.

The stronger predictive performance of capsule contact length compared to index lesion diameter might be explained by its direct relationship with tumor-capsule interaction, which is a critical determinant of local invasion potential. These findings support the integration of these quantitative mp-MRI parameters into clinical decision-making algorithms, particularly when determining treatment intensity and follow-up protocols.

The most important limitation of our study is that the biopsies were performed as transrectal ultrasound scan (TRUS)-guided biopsies, making it challenging to identify the patients who could be upgraded after surgery. For this reason, the decision for the biopsy site was made jointly together with the physicians who will perform the biopsy based on MRI images. Nonetheless, further prospective studies with MRI-guided fusion biopsies are needed to shed more light on the subject. The second limitation of our study is the short follow-up time after treatment and the fact that the small number of patients with recurrence might have provided limited data in predicting biochemical recurrence after treatment. Furthermore, the small number of patients in subgroups with different treatment options and the heterogeneity of patient characteristics resulted in a quite unbalanced dataset. Indeed, the relatively small dataset size was primarily due to the study design and inclusion criteria, which only included lesions visible on MRI. A further limitation of this research, especially in relation to biochemical and local recurrence, is the insufficient detail provided concerning the surgical interventions and radiation therapy regimens administered to the respective patient groups. Although this study's findings indicate that mp-MRI can play an active role in predicting early recurrence and selecting treatment. However, prospective studies with longer follow-up periods are needed.

CONCLUSION

In conclusion, mp-MRI has a high sensitivity in detecting clinically significant prostate cancer. This study's findings indicate that mp-MRI can be used for risk stratification and making risk-based treatment decisions in localized prostate cancer patients. Additionally, it was determined that high index lesion size, capsule contact length, and low ADC values predict poor prognosis, indicating the need for more aggressive treatments.

Conflict of interest: There is no conflict of interest among the authors.

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Antibody Labeling Kits vs Conventional Labeling: A Comparative Study in Immunofluorescence of Cell Cultures

Antikor Etiketleme Kitleri ile Geleneksel Etiketleme: Hücre Kültürlerinde Karşılaştırmalı İmmünofloresans Bir Çalışma

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ABSTRACT

Aim: This study aims to evaluate the comparative performance of antibody labeling kits and conventional antibody labeling methods regarding efficiency, accuracy, and practical usability in immunofluorescence applications on cell cultures.

Materials and Methods: Both labeling methods were applied to identical cell culture samples. Labeling efficiency, fluorescence intensity, and antibody specificity were assessed using quantitative fluorescence microscopy. Practical aspects such as ease of use, time efficiency, and cost were also systematically evaluated.

Results: Antibody labeling kits significantly reduced preparation time while increasing labeling efficiency, achieving comparable results to conventional methods in fluorescence intensity and antibody specificity. In sum, it is reported that it is higher ease of use for Antibody Labeling Kits.

Conclusion: Antibody labeling kits offer a reliable and user-friendly alternative for immunofluorescence studies. By enhancing labeling efficiency and simplifying operational processes, these kits can accelerate research workflows and improve experimental outcomes in cell-based assays.

Keywords: Antibody binding stability, quantitative fluorescence microscopy, multicolor labeling, biomolecular imaging, efficiency.

ÖZ

Amaç: *Bu çalışma, antikor işaretleme kitleri ile geleneksel antikor işaretleme yöntemlerinin immün floresans uygulamalarında hücre kültürleri üzerindeki verimlilik, doğruluk ve pratik kullanılabilirlik açısından karşılaştırmalı performansını değerlendirmeyi amaçlamaktadır.*

Gereç ve Yöntem: *Çalışmada her iki işaretleme yöntemi, aynı hücre kültürü örneklerine uygulanmıştır. İşaretleme verimliliği, floresans yoğunluğu ve antikor özgüllüğü, nicel floresans mikroskopisi ile değerlendirilmiştir. Ayrıca kullanım kolaylığı, zaman verimliliği ve maliyet gibi pratik unsurlar incelenmiştir.*

Bulgular: *Antikor işaretleme kitleri, hazırlık süresini önemli ölçüde azaltırken işaretleme verimliliğini arttırmış ve floresans yoğunluğu ile antikor özgüllüğü açısından geleneksel yöntemlere eşdeğer sonuçlar sağlamıştır. Bütün değişkenler bir arada değerlendirildiğinde, antikor İşaretleme Kitlerinin kullanım kolaylığını daha yüksek olarak bildirmiştir.*

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Sonuç: Antikor işaretleme kitleri, immün floresans çalışmaları için güvenilir ve kullanıcı dostu bir alternatif sunmaktadır. Etiketleme verimliliğini artırmaları ve operasyonel süreçleri basitleştirmeleri, bu kitlerin araştırma süreçlerini hızlandırarak hücre temelli deneylerde daha iyi sonuçlar elde edilmesini sağlayabileceğini göstermektedir.

Anahtar Sözcükler: Antikor bağlanma stabilitesi, nicel floresans mikroskopisi, çok renkli etiketleme, biyomoleküler görüntüleme, verimlilik

INTRODUCTION

Antibody labeling is a fundamental technique in immunofluorescence (IF), enabling the visualization of specific antigens within biological samples. Traditional methods, such as direct conjugation, where dyes are chemically attached to primary antibodies (1) and indirect conjugation, which utilizes secondary antibodies to bind to primary antibodies, have been foundational tools for decades (2-3) (Figure-1). Despite their robustness, these methods often require meticulous optimization to address challenges like non-specific binding and high background fluorescence.
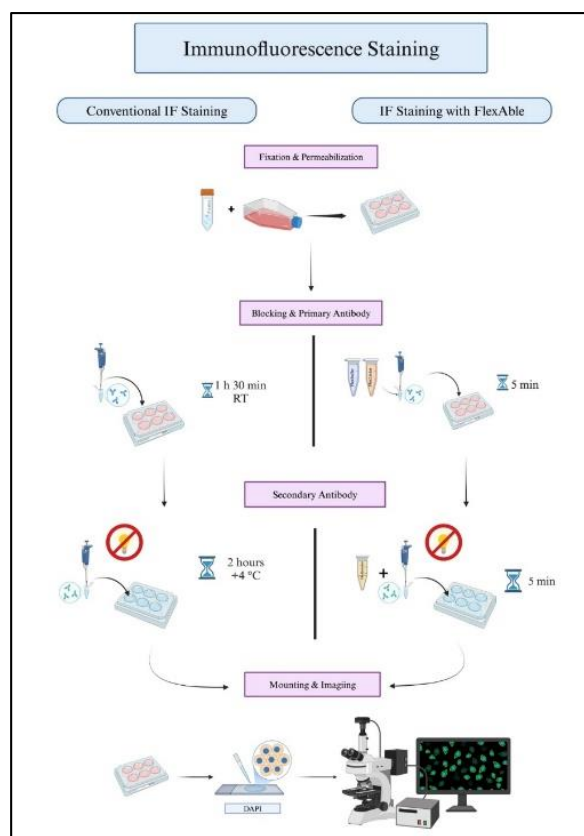
Figure-1. IF staining protocols. RT: Room temperature. Created in https://BioRender.com

Recent advancements in the field have introduced kit-based solutions that aim to simplify and enhance the efficiency of antibody labeling.

Examples include the FlexAble Antibody Labeling Kits (Proteintech, NJ, USA) (4), Lightning-Link® (Abcam, CA, USA) (5-7), and EasyLink Labeling Kits (Abcam, CA, USA) (8-11). These kits streamline the labeling process, reducing hands-on time and the complexities associated with traditional conjugation methods.

The accuracy and reliability of immunofluorescence heavily depend on the quality of antibody labeling. Efficient and precise labeling is critical for achieving high specificity and sensitivity, which are essential for accurate molecular interaction studies and protein localization analyses. Conversely, inefficient labeling can lead to weak fluorescence signals and significant data misinterpretation, ultimately hindering scientific progress. As biological research questions grow increasingly complex, there is an urgent demand for reliable, straightforward labeling techniques that align with the needs of fast-paced research environments. Kit-based solutions, such as FlexAble kits, represent a promising innovation in this area, offering potential benefits like reduced variability and increased consistency. Their impact on accelerating biomedical research and improving data reliability makes their evaluation essential.

This study aims to compare the performance, convenience, and cost-effectiveness of Antibody Labeling Kits and traditional labeling methods. Key objectives include evaluating labeling efficiency, fluorescence intensity, and antibody specificity using quantitative microscopy. Practical usability, time efficiency, and cost-effectiveness are also assessed to determine the optimal approach for immunofluorescence applications. The findings aim to guide researchers in selecting the most reliable and efficient tools for their studies.

MATERIALS AND METHODS
Subjects/Samples:

In the study, a type of human prostate cancer cell line DU145 (purchased from the American Type Cell Culture Collection-ATCC, HTB-81 ™, Manassas, VA) was used. Two different staining were performed on the cells for IF staining. First, the FlexAble CoraLite® Plus 647 Antibody

Labeling Kit for Rabbit IgG was used. For this, the FlexLinker, FlexBuffer and FlexQuencher buffers in the kit were used and the study was carried out using caspase-3 (Santa Cruz Biotechnology, INC.caspase-3 (31A1067): sc-56053) as the primary antibody and Alexa Fluor® 488 AffiniPure™ Rabbit Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc. 315-545-003) as the secondary antibody (Table-1).

Labeling Techniques:

While standard procedures were applied for conventional IF staining application, the staining protocol published by Proteintech was followed in our IF study with FlexAble Kit (4).

IF staining protocol performed with FlexAble Kit (4):

  I. DU145 cell line (were purchased from the American Type Cell Culture Collection-ATCC, HTB-81 ™, Manassas, VA) cells were fixed with 4% paraformaldehyde (PFA) for 30 minutes and then washed with phosphate buffered saline (PBS) (BioShop Canada Inc. PBS 404).

  II. For the permeabilization step, cells were kept in Triton X-100 (X100, Sigma-Aldrich) for 10 minutes and then washed again with PBS.

  III. After these procedures, cells were treated with bovine serum albumin (BSA) (BSA-15, Capricorn) for 1 hour for blocking.

  IV. Then, to equalize the total volume to 8 µL for staining with the components in the kit; 1 µL of FlexLinker was added to 0.5 µL of caspase-3 (Santa Cruz Biotechnology, caspase-3 (31A1067): sc-56053) used as primary antibody, 3.8 µL of FlexBuffer was added and incubated in the dark for 5 minutes.

  V. Then, the secondary antibody Alexa Fluor® 488 AffiniPure™ Rabbit Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc. 315-545-003) and 2 µL of

FlexQuencher were added and kept in the dark for 5 minutes.

  VI. Cover slips with cells for mounting were placed on the slides and the slides were mounted using 4′,6-diamidino-2-phenylindole (DAPI) (UltraCruz® Aqueous Mounting Medium with DAPI: sc-24941).

  VII. The slides were visualized under a fluorescence microscope for detection of findings (Figure-2).

The total processing time was approximately 1 hour (Table-2).
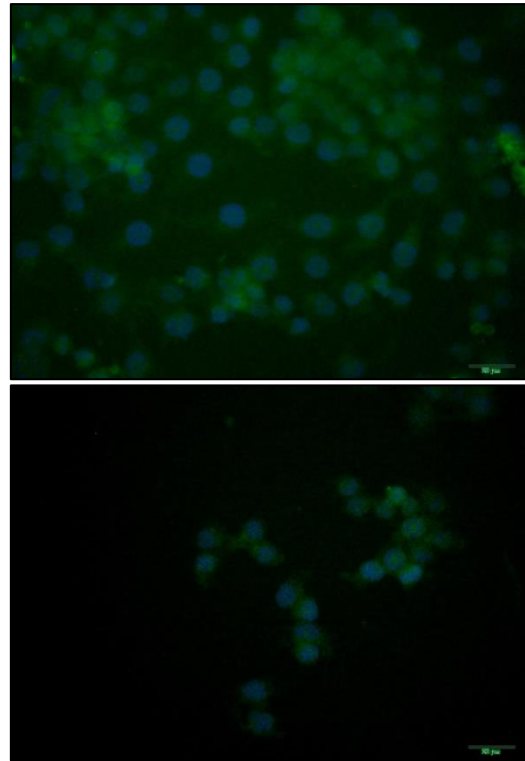
Figure-2. IF staining with FlexAble Labeling Kit on DU 145 cell line.

Table-1. Material list.

MATERIAL LIST	PRODUCT BRAND	CATALOG NO
Du 145 Cell Line	American Type Cell Culture Collection-ATCC	HTB-81
FlexAble Labeling Kit	Proteintech	KFA003
Caspase-3	Santa Cruz Biotechnology	sc-56053
Alexa Fluor® 488 AffiniPure™ Rabbit Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Laboratories, Inc.	315-545-003
Pbs	BioShop Canada Inc.	PBS404
Triton X-100	Sigma-Aldrich	X100
BSA	Capricorn	BSA-15
DAPI	UltraCruz®	sc-24941

Then, the same cell line standard IF protocol was used for comparison. Conventional IF staining protocol applied in this study:

  I. DU145 cell line cells in 6 well-plates were fixed with 4% PFA for 30 minutes and then washed with PBS (BioShop Canada Inc. PBS404).

 II. cells were treated with permeabilization buffer Triton X-100 (X100, Sigma-Aldrich) for approximately 15 minutes and then washed again with PBS.

III. Then, cells were treated with BSA (BSA-15, Capricorn), which was used as a suitable solution for blocking. Then, cells were washed again with PBS and prepared for primary antibody application.

 IV. Caspase-3 (Santa Cruz Biotechnology, caspase-3 (31A1067): sc-56053) was used as the primary antibody. After the primary antibody was applied to the cells overnight at fridge, each coverslip was washed 3 times with PBS for 3 minutes and prepared for secondary antibody application.

  V. The secondary antibody used in the cells was Alexa Fluor® 488 AffiniPure™ Rabbit Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc. 315-545-003). For effective work of secondary antibodies, antibody application was performed in a dark work area for 1 hour or 1 hour and 30 minutes, and then the cells were washed 3 times with PBS.

 VI. For the mounting stage, cells in 6 well-plates were placed on slides and mounted with DAPI (UltraCruz® Aqueous Mounting Medium containing DAPI: sc-24941).

VII. The slides were examined under a fluorescence microscope to visualize the findings (Figure-3).

The total procedure time was approximately 1 day (Table-2).

Following the completion of both IF studies, all slides were subjected to examination under a fluorescence microscope (Olympus/BX51) and subsequent analysis of the images using dedicated software (Olympus CellSens Software and Fiji by ImageJ).

Analytical Methods:

The performance of antibody labeling methods was evaluated using a comprehensive analytical approach. Labeling efficiency, fluorescence intensity, antibody specificity, and practical usability were systematically assessed to provide a robust comparison between the FlexAble Kit and conventional immunofluorescence staining methods.

Labeling efficiency was quantified using fluorescence microscopy (Olympus/BX51) with the CellSense software. Fluorescence intensity values were extracted from images and processed using ImageJ. Regions of interest (ROIs) were selected to calculate the mean fluorescence intensity (MFI), and each experiment was conducted in triplicate to ensure the reproducibility of results.

Antibody specificity was determined by analyzing the colocalization of labeled antibodies with known cellular markers. Pearson's correlation coefficient and Manders overlap coefficient were calculated using the Coloc2 plugin in ImageJ, providing quantitative metrics for the spatial overlap between fluorescence signals and target antigens (Figure-4).
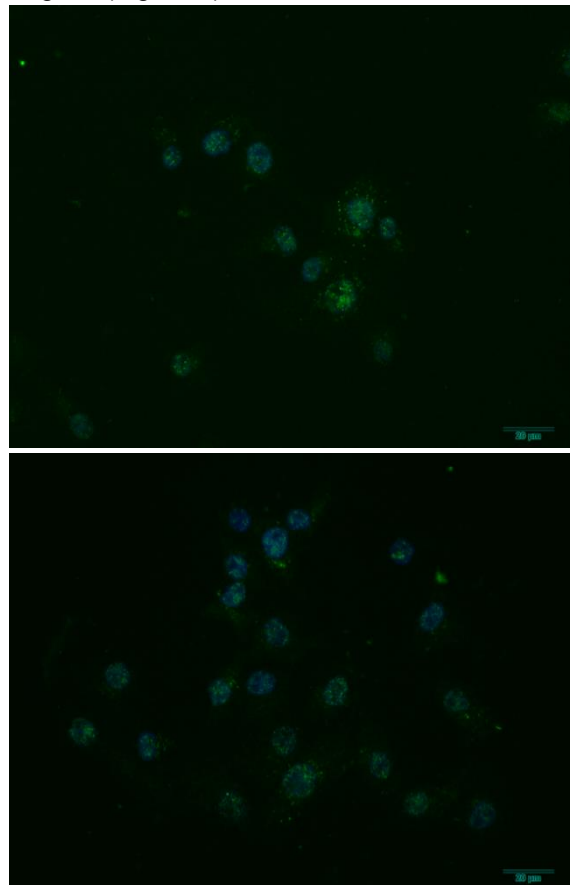
Figure-3. Conventional IF. DU 145 cell line.
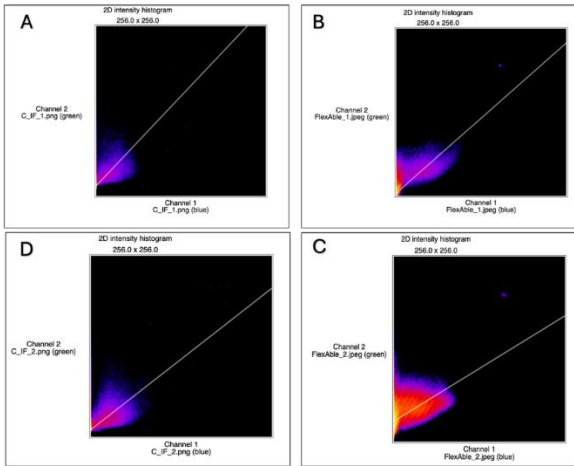
Figure-4. Comparison of histogram plots of IF staining and Coloc2 analyses. In the conventional method, the high Manders tM1 values indicate that Channel 2 shows a strong colocalization with Channel 1, but Channel 1 makes a more limited contribution to Channel 2 due to the low tM2 values. In contrast, FlexAble selectively reduced colocalization, allowing more specific analyses. FlexAble IF 2 showed a significant increase in the tM2 value, indicating that mutual colocalization improved. When the Pearson correlation values were examined, it was observed that FlexAble kept the colocalization lower in the suprathreshold regions while maintaining the correlation in the subthreshold regions. These findings indicate that FlexAble methods can be used as a more sensitive tool in cases where selective reduction or increase of colocalization is required. Channel 1(Ch1): Green, Channel 2 (Ch2): Blue. Manders' tM1 (Above auto threshold of Ch2), Manders' tM2 (Above auto threshold of Ch1).

These analyses ensured the accuracy and precision of antibody labeling. Time and cost efficiency metrics were also examined. The total time required for staining and the amount of reagents used were compared between the two methods. Procedural steps, such as blocking and secondary antibody applications, were simplified in the FlexAble Kit protocol, significantly reducing overall workflow complexity. Comparative results are summarized in Table-2.

Quantitative data were analyzed using statistical software SPSS (IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp.IBM)). The Shapiro-Wilk test was employed to verify the normality of data distribution. Comparisons of labeling efficiency, fluorescence intensity, and signal stability between methods were performed using the Student's t-test or ANOVA as appropriate, with a significance level set at $p < 0.05$. All experiments were carried out in triplicate to ensure reproducibility of the results. Data are presented as mean ± standard deviation and graphical representations were created using GraphPad Prism 5.0 to visually depict the findings.

Comparative visualizations of efficiency, stability, and workflow simplification were created to illustrate the advantages of the FlexAble Kit (Table-2) (Figure-1). These visualizations highlight its potential to streamline research workflows and enhance experimental outcomes.
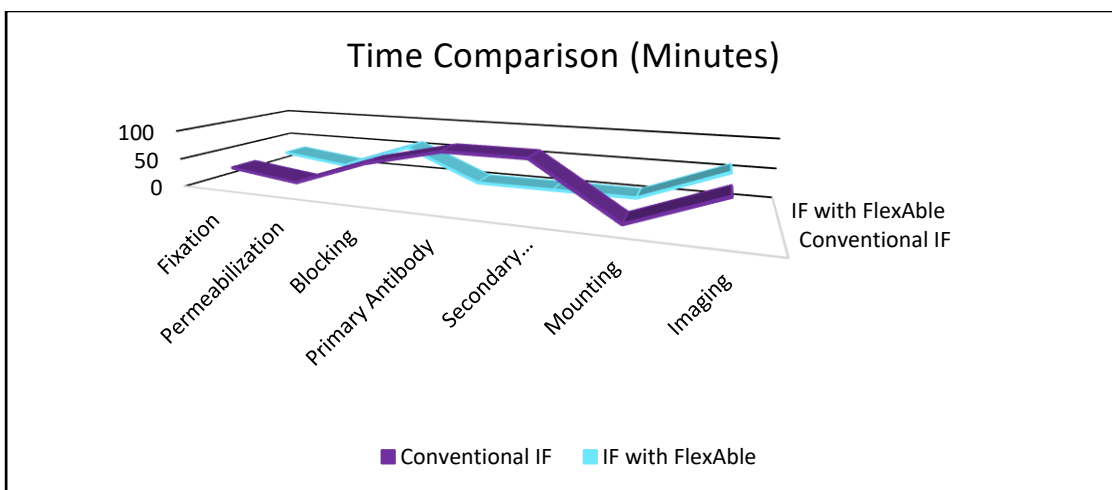
Figure-5. Comparison of the steps of two IF staining studies over time.

Table-2. Comparative Methodological Analysis.

Steps	Conventional IF	IF with FlexAble Kit
Fixation	Cells in six-well plates were fixed with 4% PFA, then rinsed with PBS.	
Permeabilization	Triton X-100 was treated for 15 min. Cells were then rinsed again with PBS.	
Blocking	BSA was incubated for one hour for blocking, then cells were rinsed again with PBS.	
Primer Antibody	Primary antibody was applied to the cells for 1 hour and 30 minutes at room temperature. Then the cells were washed with PBS.	For primary antibody application to the cells for 5 minutes at room temperature, two components from the kit were applied together with the antibody. Then the cells were washed with PBS.
Seconder Antibody	Secondary antibody was applied to the cells for 2 hours at +4ºC and in a dark environment.	A solution from the kit was applied to the cells in a blended form with the secondary antibody for 5 minutes at room temperature in a dark environment.
Mounting	The slides were mounted with mounting solution.	
Imagining	Covered slides were examined using a fluorescence microscope in a dark room.	

Table-3. Comparison of materials.

Steps	List Of Materials	Conventional IF	IF with FlexAble
Fixation	6 Well Plate	+	+
	Eppendorf Tubes	+	+
	Micropipette	+	+
	DU145 Cell Line	+	+
	%4 PFA	+	+
	PBS	+	+
Permeabilization	Triton-X 100	+	+
	PBS	+	+
Blocking	%10 BSA	+	+
	PBS	+	+
Primary Antibody	Caspase-3	+	+
	FlexBuffer	-	+
	FlexLinker	-	+
	PBS	+	+
Secondary Antibody	Alexa Fluor® 488 AffiniPure™ Rabbit Anti-Mouse IgG (H+L)	+	+
	FlexQuencher	-	+
	PBS	+	+
Mounting	DAPI	+	+

RESULTS

The FlexAble Kit used in this study offers a new application compared to IF staining in terms of reducing time, labor and materials compared to the standard IF staining protocol. We compared the data and images obtained from the staining we made using standard IF staining and FlexAble Kit from different perspectives and presented new approaches to the literature.

Efficiency and Yield: Compared to conventional IF staining, it is noteworthy that the ability to perform staining using the 3 components in the FlexAble kit reduces the number of materials and consumables required compared to the standard protocol. This leads to significant cost savings in laboratory management and studies. Secondly, there are also significant differences in antibody binding and antibody binding times between the two staining methods; for example, according to the standard IF staining method, primary and secondary antibody binding is required, and the entire protocol requires approximately 1 day of the scientist responsible for the study (depending on whether the cells are pre-fixed or not, and if the fixation stage is also performed on the same day, the time required for this process will increase even more). In the staining study performed with the FlexAble kit, the process can be completed in approximately 30 minutes to 1 hour with a single kit without spending too much time on primary and secondary antibodies and results suitable for imaging can be obtained (Figure-5).

Functionality Tests: Shows the results obtained by using labeled antibodies in certain analyses, highlighting the differences in performance. As we frequently emphasize in our study, immunofluorescence staining is a laborious application that requires a lot of time and materials and constant monitoring. The FlexAble Kit reduces the use of consumables and special materials such as primary and secondary antibodies for staining, which will be spent on an IF stain, and offers a different perspective and opportunity for scientists and researchers to conduct research effectively in a short time and cost friendly.

User Experience: In terms of usage, there is an advantage between the two protocols not only in terms of the shortening of the time between antibody use and the use of less material for the cells or tissues to be stained, but also in terms of the reduction of process steps in general between the two applications. While the entire process for a scientist performing IF staining in the standard protocol takes about one or one and a half days depending on whether the cells or tissues to be used are fixed or not, staining with the FlexAble Antibody Labeling kit takes between 30 minutes and 1 hour, and effective and high-quality images and data can be obtained in a short time without wasting time and material with applications such as blocking and secondary antibody binding. In addition, when comparing antibody prices between different brands, the fact that 10 labeling can be performed with one kit for the price of only one antibody is another major advantage for researchers (Table-3).

DISCUSSION

The results of our study indicate that the FlexAble Antibody Labeling Kits not only provides an advantage for researchers to be able to use it in multiplex multiplications which in current literature studies, applications performed with the FlexAble Kit are also included in a new way (12-17) and also significantly enhance labeling efficiency and reduce preparation time without compromising the fluorescence intensity and specificity of antibody binding, compared to the conventional direct labeling method with FITC. These findings highlight the challenges of non-specific binding and background fluorescence in traditional methods (18). FlexAble Kits address these issues by providing a streamlined workflow that minimizes such artifacts. Also reported similar improvements in reproducibility and ease of use with kit-based labeling approaches, further reinforcing our observations (19).

The streamlined workflow of FlexAble Kits is one of their most notable advantages. The one-step labeling process eliminates the need for extensive optimization typically required in traditional methods. Eng et al. emphasized the importance of such workflow improvements in enhancing experimental reproducibility (20). Similarly, it is noted that simplifying antibody preparation can significantly reduce the time in complex immunofluorescence experiments (21). FlexAble Kits integrate these improvements by including a purification step that ensures only effectively labeled antibodies are used, maintaining high specificity and reducing background fluorescence.

Another significant advantage observed in our study is the superior stability of fluorescence

signals obtained with FlexAble Kits compared to FITC-labeled antibodies. While FITC signals often degrade over time (22-23). FlexAble Kits maintained stable fluorescence signals over extended periods. This stability is particularly valuable for long-term imaging studies or multi-day experimental workflows. Moreover, Rivest et al. demonstrated that consistent signal stability is critical for high-throughput experiments (24), also a feature of the kit.

Despite these advantages, some limitations were identified. The higher cost per reaction compared to traditional methods presents a challenge, particularly for laboratories with budget constraints. Additionally, the range of fluorescent dyes offered by FlexAble Kits is less extensive than custom labeling methods. This limitation could impact their use in multi-color fluorescence applications requiring specific wavelengths. Expanding the range of dyes, including those compatible with advanced imaging techniques such as super-resolution microscopy, could address this gap. Studies emphasized the need for versatile dye options to meet diverse research requirements (25-27).

Future iterations of FlexAble Kits could focus on optimizing reaction times for even faster workflows and expanding dye compatibility. Broader evaluations across various antibodies, cell types, and experimental conditions would provide deeper insights into their versatility and performance. Dean and Palmer (2014) suggested that innovative labeling technologies could enhance the accuracy of protein localization and molecular interactions (28). Comparative studies have shown that the integration of novel labeling tools can substantially improve data reliability and reproducibility (29 - 30).

CONCLUSION

In conclusion, the FlexAble Antibody Labeling Kits represent a significant advancement in antibody labeling, offering a reliable and efficient alternative to traditional methods. Their ability to streamline workflows, maintain reproducibility, and ensure high-quality results positions them as a valuable tool in modern immunofluorescence research. Future research should validate these findings in broader contexts, including multi-labeling and clinical diagnostic applications, where precision and efficiency are critical. Expanding dye options and reducing costs could further enhance their accessibility and utility across diverse research settings. Furthermore, studies integrating FlexAble Kits into multiplex fluorescence and advanced imaging platforms would provide additional insights into their potential for transforming immunofluorescence methodologies.

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Does the configuration of the K-Wires in the coronal plane affect the time to union in supracondylar humerus fractures?

Suprakondiler humerus kırıklarında K-Tellerinin koronal düzlemdeki konfigürasyonu kaynama süresini etkiler mi?

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ABSTRACT

Aim: Supracondylar humerus fractures (SHF) are among the most common bone fractures in the pediatric population. However, there is no consensus in the literature regarding the configuration of the K wires used in this method.

Materials and Methods: Patients who underwent closed reduction using two lateral and one medial percutaneous pins for SHF were included in the study. Measurements were made on the antero-posterior elbow radiographs of the patients taken on post-operative day 0. These measurements involved the angles of each K-wire with one another, the angles of the K-wires with the fracture line, and the angles between the K-wires and the humeral shaft. Postoperative complications, splint removal and pin removal times of all patients were recorded.

Results: A total of 167 patients were included in the study. Uneventful fracture healing was achieved in all remaining patients. No significant relationship or correlation was found between the above-mentioned angular parameters and fracture union time. However, the angle between the pins placed laterally, the angle between the medial pin, and lateral pin 2, and the angle between lateral pin 2 and the humeral shaft were found to be larger in patients with complications than in patients without complications (p=0.0001, p=0.017, p=0.0001).

Conclusion: The quality of fracture reduction is the basis for postoperative functional recovery. The results of this study that the main parameter affecting union in SHF is not the configuration of the pins in the coronal plane, but the anatomical fracture reduction and stable fixation obtained.

Keywords: Supracondylar humeral fracture, children, K-wire, coronal plane.

ÖZ

Amaç: Suprakondiler humerus kırıkları (SHK), pediatrik popülasyonda en sık görülen kemik kırıkları arasındadır. Ancak bu yöntemde kullanılan K tellerinin konfigürasyonu konusunda literatürde fikir birliği yoktur.

Gereç ve Yöntem: SHK tanısıyla kapalı redüksiyon ile iki lateral ve bir medial perkütan pin fiksasyonu uygulanan hastalar çalışmaya dahil edildi. Hastaların ameliyat sonrası 0. günde çekilen ön-arka dirsek grafilerinde ölçümler yapıldı. Her bir K telinin birbiriyle olan açıları, K tellerinin kırık hattı ile olan açıları ve K tellerinin humerus şaftı ile olan açıları ayrı ayrı ölçüldü. Tüm hastaların ameliyat sonrası komplikasyonları, splint çıkarma ve pin çıkarma süreleri kaydedildi.

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Bulgular: *Çalışmaya toplam 167 hasta dahil edildi. Tüm hastalarda sorunsuz kırık iyileşmesi görüldü. Yukarıda belirtilen açısal parametreler ile kırık kaynama süresi arasında anlamlı bir ilişki veya korelasyon bulunamadı. Ancak laterale yerleştirilen pinler arasındaki açı, medial pin ile lateral pin 2 arasındaki açı ve lateral pin 2 ile humerus şaftı arasındaki açı komplikasyonu olan hastalarda komplikasyonu olmayan hastalara göre daha büyük bulundu (p= 0,0001, p=0,017, p=0,0001).*

Sonuç: *Kırık redüksiyonunun kalitesi postoperatif fonksiyonel iyileşmenin temelini oluşturur. Bu çalışmanın sonuçları, SHK'da kaynamayı etkileyen ana parametrenin pinlerin koronal düzlemdeki konfigürasyonu değil, anatomik kırık redüksiyonu ve başarılı fiksasyon olduğunu göstermektedir.*

Anahtar Sözcükler: *Suprakondiler humerus kırığı, çocuklar, K-teli, koronal plan.*

INTRODUCTION

Supracondylar Humerus Fractures (SHF) account for approximately 15% of all pediatric fractures (1). SHF are the most common elbow fractures in children and accounts for approximately 70% of elbow injuries (2). SHF occur most frequently in children between the ages of 5 and 10 [2], with the non-dominant side of male children seeing the most reported cases (3).

This fracture pattern is usually caused by hyperextension of the elbow after a fall on one's open hand; and in more than 95% of fractures the distal fragment is displaced posteriorly (4). The method used for treatment of these fractures depends on the displacement of the fractured fragments. However, there is no consensus in the literature on treatment for this fracture (5,6).

SHF are classified according to the Gartland criteria (7). Closed reduction with percutaneous pinning is the standard treatment modality for Gartland Type 2 and 3 fractures (6). The two most common percutaneous pinning methods used in the treatment of SHF are medial-lateral crossed entry pinning and lateral only entry pinning (6). There is no clear consensus in the literature regarding the comparative strengths of these methods (8). Numerous biomechanical studies have evaluated the relationship between pin configuration and stability (9).

However, few studies have been put forth on the relationship between the angular configuration of K-wires between themselves, their angular configuration with respect to the fracture line and humeral shaft, and the fracture healing time and complication rates.

The aim of this study was to evaluate the relationship between the configuration of K-wires in the coronal plane and time to union and complications in supracondylar humerus fractures treated with closed reduction using one medial and two lateral percutaneous pins.

MATERIALS and METHODS

The study was designed as a single-centered retrospective study. The study commenced after ethics committee approval was obtained. Pediatric patients operated with closed reduction percutaneous pinning method for supracondylar humerus fractures between May 2016 and June 2022 were included in the study. Inclusion criteria mandated that the patients be between 3 and 10 years of age with Gartland Type 2 and Type 3 supracondylar humerus fractures. Patients with post-fracture neurovascular deficit, congenital neurovascular problems in the injured extremity, open fractures, trans-epiphyseal injuries, as well as those who were operated on with open reduction percutaneous pinning were excluded from the study.

All pediatric patients were operated under general anesthesia without a tourniquet. All patients received surgical prophylaxis using cephalosporin during induction of anesthesia. Traction was applied with elbow flexion of approximately 20°; varus and valgus alignment disorders were corrected with forearm movements. Medial and lateral translations were corrected with thumb maneuvers. After these maneuvers, the elbow was slowly flexed and force was applied anteriorly to the olecranon using a thumb. The limb was flexed in pronation or supination, depending on the condition of the distal fracture fragment. Following reductive maneuvers, the reduction in the anteroposterior and lateral planes was evaluated with the help of C-arm X-Ray. After the reduction was approved, one 1.6 mm thick K-wire was placed in the lateral condyle and its position was examined using a C-arm X-Ray. The K-wire was moved forward until it crossed the fracture line and reached the opposite cortex. Following the placement of the K wire, one 1.6 mm thick K-wire was placed on each of the medial and lateral planes using similar instructions. After placement of all wires, fracture stability was examined both in varus/valgus stress and in full flexion/extension. Following these controls, imaging was performed again using a C-arm X-Ray. The wires were left protruding from the skin so that they could be easily removed under outpatient clinic conditions.

The procedure was terminated after applying dressing and a long arm splint.

Patients' extremities were immobilized through the application of a long arm splint for 3 weeks postoperatively. After the third week, patients were asked to exercise using full range of motion of their elbows. Patients were called for follow-up at 10-day intervals. After union was validated through these follow-ups, the K-wires were removed in an outpatient clinic setting. Final follow-up of patients was conducted at the third postoperative month.

Demographic data, operation dates and follow-up dates of all patients were recorded. The time of removal of the K-wires, complications during hospitalization and follow-ups were also recorded. Measurements were made on the radiographs taken on post-operative day 1. The angle of each K-wire among themselves in the coronal plane, the angle of each K-wire with the fracture line and the angle of each K-wire with the humeral shaft were measured.

Of the 2 different K-wires placed laterally, the one with a more proximal entry point into the bone was considered as pin number 1, and the K-wire with a more distal entry point into the bone was considered as pin number 2. The medial K-wire was considered as pin number 3. All measurements were performed by two different orthopedic surgeons who were not part of the surgical team and were not authorized to access the demographic data of the patients (Figure-1). The mean of two different measurements was used for statistical analysis.

Pre-operative, post-operative, and follow-up radiologic images of one of the patients are presented in Figure-2.
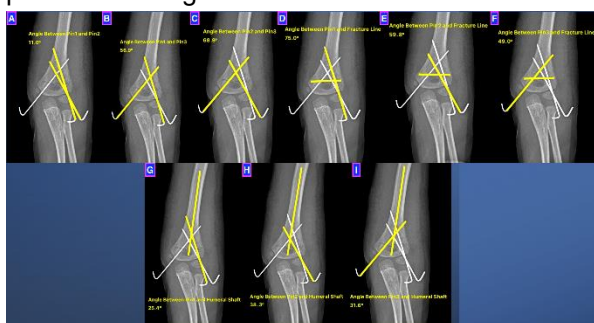
Figure-1. Angle between Pin 1 and Pin 2(A), Angle between Pin 1 and Pin 3(B), Angle between Pin 2 and Pin 3(C), Angle between Pin 1 and fracture line(D), Angle between Pin 2 and fracture line(E), Angle between Pin 3 and fracture line(F), Angle between Pin 1 and humeral shaft(G), Angle between Pin 2 and humeral shaft(H), Angle between Pin 3 and humeral shaft(I).

Figure-2. Preoperative, postoperative, and follow-up radiologic images of one of the patients.

Statistical analysis

SPSS 25. 0 package program was used for statistical analysis of the data. Categorical data was presented as numbers and percentages, and continuous data as means and standard deviation (medians and minimums-maximums where necessary). Comparisons of continuous data between groups were made according to the distribution of data. Student's T test was used for variables that met the assumption of parametric distribution, and Mann Whitney U test was used for variables that did not meet this assumption.

The correlation between variables was determined by Pearson's Correlation Coefficient. $r \geq 0.91$ indicates very high correlation between variables; $0.90 \leq r \geq 0.71$ indicates high correlation between variables; $0.70 \leq r \geq 0.51$ indicates moderate correlation between variables; $0.50 \leq r \geq 0.31$ indicates low correlation between variables; $r \leq 0.3$ indicates no correlation between variables. A statistical significance of $p < 0.05$ was considered as significant for all analyses.

RESULTS

A total of 167 patients who met the inclusion criteria were included in the study. The left upper extremities of 109 (61.9%) and the right upper extremities of 67 (38.1%) patients were operated on. The mean age of the patients was 5.5 ± 1.9 years. Complications were encountered in 14 (8%) of 167 patients. The distribution of these complications is summarized in Figure-3.
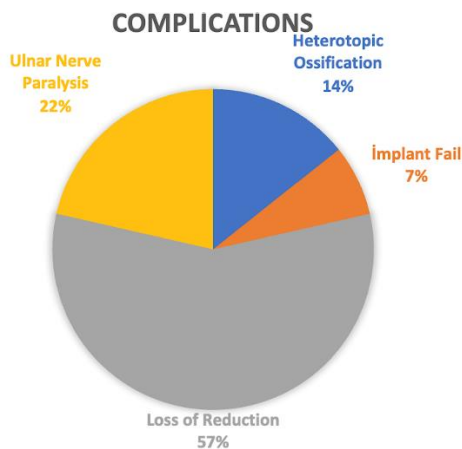
Figure-3. The distribution of the complications.

Patients with complications were analyzed separately and it was examined whether there was a relationship or correlation between complications and angular parameters of these patients. Accordingly, the angle between the lateral pins, the angle between the medial pin and the lateral pin 2, and the angle between lateral pin 2 and the humeral shaft were found to be larger in patients with complications than in patients without complications. All data related to these comparisons is provided in Table-1.

The correlation and relationship between the angular parameters and pin removal time, which was considered as the time of union, are shown in Table-2.

Table-1. Data showing the relationship between angular parameters in patients with and without complications.

Angle	No Complication Group	Complication Group	p
P1-P2	7,1±5,5	14,7±10,4	**0,0001***
P1-P3	69,6±11,1	72,1±12,9	0,434
P2-P3	73,3±11,6	81,1±12,9	**0,017***
P1-FL	61,9±14,9	61,6±17,2	0,934
P2-FL	57,7±14,6	50,1±16,5	0,065
P3-FL	46,7±14,2	43,7±17,9	0,466
P1-HS	34,4±9,9	32,8±11,6	0,572
P2-HS	38,0±9,3	46,6±9,4	**0,001***
P3-HS	35,7±8,9	39,2±11,7	0,170

Pin 1 (P1): From the lateral pins, the entry point to the bone is more proximal, Pin 2 (P2): From the lateral pins, the entry point to the bone is more distal, Pin 3 (P3): Medial pin, FL: Fracture Line, HS: Humeral Shaft, *: Statistically significant

Table-2. Data showing the relationship between boiling time and angular parameters.

Angle		P1-P2	P1-P3	P2-P3	P1-FL	P2-FL	P3-FL	P1-HS	P2-HS	P3-HS
Pin Output Time. (week)	r	0,33	-0,10	-0,01	0,08	-0,02	-0,03	-0,18	0,03	-0,01
	p	0,0001	0,180	0,899	0,269	0,738	0,970	0,018	0,694	0,889

P1: Pin1, P2: Pin2, P3: Pin3, FL: Fracture Line, HS: Humeral Shaft,

DISCUSSION

Closed reduction with percutaneous pinning is considered the gold standard treatment method for SHF (10, 11). Closed reduction using two lateral and one medial percutaneous pins for treatment of these fractures provide a high chance of success with a low chance of complications. No clear correlation between the coronal plane pin configuration used in this technique and union time has been found.

Despite numerous prior biomechanical and clinical studies, there is no consensus on the ideal K-wire configuration in pediatric SHF (12). However, the generally accepted opinion is that better stability can be achieved by using two lateral pins and one medial pin crossing the fracture line (13). All patients in our study underwent operations using two lateral and one medial percutaneous pins. In their study, Durusoy et al. examined the effect of

the angle between the fracture line and K-wires on stability in SHF with a computer-aided 3D model in order to determine the optimal K-wire configuration (14). In their study, nine different angle combinations using 30-, 45- and 60-degree angles between K-wires and fracture lines were used. According to the results of their study, as the angle between the medial pin and the fracture line increases, the load on the lateral pin and fracture displacement decreases, and as the angle between the lateral pins and the fracture line increases, the stress on the medial pin and fracture displacement increases (14). In our study, it was concluded that the angle between the lateral and medial pin and the fracture line was not associated with either fracture union time or complication rates. This result indicates that the angular configuration between the fracture line and the pins does not have a direct effect on time of union despite resulting biomechanical differences.

Lee et al. published a series of 61 patients with Gartland Type 2 and Type 3 fractures treated with only lateral pinning, some of the patients receiving diverging lateral pins and in parallel lateral pins (15). The results showed that success was obtained with K-wires in both configurations (15). In another series of 30 patients, patients were treated with either diverging or parallel lateral pins and the groups were compared in terms of time of union and clinical outcomes (16). Comparable to the results from the study of Lee et al., Gopinathan et al. concluded that there was no significant relationship or correlation between lateral pin configuration and clinical outcomes and time of union (16). Hannonen et al. also examined the pin configurations of patients who underwent divergent percutaneous pinning and concluded that the angular proximity of the lateral pins may be associated with fracture displacement. They also stated that the minimum satisfactory angle between the divergent lateral pins is 25° (17). The mean angle between the lateral pins of the patients in our study was 7.7 ± 6.3, and despite this, fracture union rates were quite satisfactory. On the other hand, there are biomechanical studies showing that divergent pin configuration may provide better stability (18). In the present study, no relationship or correlation was found between the coronal angular configurations of the two lateral pins and the medial pin and time of union. Similar to the present study, Skags et al. found that pin configuration did not affect the

permanence of reduction in Gartland Type 2 and Type 3 fractures (19).

The incidence of ulnar nerve injury due to medial pinning ranges from 0% to 9.4% (20, 21). In our study, this rate was 1.76%. Loss of reduction rate is 4% for patients treated with only lateral pins, and 2% for patients treated with both medial and lateral pins (22, 23). In our study, the loss of reduction rate was 4.56%. However, no patient required revision surgery. Some pin configuration parameters were found to significantly affect complication rates. Since there were very few patients in the complication subgroups, no solid inferences or comments on this issue could be made. Studies with a greater number of patients are needed in order to produce more conclusive data.

In this study, the relationship between coronal angular configuration of the two lateral and one medial percutaneous pins used in closed reduction technique and time of union and complications were examined. However, Bitzer et al. showed in their biomechanical analysis study that the configuration of the pins in the sagittal plane is as important as their configuration in the coronal plane in treating SHF (24). Therefore, further studies evaluating pin configuration in 3D are needed.

This study had some limitations. The retrospective design of the study was one of these limitations. The small sample size and the small number of patients in complication subgroups also limited the generalizability and interpretability of the data. Another limitation was that the measurements were taken manually, rather than by a computer.

The quality of fracture reduction is the basis of postoperative functional recovery (25). In their study on SHF patients with loss of reduction, Sankar et al. clearly concluded that intraoperative reduction is the most important factor for fracture stability regardless of the pin fixation technique (26).

CONCLUSION

The results of the present study support that the main parameter affecting union in SHF is not the configuration of the pins in the coronal plane, but the fracture reduction and stability obtained, a conclusion which is supported by data found in the literature.

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

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Levels of anxiety, depression, and quality of life in patients with fibromyalgia during the COVID-19 pandemic: A cross-sectional study

COVID-19 pandemisi sürecinde fibromiyalji sendromlu hastalarda kaygı düzeyi, depresyon ve yaşam kalitesinin değerlendirilmesi, kesitsel çalışma

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ABSTRACT

Aim: Covid-19 pandemic has been reported to cause increased levels of depression, anxiety and posttraumatic stress disorders in the general population. Fibromyalgia syndrome (FMS) patients may be more inclined to develop these problems because of reduced resilience and coping mechanisms. For this reason, we have aimed to assess FMS patients' levels of resilience, Obsession with covid-19, anxiety and quality of life and compare these values with controls living in the same household.

Materials and Methods: Patients diagnosed as having FMS according to the ACR 2016 classification criteria and a control subject living in the same household were included in the study. Subjects were asked to fill out an online questionnaire form containing pain level, Short form 36, covid-19 anxiety scale, Obsession with covid scale and resilience scale for adults. Results were compared between the FMS patients and controls.

Results: 39 FMS patients and 39 controls were included in the study. Demographic characteristics were similar between the groups (p>0.05). Subjects with FMS had lower levels of quality of life in addition to worse anxiety and obsession with Covid-19 scores (p<0.05). Surprisingly, most subsections of the resilience scale for adults were not significantly different between the groups (p>0.05), except for structured style subsection (p<0.05).

Conclusion: Patients with FMS are affected more negatively psychologically than controls during the Covid-19 pandemic. Regular follow up visits, whether face to face or through telemedicine should include assessment for pandemic related anxiety and obsession may help control pain and other FMS symptoms.

Keywords: Anxiety, covid-19, fibromyalgia, pain.

ÖZ

Amaç: *Kovid-19 pandemisi sürecinde hem hastalık korkusu hem de sosyal izolasyon nedeni ile kronik ağrısı olan bireylerde sosyal izolasyonun ağrı düzeyleri üzerine de olumsuz etkileri olduğu gösterilmiştir (Hruschak 2021). Bir kronik yaygın ağrı sendromu olan Fibromiyalji hastalarında da sosyal izolasyon nedeni ile anksiyete ve depresyon üzerine olumsuz etkiler olduğu bildirilmiştir (Aloush 2021). Bu bulgulardan yola çıkarak, bu çalışmada, fibromiyalji hastalarının pandemi sürecindeki anksiyete ve depresyon düzeylerinin değerlendirilmesi ve kontroller ile karşılaştırılması amaçlanmıştır.*

Gereç ve yöntem: *Çalışmaya her iki grupta 35 gönüllü olacak şekilde toplam 70 hasta dahil edilmesi planlanmıştır. Fibromiyalji grubuna 2015 Amerikan Romatoloji Birliği kriterlerine göre fibromiyalji sendromu kriterlerini karşılayan ve polikliniğimizden takipli olan hastalar dahil edilmiş, kontrol grubuna ise fibromiyalji hastaları ile aynı evde yaşayan birinci derece yakınları dahil edilmiştir. Hastaların demografik özellikleri, Covid-19 geçirip geçirmedikleri, yakın çevrelerinden Covid-19 geçiren olup olmadığı, mevcut ağrı düzeyleri, yaygın ağrı ölçeği ve semptom şiddeti skalası değerleri kaydedilmiştir. Ayrıca yaşam kalitesinin değerlendirilmesi amacı ile katılımcılardan Kısa form 36 (SF36), pandemiden etkilenme düzeylerini değerlendirmek amacı ile de Koronavirüs endişe anketi (KEA), Koronavirüs takıntı anketi (KTA), dayanıklılık düzeylerinin değerlendirilmesi amacı ile Yetişkinler için psikolojik dayanıklılık ölçeğini doldurmaları istenmiştir.*

Demografik ve hastalık özelliklerinin değerlendirilmesinde tanımlayıcı istatistikler, kategorik verilerin dağılımının değerlendirilmesi amacı ile ki-kare testi, nümerik verilerin gruplar arası karşılaştırılması için bağımsız örneklem t-testi uygulanmıştır.

Bulgular: *39 fibromiyalji hastası ve 39 kontrol katılımcı çalışmaya alınmıştır. Hastaların %71,8'i kadın, kontrol grubunun %50'si kadındır ve gruplar arasında cinsiyet açısından anlamlı fark mevcuttur (p<0,05). Yaş, medeni durum, ek hastalık varlığı ve çalışma durumu açısından gruplar arasında anlamlı fark saptanmamıştır (p>0,05). SF36 ölçeğinin tüm alt gruplarında, fibromiyalji hastalarında kontrollere oranla anlamlı daha kötü skorlar saptanmıştır (p<0,05). Covid endişe, covid takıntı ve psikolojik dayanıklılık skorları da fibromiyalji tanılı hastalarda, kendileri ile aynı evde yaşayan kontrollere oranla anlamlı daha kötü saptanmıştır (p<0,05).*

Sonuç: *Fibromiyalji hastalarında sağlıklı bireylere oranla yaşam kalitesi ve anksiyete düzeylerinin daha kötü olduğu bildirilmiştir (Baxter, 2014). Çalışmamızın bulguları, fibromiyalji hastalarının Kovid-19 pandemisi sırasında anksiyete ve yaşam kalitesi düzeylerinin daha kötü seyrettiğini göstermektedir. Aynı zamanda fibromiyaljili bireylerde Covid-19'a dair hasta olma korku ve takıntısının da kontrollere oranla daha yüksek olması, fibromiyaljik bireylerde fonksiyonel kayıpları şiddetlendirebilir. Kovid-19 pandemisinin devam ettiği süreçte, fibromiyalji hastalarına bakım veren hekimlerin bu endişe ve takıntı düzeylerini de değerlendirmesi ve gerektiği durumlarda bu endişelere yönelik psikolojik destek sağlanması, hastaların hem anksiyete düzeylerinde azalmaya hem de primer ağrı kontrolüne yardımcı olabilir.*

Anahtar Sözcükler: *Endişe, covid-19, fibromiyalji, ağrı.*

INTRODUCTION

The last 4 years have been marked by the Coronavirus disease 2019 (Covid-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. At the time of writing of this article, more than half a billion people have been infected and worldwide death toll is more than 6 million people (1).

Covid-19 survivors have been reported to suffer from a plethora of psychological alterations such as posttraumatic stress disorder, depression, anxiety and insomnia (2). But regardless of having been infected, population wide reports have revealed that up to 53% of individuals were affected psychologically by the pandemic (3). Another study reported that general rate of negative emotions such as depression, anxiety, and anger increased, while positive emotions and life satisfaction decreased during the pandemic (4).

Fibromyalgia syndrome (FMS) is a chronic widespread pain condition characterized by fatigue, sleep disturbances as well as cognitive and somatic symptoms. Underlying mechanism of FMS is complicated and most often explained by central sensitization with symptoms showing a close correlation with stress, anxiety and physical stressors (5, 6).

There are a number of studies assessing the effect of Covid-19 pandemic on patients with FMS. Most of these studies focus on the effects of social isolation and lack of exercise on disease symptoms such as pain, fatigue, and insomnia while others report the effects of the pandemic on anxiety and depression in patients with FMS (7–10). Differences in level of resilience may explain why some individuals are more severely affected by stress and anxiety. In this study, we aimed to assess and compare levels of resilience, covid related anxiety, obsession with Covid-19 and quality of life in FMS patients with controls, who were living in the same household, in order to control the effects of environmental stressors.

MATERIALS and METHODS

Before the commencement of the study, ethics approval was sought and obtained from the local ethics committee of our university hospital (---- University Approval number: 21-1T/52). Study was carried out in accordance with the Helsinki Declaration. Patients with a diagnosis of FMS and one of their family members who lived in the same household were included in the study, constituting group 1 and group 2 respectively. The reason for selecting controls from the same household was to be able to better control the probable effects of the pandemic and isolation on subjects.

The number of study participants was calculated by a-priori power analysis. With an alpha level of 0.05 and effect size of 0.8 for anxiety and 0.95 power, the minimum required number of subjects in each group was calculated to be 35.

Inclusion criteria

-A diagnosis of FMS according to the American college of rheumatology (ACR) 2016 classification criteria
-Older than 18 years of age

Exclusion criteria

-Illiteracy
-A history of Covid-19 infection in the last month
-Change in medication to treat FMS in the last 3 months
- Living alone (lack of a control subject)
-Severe neurological or psychiatric disorder that might hinder subjects' ability to answer the study questions

Recruiting Process

Subjects were informed and asked to participate in the study during routine outpatient visits. Those patients who lived alone were excluded. Patients who agreed to take part in the study were referred to the investigators and were further informed about the specifics of the study. All subjects were asked to sign the informed consent form. After enrollment, patients were questioned regarding their symptoms, personal and family history. They underwent a complete musculoskeletal examination. ACR 2016 FMS classification criteria were assessed and recorded by the investigator. After the examination, patients were sent by phone a link to the online study questionnaire and were asked to answer the questions for themselves. They were also requested to ask a partner/family member who lived in the same household to answer the same questions.

Study parameters

ACR 2016 FMS Classification criteria: Symptom severity scale and widespread pain index were assessed by the investigator during history taking and physical examination and recorded in the study form (11). Subjects were also included in the study if they met the classification criteria.

Health Survey Questionnaire Short Form 36 (SF-36): Turkish version of this self-reported survey assessing different subcategories of quality of life was completed by all participants (12). Subcategories consist of physical functioning, physical role, emotional role, vitality, mental health, social function, bodily pain, and general health and score range between 0-100. SF-36 has previously been used in patients with FMS to assess quality of life and higher scores indicate better functioning (13).

Coronavirus anxiety scale (CAS): This measure was developed by Lee et al. (14) to screen for dysfunctional anxiety related to the Covid-19 pandemic. Each 5 items are scored from 0 to 4. Higher scores and an overall score higher than 9 may indicate high anxiety levels (15).

Obsession with covid scale (OCS): A companion scale to CAS, OCS assessed the levels of dysfunctional and persistent thinking related to Covid-19. This 4 item scale identifies individuals with scores higher than 7 as having dysfunctional Covid-19 thinking (16, 17).

Resilience scale for adults (RSA): This scale was developed to assess individuals' levels of resilience and resistance to developing negative mental outcomes as a result of trauma and stress (18). RSA was found to be a reliable instrument to assess protection against trauma that consists of six factors, namely perception of self, planned future, social competence, structured style, family cohesion and social resources. Turkish version of the inventory was used for this study (19).

Statistical analysis

Statistical Package for the Social Sciences (SPSS ver. 20.0) was used for statistical analyses. Demographic information and disease characteristics were presented using descriptive statistical methods. Comparison for numeric variables between groups were carried out using independent samples t-test and categorical variables were compared using chi-square test. Correlations between parameters were analyzed by Pearson correlation analysis. A p value lower than 0.05 was accepted as significant.

RESULTS

Between October 2021 and February 2022, 39 patients with FMS and 39 controls were enrolled in the study. Demographic characteristics from both groups are presented in Table-1. Subjects were similar in age, marital status, employment status, number of comorbidities, and a history for infection with SARS-CoV-2.

FMS patients had a mean disease duration of 8 years. Their pain levels, widespread pain and symptom severity scale measurements are presented in Table-2.

FMS patients had significantly worse scores for all subcategories of SF-36, except physical functioning (p<0.05). Covid-19 anxiety and obsessions scores were also significantly worse in patients with FMS (p<0.05). Surprisingly, except for structured style subcategory, resilience scale for adults was found to be similar in both groups (p>0.05). Results of comparison analyses between the two groups regarding quality of life, Covid-19 related anxiety, Covid-19 obsession and resilience are presented in Table-3.

In FMS patients, widespread pain index scores were found to correlate significantly with CAS (p<0,05, r: 0,6). Age showed a negative correlation with bodily pain and physical functioning sub-scores of SF-36, which indicated less pain and better function in younger patients (p<0,05, r: -0,7 and r: -0, 6 respectively). We did not detect a statistically significant correlation between resilience scale for adults scores and other study parameters. For brevity, only statistically significant correlations are presented in Table-4.

Table-1. Demographic characteristics of study participants

	FMS group (n:39)	Control group (n:39)	p
Age, years, Mean ± SD	45.7±9.2	40.3±14.7	0.051[a]
Marital status, n (%)			
Single	6 (15.4)	12 (30.8)	
Separated/divorced/widowed	6 (15.4)	2 (5.1)	0.17[b]
Married	27(69.2)	25 (64.1)	
Sex, female, n(%)	28 (71.8)	20 (51)	0.06[b]
Employment, n(%)			
Employed	21 (53.8)	27 (69.2)	
Looking for a job	1 (2.6)	5 (12.8)	
Retired	4(10.3)	2(5.1)	0.24[b]
Homemaker	13 (18)	5 (12.8)	
Comorbidity, n(%)			
Hypertension	8 (20.5)	6 (15.3)	
Diabetes	2 (5.1)	1 (2.6)	
Other	10 (25.6)	14 (35.8)	0.19[b]
None	21 (53.8)	16 (41)	
History of Covid-19 infection, yes, n (%)	1 (2.6)	4 (10.2)	0.17[b]
Contact who has had Covid-19			
Significant other	1 (2.6)	2(5.1)	
Child	0	1 (2.6)	
Sibling	4 (10.3)	2 (5.1)	
Other relative	12 (30.8)	10 (25.6)	0.61[b]
Friend	7 (18)	12 (30.8)	
Neighbor	1 (2.6)	1 (2.6)	

FMS: Fibromyalgia syndrome, SD: Standard deviation, n: number, a: independent samples t test, b:chi square test

Table-2. Disease characteristics of group 1 (FMS patients, n:39)

FMS disease duration, years, mean ± SD	8±9.5
VAS pain, mean ± SD	7,0 ± 1,4
Widespread pain index, mean ± SD	8,1 ±3,2
Symptom severity scale, mean ± SD	9,0 ± 3,5
FMS: Fibromyalgia syndrome, SD: Standard deviation, VAS: Visual analog scale	

Table-3. Comparison of parameters between FMS patients and controls

	FMS group (n:39)	Control group (n:39)	p
SF-36 subgroup scores, %, mean ± SD			
Physical functioning	51.7±22.3	65.5 ± 20.3	0.06
Physical role	33.3 ±40.2	78.2± 24.4	0.00**
Emotional role	54.6 ±25.9	74.3 ± 22.2	0.00**
Vitality	40.2 ±9.5	55.1 ±16.4	0.00**
Mental health	40.5 ±19.2	65.2±21.1	0.00**
Social function	43.1 ±17.8	79.4±15.5	0.00**
Bodily pain	44.7 ±17.6	76.2±16.1	0.00**
General health	35.0±20.3	77.6 ±17.5	0.00**
Covid-19 anxiety scale, mean ± SD	3.0±2.9	2.4±2.0	0.03*
Obsession with Covid-19 scale, mean ± SD	4.2 ±2.7	3.5±2.8	0.04*
Resilience scale for adults, mean ± SD			
Perception of the self	2.6±0.4	3.5±0.5	0.82
Planned future	1.6 ± 0.2	2.1±0.3	0.14
Structured style	2.1±0.3	2.4±0.3	0.02*
Social competence	2.4±0.3	2.8±0.4	0.21
Family cohesion	3±0.4	3.9±0.6	0.82
Social resources	3.7±0.5	4.2±0.6	0.53

FMS: Fibromyalgia syndrome, SD: Standard deviation, n: number, SD: standard deviation, SF-36: Short form 36, * p<0.05, ** p<0.005, independent samples t test.

Table-4. Results of correlation analysis

	Age	CAS	OCS
	r	r	r
SF-36 sub scores			
Physical functioning	-0.6*	-0.2	-0.4
Physical role	-0.3	-0.1	-0.2
Emotional role	0.1	-0.1	-0.3
Vitality	-0.3	-0.2	-0.2
Mental health	0.1	-0.3	-0.4
Social function	0.2	-0.2	-0.3
Bodily pain	-0.7*	-0.3	-0.1
General health	-0.2	-0.2	-0.1
WPI	0.4	0.6*	0.2
SSS	0.3	0.3	0.2

P: Pearson coefficient, FMS: fibromyalgia syndrome, SF-36: Health Survey Questionnaire Short Form 36, CAS: Covid-19 anxiety scale, OCS: Obsession with Covid-19 scale, WPI: Widespread pain index, SSS: Symptom severity scale, VAS: Visual analog scale, * p<0.05

DISCUSSION

In this study, we have enrolled as controls, subjects sharing the same household as the FMS patients. to assess the effects of the pandemic on FMS patients and at the same time control for other factors that may play a role in pandemic-related anxiety such as social isolation and loss of a family member, assuming family members sharing the same home would also be affected by the same factors to a degree. Resilience may play an important role in developing mental health conditions such as depression, post-traumatic stress disorder or anxiety after a stressful life event. The discussion of whether resilience is a character trait or a dynamic trait continues but it is widely accepted that resilience is a protective factor making individuals less vulnerable to adverse life events (20). FMS patients have been reported to possess reduced resilience against stressful events (21).

Surprisingly for us, we did not detect a difference in most subcategories of the Resilience scale for adults. This may partly be explained by subjects sharing the same household as the controls, therefore being subject to similar environmental factors and similar quarantine and pandemic conditions. Genetic background of resilience might in part explain this similarity between the groups, and although controls did not report widespread pain, other genetic similarities could account for similar levels of resilience between our FMS and control groups.

Many studies have detected the negative effect of FMS on quality of life. Galvez-Sánchez et al. (6) reported that FMS patients had lower scores in all subcategories of SF-36, compared to healthy adults. Pain, depression and anxiety levels all correlated with lower quality of life, and all mutually influenced each other. Similarly, Akay et al. (22) reported that pain intensity, anxiety and alexithymia were related to lower quality of life and disability. In this study, we found that greater widespread pain scores correlated with worse covid related anxiety scores. Our patients had lower scores on all sub scores of the SF-36 scale, except physical functioning. This was an unexpected result. FMS patients usually report worsening symptoms with exercise and physical activity. Our results may have been caused by a lack of exercise because of the quarantine and lockdown regulations during the pandemic, which reduced the difference in physical functioning in FMS patients as well as the controls. Regular

exercise tailored to the patients' needs has been shown time and again to provide favorable outcomes in decreasing pain levels in FMS (23–25). In this period of decreased physical activity, all patients should be encouraged to stay physically active in order to combat increased pain levels during lockdown and social isolation.

We found that patients' greater spread of pain correlated with higher levels of covid-19 related anxiety. Pain and anxiety are parts of a vicious cycle, each aggravating the other. Anxiety also negatively impacts patients' sleep quality, which further worsens pain and central sensitization. Covid-19 related anxiety may also cause patients to delay seeking medical attention and to fear going outside to exercise. Telemedicine assessment of anxiety and referral of those patients with high anxiety levels may help control pain and other related somatic symptoms (26). Physical exercise is also beneficial for the control of anxiety and other psychological symptoms of FMS, which in turn may help control widespread pain levels (27, 28). We also found that older patients had worse pain and physical function scores. Older individuals are already at more risk for social isolation and anxiety. Worsening of pain and physical function may exacerbate this situation. All patients with FMS should be questioned regarding their exercise habits and supported in finding the best way to stay active during and after the pandemic.

Limitations of the study

Because we have enrolled patients who lived with a significant other or family member to control for environmental and local stressors, we were not able to assess if living alone had any effect on any of the study parameters.

Our sample size may have been too small to detect the differences in resilience between the groups.

We carried out our study during the pandemic, but we do not have data regarding subjects' pre-pandemic pain and anxiety levels. We tried to control this by having a control group with similar environmental factors and restrictions.

Although we carried a power analysis a-priori using variance for covid anxiety scale, we did not do a power analysis for resilience. The reason for our inability to detect difference in resilience levels may be due to the sample size being too small, in other words, we might have made a type 2 error for resilience.

CONCLUSION

Symptom severity of FMS is closely correlated with levels of anxiety, depression and physical stressors. During the Covid-19 pandemic, social isolation, decreased levels of exercise, anxiety and obsession with Covid-19 and difficulty getting healthcare may all play a role in aggravating pain and other FMS related symptoms. Assessing individuals with FMS about their fears and anxieties around Covid-19 may help physicians better care for patients' somatic and psychological needs. Referring those patients with significant anxiety to proper psychological counseling and therapy and encouraging patients to exercise might play a role in disease control and increasing quality of life.

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

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Kopya sayısı varyasyonlarının makine öğrenmesi algoritmaları kullanılarak biyoinformatik analizi

Bioinformatic analysis of copy number variations using machine learning algorithms

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ÖZ

Amaç: Kopya sayısı varyasyonları, insan genomunun yaklaşık yüzde ikisinde bulunan belirli DNA bölgelerinin kayıp veya kazançlarıdır. Yapısal varyasyonlar arasında yer alan bu grup, sağlıklı popülasyonda bulunabileceği gibi ilgili bölgenin kayıp veya kazançları klinik tablolarla da ilişkilendirilebilir. Tespit edilen kopya sayısı varyasyonunun klinik olarak yorumlanması, aile çalışmasını da gerektiren karmaşık bir süreçtir. Klinik ve genetik verilerin yorumlanması sürecinde her zaman doğru bilgiye ulaşılamamaktadır. Kullanımı artan makine öğrenme algoritmaları giderek tıp alanında da kullanılmakta ve özellikle büyük veri setlerinin bulunduğu genetik gibi alanlarda giderek önem kazanmaktadır. Bu çalışma ile kopya sayısı varyasyonlarının klinik değerlendirilmesinde makine öğrenme algoritmalarının kullanımı amaçlanmıştır.

Gereç ve Yöntem: Araştırmada öncelikle 11989 varyant bulunan ISCA konsorsiyumu verileri ile pilot analiz gerçekleştirilmiş, sonrasında ClinVar veri tabanından elde edilen 63156 varyantlı veri seti kullanılmıştır. Beş ana sınıfta (Benign, Muhtemel Benign, VUS, Muhtemel Patojenik ve Patojenik) bulunan varyantlar, Microsoft Azure Machine Learning Studio platformunda, %70 eğitim ve %30 test verisi olarak ayrılmış ve çeşitli makine öğrenmesi algoritmaları (Çok Sınıflı Karar Ağaçları, Lojistik Regresyon ve Sinir Ağı) kullanılarak analiz gerçekleştirilmiştir.

Bulgular: ISCA veri seti ile yapılan modelde çok sınıflı karar ağacı ile ortalamada 0,96 doğruluğa ulaşılırken, ClinVar veri setinde yine çok sınıflı karar ağacı ile 0,86 doğruluğa ulaşılmıştır. Bu modelde patojenikler %74.8, benignler %77.6 ve önemi bilinmeyen varyantlar %62.6 oranında doğru tahmin edilmiştir. Çalışmada sık karşılaşılan varyantlar daha yüksek başarı ile tanımlanmış ve örneklemin benign ve patojenik olarak iki sınıflı haline getirilmesi durumunda ise ortalama ve toplamda 0.90 doğruluğa ulaşılmıştır.

Sonuç: Bu çalışma, kopya sayısı varyantlarının klinik değerlendirilmesinde kullanılabilecek ve tanıyı otomatikleştirebilecek öncül bir makine öğrenme modeli oluşturulabileceğini göstermiştir.

Anahtar Sözcükler: Kopya sayısı varyasyonları, makine öğrenme, genetik, biyoinformatik.

ABSTRACT

Aim: Copy number variations (CNVs), comprising approximately two percent of the human genome, represent specific DNA segment deletions or duplications. While these structural variations may be present in healthy populations, they can also manifest clinically significant implications. The clinical interpretation of identified CNVs constitutes a complex process necessitating comprehensive family studies.

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The interpretation of clinical and genetic data often presents challenges in achieving definitive conclusions. Machine learning algorithms have emerged as increasingly valuable tools in medical applications, particularly in genetics where large-scale datasets predominate. This investigation aimed to evaluate the implementation of machine learning algorithms for the clinical assessment of copy number variations.

Materials and Methods: *The study methodology comprised an initial pilot analysis utilizing ISCA consortium data (n=11,989 variants), followed by a comprehensive analysis of ClinVar database variants (n= 66803). The variants were stratified into five clinical classification categories (Benign, Likely Benign, VUS, Likely Pathogenic, and Pathogenic). Analyses were conducted using the Microsoft Azure Machine Learning Studio platform, implementing various machine learning algorithms (Multiclass Decision Trees, Logistic Regression, and Neural Network) with a 70:30 training-testing data partition.*

Results: *The ISCA dataset analysis demonstrated an average accuracy of 0.96 utilizing multiclass decision trees, while the ClinVar dataset achieved 0.86 accuracy with the same algorithmic approach. The model exhibited predictive accuracies of 74.8%, 77.6%, and 62.6% for pathogenic, benign, and variants of unknown significance, respectively. Frequently occurring variants demonstrated superior predictive accuracy, and binary classification (benign/pathogenic) yielded an enhanced average accuracy of 0.90.*

Conclusion: *This investigation demonstrates the feasibility of developing a preliminary machine learning model for the clinical evaluation and potential automated classification of copy number variants.*

Keywords*: Copy number variations, machine learning, genetics, bioinformatics .*

GİRİŞ

İnsan genomu 23 çift kromozom üzerinde yaklaşık 20.000 gen ve 6 milyar baz çifti içerir. Bireyler arasındaki genomik farklılıklar %0.01'den azdır. Bu farklılıklar DNA baz değişiklikleri (tek nükleotid polimorfizmleri (SNP), mikrosatellitler, minisatellitler, değişken sayıda ardışık tekrarlar (VNTR)), transpozon yapılar (Alu elementleri gibi), yapısal değişiklikler (delesyon, duplikasyon, inversiyon, oryantasyon değişikliği) ve epigenetik değişimler gibi farklı şekillerde ortaya çıkar. Kromozomlardaki ortalama 100 Kb'den büyük DNA bölgelerinin beklenilen kopya sayısından farklı olarak (kayıp ya da kazanç) halinde bulunduğu durumlar kopya sayısı varyasyonları (CNV) olarak adlandırılır (1). CNV tanımı genel olarak oryantasyonel bilgi içermeyen kayıp ve kazanç bölgelerini tanımlar. Yaklaşık 20 Kb'dan küçük CNV'ler hemen her bireyde bulunurlar ve karmaşık genomik yeniden düzenlemeler sonucu oluşur (2). GTG bantlama, DNA dizileme teknikleri ve PCR gibi zenginleştirme işlemleri gen bölgelerinin sayısal ölçümünü zorlaştırmaktadır. Floresan In Situ Hibridizasyon (FISH) tekniği ise yalnızca 20-100 Kb uzunluğundaki spesifik bölgeleri inceleyebilmektedir. CGH ve sonrasında geliştirilen arrayCGH/Oligo Array teknolojileri sayesinde, tüm genomun kopya sayısı analizleri daha erişilebilir ve yaygın hale gelmiştir (3). Ayrıca yeni nesil DNA dizileme teknikleri (Masif paralel sekanslama) ile de genomik olarak kopya sayısı varyasyonları yüksek hassasiyetle tespit edilebilir.

Çoğu CNV klinik açıdan önemsiz olmakla birlikte, bazıları gelişimsel gerilik ve doğumsal anomaliler gibi ciddi klinik durumlara neden olabilir. İyi bilinen DiGeorge(22q), Cri-du Chat(5p), Prader Willi (15p) vb. mikrodelesyon sendromlarının yanı sıra birçok mikrodelesyon sendromları array tabanlı yeni nesil teknolojilerle tanımlanmıştır (4). Bu varyasyonların klinik öneminin belirlenmesi uzman hekimlerin değerlendirmesini gerektirir (5). Kopya sayısı varyasyonlarının klinik yorumlanması karmaşık bir süreçtir. Bu süreçte, tespit edilen varyasyonlar DGV ve DECIPHER gibi uluslararası veri tabanlarındaki varyasyonlarla karşılaştırılır (6,7). Ancak yeni ortaya çıkan (*de novo*) varyantlar bu veri tabanlarında bulunmayabilir veya tanımlı varyantlarla tam eşleşme göstermeyebilir. Bu durumda, ebeveyn analizleri yapılır ve kalıtsal varyantlar genellikle benign olarak değerlendirilir. Ayrıca, her gen için hesaplanan haployetmezlik skorları, kopya sayısı değişikliklerinin klinik etkilerini değerlendirmede önemli bir araç(4) olarak kullanılmaktadır. Varyant sınıflamasında American College of Medical Genetics (ACMG) tarafından önerilen beşli sınıflandırma Benign, Muhtemel Benign, Önemi bilinmeyen varyant (VUS), Muhtemel Patojenik ve Patojenik yaygın olarak kullanılır (8). Kopya sayısı varyasyonlarının klinik yorumlanmasında mevcut algoritmalar her zaman kesin sonuçlar veremeemektedir.

Makine öğrenmesi teorileri yirminci yüzyılın ilk yarılarında ortaya çıkmış, ancak teknolojik sınırlamalar nedeniyle pratik uygulamaları

günümüze kadar gecikmiştir. Özellikle gelişmiş işlemci kapasiteleri ve modern yazılımlar sayesinde çeşitli alanlarda yaygın kullanım imkanı bulmuş ve farklı algoritmalarla uygulanabilir hale gelmiştir. Öğrenme, deneyimlerin bilgi ve tecrübeye dönüşümü olarak tanımlanırken, makine öğrenmesi verilerdeki anlamlı ilişkilerin otomatik olarak tespit edilmesi sürecidir. Geleneksel programlamadan farklı olarak, karmaşık ilişkileri öğrenme algoritmaları aracılığıyla yazılıma dönüştüren bu teknoloji, son yıllarda otonom araçlardan görüntü işlemeye kadar geniş bir kullanım alanı bulmuştur (9). İnsan kapasitesini aşan büyük ve karmaşık veri setlerinin analizi için özellikle sağlık ve tıp alanında yaygın olarak kullanılmaya başlanmış, radyolojik görüntüleme, kanser dokusu değerlendirmesi ve kompleks hastalıklarda risk faktörlerinin belirlenmesi gibi çeşitli alanlarda önemli uygulamalar geliştirilmiştir (10, 11).

Makine öğrenmesi yaklaşımları, veri etkileşim biçimlerine göre farklı kategorilerde incelenir. Gözetimli öğrenmede, önceden etiketlenmiş veriler üzerinden eğitim ve test setleri oluşturularak sistem değerlendirilir. Gözetimsiz öğrenmede ise etiketlenmemiş veriler üzerinden algoritmaların kendi içinde fonksiyon tespiti yapması sağlanır. Her iki yöntemin de kendine özgü avantaj ve dezavantajları vardır. Takviyeli (reinforcement) öğrenme, sistemin ürettiği sonuçlara doğru/yanlış şeklinde geri bildirim verilen bir ara form olarak tanımlanır. Öğrenme süreçleri ayrıca aktif ve pasif olarak da sınıflandırılır; aktif öğrenmede sistem parametrelerle doğrudan etkileşim kurabilirken (örneğin otonom araçlar), pasif öğrenmede hazır veriler üzerinden analiz yapılır (örneğin radyolojik görüntü değerlendirmesi). Online öğrenme sayesinde sistem, öğrenme sonuçlarını anlık olarak gözlemleyebilmektedir (12, 13).

Gözetimli öğrenme, önceden etiketlenmiş veriler üzerinden eğitim yapan bir makine öğrenmesi yaklaşımıdır. Bu sistemde, eğitim verileri ve bunlara ait sınıflar/kategoriler önceden belirlenmiş durumdadır. Sistem, bu etiketli verilerden öğrendiklerini kullanarak test verilerini yorumlar ve el yazısı tanıma veya görsel sınıflandırma gibi çeşitli alanlarda yaygın olarak uygulanır. Başarılı bir gözetimli öğrenme için geniş, etiketlenmiş veri setlerinin varlığı şarttır ve bu sistemler temel olarak sınıflandırma ve regresyon algoritmaları olmak üzere iki kategoride incelenir (12). Sıklıkla kullanılan algoritmalar: En Yakın Komşuluk (k-Nearest Neighbors (KNN)), Destek Vektör Makineleri (Support Vector Machine (SVM)), Karar Ağaçları (Decision Trees (DTs)), Doğrusal Regresyon (Linear Regression), Lojistik Regresyon (Logistic Regression), Naif Bayes (Naive Bayes), Yapay Sinir Ağları (Artificial Neural Network (ANN))'dır (14). Makine öğrenmesi yöntemleri arasında, KNN mesafe bazlı sınıflandırma yaparken, SVM destek fonksiyonları kullanır, karar ağaçları veriyi dallanmalar halinde gruplar, regresyon analizleri sayısal/kategorik ilişkileri modeller ve yapay sinir ağları biyolojik sinir sistemini taklit eden matematiksel modellerdir. Bu algoritmaların her biri farklı veri türleri ve problemler için optimize edilmiş olup, spesifik avantaj ve kısıtlamalara sahiptir (15). Gözetimsiz Öğrenme ise gözetimli öğrenmeden farklı olarak verilerde herhangi bir etiketlendirme bulunmaz ve sistem veriler arasında bağlantılar bulup birbirine yakın verilerin anlamlılıklarının arar. Bu amaçla Kümeleme (Clustering), Birliktelik Kuralı (Association Rule Mining), Boyut Azaltma (Dimensionality Reduction) gibi yöntemler kullanılır (16, 17).

Özellikle *de novo* varyantların değerlendirilmesinde klinik yorumlar belirsiz kalabilmekte ve farklı yaklaşımlar gerekebilmektedir. Günümüzde varyant değerlendirmeleri çoğunlukla manuel olarak, klinisyenlerin bireysel değerlendirmeleriyle yapılmaktadır. Bu nedenle, sürecin otomatikleştirilmesi ve yapay zeka destekli, yüksek doğrulukta klinik yorumlama sistemlerinin geliştirilmesi önemli bir ihtiyaç olarak görülmektedir. Bu çalışma ile makine öğrenme tekniklerinin bireyde tespit edilen kopya sayısı varyantının klinik etkisinin değerlendirilmesinde kullanılabilirliğini sorusunu araştırmayı planladık. Bu çalışma ile açık veri tabanlarında bildirilmiş kopya sayısı varyasyonları ile makine öğrenme teknikleri kullanarak ilgili CNV klinik sınıfının tahmin edilmesi amaçlanmıştır.

GEREÇ ve YÖNTEM

Öncelikle çalışma tasarımının denenmesi amacıyla 11989 varyantın bulunduğu ISCA (International Standards for Cytogenomic Arrays) konsorsiyumunun verileri kullanılarak pilot analiz gerçekleştirilmiştir (18, 19). Veriler dbVar veri tabanından CSV dosyası olarak alınmıştır. (20). Pilot çalışma sonuçları sonucu çalışmaya ClinVar veritabanına girilmiş 63156 varyantın bulunduğu nstd102 (Clinical Structural Variants), GRCh38 (hg38) versiyonu cinsiyet kromozomları hariç

tutularak çalışmada kullanılmıştır (21). Veri setlerindeki veriler klinik özelliklerine göre sınıflandırılmış ve hangi kromozomda bulundukları, genomik lokasyonları ve kayıp kazanç durumu bilgileri kullanılmıştır. Bu veriler içerisinden klinik özelikleri belirten Benign, Muhtemel Benign, Önemi bilinmeyen varyant (VUS), Muhtemel Patojenik ve Patojenik sınıfları kullanılmıştır. Veri setinde bulunan farklı tanımlanmış varyantlar uyumlu sınıf etiketine dönüştürülmüş, dönüştürülemeyen 535 varyant dışlanarak 66268 varyant ile çalışmaya devam edilmiştir. Oluşturulan veri setinde 35494 kopya sayısı kaybı ve 30774 kopya sayısı kazancı bulunmaktadır (Tablo-1). CNV'ler boyutlarına göre değerlendirildiğinde ortanca değer 132406 bç iken ortalama değer 2046347 bç olarak bulunmuştur. Veriler Microsoft Azure Machine Learning Studio kullanılarak analiz edilmiştir (22). Verilerden öncelikle gerekli sütunlar ayrılmış, sonrasında boş veriler temizlenmiştir (Clean Missing Data fonksiyonu ile). Verilerin analizi için ilgili varyantın bulunduğu kromozom bilgisi, kayıp kazanç bilgisi, kromozomal pozisyon başlangıç ve bitiş noktası ve klinik durum bilgisi kullanılmıştır. Örneklem %70 eğitim ve %30 test verisi olacak şekilde randomizasyon fonksiyonu ile iki gruba bölünmüştür. Eğitim modeli klinik etikete göre oluşturulmuştur. Analiz sırasında farklı makine öğrenme algoritmaları denenmiş ve sonuçlar birbirleriyle karşılaştırılmıştır. Eğitim modeli olarak: Çok Sınıflı Karar Ağacı-Forest (8 dal), Çok Sınıflı Karar Ağacı-Forest (16 dal), Çok Sınıflı Karar Ağacı-Forest (32 dal), Çok Sınıflı Karar Ağacı-Jungle, Çok Sınıflı Lojistik Regresyon ve Çok Sınıflı Sinir Ağı kullanılmıştır. Eğitim verisi test verisi ile karşılaştırılmış (Score Model) ve modelin başarısı değerlendirilmiştir (Evaluate Model). Ayrıca Sonuçlar Microsoft Excel kullanılarak değerlendirilmiştir ve grafikler oluşturulmuştur.

Tablo-1 Veri seti etiketlerinin dağılımı.

	Kazanç	Kayıp	INDEL	Toplam
Benign	10678	13427	5	24110
Muhteml Benign	2927	1889	18	4834
VUS	12454	7922	226	20602
Muhtemel Patojenik	854	1517	31	2402
Patolojenik	3861	10739	255	14855

BULGULAR

ISCA verileri ile yapılan çalışma sonucunda toplamda %89.241 ve ortalamada %96.4137 doğruluğa ulaşılmıştır. Plot veri setinde patojenik olarak tarif edilen örnekler %93,2 oranında patolojik %5,6 belirsiz etki, %1,2 benign olarak işaretlenirken, benign örnekler ise %90,3 oranında benign, %5,3 patojenik ve %4,4 belirsiz etki olarak işaretlenmişlerdir. Çok sınıflı lojistik regresyon (Multiclass Logistic Regression), çok sınıflı karar ormanı-Jungle (Multiclass Decision Jungle) ve çok sınıflı karar ormanı-forest (Multiclass Decision Forest) algoritmaları denenmiş ve bunlar içerisinde çok sınıflı karar ağacı en yüksek doğruluğa ulaşmıştır (Tablo-2). Pilot çalışmada veri etiketleri kendi içerisinde değerlendirilmemiştir ayrıca alt grupların 5 ten fazla olmasına rağmen modelin ortalama başarısı %96'nın üzerinde olarak değerlendirilmiştir.

Pilot çalışma sonrası ClinVar veri seti için yapılan analiz de farklı yöntemler birbirleri ile karşılaştırılmış ve bunlar arasında çoklu karar ağacı-forest (32) yaklaşık %86 ortalama doğruluk ile en başarılı yöntem olarak değerlendirilmiştir (Tablo-3). Yine bu analizler sonrası elde edilen doğru tahminlerin dağılımı Şekil-1'de gösterilmiştir.

Tablo-2 Pilot çalışma değerlendirmesi.

	Çok Sınıflı Lojistik Regresyon	Çok Sınıflı Karar Ormanı-Jungle	Çok Sınıflı Karar Ormanı-Forest
Toplam Doğruluk	0.46094	0.727829	0.89241
Ortalama Doğruluk	0.820313	0.909276	0.964137

Tablo-3. Eğitim verilerinin farklı algoritmalarla analizleri (NaN:verilerde numerik değer bulunmadığı için analiz edilememiştir.)

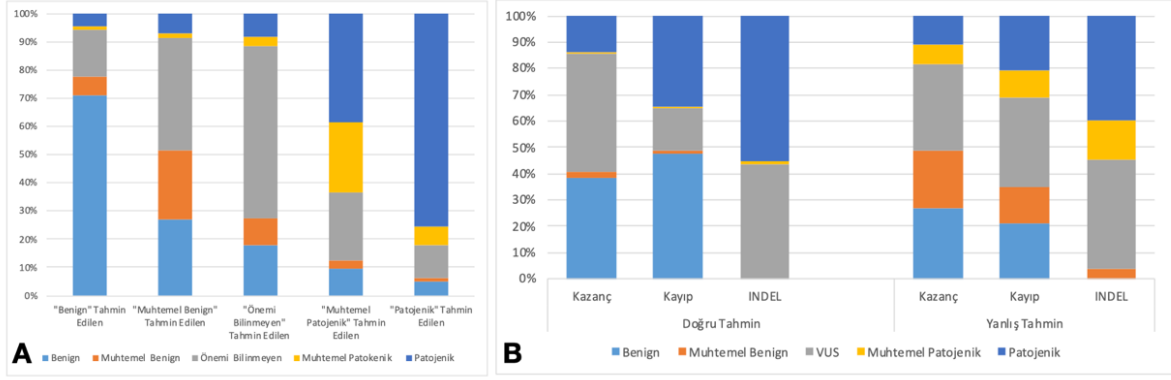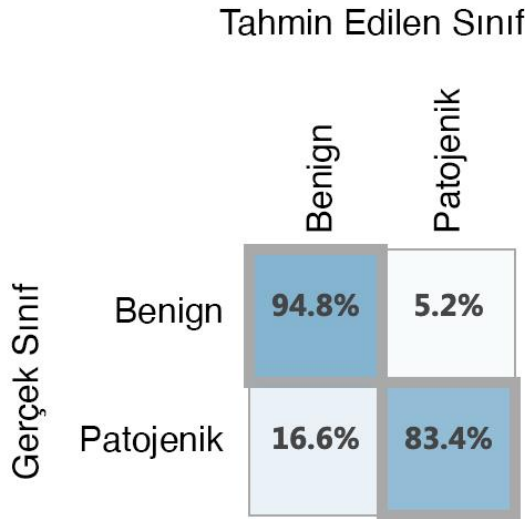|	Çok Sınıflı Karar Ağacı-Forest (8 dal)	Çok Sınıflı Karar Ağacı-Forest (16 dal)	Çok Sınıflı Karar Ağacı-Forest (32 dal)	Çok Sınıflı Karar Ağacı-Jungle	Çok Sınıflı Lojistik Regresyon	Çok Sınıflı Sinir Ağı
Toplam Doğruluk	0.637443	0.650167	0.657602	0.52188	0.432813	0.480415
Ortalama Doğruluk	0.854977	0.860067	0.863041	0.808752	0.773125	0.792166
Mikro-ortalama hassasiyet	0.637443	0.650167	0.657602	0.52188	0.432813	0.480415
Makro-ortalama hassasiyet	0.49486	0.510174	0.514232	NaN	NaN	NaN
Mikro-ortalama hatırlama	0.637443	0.650167	0.657602	0.52188	0.432813	0.480415
Mikro-ortalama hatırlama	0.479561	0.488108	0.491519	0.341952	0.268661	0.320082
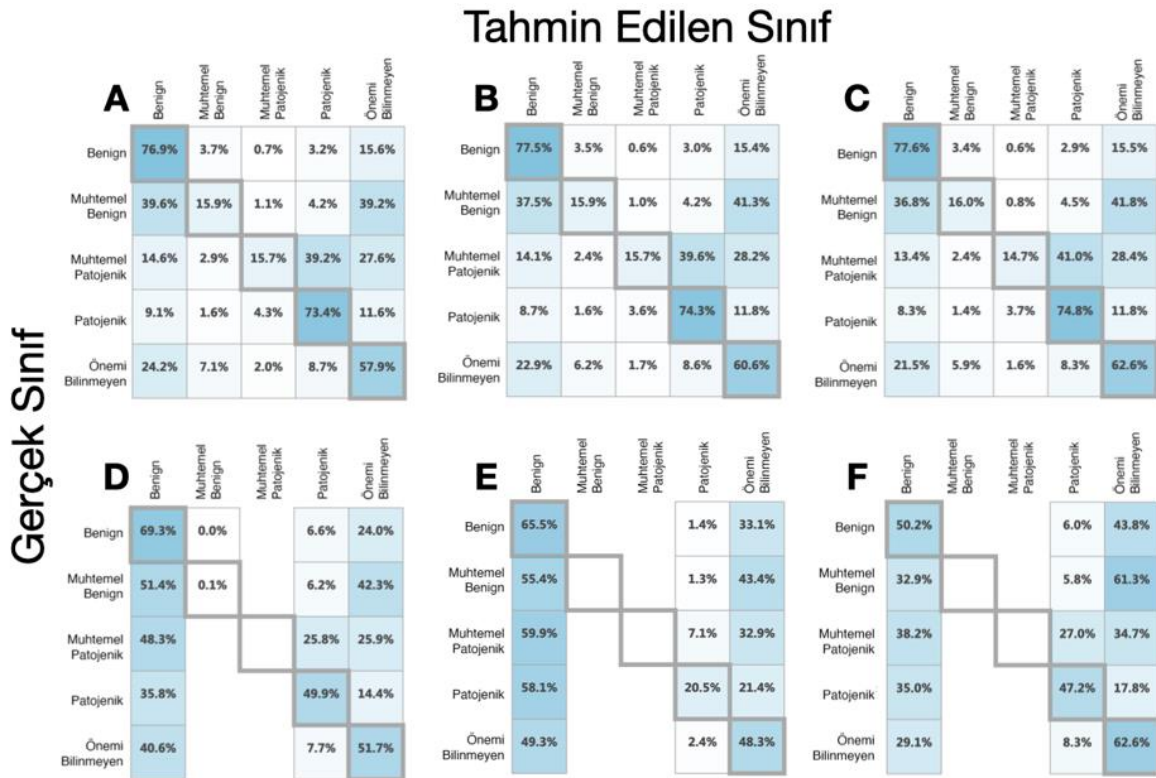
Şekil-1. A) Çok Sınıflı karar ağacı-forest (8 dal), B) Çok sınıflı karar ağacı-forest (16 dal), C) Çok sınıflı karar ağacı-forest (32 dal), D) Çok sınıflı karar ağacı-jungle, E) Çok sınıflı lojistik regresyon, F) Çok sınıflı sinir ağı doğru tahminlerin dağılımı

Şekil-2. Tahminlerin etiketlere göre dağılımı A) Gerçek etiketlerin tahmin edilen etiketler içerisindeki oranları B) Doğru ve Yanlış tahminlerin CNV türüne göre oranları

Çok sınıflı karar ağacı-forest (32 dal) analizinde muhtemel benign örnekler benign yönünde değerlendirilirken (%36,8) muhtemel patojenik örnekler patojenik (%41,0) yönünde kaymıştır. Benign ve patojenik kategorilerdeki tahmin başarısı diğer gruplara kıyasla daha yüksek bulunmuştur. Çalışmada incelenen varyantların çoğunluğu 10Kb-10Mb aralığında yer almakta olup, varyant boyutu arttıkça tahmin doğruluğunun yükseldiği, özellikle 100 baz çiftinden küçük varyantlarda yanlış tahmin oranının daha fazla olduğu tespit edilmiştir. (Şekil-2).

Tahmin Edilen Sınıf

Şekil-3 İki sınıflı örneklem çoklu sınıf karar ağacı-forest(32 dal).

Örneklemdeki benign ve patojenik değerler dışındaki veriler dışlandıktan sonra analiz tekrarlanmış ve çok sınıflı karar ağacı-forest (32 dal) ile ortalama ve toplamda 0.905176 keskinliğe ulaşılmıştır (Şekil-3). İki sınıflı karar ağacı-forest algoritması 0.897 keskinlikte sonuç vermiştir. İki sınıflı sinir ağı analizinde 0,730 keskinliğe ulaşılırken iki sınıflı destek vektör makinesi (SVM) ile 0.669 keskinliğe ulaşılmıştır.

TARTIŞMA

Kopya sayısı varyantlarının (CNV) klinik değerlendirmesi önemli zorluklar içermektedir. Modern array teknolojileri ve yeni nesil dizileme yöntemleri CNV tespitini kolaylaştırsa da, özellikle pozisyonel ve oryantasyonel bilgi eksikliği gibi kısıtlılıklar devam etmektedir (3). Gen içeren bölgelerdeki CNV'lerin değerlendirilmesi, haplo-yetmezlik skorları ve gen/dozaj ilişkisi sayesinde nispeten kolay olsa da, düzenleyici genom bölgelerindeki CNV'lerin (tüm analizlerin %95'i) değerlendirilmesi oldukça zordur (23). Bu zorlukları aşmak için çeşitli değerlendirme kriterleri ve skorlama sistemleri önerilmiştir (24) . Değerlendirme süreci genellikle veri tabanı taraması ile başlar, *de novo* varyantlarda aile çalışmaları ve segregasyon analizleri önem kazanır (25). Ancak süreç halen uzman değerlendirmesi gerektiren, zaman alıcı ve maliyetli bir yapıdadır.

Makine öğrenme teknikleri, teknolojik gelişmeler ve algoritmaların iyileştirilmesi sayesinde günlük yaşamın önemli bir parçası haline gelmiştir (26). Özellikle sağlık sektöründe geniş uygulama alanı bulan bu teknolojiler, büyük veri analizinin yoğun olduğu genetik alanında da kendine önemli bir yer edinmiştir (27). Makine öğrenme teknikleri, genetik varyantların değerlendirilmesinden kanser gibi karmaşık hastalıkların araştırılmasına kadar geniş bir yelpazede kullanılmaktadır (28–30). Ayrıca, yeni nesil dizileme verilerinden kopya

sayısı varyantlarının belirlenmesinde de bu tekniklerden yararlanılmaktadır (31).

Bu araştırma, klinik önemi belirsiz olan genetik varyantların değerlendirilmesinde makine öğrenme modellerinin kullanımını hedeflemiştir. Çalışmada, açık erişimli bir veri tabanı olan dbVar'ın nstd102 veri seti kullanılmıştır (32). dbVar, insan genomunda yaklaşık 6 milyon genomik bölgeyi kapsayan 35 milyondan fazla varyant içermekte olup, 100'ün üzerinde bilimsel çalışmadan elde edilen verileri barındırmaktadır. Bu verilerin önemli bir kısmı popülasyon çalışmalarından elde edilmiş olup, bazı verilerde klinik bilgiler mevcut değildir (33–35). İnsan genomunun büyüklüğü göz önüne alındığında, 66 bin varyantlık veri seti oldukça sınırlı bir kapsamı temsil etmektedir. Veri setinde yaygın mikrodelesyon/duplikasyon sendromları iyi temsil edilirken, nadir görülen yapısal değişiklikler için yeterli veri bulunmamaktadır. Ayrıca, veri setinin en önemli eksikliği doğrudan gen bilgisi içermemesidir. Her ne kadar kromozomal lokasyonlar gen içeriği hakkında fikir verse de, etkilenen gen ve gen bölgelerinin analize dahil edilmesi çalışmanın etkinliğini önemli ölçüde artıracaktır. Özellikle mikrodelesyon sendromlarında, kritik gen bölgelerinin kaybı klinik tabloyu belirlediğinden, genomik bölge analizlerinin gen/ekzon bilgisini içermesi sonuçların doğruluğunu güçlendirecektir (36). Ayrıca ilgili bölgenin pozisyonel durumu, epigenetik derişikliklerin olabilmesi, cinsiyetler arası farklılıklar gibi birçok etken bu çalışmanın başarısını kısıtlamaktadır.

Kromozomal dağılımlar incelendiğinde, otozomal kromozomların çoğunlukla dengeli bir dağılım gösterdiği, ancak 15, 16, 17, 19 ve 22. kromozomlarda beklenenden daha fazla varyasyon bulunduğu gözlemlenmiştir. Bu kromozomlardaki farklılıkların, buralarda sık görülen mikrodelesyon/duplikasyon sendromlarıyla ilişkili olduğu düşünülmektedir (37). Doğru tahminlerin kromozomal dağılımında ise sadece 15, 16 ve 17. kromozomlarda belirgin farklılıklar tespit edilmiş olup, bu durumun da yine bu bölgelerdeki mikrodelesyon/duplikasyon sendromlarının sıklığıyla bağlantılı olabileceği öne sürülmüştür.

Pilot çalışmada yüksek doğruluk oranları (%96) elde edilmesine rağmen, daha geniş veri setinde farklı yöntemler denenmesine karşın doğruluk oranları daha düşük seyretmiştir. Pilot çalışmadaki veri seti daha küçük olmasına rağmen, iyi kürate

edilmiş ve sınırlı kaynaktan gelen varyantlardan oluşurken, ClinVar veritabanı daha çok kaynaktan gelen ancak daha az denetlenmiş veriler içermektedir. ClinVar'ın klinik kullanımda güvenilirliği sıklıkla sorgulanmakta, özellikle nokta mutasyonlarındaki çelişkili kayıtlar nedeniyle yorumlama zorlukları yaşanmaktadır (38).

Yapılan analizlerde, varyant sınıflandırmalarının çoğunlukla "önemi bilinmeyen varyant" kategorisine kayma eğilimi gösterdiği tespit edilmiştir. Uluslararası kılavuzlar, varyantların klinik etkisi kesin olarak kanıtlanana kadar beş basamaklı bir sınıflandırma sisteminin kullanılmasını önermektedir (39, 40). Ancak bu çok kategorili sistem, doğru değerlendirmeyi zorlaştırmaktadır. Araştırma sonuçları, benign ve patojenik kategorilerdeki tahminlerin daha başarılı olduğunu, ancak ara kategorilerde sınıflar arası kaymaların daha fazla olduğunu göstermiştir. Özellikle, muhtemel benign, muhtemel patojenik ve önemi bilinmeyen varyantlar çıkarıldığında analiz başarısının arttığı gözlemlenmiştir. Makine öğrenme algoritmaları arasında, iki sınıflı ve çok sınıflı karar ormanları, SVM ve sinir ağı yöntemlerine kıyasla daha yüksek performans sergilemiştir.

Yapay zeka ve makine öğrenme teknolojileri, özellikle tıp alanında giderek daha fazla önem kazanmaktadır. Artan bilimsel veri hacmi ve karmaşık parametreler, insan yorumlama kapasitesini aşmakta ve tıbbi uygulamaların dijitalleşmesini zorunlu kılmaktadır (41). Tıbbi görüntüleme, laboratuvar ve nörolojik kayıtlar gibi sistemlerden elde edilen büyük veriler, yapay zeka algoritmaları ile analiz edilmekte ve bu uygulamalar klinik verilerin doğru yorumlanması ve maliyet etkinliği açısından değerli sonuçlar vermektedir (42,43). Microsoft Azure Machine Learning Studio gibi platformlar, kullanıcı dostu arayüzleriyle programlama deneyimi olmayan kişiler için bile makine öğrenme süreçlerini kolaylaştırmaktadır.

Bu çalışma, özellikle mental retardasyon ve çoklu konjenital anomali hastalarının tanısında kullanılan kopya sayısı varyasyonlarının analizi için veri analiz yöntemlerinin geliştirilmesini amaçlamıştır. Gelişen teknoloji ve dizi analizi uygulamaları, mikroarray teknolojileri sayesinde kopya sayısı varyantlarının tespiti kolaylaşmakla birlikte, genomik verilerin tam olarak anlaşılamaması ve değerlendirme zorlukları halen devam etmektedir. Çalışma, makine öğrenme algoritmalarının klinik uygulamalarda

kullanılabilirliğini göstermiş olup, modelin optimize edilmesi için veri setlerinin genişletilmesi, standart verilerin oluşturulması ve çeşitli parametrelerin iyileştirilmesi gerekmektedir. Gelişen teknoloji ve artan genetik veri birikimiyle birlikte, bu çalışmanın genetik tanı ve tedavide dijitalleşme sürecine önemli katkılar sağlaması beklenmektedir.

Çıkar çatışması: Bu çalışmada yazarlar arasında çıkar çatışması bulunmamaktadır.

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Orbital duvar kusurlarının düzeltilmesinde zigomatik ark transpozisyonu

Zygomatic arch transposition in correction of orbital wall defects

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ÖZ

Amaç: Travmalar, enfeksiyonlar, tümör invazyonları ve/veya iyatrojenik geniş kemik rezeksiyonları, orbita duvarı defektlerinin bilinen ana nedenleridir. Orbital duvar defektlerinin rekonstrüksiyonunda estetik problemlerin yanında, enoftalmi veya distopi gibi klinik açıdan anlamlı fonksiyonel oftalmik sorunların çözülmesi önemlidir.

Gereç ve Yöntem: Bu çalışma Bahçeşehir Üniversitesi (BAÜ) Rhoton Anatomi Laboratuvarından (İstanbul, Türkiye) temin edilen 4 kadın, 4 erkek ve 16 taraf orbital alan üzerinde yapıldı. Konumlandırma için Mayfield kafa tutucuları kullanıldı. Orbital duvar defektlerinin değiştirilmesinde ve zigomatik ark (ZA) otogreftlerinin uyum içinde sabitlenmesi için titanyum mini plak ve mini vida sistemleri kullanıldı.

Bulgular: Anatomik noktaların en iyi şekilde görüntülenmesi için en uygun konumlandırma prensipleri tanımlandı. ZA diseksiyonu için Al-Mefty'nin kranio-orbito-zigomatik yaklaşım insizyonunun Gillie ile modifikasyonu yapıldı. Mini plak/vida sistemleriyle tutturulmuş ZA otogreftinin başarılı ve uyumlu bir şekilde değiştirildi.

Sonuç: Farklı insizyon tekniklerinin modifikasyonları daha iyi bir görüntü elde edilmesini sağlayabilir. Komplikasyonları önlemek için seçilen anatomik bölgelerin ve komşulukların ayrıntılı anatomik tanımlarının yapılması önemlidir. ZA transpozisyonu, otogreftlerin bilinen diğer avantajlarıyla birlikte uygulanabilir, ekonomik bir teknik olabilir ve ayrıca acil durumlarda çok kullanışlı olabilir.

Anahtar Sözcükler: Orbital rekonstruksiyon, zigomatik ark, cerrahi teknik, nöroşirurji, anatomi

ABSTRACT

Aim: Traumas, infections, tumor invasions, and/or iatrogenic extensive bone resections are well-known causes of orbital wall defects. In addition to addressing aesthetic issues in the reconstruction of orbital wall defects, resolving clinically significant functional ophthalmic problems such as enophthalmos or dystopia is important.

Materials and Methods: This study was conducted on 4 female and 4 male cadavers, totaling 16 orbits, obtained from the BAU, Rhoton's Anatomy Laboratory (Istanbul, Turkey). Mayfield head holders were used for positioning. Titanium mini-plate and mini-screw systems were used for replacing orbital wall defects and securing zygomatic arch (ZA) autografts in place.

Results: Optimal positioning principles were defined for visualizing anatomical landmarks. The cranio-orbito-zygomatic approach incision by Al-Mefty was modified with Gillie for dissection of the ZA. The ZA autograft secured with mini-plate/screw systems was successfully and compatibly replaced.

Conclusion: *Modifications of different incision techniques can lead to better visualization. Detailed anatomical descriptions of selected anatomical regions and neighborhoods are important to prevent complications. ZA transposition, along with other known advantages of autografts, can be applied as an economical technique and can also be very useful in emergency situations.*

Keywords: *Orbital reconstruction, zygomatic arc, surgical technique, neurosurgery, anatomy.*

GİRİŞ

Zigomatik-orbital kompleks yaralanmaları, travmalar sonucu oluşan, sıklıkla görülen, ciddi estetik ve fonksiyonel sekellere sahip yüz lezyonlarıdır. Travmalar, enfeksiyonlar, tümöral invazyonlar ve/veya iyatrojenik geniş kemik rezeksiyonları, orbital duvar defektlerinin bilinen ana nedenleridir. Orbital duvar defektlerinin rekonstrüksiyonu, cerrahlarda yalnızca kozmetik problemler açısından değil, aynı zamanda enoftalmi veya distopi gibi klinik açıdan anlamlı fonksiyonel oftalmik rahatsızlık riskleriyle karşı karşıya kalma açısından da endişe yaratmaktadır (1,2). Çoğunlukla estetik sınırlamalarla ilişkili olabilecek yüzün atrofik bir bölgesi ile kemik gelişiminde bir kusura neden olurlar.

Travmatik orbita yaralanmaları yüksek oranda gözlenir ve tüm orta yüz kırıklarının %23'ünü oluşturur (3). Orbital bileşenlerin yer değiştirmesi sadece estetik kusurların yanı sıra binoküler hasarlara da (enoftalmi, kalıcı diplopi vb.) yol açar. Göz küresinin doğal pozisyonunu düzeltmeyi amaçlayan cerrahi tedavi, kırık bölgede kas kasılması olmasa dahi neredeyse tüm vakalarda zorunlu bir işlemdir.

ZA kompleksini ilgilendiren kırıklarda lateral orbital duvar genellikle diyastaz ve yer değiştirme gösterir ve zigomatik-orbital kompleksin redüksiyonu sağlandığında normal anatomisine döner (4). Ancak yüksek enerjili yaralanmalarda bu duvar parçalanır (5) ve primer müdahalede rekonstrüksiyon gerekir. Lateral duvarın anatomisi, iyileşmemiş lateral duvar kırıklarında enoftalmi gelişmesinde olduğu gibi, postoperatif orbita hacminde önemli bir rol oynar (6).

Orbito-zigomatik rekonstrüktif cerrahi, karmaşık anatomisi nedeniyle cerrahi açısından zorlu bir yönetim gerektirir. Rekonstrüksiyon sürecinde zigomatik kemik veya orbita kenarlarını oluşturan diğer kemik yapılar ile kontur çizgilerinin tam olarak yeniden tanımlanması gereklidir (7,8).

Bu çalışmamızda orbital hasarların rekonstrüksiyonunda zigomatik ark (ZA) transpozisyonu cerrahisini yeni bir cerrahi uygulama metodu ile değerlendirmeyi amaçladık.

GEREÇ ve YÖNTEM

Bu çalışma Bahçeşehir Üniversitesi (BAÜ) Rhoton Anatomi Laboratuvarından (İstanbul, Türkiye) temin edilen 4 kadın, 4 erkek, 16 taraf orbital alan üzerinde yapıldı.

Cerrahi yöntem

İnsizyon tekniği, ZA anatomisi ve çevresinin tanımlanması amacıyla dört taraflı 2 adet sabit kafalı kadavra kullanıldı. Konumlandırma için Mayfield kafa tutucuları ile kafatası üç noktadan sabitlendi. Kadavra sırtüstü yatırıldı ve baş ekstensiyona getirildi (Şekil-1). Scalp flebi kaldırılarak kafa tabanına erişim sağlandı. Buna ek olarak, temporal kas eleve edildi ve orbita çevresindeki kemik yapılar (supraorbital marj, lateral orbital duvar) görüldü. Kraniektomi yapılarak intrakraniyal kompartmana erişim sağlandı. ZA'ya erişmek için saçlı deri içinde minimal bir insizyon yapıldı. Yumuşak doku diseke edilerek temporal fasiyal katmanlara ulaşıldı. Bunun ardından ZA'nın anatomisi milimetrik cerrahi cetvel ölçüleri eşliğinde tanımlandı (Şekil-2).


Şekil-1. Soldan yaklaşımla zigomatik arka ulaşmak için insizyon hattı crus helicis'ten tragus'u takip eden lineer çizgi üzerinde meatus acuticus externus'un 10-13 mm altında, apex auriculae'nin yaklaşık 3,5 cm aşağısına kadar uzatılmıştır. (Mavi çizgi temporal saç çizgisi, siyah ok apex auriculae, beyaz ok crus helicis hizasını göstermektedir.)
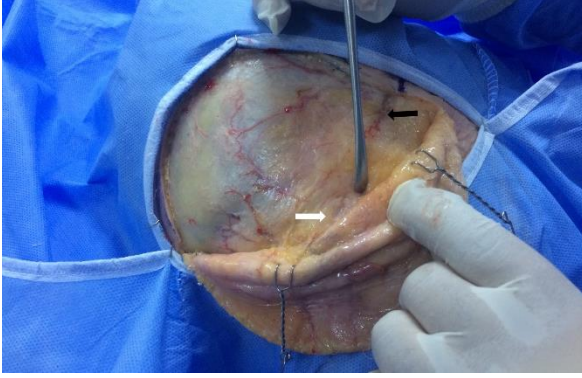
Şekil-2. Yüzeyel temporal fasya, temporal arter dalları korunarak ve cilt altı yağ dokusunun altında diseke edilerek ciltten ayrılmıştır. Bu sayede, orbitozigomatik bileşkenin alt sınırı net bir şekilde görülebilir hale getirilmiştir. Siyah ok, orbitozigomatik bileşkeyi, beyaz ok ise yüzeysel temporal arterin ikiye ayrıldığı noktayı (bifurkasyon) göstermektedir.

Şekil-3. Gillies elevatörü kullanılarak temporal fascia altından zigomatik arka yerleştirilmesi.
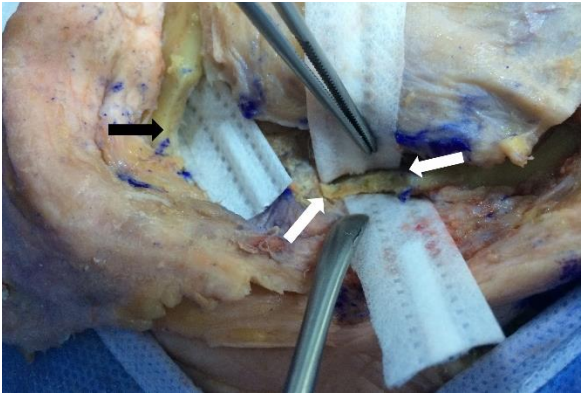
Şekil-4. Sol taraf superolateralden bakışta, zigomatik arkı saran temporal fascia katları dikkatlice diseke edildikten sonra (siyah ok) zigomatik ark ön ve arka sınırları, infratemporal fossaya uzanan venöz yapılar pediler vasıtasıyla künt diseksiyonla lateralize edilerek ortaya konulmuştur. Defekt boyutu ve ihtiyaç olan greftin ölçülebilir mukayesesine de izin vermektedir (Beyaz oklar ile gösterilmiştir).

Daha sonra, bir Gillies elevatörü kullanılarak temporal fascia altına girildi ve elevatör ZA'nın altına yerleştirildi (9) (Şekil-3). Elevatör ZA'yı yukarı ve dışa doğru kaldıracak şekilde kullanıldı. Gillies yaklaşımına ek olarak Al-Mefty tekniğinin prensipleri uygulanarak, kafa tabanı ve orbita bölgesine erişildi (10).

ZA Otogreftleme

ZA bölgesinde uygun bir insizyon yapıldı ve gerekli büyüklükte bir zigomatik kemik grefti (otogreft) dikkatli bir şekilde çıkarıldı (Şekil-4). Bu işlem sırasında, kemik greftinin etrafındaki yumuşak dokulara ve sinir yapılarına zarar vermemek için dikkatli olundu. Bu yöntemde orbital duvar defektine erişimin sağlandığı ve defekt oluşan alanın rahatlıkla görüldüğü saptandı. Zigomatik kemik greftinin çıkarıldığı alanın kenarları düzeltildi ve greftin yerleştirilmesi için uygun hale getirildi (11) (Şekil-5 ve Şekil-6). Alınan otogreft, orbital duvar defektine uyacak şekilde şekillendirildi. Greft, defektin üzerine doğru bir şekilde yerleştirildi ve anatomik uyum sağlanması için gerekli düzeltmeler yapıldı.


Şekil-5. Zigomatik kemik greftinin milimetrik büyüklüğü.

Şekil-6. Greftin yerleştirilmesi için uygun hale getirilmesi ve transpozisyona hazırlanması.

BULGULAR

Anatomik yer işaretlerinin en iyi şekilde ortaya çıkarılması için en uygun konumlandırma ilkeleri tanımlanmıştır (12,13). ZA'yı diseke etmek amacıyla Al-Mefty'nin kranio-orbito-zigomatik yaklaşım insizyonunun Gillie yöntemi ile modifikasyonu yapıldı. ZA'nın anatomisi milimetrik cerrahi cetvel ölçüleri eşliğinde detaylı şekilde tanımlandı. Orbital duvar defektlerinin düzeltilmesinde ZA otogreftlerinin uyum içinde tutturulması için titanyum mini plak ve mini vida sistemleri kullanıldı (Şekil-7). Mini plaklar, otogreftin kenarlarına yerleştirildi ve uygun uzunluktaki mini vidalarla hem greft hem de çevre kemik dokusu üzerine sabitlendi. Otogreftin sabitlenmesinin ardından yapılan insizyon dikkatlice kapatıldı.
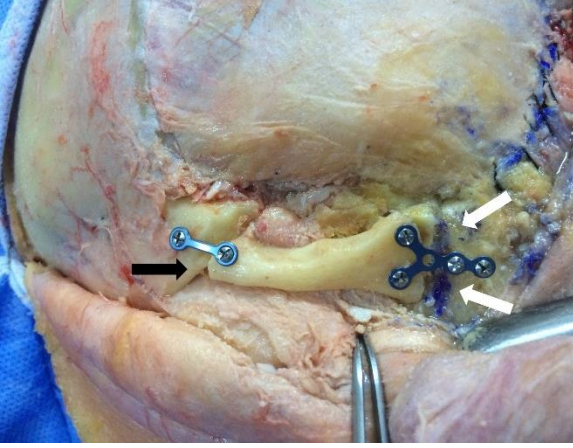
Şekil-7. Sol anterolateralden bakışta, zigomatik arktan alınan kemik greftinin, optimal orbita dış duvar konturunun rekonstrüksiyonunu sağlamak üzere, mikro plak (siyah ok) ve mikro vida sistemleri (beyaz oklar) ile tespit edilmesi.

TARTIŞMA

Bu çalışmamızda orbital defekt onarımında kullanılabilecek pratik ve güvenli bir yöntem olan bikoronal insizyon eşliğinde Gillies yaklaşımının yeni bir modifikasyonunu sunuyoruz. Flep öne doğru çekildiğinde, temporal saç çizgisi alanının altında yer alan derin temporal fasya içerisinde basit bir kesi yapılabilir ve böylece elevatörle benzer bir diseksiyon yapılabilir (9,14). Bu teknik ile tüm yumuşak doku bağlantıları korunabilir, operasyon süresinde ve maliyette azalma sağlanabilir.

Gillies yaklaşımı başlangıçta tüm zigomatiko-maksiller kompleks (ZMC) kırıkları için tanımlanmış olmasına rağmen, bugün esas olarak çökmüş ZA kırıkları için kullanılmaktadır.

Zigomatiko-frontal bölge, zigomatiko-maksiller destek ve infraorbital kenar kırıkları daha sıklıkla supratarsal, intraoral ve trans-konjonktival yaklaşımların bir kombinasyonu yoluyla mini plaklarla sabitlenir. Ayrıca, ark kırıkları kalvariyal, supraorbital kenar ve nazoorbitoetmoid kırıklarla ilişkili olabilir ve yeterli görüntüleme için bikoronal insizyon gerektirir.

Gillies yaklaşımı, skarın gizlenmesi, fasiyal bağlantıların korunması ve fasiyal sinirin ön dalının korunması gibi önemli avantajlar sayesinde uygun hastalarda sıklıkla tercih edilir. Uzun vadeli çalışmalar güvenilir stabilite ve mükemmel estetik sonuçlar ortaya koymuştur (15,16).

ZA kırıkları kalvariyal, supraorbital kenar ve nazo-orbitoetmoid kırıklara eşlik edebilmektedir. Birçok makale, travma sonrası yüz rekonstrüksiyonunda kişiye özel implantların kullanımının iyi klinik sonuçlara sahip olduğunu bildirmektedir (17,18). Bilgisayar destekli cerrahi, nadir görülen malformasyonlar gibi karmaşık vakalarda özellikle yararlı olabilir. Bazı vaka derlemelerinde bilateral orbito-zigomatik rekonstrüksiyon için cerrahi öncesi planlama ve otolog kemik greftleri veya alloplastik özel implantları araştırılmıştır (19–21).

Gillies yaklaşımında, temporalis kasının yüzeyine yüzeysel olarak, derin temporal fasyanın altına bir elevatör yerleştirilir. Ardından elevatörün arkın altından kaydırılarak redüksiyona kaldırıldığı temporal saç çizgisi kesisini yapılır (14). Gillies yaklaşımı sıklıkla tercih edilir çünkü uygun hastalarda daha pratik ve daha küçük kesi alanından cerrahi alana ulaşım rahatlıkla sağlanabilir. Belirgin bir yara izi bırakmaz ve fasiyal sinir hasarı veya intrakraniyal doğrudan travma olasılığı düşüktür (22).

Al-Mefty'nin kraniyo-orbito-zigomatik yaklaşım insizyonunun Gillies metodu ile modifikasyonu, kafa tabanı, orbita ve zigomatik bölge cerrahisinde önemli avantajlar sunar. Bu iki yaklaşımın birleştirilmesi, hem geniş cerrahi erişim hem de minimal invaziv yaklaşım ile estetik sonuçlar açısından önemli faydalar sağlayabilir. Bu modifikasyon, özellikle yüz bölgesinde çalışırken, cerrahi izlerin daha az görünür olmasını sağlayabilir. Minimal invaziv tekniklerin kullanımı, postoperatif ağrıyı, şişliği ve iyileşme süresini azaltabilir. Ayrıca, bu yöntem komşu sinir ve damar yapılarına zarar verme riskini de minimize eder.

SONUÇ

ZA transpozisyonu tekniğinde, ZA'nın uygun bir segmenti seçilir, dikkatlice çıkarılır ve orbital duvar defektinin onarımı için transpoze edilir. Gerektiğinde, titanyum mini plak ve vida sistemleri kullanılarak sabitlenir (11). Bu yöntem, özellikle yeterli otolog greft materyali bulunmayan veya allogreft ve sentetik malzemelerin kullanımıyla ilgili potansiyel risklerin önlenmek istendiği durumlarda tercih edilebilir. Bu yöntemde, hastanın kendi kemik dokusu, greft materyali olarak kullanılır. Bu yöntem otolog greftlerin avantajlarını taşır. Bu avantajlar arasında; greft reddinin olmaması, düşük enfeksiyon riski ve biyouyumluluk sorununun olmaması sayılabilir (23). ZA, yüz anatomisinde kolayca erişilebilir bir bölgedir ve greftin alınması nispeten basit bir işlemdir. Bu, özellikle acil durumlarda hızlı müdahale gerektiren durumlar için avantajlıdır. Bu işlemde zigomatik kemiğin sadece küçük bir kısmı kullanılır ve bu bölge hızlı iyileşme kapasitesine sahiptir.

ZA transpozisyonunun, otogreftlerin bilinen diğer avantajlarıyla birlikte uygulanabileceğini, ekonomik bir teknik olduğunu ve ayrıca acil durumlarda çok kullanışlı olabileceğini düşünmekteyiz.

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İlk trimester kistik higroma tanısı konulan olguların retrospektif analizi; tek merkez tecrübeleri

Retrospective analysis of cases diagnosed with first trimester cystic hygroma; single center experiences

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ÖZ

Amaç: Kistik higroma antenatal yaşamın ilk trimesterinde veya erken ikinci trimesterde tanı konulan, sıklıkla boyun, daha nadir mediasten veya abdomen çevresinde sıvı koleksiyonu ile izlenen konjenital bir anomalidir. Bu çalışmamızın amacı kliniğimizde tanı konulan kistik higroma olgularını değerlendirmek yapısal anomaliler, genetik anomaliler açısından sonuçlarını analiz etmektir.

Gereç ve Yöntem: Kliniğimize son iki yılda refere edilmiş hastalar arasından kistik higroma tanısı alan 22 hasta çalışmamıza dahil edilmiştir. Hastalarımızdan invaziv tanı testine izin veren 13 hastaya uygun hafta aralığına göre koryon villus örneklemesi veya amniyosentez uygulanmıştır. İzin vermeyen hastalar antenatal ultrasonografi takibine alınmıştır.

Bulgular: Genetik veya yapısal anomalisi olan 6 hastaya tıbbi tahliye uygulanmış 5 tanesi spontane abort veya missed olmuştur. Vakalardan bir tanesine amniyosentez işlemiyle kromozomal değerlendirme ve microarray analizi yapılmış ve normal bulunmuştur, antenatal takipte rezolüsyona uğramış ve miadında sağlıklı bir bebek doğurmuştur.

Sonuç: Konjenital kistik higroma tanısı konulan bebekler yapısal anomaliler, konjenital kalp hastalıkları, iskelet displazileri ve genetik hastalıklar açısından risk altındadır bu nedenle bu olguların perinatoloji genetik ve yenidoğan birimleri tarafından dikkatli takip edilmesi gerekmektedir. Genetiği normal olan bebeklerin prognozunu yapısal anomalilerin varlığı belirlemektedir.

Anahtar Sözcükler: Kistik higroma, konjenital anomaliler, kromozomal anomali.

ABSTRACT

Aim: Cystic hygroma is a congenital anomaly diagnosed in the first trimester or early second trimester of antenatal life, often accompanied by fluid collection around the neck, less commonly around the mediastinum or abdomen. The aim of this study is to evaluate cases of cystic hygroma diagnosed in our clinic and to analyze their results in terms of structural anomalies and genetic abnormalities.

Materials and Methods: Twenty-two patients diagnosed with cystic hygroma among those referred to our clinic in the last two years were included in our study. Chorionic villus sampling or amniocentesis was performed on 13 patients who consented to invasive diagnostic testing, according to the appropriate week interval. Patients who did not consent were placed under antenatal ultrasound follow-up.

Results: Medical termination was applied to 6 patients with genetic or structural anomalies, and 5 of them resulted in spontaneous abortion or missed abortion. Chromosomal evaluation and microarray analysis were performed on one of the cases through amniocentesis, which was found to be normal. The case underwent resolution during antenatal follow-up and delivered a healthy baby at term.

Conclusion: Babies diagnosed with congenital cystic hygroma are at risk for structural anomalies, congenital heart diseases, skeletal dysplasia, and genetic disorders, therefore requiring careful monitoring by perinatology, genetics, and neonatal units. The presence of structural anomalies determines the prognosis of babies with normal genetics.

Keywords: Cystic hygroma, congenital anomaly, chromosomal anomaly.

GİRİŞ

Konjenital anomaliler perinatal mortalite ve morbidite oranını artıran en önemli sebeplerden biridir(1). Kistik higroma sıklıkla (%75-90) boyun arkasında posterior servikal üçgende görülen, kistik lenfanjiom olarak da bilinen benign konjenital anomalilerden biridir. Ayrıca aksilla, mediastinum retroperitonal bölge ve torasik duvarda da görülebilir (2–4). Önceki çalışmalarda görülme sıklığı değişen oranlarda bildirilmiş. 1/285 den 1/6000 e kadar değişik yüzdelerde görülme sıklığından bahsedilmiştir (5–8).

Tanı ultrasonografi aracılığı ile konulur. Sıklıkla boyun arka tarafında retroservikal üçgende Nuchal translusens- Ense saydamlığı (NT)ölçümü yapılırken izlenir. Değerlendirme için aksiyal ve sagittal kesitler kullanılır. Bir veya birden fazla septa içeren kistik oluşum olarak izlenir (9).

Yapılan çalışmalarda sıklıkla kromozomal anomaliler ile ilişkilendirilmiştir. Anaploidi insidansı%60'a varan çeşitli oranlarda bildirilmiştir (5,9–13). Anaploidiler arasında en sık görülen Turner, Trizomi 21 Trizomi 18 Trizomi 13 ve triploidiler sayılabilir (1,12–14).

Antenatal girişimsel genetik tanı testleri yapıldı ve kromozomal bozukluk tespit edilmediyse microarray inceleme ilave edilmelidir (6, 15). Noonan sendromu kistik higromada tespit edilen rasopatilerden biridir (6, 15). Kromozomal anomali tespit edilmeyen olgularda detaylı bir organ taraması ilerleyen haftalarda mutlaka yapılmalıdır (16). Kardiyak anomaliler en sık rapor edilen yapısal anomalilerdir (10,17). İskelet displazileri (Robert's sendromu, Kampomelik displazi) SSS (santral sinir sistemi) anomalileri gibi yapısal anomaliler de rapor edilmiştir (16, 18, 19).

Beşinci gebelik haftasının sonunda lenfatik sistemin gelişmeye başladığı bilinmektedir. Kistik higroma lenfatik damar yolları içinde ve boyun lenfatik keselerinin büyümesi, lenfatik ve venöz sistem arasındaki bağlantı başarısızlığı sonucu sıvı birikimiyle ortaya çıkar. Kistik higromalar, alternatif bir drenaj yolu gelişirse veya jugüler damarlar ile juguler lenfatik keseler arasında bir bağlantı kurulabilirse çözülebilir (20). Genetik anomali tespit edilmeyen, yapısal anomalisi olmayan fetuslarda zamanla çözülme görüldüğünü bildiren çalışmalar

yayınlanmıştır(21). Bizde bu çalışmamızı, tersiyer bir merkez olan Elazığ Fethi Sekin Şehir Hastanesi perinatoloji kliniğine refere edilen ve kistik higroma tanısı doğrulanan hastalarımızın gebelik sonuçlarını değerlendirmek için planladık.

GEREÇ ve YÖNTEM

Bu çalışma tersiyer bir merkez olan Elazığ Fethi Sekin Şehir Hastanesi perinatoloji kliniğine Mayıs 2022- Aralık 2023 tarihleri arasında refere edilen ve kistik higroma tanısı doğrulanan hastalarımızın gebelik sonuçlarını değerlendirmek için tasarlanmıştır. Retrospektif bir kesitsel çalışma olarak yürütülmüştür. Kistik higroma olguları literatürde de belirtildiği üzere, ultrasonografi aracılığı ile sıklıkla boyun arka tarafında retroservikal üçgende NT (Nuchal translusens- Ense saydamlığı) ölçümü yapılan kesitte (sagittal) ve aksiyal kesitler kullanılarak. bir veya birden fazla septa içeren kistik oluşum şeklinde tanı almıştır (9) (Şekil-1,2).

Şekil-1. Kistik higroma tanılı 13 haftalık fetüs cranium'unun transvers kesitte görüntüsü. Septalı görünümde kistik higroma başın arka kısmında dikkat çekmektedir.

Şekil-2. Sagittal planda 12 haftalık bir gebelikte kistik higroma görünümü

Bilgisayar kayıtları, ultrasonografi verileri taranarak kistik higroma tanısı koyduğumuz hastalarımızın verilerine ulaştık. NT ölçümü yapılan kesitte fetal boyunla sınırlı, bölmeli olmayan bir sonolusens alanı olarak tanımlanan ense saydamlığının aksine, fetal vücut ekseni boyunca uzanan açıkça görülebilen septasyonlarla genişlemiş bir sonolusens olarak belirledik. Bu kriterler sınırında 6040 hastanın geriye yönelik kayıtları incelendiğinde 22 kistik higroma tanılı hastaya ulaşıldı ve kayıtlardan; hastaların yaşı, gebelik ve doğum sayıları, tanı konulan haftaları, girişimsel işlem sonuçları, takiplerde ortaya çıkan yapısal anomalileri, tıbbi terminasyon, abort veya canlı doğum sonuçları veri olarak kaydedildi. Kistik higroma tanısı koyduğumuz hastaları genetik hekimi ile konsülte etmiştik ve uygun hafta aralığına göre hastanın kabul etmesi durumunda amniyosentez veya koryon villus örneklemesi (CVS) uygulamıştık. Bu kayıtlarla hastalarımızın genetik tanı sonuçlarını kayıt altına aldık. İnvaziv işlem istemeyen hastaları veya genetik testleri normal olarak sonuçlanan hastaları perinatoloji polikliniğinde takibe aldık, ilerleyen haftalarda yapısal anomalileri tespit etmek için anomali taraması yaptık. Genetik anomaliler veya majör anomaliler nedeniyle aile istemi olursa tıbbi tahliye yaptık, gebeliğine devam etmek isteyen hastaların bebeklerinde hastanemiz dışında abort veya intrauterin ölüm olmuşsa bu bilgileri 'E nabız' isimli bakanlık sağlık kayıtlarından veya telefonla arayarak ulaşıp kaydettik. Canlı olarak miada ulaşan fetusların doğum bilgileri, neonatal, genetik muayene sonuçları da hastane kayıtlarından ve yine aynı ülke sağlık uygulamasından bulunup verilere kaydedilmiştir. Veri toplama süreci Helsinki bildirgesi ilkelerine uygun olarak sürdürülmüştür. Çalışmamızın etik onayı Türkiye, Elazığ' da bulunan Bölgesel Etik Komitesinden alınmıştır (Protokol No. 23402 2024/03).

İstatistiksel analiz

Araştırmadaki verilerin analizi, SPSS 25.0 programıyla (SPSS Inc., Chicago, IL) yapıldı. Hastalara ilişkin verilerin demografik dağılımları tanımlayıcı istatistiklerle (ortalama, standart sapma, minimum ve maksimum) oluşturuldu.

BULGULAR

22 kistik higroma olgusu çalışmaya dahil edilmiş ve istatistiksel analizi yapılmıştır. Bu sonuçlara göre hastaların demografik özellikleri Tablo-1'de gösterilmiştir.

Tablo-1. Demografik ve Klinik Özellikler

|	EN AZ	EN ÇOK	ORTALAMA
YAŞ	21	43	30,64 ± 6,3
GRAVİDA	1	5	2,41 ± 1,22
PARİTE	0	3	1,09 ± 0,97
TANI HAFTASI	11	18	12,2 ± 1,54

22 hastamızın 9'u (%40,9) herhangi bir girişimsel işlemi istemezken uygun hafta aralığına göre 10(%45,4) hastamıza koryon villus örneklemesi (CVS), 3 (%13,6) hastamıza amniyosentez (AS) uygulanmıştır. İnvaziv işlem ile elde edilen veya postpartum tanı konulan genetik hastalıklar tablo 2 de gösterilmiştir. 5 (%22,7) Trizomi 21 (Down sendromu), 3 (%13,6) Turner sendromu, 1 Trizomi 13, 1 mozaik Klineferter, 1 iskelet displazi tanısı konulmuştur. 4 olgumuzda herhangi bir kromozomal anomali olmaksızın yapısal anomaliler gelişmiştir. Bunlar holoprosensefali, diyafram hernisi, atrioventriküler septal defekt (AVSD), Fallot tetralojisi ve serebellar hipoplazisidir.

Tablo-2 de listelendiği gibi 13 hastamıza girişimsel işlem uygulanmıştır. 7 hastamızın sonucu anöploidi gelmiştir. 4 vakada trizomi 21 tespit edilmiş 3 hasta tıbbi terminasyon istemiş, 1 hasta gebeliğine devam etmeyi tercih etmiş fakat takiplerinde 17. haftada missed abortus gelişmiştir. 1 vakada trizomi 13 tanısı konulmuş aile tıbbi tahliyeyi kabul etmemiş ve takiplerde 16. Haftada abort olmuştur. Tanı konulan seks kromozom bozukluklarından Klineferter olan olgu canlı olarak 35. haftada doğmuş, Turner olan olgu 15. Haftada abort olmuştur. 6 hastamızın kromozom analizinde sayısal bir sorun tespit edilmemiştir. İlerleyen haftalarda yapılan ultrasonografik incelemelerde tespit edilen bazı yapısal anomaliler olmuştur. Bir vakada holoprosensefali tespit edilmiş olgu tıbbi tahliye istemiş, bir olguda diyafram hernisi görülmüş 17. Haftada missed abortus gelişmiş, bir olguda diyafram hernisi AVSD tespit edilmiş aile ileri inceleme ve tahliye kabul etmemiş 38 w da 2300 gr olarak doğup postpartum ex olmuştur. Bir olguda cerebellar hipoplazi ve corpus callosum hipoplazisi görülmüş ,1 olguda ise takiplerde kistik higroma görüntüsü kaybolmuş miadında sağ sağlıklı doğum gelişip postpartum ilk takiplerinde sorun tespit edilmemiştir. Bir olguda ise takiplerde (15. Haftada) uzun kemiklerde eğrilik kısalık dar torax görülmesi üzerine iskelet displazisi tanısıyla gebelik sonlandırılmıştır. Sosyal olanaklardan ötürü amniyon veya abort materyalinden ileri genetik inceleme yapılamamıştır.

Herhangi bir antenatal tanı testini kabul etmeyen 9 hastamız olmuştur. Bu hastalarımızın yapılan takiplerinde 1 olguda kalpte Fallot tetralojisi gelişmiş, 1 olguda AVSD gelişmiş bu olgu postpartum Down sendromu tanısı almış ex olmuştur. 2 olguya postpartum Turner sendromu tanısı konulurken 1 olguda 16 haftada missed abortus gelişmiştir. 1 olguya kistik higromaya ek ectopia kordis nedeniyle tıbbi tahliye uygulanmış, 3 olgunun 2'si intrauterin gelişme kısıtlılığı olarak 1'i de miadında sağlıklı olarak canlı dünyaya gelmiştir. Postpartum ilk muayenelerinde özellik tespit edilmemiştir. Tablo-3'de kistik higroma olgularının gebelik sonuçları gösterilmiştir.

Tablo-2. Kistik higroma olgularının genetik ve obstetrik sonuçları.
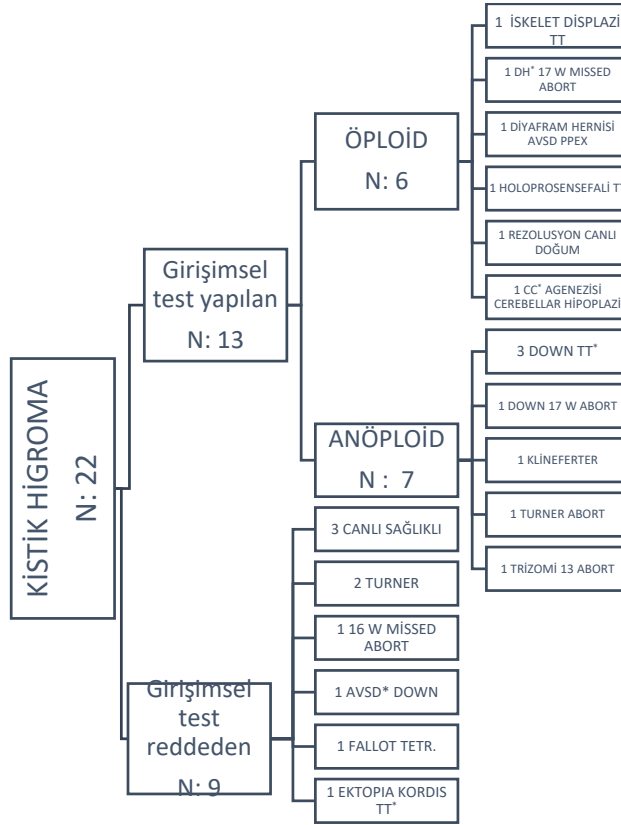
*TT : Tıbbi tahliye, *DH: Diyafragma hernisi, *CC: Corpus Callosum, *AVSD: Atrioventriküler septal defekt

Tablo-3. Kistik higromalı fetusların gebelik sonuçları:

	Kistik higroma N: 22
Tıbbi terminasyon	6 %27,2
Abortus /intrauterin ex	5 %22,7
Kromozomal anomalili canlı doğum	4%18,1
Yapısal anomalili canlı doğum	3%13,6
Anomalisiz canlı doğum	4%18,1

TARTIŞMA

Kistik higroma boyun arka üçgeninde yer alan lenfatik ve venöz sistemdeki geçişte bozukluk ile oluşan bir lenfanjiom türüdür. Sıklıkla 11-14 hafta arasında NT ölçümü yapılacakken mid-sagittal kesitte, septalı kistik oluşum olarak görülür. Detaylı ultrasonografi ve genetik inceleme endikasyonu vardır.(16) Yapılan son çalışmalarla da doğrulandığı üzere fetal kistik higroma kromozomal anomaliler ve yapısal anomaliler

açısından risk oluşturmaktadır (22–24). Bizim çalışmamızdan elde ettiğimiz sonuçlara göre 22 Kistik higroma olgusunun sadece 4'ü yapısal veya kromozomal bir anomali olmadan canlı sağlıklı doğmuştur.

Yapısal anomaliler, ciddi genetik hastalıklar ve sendromlarla ilişkili olması sebebiyle kistik higromanın erken tanısı oldukça önemlidir (25). Erken gebelik haftalarında tanı konulan ve kromozomal veya yapısal anomali tespit edilen kistik higroma olgularında terminasyon yapılmadığı taktirde hidrops, intrauterin ölüm ve abort olma olasılığı çok yüksektir (16, 18). Kistik higroma tespit edilen olguların sağ sağlıklı miadında doğuma ulaşma oranı oldukça düşüktür, yapılan çalışmalarda %5 veya daha altında oranlar verilmiştir (16). Bizim çalışmamızda bu oran %18 olarak bulunmuştur.

Ayrıca çalışmamızın bulguları, kistik higromanın teşhis ve yönetiminde önemli bilgiler sağlamaktadır. Bulgularımızı mevcut literatürle karşılaştırdığımızda, bazı benzerlikler ve farklılıklar dikkat çekmektedir

Daha önceki çalışmalarda %65'e kadar farklı oranlarda verildiği gibi en sık tespit edilen problem kromozomal anomali olmuştur (5, 11, 14, 24). Olgularımızın %45 ' inde (n:10) kromozomal bozukluk tespit edilmiştir. Schceurs ve arkadaşlarının çalışmasında en sık kromozomal anomali Turner olarak gösterilse de birçok çalışmada en sık tespit edilen kromozomal anomali Down sendromu olmuştur (5,10,14). Bizim çalışmamız da bu çalışmaları destekler nitelikte olup tespit ettiğimiz en sık kromozomal anomali Trizomi 21 olmuştur (5/10).

Öploid olgularda prognozu belirleyen en önemli durum yapısal anomalilerin varlığıdır (11) ve bunlar arasında en sık görülen kardiyak anomalilerdir, %62 'den %72 'ye varan oranlarda sıklık bildirilmiştir (17, 26). Literatürü destekler şekilde biz de yapısal malformasyonlar arasında en sık kardiyak anomalileri tespit ettik (%50); 1 olgumuzda AVSD, 1 olgumuzda Fallot tetralojisi tanısı konulmuştur.

Bazı vaka sunumlarında ve çalışmalarda değinildiği üzere kistik higroma takiplerde yeniden lenfatik -venöz bağlantı kurulursa, rezolüsyona uğrayıp kaybolabilir (9, 27). Bu genetik ve yapısal anomalisi olmayan fetuslarda görülebilir. Bizim olgularımızdan 4'ünde (%18) takiplerinde kistik higroma görünümü kaybolmuş ve miadında canlı doğum gerçekleşmiştir. Bu olguların 1'inin antenatal, 3'ünün postnatal genetik incelemeleri vardır ve kromozomal anomali tespit edilmemiştir. Çalışmamızın güçlü yönleri; belirli bir zaman diliminde tek bir merkezde toplanan büyük bir hasta grubunu kapsamaktadır, tıbbi kayıtların ayrıntılı bir şekilde incelenmesi, tanı ve yönetim süreçleri hakkında derinlemesine bilgi sağlamıştır, hastaların postnatal sonuçlarını değerlendirme olanağı uzun dönem sonuçlar hakkında literatüre katkı sağlamıştır. Çalışmanın zayıf yönleri ise sırasıyla şöyle sıralanabilir;

Çalışmanın retrospektif doğası, veri toplama ve analizde bazı sınırlamalara yol açabilir. Özellikle eksik veya hatalı kayıtlar, sonuçların doğruluğunu etkileyebilir.

Çalışmanın tek bir merkezde yapılmış olması, sonuçların farklı popülasyonlara genellenebilirliğini kısıtlayabilir. Ultrasonografi sonuçlarının yorumlanmasında subjektif faktörler rol oynayabilir. Bu da tanı ve yönetimde farklılıklara yol açabilir.

SONUÇ

Çalışmamızın sonuçları literatürle uyumludur ve kistik higromalı fetüslerde anormal karyotip ve yapısal anomalilere ilişkin risk yüksektir ve canlı doğum oranı düşüktür. Ultrasonografi sırasında kistik higroma saptanan fetüslerde daha ileri tetkiklerin (amniyosentez veya koryon villus örneklenmesi) yapılması gereklidir. Kistik higromalı gebelerde kromozomal veya yapısal anomalilerin yüksek riski nedeniyle ve gebelik sonucunda isteğe bağlı terminasyon veya intrauterin ölümle sonuçlanabileceği bilgisi aileye verilmeli ve bu ailelere genetik danışmanlık sağlanmalıdır.

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Case report: management of Phytolacca americana " dragon berries " poisoning

Vaka sunumu: Phytolacca americana " Şekerci Boyası" zehirlenmesinin yönetimi

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ABSTRACT

Phytolacca americana, commonly known as "Phytolacca" in our country, is a toxic plant. A 43-year-old male patient presented to the emergency department with abdominal pain, nausea, vomiting, and chest pain after consuming tea made from this plant. Examination and tests revealed elevated lactate, C-reactive protein, and white blood cells. Supportive treatment was administered, leading to symptom improvement. The literature indicates that all parts of this plant are toxic to humans and mammals, highlighting the importance of early recognition and supportive care. The FDA advises against the use of this plant.

Keywords: Phytolacca americana, toxidrome, cholinergic syndrome.

ÖZ

Ülkemizde yaygın olarak "Şekerci Boyası" olarak bilinen Phytolacca americana zehirli bir bitkidir. 43 yaşında erkek hasta bu bitkiden yapılan çayı tükettikten sonra karın ağrısı, bulantı, kusma ve göğüs ağrısı şikayetiyle acil servise başvurdu. Muayene ve testler yüksek laktat, C-reaktif protein ve beyaz kan hücrelerini ortaya çıkardı. Destek tedavisi uygulandı ve semptomlarda iyileşme sağlandı. Literatürde bu bitkinin tüm kısımlarının insanlar ve memeliler için toksik olduğu belirtiliyor ve bu da erken teşhis ve destekleyici bakımın önemini vurguluyor. FDA bu bitkinin kullanımına karşı tavsiyede bulunuyor.

Anahtar Sözcükler*: Phytolacca americana, toksidrom, kolinerjik sendrom.*

INTRODUCTION

In our country, the plant known as "Phytolacca" and scientifically named Phytolacca americana, grows to a height of 1-3 meters and produces green flowers and purple-colored berries. It is found growing in the Middle East region, including our country (1). This plant, known to be toxic to both humans and dogs, has historically been used in traditional medicine and has been the subject of some biomedical research (2). The Phytolacca americana plant grows in various regions of Turkey. This herbaceous plant, found at altitudes between 0-500 meters, is typically located at the foothills of mountains and within fields and shrubs. According to a study conducted in our country, it has been reported that this plant is collected in the Black Sea region (Ordu, Rize, Giresun), the Marmara region (Istanbul), and the Mediterranean region (Antalya). As demonstrated in this study, it has a widespread habitat in Turkey (Figure-1) (1). This text will discuss the conditions observed after the consumption of "Phytolacca" which exhibits toxic effects in humans.

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Figure-1. Phytolacca americana.

CASE

A 43-year-old male patient presented to the emergency department with complaints of abdominal pain, nausea, vomiting, and chest pain after drinking tea made from the P. americana plant. Upon admission, the patient was conscious, cooperative, and oriented, with a Glasgow Coma Score of 15/15. The patient's medical history revealed no chronic diseases or known allergies. At the time of admission, the patient's systolic blood pressure was 124 mmHg, diastolic blood pressure 80 mmHg, pulse 107 beats per minute, and partial oxygen saturation 98%.

Physical examination of the patient revealed no pathological cardiac murmurs, and breath sounds were normal. Abdominal auscultation indicated rapid bowel sounds and tenderness without defense or rebound tenderness. During follow-up, the patient's electrocardiogram showed a normal sinus rhythm at 99 beats per minute, with no ST changes. Blood tests showed an increase in lactate (3.2 mmol/L), elevated C-reactive protein (23.39 mg/L), and leukocytosis (16.51x10^9/L) with a left shift (88.8% neutrophils). No significant changes were observed in coagulation parameters or troponin levels.

During follow-up, the patient experienced nausea, vomiting, and fecal incontinence. A brain computed tomography scan revealed no significant findings. Due to the existing symptoms and irritability, supportive treatment was initiated.

A proton pump inhibitor (pantoprazole 40 mg) and metoclopramide 10 mg were administered, and hydration was ensured. The patient, primarily presenting with cholinergic symptoms, was hospitalized for observation. During follow-up, no changes were observed in biochemical values, and the patient's symptoms subsided. The patient was discharged with a recommendation for outpatient follow-up.

DISCUSTIONS

A review of the literature indicates that this plant, with toxic effects in all parts, frequently causes poisonings primarily in its native region of the Americas (3-5). "Phytolacca" which exhibits toxic effects on various mammalian species including humans, primarily shows cholinergic effects in our case as well (3). In an observational study, it was frequently observed that poisoning occurred predominantly in males and ended tolerably. Additionally, the most common findings were gastrointestinal symptoms. Cutaneous symptoms were also observed. Supportive treatment is an important follow-up parameter for this patient group, which showed good results during follow-up (4).

The primary treatment for such cases is supportive care. Despite being listed by the U.S. Food and Drug Administration (FDA) as a substance to be cautious with and avoided (6), this plant and its extracts are known to grow in our country. Consumption of this plant can result in cases ranging from asymptomatic to fatal. Early recognition and initiation of supportive treatment are crucial.

Ethical Approval: Ethics Committee approval is not required as is a case report. Informed consent has been received from the patient.

Conflict of interest: Authors declared no conflict of interest.

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A rare cause of sudden and severe shoulder pain: Parsonage-Turner Syndrome a case report

Ani başlangıçlı ve şiddetli omuz ağrısının nadir bir nedeni: Parsonage-Turner Sendromu olgu sunumu

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ABSTRACT

Parsonage-Turner syndrome is a peripheral neuropathy characterized by acute onset shoulder pain, sensory disturbances and weaknesses. The present report discusses a 46-year-old male patient presented to our clinic with acute pain in the right shoulder and notable difficulty in moving the right arm. Given the progression of his symptoms and the intensity of his pain, a clinical suspicion of Parsonage-Turner syndrome was raised. The diagnosis was subsequently confirmed through electromyography. The patient was prescribed pregabalin and enrolled in a physiotherapy program which resulted in a gradual improvement in his symptoms. The differential diagnosis of shoulder pain can be challenging because many conditions exhibit similar symptoms. Key considerations include rotator cuff pathology, cervical radiculopathy, calcific tendinitis, and amyotrophic lateral sclerosis. To accurately distinguish among these conditions, it is imperative to obtain a comprehensive medical history and conduct a thorough physical examination. While there is no specific treatment recommended, managing symptoms effectively and providing pain relief are crucial. Appropriate rehabilitation protocols can help prevent complications. A significant challenge with this syndrome is that many doctors remain largely unaware. Therefore, this case report aims to increase awareness about this condition.

Keywords: Parsonage turner syndrome, brachial neuritis, shoulder pain.

ÖZ

Parsonage-Turner sendromu, akut başlangıçlı omuz kuşağı ağrısı, duyusal bozukluklar ve kas güçsüzlüğü ile karakterize bir periferik nöropatidir. Bu olgu sunumunda, 46 yaşında akut başlangıçlı ve şiddetli omuz ağrısı ile başvuran ve akabinde kas güçsüzlüğü gelişen bir hasta tartışılmaktadır. Motor kaybın progresyonu ve ağrısının yoğunluğu göz önüne alındığında, Parsonage-Turner sendromu ön tanısı ile yapılan elektromiyografi ile doğrulandı. Pregabalin ile medikal tedavisi düzenlenen hasta rehabilitasyon programına alındı. Omuz ağrısının ayırıcı tanısı, rotator manşet patolojisi, servikal radikülopati, kalsifik tendinit, amiyotrofik lateral skleroz, Parsonage-Turner sendromu gibi benzer semptomlara sebep olan birçok hastalık sebebi ile zor olabilir. Ayırıcı tanı yapabilmek için doğru anamnez ve kapsamlı fizik muayene esastır. Önerilen belirli bir tedavi olmasa da semptomları etkili bir şekilde yönetmek ve ağrıyı kontrol altına alabilmek çok önemlidir. Uygun rehabilitasyon protokolleri, komplikasyonları önlemeye yardımcı olabilir. Bu sendromla ilgili olarak hekimlerdeki farkındalık düzeyi yeterli değildir. Bu olgu sunumu ile bu farkındalığın artırılması amaçlanmaktadır.

Anahtar Sözcükler: *Parsonage-Turner sendromu, brakial nörit, omuz ağrısı.*

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INTRODUCTION

Parsonage-Turner syndrome (PTS), also known as neuralgic amyotrophy or brachial neuritis, is a rare neurological condition that was described in 1948 (1). PTS is more frequent in men than women and is starts with unilateral and sudden onset shoulder pain. Patchy motor weakness and sensory loss arise approximately within days and weeks in the upper extremity (2). Although the annual incidence of PTS was reported as 2/100.000 in old publications, it is stated that the annual incidence has increased to 1/1000, thanks to the increased awareness among physicians (3). This syndrome usually affects adults between ages 20-60. Despite identifying some triggering conditions such as the post-surgical, post-infection, and post-vaccination period, the pathophysiology of the syndrome remains unknown. The diagnosis is made from the history and physical examination findings and confirmed by clinical neurophysiology testing. Written consent was obtained from the patient stating that her medical data could be published.

Case

A 46-year-old male patient, he initially presented to his family physician with sudden onset of right shoulder pain. Over the following 2 weeks, the dull ache spread into the right shoulder, scapula and hand. Over the next few days, the patient's pain increased, and weakness began in the shoulder. He also developed difficulty moving the thumb and index finger of his right hand. As illustrated in Figure-1, physical examination showed atrophy of the right deltoid muscle. There was no restriction in the passive range of motion of the right shoulder, however pain was exacerbated with shoulder movement. Manual muscle testing of supraspinatus and deltoid on the right side were graded 1/5. The patient's elbow joint range of motion and muscle strength were normal. Physical examination showed weakness of the flexor pollicis longus and flexor digitorum profundus to the index finger. The OK sign test was positive in the right hand (Figure-1). Hypoesthesia was detected on the lateral side of the right arm. There was no history of trauma and infection. Seven months before the onset of symptoms, the patient was administered the third dose of the BNT162b2 COVID-19 vaccine. The patient's medical history included diagnoses of diabetes, coronary artery disease, anxiety disorder, obsessive compulsive disorder. Although routine biochemical examinations were within normal limits, the HbA1c value was %12.5 and fasting blood sugar level of 297 mg/dL. Chest X-ray excluded compression mass in the upper lungs which may compress the brachial plexus creating similar symptomatology. No pathology was found in the brain-cervical-brachial plexus and shoulder magnetic resonance imaging. In needle electromyography (EMG), there were no motor unit action potentials in the deltoid muscle and flexor pollicis muscle, in addition, fibrillation and positive sharp waves, which are signs of acute denervation, were observed. The patient was diagnosed with PTS by physical exam and EMG results. Despite taking various medications, such as paracetamol and ibuprofen, there was no relief or improvement in the symptoms during this time. The pain is usually expected to resolve within a week or two, but due to the patient's ongoing severe neuropathic pain, tramadol 50 mg and pregabalin 75 mg twice daily were added to his treatment. A physical therapy program was designed for the patient, including electrical stimulation of the deltoid muscle, analgesic electrotherapy, superficial heat therapy and joint range of motion exercises. After two months his pain was relieved, but muscle weakness continued.
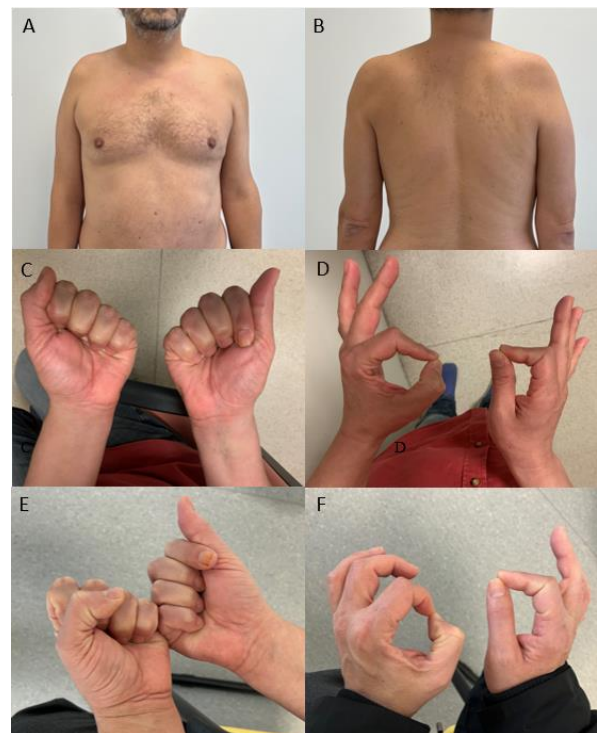

Figure-1. Images A and B were taken during diagnosis; moderate atrophy of the right shoulder girdle with loss of right deltoid bulk compared to the left arm. Images C and D were taken during diagnosis; images E and F were taken during the first-year follow-up. The OK sign test was positive in the right hand, with reduced flexion in the first interphalangeal joint and the second distal interphalangeal joint, as compared with the corresponding joints of the nonaffected hand.

An EMG test was performed again during the 1st year follow-up to determine the patient's prognosis. Nerve conduction studies indicated a significant decrease in the amplitude of the left median and ulnar compound muscle action potential response. At the time of diagnosis, the muscles examined showed total denervation, which persisted according to the results of the control needle EMG.

DISCUSSION

PTS can be diagnosed due to the intense neuropathic pain at the beginning, the rapid development of mono or multifocal paresis and atrophy, mainly in the upper extremity, and slow healing in months to years (4). The symptoms of PTS, a peripheral neuropathy, include acute, intense upper arm pain and muscle weakness (5).

The reported yearly incidence of this syndrome is 1 in 1.000, but a recent study found that because it is difficult to diagnose at an early stage, it is commonly disregarded and misdiagnosed (6). The disease may be hereditary or idiopathic. In the idiopathic type, 50% of patients have been exposed to a prior event (infection, partum, heavy exercise, previous operation or systemic disease) that activates lymphocytes sensitized to the brachial plexus in people who have a genetic predisposition (7). It is unlikely that the patient's PTS was caused by vaccination, as he had received the vaccine a few months earlier. Our patient had a type 2 diabetes mellitus for 10 years, with poor metabolic control. We report the case of a patient affected by this syndrome, which had no triggering factor except diabetes mellitus. Laboratory tests are usually normal in PTS patients, as in our case. Recovery from muscle weakness may take several months to several years, estimated rate of recovery is 36%, 75%, and 89% within 1, 2, and 3 years, respectively. Contrary to the literature, there was no improvement in the patient's condition in the first year of follow-up, and weakness in both the shoulder girdle and fingers continued similarly. Most cases are unilateral, but there are also cases where bilateral involvement has been reported (8).

Diagnosis of PTS based on clinical history, physical examination, EMG results, and imaging findings. EMG, which usually demonstrates patchy nerve loss throughout the whole plexus or acute denervation in just one nerve. EMG should be performed 2-3 weeks after the first appearance of symptoms, since there is no degenerative action potential during the acute period (9).

The treatment for PTS is generally conservative and there is no definitively recommended treatment. Neuropathic pain is a typical symptom of PTS and can be difficult to manage. Initial treatment typically includes opioids, anti-inflammatory medicines, and antiepileptic drugs. It has been reported in the case series that corticosteroids and İntravenous immunoglobulin (IVIG) may be effective in the acute period (10).

CONCLUSION

PTS is a particularly difficult syndrome to diagnose. The diagnosis is determined by ruling out other disorders with similar symptoms, such as rotator cuff pathology, cervical radiculopathy, calcific tendinitis, amyotrophic lateral sclerosis, cervical spine osteoarthritis, and adhesive capsulitis. The primary issue with this syndrome is that many doctors are still largely unaware of PTS. For this reason, this case report aims to raise awareness about PTS.

Ethics and competing interest

Consent for publication: Informed consent was obtained from the patient included in the study.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author.

Conflict of interest: The author has no competing interest.

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A Rare complication of epileptic seizure: two cases of neurogenic pulmonary edema

Epileptik nöbetin nadir bir komplikasyonu: iki nörojenik pulmoner ödem olgusu

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ABSTRACT

Neurogenic pulmonary edema is a form of alveolar edema that results from an increased adrenergic response secondary to central nervous system injury. It is characterized by elevated catecholamine levels and increased pulmonary hydrostatic pressure. The causes of neurogenic pulmonary edema include traumatic brain injury, stroke, intracranial hemorrhage, acute hydrocephalus, meningitis, drug overdose, and epilepsy. Non-specific symptoms such as shortness of breath, cough, and pink frothy sputum may occur due to pulmonary edema. We present two cases of patients, aged 43 and 32, both diagnosed with epilepsy, who presented to the emergency department with respiratory complaints following epileptic seizures. Diagnostic evaluations confirmed neurogenic pulmonary edema, and clinical improvement was observed following symptomatic treatment.

Keywords: Neurogenic pulmonary edema, epilepsy, central nervous system injury.

ÖZ

Nörojenik pulmoner ödem, santral sinir sistemi hasarına sekonder olarak gelişen artmış adrenerjik cevap, katekolamin seviyesinde artış, pulmoner hidrostatik basınç artışı sonucu meydana gelen alveolar ödemdir. Nörojenik pulmoner ödem nedenleri arasında, travmatik beyin hasarı, felç, intrakranial kanama, akut hidrosefali, menenjit, aşırı doz ilaç alımı ve epilepsi yer alır. Pulmoner ödeme bağlı nefes darlığı, öksürük, pembe köpüklü balgam gibi nonspesifik semptomlar izlenebilir. Biz 43 ve 32 yaşlarında, epilepsi tanılı iki hastanın epileptik nöbet sonrası gelişen solunumsal yakınmalar ile acil servise başvurup yapılan tetkikler sonucu nörojenik pulmoner ödem tanısı alan ve sonrasında semptomatik tedaviyle klinik düzelme görülen iki vakayı sunuyoruz.

Anahtar Sözcükler: *Nörojenik pulmoner ödem, epilepsi, santral sinir sistemi hasarı.*

INTRODUCTION

Neurogenic pulmonary edema is a critical clinical condition characterized by the sudden onset of pulmonary edema following injury to the central nervous system, and it is a relatively rare occurrence (1). It can develop as a result of various central nervous system disorders, including head trauma, subarachnoid hemorrhage, multiple sclerosis, strokes, cervical spine injury, central nervous system tumors, and epilepsy (2). Although the exact pathogenesis remains unclear, the increased catecholamine levels resulting from the adrenergic response to central nervous system injury, along with elevated pulmonary hydrostatic pressure and increased capillary permeability, are considered to be the underlying cause (1).

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The most frequently observed findings include shortness of breath, cough, hypoxemia, pink frothy sputum, rales on auscultation, and bilateral pulmonary infiltrates on chest radiography. While pulmonary edema can develop within minutes to hours following central nervous system injury, it may be resolved with the appropriate symptomatic treatment within 48-72 hours (3). This case series presents two cases of neurogenic pulmonary edema that developed subsequent to epileptic seizures.

Written consent was obtained from the patient stating that medical data could be published.

CASE REPORT 1

A 43-year-old female patient diagnosed with epilepsy experienced three consecutive epileptic seizures following numbness on the left side of her body. The patient presented to the emergency department with complaints of shortness of breath, chest pain, and a small amount of pink sputum following the seizures. Upon admission to the emergency department, the patient's body temperature was 36.8°C, heart rate was 87 beats per minute, blood pressure was 121/68 mmHg, and peripheral oxygen saturation was measured at 82% while receiving 6 liters per minute of oxygen inhalation. Laboratory results revealed a C-reactive protein level of 0.62 mg/dL, creatinine level of 1.1 mg/dL, white blood cell count of 12,900/mL, hemoglobin level of 8.9 g/dL, and platelet count of 221,000/μL. Arterial blood gas analysis showed a pH of 7.42, pO2 of 69.4 mmHg, pCO2 of 37 mmHg, and an oxygen saturation (SO2) of 92%. The patient reports experiencing seizures intermittently over the past five years, accompanied by shortness of breath following the seizures. Upon respiratory examination, bilateral rales were audible at the bases during auscultation. Chest radiography revealed an increase in central-weighted heterogeneous opacity, more pronounced in the right lung (Figure-1a). Computed tomography of the chest revealed widespread areas of increased density in a central-weighted alveolar pattern, particularly pronounced in the right upper lobe, accompanied by bilateral peripheral focal ground-glass opacities (Figure-1b). Echocardiography demonstrated an ejection fraction of 65% and identified grade 1 mitral regurgitation. The cardiological assessment did not indicate the presence of heart failure. Neurological examination revealed no evidence of pathological findings. The neurology team-initiated treatment for epilepsy with levetiracetam.

The patient admitted to the Chest Diseases Clinic was initiated on inhaled oxygen therapy and broad-spectrum antibiotic treatment. During the subsequent follow-up period, the patient exhibited a notable improvement in respiratory symptoms, accompanied by a marked enhancement in lung radiological findings by the conclusion of the first week of follow-up (Figure-1c).

Approximately 9 months after discharge, the patient presented to the emergency department following another epileptic seizure, accompanied by shortness of breath and hemoptysis of about 200 mL. The physical examination findings and laboratory parameters were similar to those of the previous admission. Chest radiography revealed an increase in bilateral heterogeneous opacities, more pronounced in the central region of the right lung (Figure-1d). Computed tomography of the chest revealed the presence of diffuse, scattered areas of increased nodular density, which were particularly prominent in the middle and lower lobes. Additionally, focal ground-glass opacities were observed (Figure-1e).

The patient was monitored in the Chest Diseases Clinic with oxygen inhalation therapy. No pathological findings were observed on the computed tomography of the brain. The epilepsy treatment was adjusted. During follow-up, the patient's hemoptysis had completely regressed, and significant improvement was noted in respiratory symptoms and in the lesions on chest radiography (Figure-1f). The patient's condition is being monitored stably with regular follow-ups in the Chest Diseases and Neurology departments.
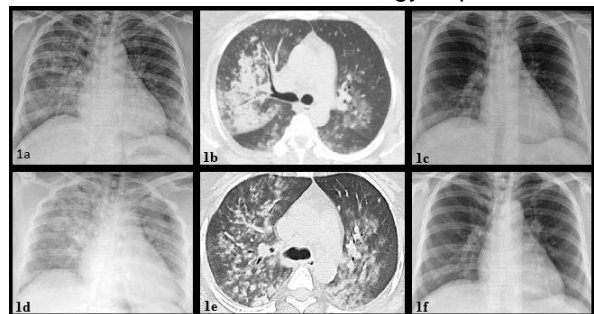

Figure-1. Figures-1a and 1b show the chest X-ray and tomography findings taken at the patient's first application; Figure-1c shows the chest X-ray findings taken at discharge. Figures-1d and 1e show the chest X-ray and tomography findings taken at the re-application 9 months after discharge; and Figure-1f shows the discharge X- ray.

CASE REPORT 2

A 32-year-old female patient with a known diagnosis of epilepsy was brought to the emergency department after fainting while at work. She was unable to recall the events preceding or following the incident. According to her relatives, she experienced convulsions during the episode. The patient had previously reported occasional shortness of breath and palpitations with exertion. She is currently undergoing treatment with metoprolol for mitral regurgitation. The patient has a documented history of smoking of 15 pack-years and is an active smoker. The patient's vital signs at the time of initial presentation to the emergency department were recorded as follows: body temperature 36.9°C, heart rate 119 beats per minute, blood pressure 135/90 mmHg, and peripheral oxygen saturation of 78% (room air). On respiratory examination, bilateral rales were auscultated in the middle and lower lung zones. There was no evidence of edema in the pretibial region. Neurological examination revealed a Glasgow Coma Scale (GCS) score of 15, with the patient being conscious, oriented, and cooperative. Motor strength was assessed as 5/5 in all four extremities. The echocardiogram demonstrated a normal ejection fraction and identified grade 1 mitral regurgitation. Upon examination of the laboratory values, C-reactive protein was 0.17 mg/dL, creatinine was 0.93 mg/dL, BNP was 486 pg/mL, troponin was 12 ng/L, leukocyte count was 8.980/mL, hemoglobin was 14.1 g/dL, and platelet count was 219.000/μL. The pH of the arterial blood gas was 7.40, with a PO2 of 44 mmHg, a PCO2 of 25 mmHg, and a SO2 of 79%. Computed tomography of the chest demonstrated ground-glass opacities and consolidated areas in all lobes and segments of both lungs (Figures-2a-2b). The brain computed tomography did not reveal any findings suggestive of acute intracranial stroke, hemorrhage, or mass effect. The patient was admitted to the intensive care unit with a diagnosis of neurogenic pulmonary edema. Symptomatic treatment was initiated. During follow-up, the patient did not report any respiratory complaints, required no supplemental oxygen after 48 hours, and showed significant regression on chest radiography. The patient was discharged with recommendations for follow-up in the neurology and pulmonology outpatient clinics (Figure-2c).

Figure-2. Figures-2a and 2b show the tomography findings taken at the patient's first application; Figure-2c shows the chest X-ray findings taken at discharge.

DISCUSSION

Both cases presented with a diagnosis of epilepsy and neurogenic pulmonary edema, which developed following epileptic seizures. A review of the literature revealed that pulmonary edema occurring after epileptic seizures is rare but can increase mortality (4).

The pathophysiology of neurogenic pulmonary edema has not been fully elucidated. The most widely accepted mechanism is non-cardiogenic alveolar edema resulting from increased adrenergic response secondary to elevated intracranial pressure, leading to increased catecholamine levels and elevated pulmonary hydrostatic pressure (5, 6). The most common causes of neurogenic pulmonary edema are head trauma, subarachnoid hemorrhage, encephalitis, brain tumors, stroke, and epileptic seizures (7). Due to the absence of established diagnostic criteria, diagnosis can be challenging. In cases, acute onset of dyspnea, tachypnea, pink frothy sputum, tachycardia, and a decrease in peripheral oxygen saturation are typically observed, often accompanied by neurological symptoms and signs. In the presence of a neurological condition that increases intracranial pressure, arterial blood gas analysis typically reveals hypoxemia, and chest radiography shows centrally weighted opacities (1). Due to the absence of specific diagnostic criteria for neurogenic pulmonary edema, it is essential to exclude other potential causes of pulmonary edema (3). The fundamental principles of treatment for neurogenic pulmonary edema include ensuring adequate oxygenation and providing sufficient ventilatory support with positive end-expiratory pressure, as well as reducing intracranial pressure. Although there is limited data regarding mortality, a study involving 21 cases reported a mortality rate of 10%. Similar to our two cases, most patients improve within 48 to 72 hours with supportive treatment (8).

CONCLUSION

Neurogenic pulmonary edema is a relatively uncommon condition, and acute pulmonary edema is predominantly of cardiogenic origin. However, in patients with central nervous system pathology and no cardiac pathology to explain the condition, the presence of accompanying dyspnea, cough, hypoxemia, and bilateral opacities on chest radiography should raise suspicion for neurogenic pulmonary edema.

Conflict of interest: None of the authors have conflict of interest to declare.

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Glioblastoma multiforme'nin ölüm sarkıtları: metastaz
Brinicles of glioblastoma multiforme: metastasis

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ÖZ

Glioblastoma multiforme (GBM), beyin tümörleri arasında en sık görülen ve en agresif malign tümör olarak bilinir. Yüksek dereceli malign glial hücrelerden kaynaklanan bu tümör, beyin içinde lokalize olma eğilimindedir. Bununla birlikte, GBM'nin ekstrakraniyal metastazları son derece nadir görülmektedir. Genellikle hastalığın ileri evrelerinde, tedaviye dirençli vakalarda akciğerler, karaciğer ve lenf nodülleri gibi organlarda ortaya çıkabilmektedir. Ancak metastazlar, nadiren de olsa kemik, deri ve diğer organlarda da ortaya çıkabilir. Ekstrakraniyal metastazların varlığı, hastalığın biyolojik olarak daha agresif bir hal aldığını gösteren önemli bir prognostik faktördür. Ekstrakraniyal metastazlar beynin dışında bazı organlarda semptomlarla ortaya çıkmaktadır ki bu metastazın tedavisi ve sürecini oldukça zorlaştırır. Kan beyin bariyerinin varlığı nedeniyle ekstrakraniyal metastazların gerçekleşme ihtimali mümkün görünmezken, günümüzde bazı klinik vakalarda ekstrakraniyal metastazlarının görülme oranının %0.5-2 arasında olduğu değerlendirilmiştir.

Klinik çalışmalar, ekstrakraniyal metastazları olan GBM hastalarının daha kısa yaşam sürelerine sahip olduğunu ve tedaviye daha az yanıt verdiklerini ortaya koymaktadır. Bu durum, metastazların tedaviye karşı dirençli doğası, hasta sağkalımının kısa olmasından dolayı metastazların fark edilemeyişi ve hastalığın kontrol altına alınmasının zorluğundan kaynaklanmaktadır.

Tanı ve tedavi süreçlerinde, ileri görüntüleme teknikleri ve sistemik tedavi yöntemleri önemlidir. Ancak günümüzde GBM metastaz tedavisi için standart bir protokolü bulunmamaktadır. Bununla birlikte, GBM'nin ekstrakraniyal metastazları üzerine daha fazla klinik araştırma yapılması, tedaviye yönelik daha etkili stratejilerin geliştirilmesine olanak sağlayacaktır.

Anahtar Sözcükler: Glioblastoma multiform, beyin tümörü, ekstrakraniyal metastaz.

ABSTRACT

Glioblastoma multiforme (GBM) is know as most common and aggressive malignant tumor among brain tumors. Originating from high-grade malignant glial cells, this tumor tends to be localized within the brain. However, extracranial metastases of GBM are extremely rare. Extracranial metastases are generally observed in organs such as lungs, liver and lymph nodes in treatment-resistant cases, usually in advanced stages of the disease. However, metastases may rarely occur in bone, skin and other organs. The presence of extracranial metastases is an important prognostic factor, indicating that the disease has become more biologically aggressive. Extracranial metastases occur with symptoms in some organs other than the brain, making the metastasis treatment and process very difficult. While the possibility of extracranial metastases seems unlikely due to the presence of the blood-brain barrier, it has been evaluated that the the incidence of extracranial metastases is between 0.5-2% in some clinical cases.

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Clinical trials have demonstrated that GBM patients with extracranial metastases have reduced survival and are less responsive to treatment. This is attributable to the refractory nature of metastases and the potential for metastases to go undetected due to the limited survival of patients, which complicates effective disease management.

The utilization of advanced imaging techniques and systemic treatment modalities are of paramount importance in the diagnosis and treatment processes. However, there is currently a paucity of standardised protocols for the treatment of GBM metastases. Further clinical research on extracranial metastases of GBM will facilitate the development of more effective treatment strategies.

Keywords: *Glioblastoma multiforme, brain tumors, extracranial metastasis.*

GİRİŞ

Ölüm sarkıtları (brinicles) çok nadir olarak görülen ve dokunduğu her canlıyı hızlı bir şekilde öldüren doğa olayıdır. Denizin yüzeyindeki buz kütlesinden ayrılan aşırı soğuk tuzlu su, etrafındaki suyu dondurarak deniz tabanına ulaşırken sualtında devasa buz sarkıtları oluşur (1). Bu nadir doğa olayında deniz suyundan çok daha yoğun olan aşırı soğuk su, buz kütlesinin içinden kaçabilecek bir yol bulduğunda yoğunluğu nedeniyle aşağı doğru hareket eder ve denizin dibine doğru iner. Dipte bulunan deniz canlıları ile temas etmesi halinde, temasta bulunduğu tüm canlıları hızlı bir şekilde öldürür ve yayılmaya devam eder (2).

Glioblastoma Multiforme (GBM) da diğer tüm gliomalar arasında en yüksek ölüm oranına sahip olan cerrahi, kemoterapi, radyoterapi gibi standart tedavilere direnç gösteren en agresif ve en sık tekrarlayan glioma türlerinden biridir (3). Primer beyin tümörleri arasında yaygın görülmesine rağmen, GBM'nin ekstrakraniyal metastazları oldukça nadirdir ve yalnızca %0,4-%2 oranındadır (4, 5). Bu düşük oranlarla beraber metastatik glioblastoma için prognoz, tanıdan sonra 6 aylık sağkalım oranı ile ilişkilendirilmiştir (5). Beyindeki kan-beyin bariyerinin varlığı ve genel olarak düşük medyan sağ kalım nedeniyle GBM'lerin MSS dışında metastaz yapmadığı düşünülmekteydi ancak Davis L., 1928 yılında ekstrakraniyal GBM metastazının ilk tanımını yaparak GBM dünyasında yeni bir kapıyı aralamıştır (6).

Bu nadir gözlenen metastaz, hastaların kısalmış yaşam süreleri nedeniyle glioblastoma hücrelerinin ekstrakraniyal organlarda yerleşmesi için yeterli zamanın olmadığı düşüncesiyle hep yüzeysel olarak yorumlanmıştır. Ayrıca tümör hücrelerinin bulundukları nöral ortamın dışına sızmasını ve hayatta kalmasını engelleyen bazı içsel biyolojik engellerin de olduğu bilinmektedir. Bunlar;

(i) Beyin ve omurilik içinde sistemik yayılmaya izin veren lenfatik sistemin olmaması,

(ii) İntrakranial venler etrafında bulunan ve tümör hücrelerinin penetrasyonunu engelleyen yoğun dura materin varlığı,

(iii) Glioblastoma hücrelerinin sağkalımını ve proliferasyonunu kolaylaştıran besleyici bir stromanın diğer organlarda olmaması şeklinde sıralanabilir.

Bununla birlikte, bu engellere rağmen, literatürde ekstrakraniyal glioblastomalara nadiren de olsa rastlanmış ve klinik veriler bildirilmiştir (7).

Ekstrakraniyal metastazlar ile ilgili 1958-2024 yılları arasında 102 klinik vaka bildirilmiş ve bu vakalarla ilgili cinsiyet, yaş grubu gibi bazı demografik özelliklerin seçici olduğu yorumlanmıştır. Bu vakalardan 40 tanesi kadın bireylere aitken, 71 tanesinin de erkek bireylere ait olduğu rapor edilmektedir. Bu vakalardan yalnızca 10 tanesi 18 yaşından küçük bireylerde tanımlanırken, 90 tanesi yetişkin bireylerde rastlanan GBM metastaz vakasıdır (8, 9).

GBM'deki bu metastaz mekanizması henüz tam olarak bilinmemekle birlikte, birkaç hipotez öne sürülmektedir. Bunlar arasındaki en olası yol; tümörün dural damarlar yoluyla doğrudan ekstrameningeal dokuya erişimidir (10). Bu hipotezi destekler nitelikteki kanıtlar, metastazdan en sık etkilenen organlar olan akciğerler ve lenf düğümlerindeki tohumlanma paternine dayanmakta ve tümörün hematolojik veya lenfatik yolları tercih ettiğini düşündürmektedir. Paget'in önerdiği gibi, "tohum" bir tümör hücresinin ana tümörden yeni ve uzak bir yere göç etme ve kolonileşip büyümek için uygun bir ortamda (toprak) yeniden var olma yeteneğini temsil etmektedir (11) ve bu "Tohum ve Toprak" hipotezi, metastatik yayılma için bir mekanizma olarak bugün hala geçerliliğini korumaktadır. Son zamanlarda GBM hastalarının %20-39'unda kanda dolaşan tümör hücrelerine (CTCs) rastlanmıştır. Bu durum tümör hücrelerinin kan-beyin bariyerini aşma ve sonrasında immün sistemden de kaçarak kan dolaşımında hayatta kalma potansiyeline sahip olduğunu göstermektedir (10).

Şu anda ekstrakraniyal GBM metastazları için standart bir tedavi protokolü mevcut değildir. Bununla birlikte, Lun ve arkadaşları, farklı tedavi yöntemlerinin ardışık olarak eklenmesiyle metastatik hastalarda sağ kalımın artmasına yönelik bir eğilim gözlemlemiştir (7). Ameliyat + radyasyon + kemoterapi + beyin omurilik sıvısı (BOS) şantı uygulanan hastalar, yalnızca ameliyat, yalnızca radyasyon, ameliyat + radyasyon ve ameliyat + radyasyon + kemoterapi uygulananlarla karşılaştırıldığında metastazdan ölüme kadar en uzun ortalama sağ kalım süresine sahip olduğu bildirilmektedir (7).

Ekstrakraniyal metastazların tanısı, genellikle ileri görüntüleme teknikleri kullanılarak yapılmaktadır. Manyetik rezonans görüntüleme (MRG) ve bilgisayarlı tomografi (BT), beyin içi lezyonların değerlendirilmesinde yaygın olarak kullanılırken, ekstrakraniyal metastazların tespiti için genellikle PET-CT gibi daha hassas yöntemler tercih edilmektedir. Bu görüntüleme yöntemleri, metastatik lezyonların erken tespitine ve hastalığın yayılma düzeyinin belirlenmesine yardımcı olmaktadır (12).

Bu bilgilerin yanı sıra klinikten gelen verilere göre ekstrakraniyal metastazların en yaygın bölgeleri; lenf düğümleri, akciğerler, plevra, yumuşak dokular, bezler, karaciğer ve kemik dokuları olarak görülmektedir (4). Ancak daha sıra dışı metastatik glioblastoma örnekleri kalp, adrenal bez, böbrek, diyafram, mediasten ve pankreasta da bildirilmektedir (13).
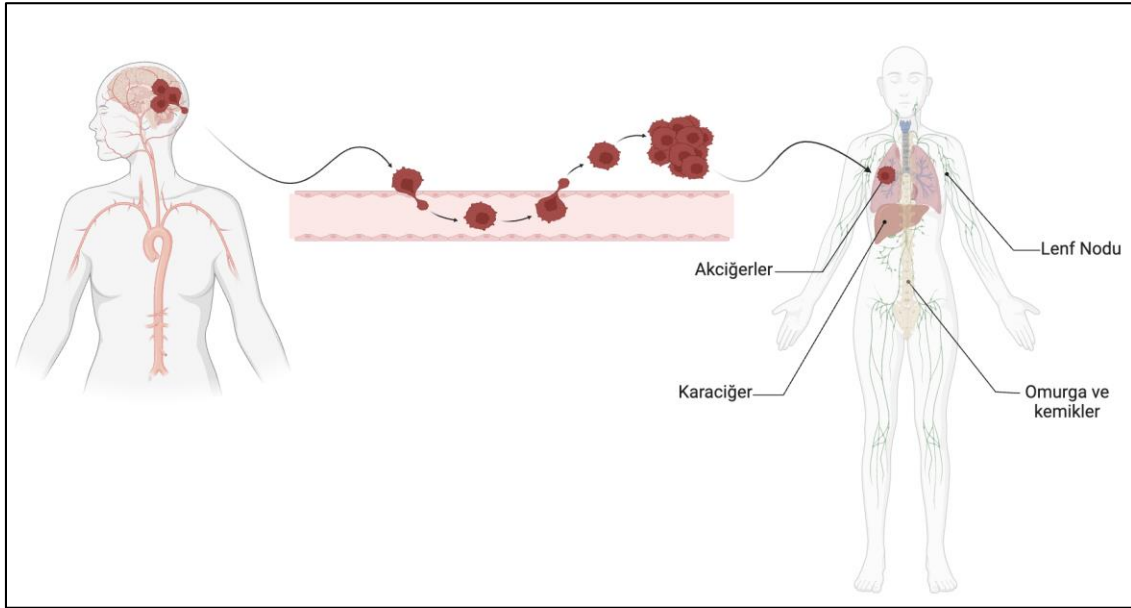
Şekil-1. Glioblastoma Multiform metastazlarının yaygın olarak gerçekleştiği organlar gösterilmektedir. BioRender ile çizilmiştir.

Akciğer Metastazı

Metastazların primer odak bölgesinin hastalığın prognozunu etkilediği görülmektedir ve bu bağlamda akciğer metastazı diğer metastazlar arasında en olumsuzu olup; hastalar 2,7 aylık bir sağkalım süresine sahiptir (7,14). Epidemiyolojik olarak, daha genç hastalar ekstrakraniyal metastaz geliştirmeye daha yatkındır ve vakaların yaklaşık %0,2 ila %1'inde bu metastazlar görülmektedir (15). 2015 yılında Ray ve arkadaşları tarafından üç farklı vaka üzerinde yapılan çalışmada, 29 yaşındaki kadın hastanın beyninde bulunan 7 cm boyutundaki kitlenin total rezeksiyon ile beraber adjuvan ve ışın tedavisi uygulamasından yaklaşık 17 ay sonra tümörün tekrar nüksettiği görülmüştür. Ardından ameliyat öncesi yapılan göğüs röntgeninde, akciğerde asemptomatik bilateral, çoklu yuvarlak hiperdansite alanlarının yapılan biyopsiyle birlikte hastanın metastatik GBM'e sahip olduğu bildirilmiştir (14).

Ayrıca primer tümöre sahip olmayan ancak GBM'li bir donörden yapılan bilateral akciğer nakli sonrasında metastatik GBM'in geliştiğine dair iki rapor bulunmaktadır. Bu da ölüm anında fark edilmeyen GBM mikrometastazlarının olabileceğinin kanıtı olabilir (16).

Yapılan kraniyotomiler, MSS'nin doğuştan gelen savunma mekanizmasını değiştirmekte ve tümör hücrelerinin hematojen olarak kan damarlarına girmesini kolaylaştırmaktadır. Bu olay, diğer organlara metastazın yolunu da açabilmektedir (17).

Lenf Düğümleri Metastazı

Ekstrakraniyal GBM'nin; hematojen yol ile dağılımının yanı sıra lenfojen veya serebrospinal sıvı aracılı ventriküler şant yoluyla yayılımı da söz konusudur (18). Embriyolojik gelişim sırasında, lenf düğümleri ve lenfojen doku parotis bezinin içine dahil olmaktadır. Parotis bezine gerçekleşen metastazlar en sık lenfojen yayılımla ve nadiren hematojen yayılım ile gelişirken, parotis ve servikal lenf nodu metastazları ağrı ve bezlerdeki şişme ile karakterize olarak meydana gelmektedir (19).

Literatürde lenf nodu metastazı olan hastaların çoğu birden fazla sayıda kraniyotomi geçirmekte ve lenf nodu metastazının, beyin anatomik yapılarının cerrahi olarak tahrip edilmesi nedeniyle meydana geldiği ve tümör hücrelerinin meningeal ve/veya kafa derisi lenf damarlarına erişmesine izin verdiği için yayılıma olanak sağladığı düşünülmektedir (20). Ancak, ameliyat öncesi lenf nodu veya uzak metastazların olduğu bazı vakalarda da bildirilmektedir (21). Literatürdeki bir vaka raporunda hastanın, 2 yıldan uzun süredir adjuvan temozolomid tedavisi almış ve yüksek interlökin-2 reseptörü sergilediği belirtilmiştir. Bu nedenle başlangıçta mediastinal lenf düğümlerindeki metastatik GBM'den ziyade uzun vadeli temozolomid tedavisiyle ilişkili sekonder lösemiden şüphelenilmiştir (22). Yapılan son takip MRI'sında intrakraniyal tümör tekrarlamamış ve bu durum meydana gelen GBM lenf nodu metastazlarının tespitini geciktirmiştir. Ek olarak buradan da metastaz yaparak akciğerlere ilerleyerek hastanın yaşam kaybına neden olmuştur (20).

Kemik Metastazı

Omurgada meydana gelen GBM metastazları en sık torasik omurlarda görülürken, lomber, servikal ve sakral bölgelerde de görülebilmektedir (23). Omurga içinde ortaya çıkan GBM metastazları litik veya sklerotik olabilmektedir (24). Kemik metastazları bulunan hastaların %70'inde ilk semptom şiddetli sırt ağrısıdır (25). Bazı durumlarda tümör infiltrasyonu segmental instabiliteye, patolojik kırıklara ve kifotik deformitelere neden olabilmekte ve bu durumlar da ağrıyı dayanılmaz hale getirebilmektedir. Diğer semptomlar arasında radiküler ağrı, bağırsak ve mesane disfonksiyonu, uyuşma, karıncalanma, güç kaybı, felç ve kilo kaybı gibi durumlara da rastlanmaktadır (4).

10 yaşında bir kız çocuğunun ekstrakraniyal metastazını sunan bir klinik vakada, 2 aydır süren baş ağrısı ve baş dönmesi şikâyeti ile hastaneye başvurmuş ve tümörün çıkarılması için kraniyotomi yapılmıştır. Pediatrik yüksek dereceli GBM hastalarında adjuvan kemoterapi için standart tedavi olmadığından, tedavi kararları pediatrik nöro-onkoloji ekibi tarafından sınırlı literatüre dayanarak verilmiştir. İlk ameliyattan 4 ay sonra bel ağrısı gelişen hastada omurganın MRI'sında L1 seviyesinde gelişmiş bir lezyonla birlikte bir kompresyon kırığı olduğu görülmüş ve tüm vücut kemik taramasıyla GBM'nin osteolitik kemik metastazı olduğu doğrulanmıştır. İlk kritonomiden 5 ay sonra yeniden nüks eden GBM ile birlikte 11. ayda ikincil metastazlar akciğer ve lenf nodlarında gelişmiştir (15).

Karaciğer Metastazı

Karaciğer metastazları diğer metastazlara göre nispeten daha nadirdir. Karaciğer metastazlı GBM ile ilgili tüm mevcut literatür incelendiğinde, karaciğer metastazlarının neredeyse tamamı primer GBM'yi çıkarmak için yapılan ilk cerrahiden sonra görülmüştür. Karaciğer metastazları olan tanımlanmış vakaların çoğunda da tek veya birkaç metastatik tümör olduğu belirtilmiştir (13).

Literatürde yer alan bir diğer vakada GBM tanısı konulan ve ilk rezeksiyon sonrası tümör nüksü gerçekleşen hasta, şiddetli karın ağrısı şikayetiyle hastaneye başvurduğu karın ve pelvisin BT taraması sonucunda, çeşitli boyutlarda çok sayıda karaciğer lezyonu gözlenmiş ve bu karaciğer lezyonlarından birinin biyopsisi sonucunda histopatolojik olarak, GBM metastazlarıyla uyumlu karaciğer dokusu olduğu doğrulanmıştır—(23). Laboratuvar histopatoloji sonuçları ve görüntüleme bulgularına dayanarak bu hastaya beyinde yaygın karaciğer ve kemik metastazları bulunan GBM tanısı konulduğundan bahsedilmektedir (22).

TARTIŞMA

GBM, beyin parankiminde hızla büyüyen, heterojen yapıya sahip, yüksek dereceli bir malign tümördür. Bu tümör, beyin içinde lokalize olma eğilimindedir, ancak nadiren ekstrakraniyal organlara metastaz yapabilmektedir. Ekstrakraniyal metastazlar, GBM'nin ileri evrelerinde ortaya çıkar ve genellikle hastalığın

kötü prognozuna işaret eder. Metastatik yayılımın mekanizmaları ve bu metastazların hedef organları konusu hâlâ çok sayıda belirsizlik içermektedir.

GBM hücrelerinin periferik kan yoluyla vücudun diğer bölgelerine yayılma mekanizması, özellikle bu tümörlerin diğer ekstrakraniyal organlara metastaz yapma süreci hâlâ tam olarak anlaşılabilmiş değildir. "Tohum ve toprak" hipotezi, bazı tümör hücrelerinin belirli organlarda veya organ bölümlerinde seçici olarak metastaz yapma eğiliminde olduğunu ileri sürmektedir. Bu hipotez, tümör hücrelerinin yalnızca o organın mikro çevresine uygun bir ortamda büyüme gereksiniminden ya da tümör hücrelerinde, organlara özgü endotel hücrelerindeki reseptörlere bağlanan belirli yüzey belirteçlerinin bulunmasından kaynaklanabileceğini öne sürmektedir (26).

Bir çalışmada, farklı kanser türleri taranarak, GBM'nin; akciğer (26 gen) ve karaciğer kanseri (18 gen) ile benzer genlerde aşırı ekspresyon gösterdiği belirlenmiştir. Bu bulgular, GBM hücrelerinin bu organlara metastaz yapmayı tercih edebileceğini ve bu organlara özgü doku seçici genlerin aşırı ekspresyonunun metastazda rol oynayabileceğini göstermektedir. Ayrıca, dolaşımdaki GBM hücrelerinin mezenkimal bir ekspresyon profili sergilediği de belirtilmektedir. Bazı araştırmacılar, bu hücrelerin kan dolaşımına geçiş kabiliyetinin mezenkimal özelliklerinden kaynaklandığını ileri sürmüşlerdir (27).

Glioblastomaların yaklaşık %40'ında EGFR (epidermal büyüme faktörü reseptörü) gen amplifikasyonu gözlemlenmiştir. Dolaşımdaki GBM hücrelerinin bazılarında EGFR amplifikasyonu belirlenmiş ve bu durumun da EGFR sinyal yollarının GBM'nin ekstrakraniyal yayılımını destekleyebileceği fikri ortaya çıkmıştır (28).

Bağışıklık sistemi de GBM'nin ekstrakraniyal metastazlarında rol oynayabilmektedir. Glioblastomalı organlardan alınan nakillerin alıcılarında ekstrakraniyal metastazların görülmesi bu durumu desteklemektedir.

Glioblastomalı organların %12,5 ile %25 oranında tümörü alıcıya aktardığı bildirilmiştir (27). Bu bağlamda yapılan bir çalışmada, GBM'li hastalardan organ nakli yapılan 11 hastanın 3'ünde karaciğer metastazı, 3'ünde böbrek metastazı ve 5'inde akciğer metastazı geliştiği bildirilmektedir (29).

GBM'nin ekstrakraniyal metastazlarının tedavisiyle ilgili mevcut bilgiler sınırlıdır. Bununla birlikte, tedavi seçenekleri arasında temozolomid, karboplatinli bevacizumab (Avastin) ve karboplatinli docetaxel gibi tedavi kombinasyonları yer almaktadır. Ancak, bu tedavi seçeneklerinin etkinliği sınırlıdır. GBM metastazının tedavisinin araştırılmasındaki zorluk, ideal hayvan modellerinin eksikliğidir. Mevcut fare modelleri, insan kan-beyin bariyerini ve bağışıklık sistemlerini doğru bir şekilde taklit etmemektedir (13). İnsan beyninin karmaşık yapısı, yoğun hücre popülasyonu ve bağışıklık yanıtları ile şekillenen mikro çevrenin varlığı gibi nedenler, mevcut hastalığın taklit edilmesi ve seyrinin tahminini oldukça zorlaşmaktadır.

SONUÇ

GBM'nin ekstrakraniyal metastazları nadir görülmekle birlikte, hastaların prognozunu olumsuz yönde etkileyen önemli bir klinik durumdur. Ölümcül beyin herniasyonundan önce klinik olarak bu metastazları tanımaya vakit kalmamaktadır. GBM'nin metastatik potansiyeli, genetik ve biyolojik faktörlerle şekillenmekte ve metastazların varlığı, hastalığın daha agresif hale geldiğini göstermektedir. Tanı ve tedavi süreçlerinde ileri görüntüleme teknikleri ve sistemik tedavi seçenekleri önemini korumaktadır ancak tedaviye karşı gelişen direnç ve metastaza yönelik sistemik bir tedavi planının olmayışı hala büyük bir zorluk teşkil etmektedir. Ekstrakraniyal metastazların yönetimi ve tedaviye yönelik daha etkili yaklaşımların geliştirilmesi için daha fazla araştırma yapılması; bu tümörle savaşmak için büyük önem arz etmektedir.

Çıkar çatışması: Bu çalışmada yazarlar arasında çıkar çatışması bulunmamaktadır.

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Glioblastoma multiforme tedavisindeki birincil engel: kan beyin bariyeri

The primary obstacle in glioblastoma multiforme treatment: the blood-brain barrier

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ÖZ

Dünya Sağlık Örgütü tarafından derece 4 astrositom olarak sınıflandırılan Glioblastoma Multiforme (GBM), merkezi sinir sisteminin en agresif ve yaygın görülen primer beyin tümörüdür. Klinikteki GBM hastaları için mevcut tedavi; rezeksiyonun ardından eş zamanlı uygulanan radyoterapi ve kemoterapiyi içermektedir. GBM tedavisindeki bu yaklaşımların etkinliği; tümör heterojenliği, glioma kök hücreleri, DNA hasar onarım mekanizmaları ve kan-beyin bariyeri gibi faktörler nedeniyle yetersiz kalmaktadır. Temozolomid, lipofilik bir ajan olması nedeniyle kan-beyin bariyerini kolaylıkla geçebilmekte ve bu özelliği sayesinde glioma tedavisinde etkili bir ajan olarak kullanılmaktadır. Bununla birlikte, hastaların büyük bir kısmında nüks meydana gelmekte ve bu hastalar temozolomide uzun süre maruz kaldığı için tedaviye direnç geliştirmektedir. Günümüzde, nüks eden GBM hastaları için farklı bir tedavi yaklaşımı söz konusu değildir. Bu yüzden, hasta sağkalımını uzatacak yeni ajanların araştırılması son derece önemlidir.

Terapötik ajanların merkezi sinir sistemine iletimi, kan-beyin bariyeri tarafından engellenmekte ve beyindeki hedeflenen bölgelere erişim, GBM için yeni ilaçların geliştirilmesinde en büyük zorluklardan birini oluşturmaktadır. Beyin tümörleri tedavisinde kan-beyin bariyerinin ilaç geçirgenliği heterojen bir yapı sergilemektedir. Sonuç olarak, bu bariyer geçirgenliğinin modüle edilmesi ve ilaç biyoyararlanımını artırmayı hedefleyen çeşitli stratejiler geliştirilmelidir. Kan-beyin bariyerinin aşılması, hedefe yönelik tedavi yaklaşımlarında dikkate alınması gereken önemli bir konudur. Bu derleme, kan-beyin bariyerinin GBM ile ilişkisini inceleyerek, konuya dair güncel bilgileri ayrıntılı bir şekilde sunmayı amaçlamaktadır.

Anahtar Sözcükler: Glioblastoma multiform, kan-beyin bariyeri, tedavi yöntemleri.

ABSTRACT

Classified as grade IV astrocytoma by the World Health Organization, Glioblastoma Multiforme (GBM) is the most aggressive and common primary brain tumor of the central nervous system. The current standard treatment for GBM patients in clinical practice involves resection followed by concurrent radiotherapy and chemotherapy. The effectiveness of these approaches in GBM treatment remains limited due to factors such as tumor heterogeneity, glioma stem cells, DNA damage repair mechanisms, and the blood-brain barrier. Temozolomide, due to its lipophilic nature, easily crosses the blood-brain barrier, making it an effective agent in the treatment of gliomas. However, a significant proportion of patients experience recurrence, and due to prolonged exposure to temozolomide, they develop resistance to the treatment. There is currently no alternative therapeutic approach for recurrent GBM patients. Therefore, the investigation of novel agents is of paramount importance to prolong patient survival.

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The permeability of the blood-brain barrier to therapeutic agents in the treatment of brain tumors exhibits heterogeneity. Consequently, various strategies aimed at modulating this barrier permeability to enhance drug bioavailability should be developed. Overcoming the blood-brain barrier is a critical consideration in targeted therapeutic approaches and must be addressed in the development of effective treatments. This review aims to provide a comprehensive analysis of the relationship between the blood-brain barrier and GBM, presenting the most recent findings and insights on the subject.

Keywords: *Glioblastoma multiforme, blood-brain barrier, treatment methods.*

GİRİŞ

Glioblastoma Multiform (GBM), yıllık 100.000 popülasyonda 3,19 insidans ile yetişkinlerde en sık görülen primer beyin tümörüdür (1). Gliomaların derecelendirilmesi için en yaygın kullanılan sınıflandırma, Dünya Sağlık Örgütü (DSÖ) tarafından yapılmaktadır. Bu sınıflandırmada gliomalar, histolojik özelliklerine göre derece 1 ve 4 arasında olmak üzere dört kategoriye ayrılmaktadır. Derece I, solid tümörlerdir ve benign özellikte oldukları için bu tümörlerin cerrahi tedavisi mümkündür. Derece II tümörler, genellikle düşük dereceli gliomaları oluşturmaktadır. Derece III gliomalar ise oldukça agresif ve invazif olup, hızla büyümektedirler. İzositrat Dehidrojenaz (IDH)-vahşi tip tümör olarak tanımlanan GBM, derece IV astrositom olarak kabul edilmektedir (2). GBM, *de novo* ortaya çıkan birincil GBM'ler ve düşük dereceli astrositomlardan oluşan ikincil GBM'ler olmak üzere iki alt gruba ayrılmaktadır (3). Birincil GBM'lerin görülme oranı %90'dır ve bu gruptaki tümörler genellikle hastalık öncesi klinik bulgu vermeden oldukça hızlı yayılmaktadır. GBM'lerin %10'luk kısmını ise akut *de novo* olarak gelişim gösteren ikincil GBM'ler oluşturmakta ve bu tümörler daha düşük dereceli tümörün ilerlemesi ile ortaya çıkmaktadır (4). Birincil GBM'ler erkeklerde, ikincil GBM'ler ise kadınlarda daha sık görülmektedir (5).

Glioma, nöronların destek hücreleri olarak bilinen glia hücrelerinin malign karakter kazanmasıyla ortaya çıkmakta ve yıldız şeklinde ışınsal karakterde büyüyerek beyin dokusuna yayılmaktadır. GBM, tüm merkezi sinir sistemi (MSS) tümörlerinin yaklaşık %15'ini ve primer beyin tümörlerinin de %45'ini oluşturmaktadır (6). Çocuklarda nadir görülmekte ve çoğunlukla 55-65 yaş aralığında ortaya çıkmaktadır. Hastalığın görülme sıklığı 75-84 yaşlarda zirve yaparak 85 yaştan sonra azalmaktadır (7). 2021 yılı DSÖ verilerine göre bu tümör her 100 kişiden birinde görülmekle birlikte, erkeklerde kadınlara göre daha fazla rapor edilmiştir. Bunlara ilaveten yapılan çalışmalar, GBM insidansının beyaz ırklarda artış gösterdiğini bildirmektedir (8). Literatürde hastalığı oluşturan risk faktörlerine dair kesin bir bilginin yer almamasına rağmen iyonlaştırıcı radyasyonun ilk sırada olduğu bilinmektedir (9). Sigara içme, uyuşturucu kullanma ve alkol tüketme gibi kişinin yaşam tarzını etkileyen faktörlerden dolayı GBM'e yakalanma riskini etkileyen bir kanıt henüz yoktur. GBM hastalarının klinik belirtileri, tümörün yeri ve derecesine göre değişmektedir. Bu hastalarda genellikle baş ağrısı, havale, hafıza kaybı ve davranış değişiklikleri ortaya çıkmaktadır (10). Bu belirtiler tümörün hızla büyümesi sonucu, beyin içi basıncın artmasıyla meydana gelir. Tümör ilerledikçe tümörün beyinde bası yaptığı bölgeye göre bazı beden fonksiyonlarında kayıplar olabilmektedir.

Hızlı hücresel proliferasyon, yaygın anjiyogenez ve invazyon özelliklerine karşın bu tümörler çok nadir metastaz yapmaktadır. Klinikteki tedavi, cerrahi rezeksiyonu takiben eş zamanlı kemoterapi ve radyoterapi tedavisinin uygulanmasıdır (11). Rezeksiyon, tümörü çıkartmayı veya küçülterek kanser belirtilerinden bir an önce kurtulmayı amaçlar. Fakat GBM'in maksimum cerrahi rezeksiyonu zordur. Çünkü bu tümörler sıklıkla invaziftir ve genellikle beynin konuşmayı, motor fonksiyonunu ve duyularını kontrol eden alanlarında bulunmaktadır. Buna ilaveten, GBM hücrelerinin çevre yapılara infiltre olması, anatomik pozisyonu, karmaşık damar sistemi, tümörün yeri ve sağlam beyin dokusuna zarar verme korkusu da tam rezeksiyonu zorlaştırmaktadır. Tümör rezeksiyonu sonrasında alınamayan mikroskopik bir tümör kalıntısı bile hastalığın rekürrensine neden olabilmektedir. Bu yüzden total cerrahi sonrası kalan tümör hücrelerinin prolifere olmasını önlemek için hastalara eş zamanlı radyoterapi ve kemoterapi tedavisi uygulanmaktadır (12). Radyoterapideki güncel yaklaşım; hastalara günde 2 Gray (Gy) fraksiyonda, haftada 5 gün olmak üzere toplam 60 Gy olacak şekilde 6 haftalık bir tedavi sürecini kapsamaktadır. Radyoterapi uygulamasının, GBM

hastalarında nörolojik yaşam kalitesini artırdığı bilinmektedir (13). Bu sebeple radyoterapi, glioma için önemli bir terapötik tedavi olarak kabul edilir. Fakat hastaların yaşam kalitesini iyileştirmek için tek başına radyoterapi tedavisi yerine kemoterapötik ajan olan temozolomid (TMZ) ile kombine tedavisi hastalarda daha uzun sağkalım ile sonuçlanmıştır (14). Cerrahi ve farmakolojik tedaviler gibi önemli ilerlemelere rağmen malign gliomalar, kötü bir prognoza sahiptirler. Bu hastalar klinikte uygulanan tedaviye zamanla direnç geliştirirler. Bu yüzden de hasta sağkalımı 12-15 ay ile sınırlı ve bu hastalarda 5 yıllık sağkalım oranı %6'dan azdır (2). Hastalar bu kadar sınırlı yaşam süresine sahip oldukları için klinikteki ilk amaç hastaların sağkalım süresini uzatmaya yöneliktir. Hastanın tedavisini; MSS'deki tümör konumu, heterojen hücre popülasyonu, invazif yapı, glioblastoma kök hücreleri, tümör mikroçevresi ve tedavide oluşan direnç gibi çeşitli engeller kısıtlamaktadır (15). Özellikle beyinde bulunan kan-beyin bariyeri (KBB) tedaviyi kısıtlayan en büyük engeldir. İlaçların KBB'den geçebilmeleri için genellikle 400kDa'dan küçük ve lipofilik yapıda olmaları gerekmektedir (16). Bu seçici geçirgen bariyer, ilaçların MSS'ye ulaşmasını engelleyerek kanser tedavisinin etkinliğini azaltmaktadır. Bu derlemede, beyinde bulunan KBB varlığının tedavideki etkinliği nasıl azalttığı ve bu bariyerin GBM ile ilişkisinin ne yönde olduğu güncel literatür eşliğinde ele alınacaktır.

Güncel Tedavi Yaklaşımları ve Bu Tedavilerin Zorlukları

GBM tanısı alan bireyler için etkili tedavi yöntemleri oldukça sınırlıdır. MSS'nin en sık görülen malign tümörü olan GBM'in tedavisi; maksimum cerrahi rezeksiyonu ve radyoterapi ile TMZ uygulamasını içeren multimodal yaklaşımlardan oluşmaktadır (17). Bu tedavilere rağmen; hastaların ortalama sağkalım süresi 12-15 ay ile oldukça kötü bir prognoz sergilemektedir. Son on yılda, yüksek dereceli gliomaların (III ve IV. derece) ortalama sağkalım oranlarında ilerleme kaydedilememiştir. Klinikte GBM tanısı alan hastalara ilk olarak rezeksiyon yapılmaktadır. "Nöroşirürji'' terimi ilk kez 1904 yılında kullanılmış ancak ilk glioma rezeksiyonu 1884 yılında Rickman Godlee tarafından gerçekleştirilmiştir. Nöroşirürjik tedavinin amacı, tümör kitlesinin total rezeksiyonudur; ancak bu durum, özellikle net bir sınır göstermeyen GBM'lerde her zaman başarı gösterememektedir (18). Beyin tümörleri rezeksiyonunda, belirgin inter- ve intratümöral heterojeniteden dolayı tümör sınırlarının sağlıklı beyin dokusundan ayırt edilememesi önemli bir sorundur. Glioma tedavisinin temeli, doku tanısına dayanmakta olup, histopatolojik incelemeyi tamamlamak amacıyla moleküler analizlerle desteklenmektedir. Yüksek dereceli glioma hastalarının, ortalama sağkalım süreleri; tanı anında gerçekleştirilen tümör rezeksiyonuna bağlı olarak değişim göstermektedir (17). Rezeksiyon sonrası hastalara, tümör hücrelerinin prolifere olmasını engellemek için birinci basamak tedavi olarak TMZ uygulanmaktadır. Kemoterapötik bir ajan olan TMZ, 2005 yılında ABD Gıda ve İlaç Dairesi (FDA) onayından sonra GBM tedavisinde kullanılan DNA alkilleyicidir (19). Stupp'un GBM hastalarıyla yaptığı bir çalışmada, hasta sağkalım süresinin TMZ ile radyoterapinin birlikte uygulandığı gruplarda yalnızca radyoterapi uygulanan gruba kıyasla 2,5 ay arttığı gösterilmektedir. Sonuç olarak, TMZ'nin radyoterapiyle birlikte uygulandığı kombine gruplarda hasta sağkalım süresi 12,1 aydan 14,6 aya kadar artmıştır. Buna ilaveten, iki yıllık sağkalım oranı ise %10,4'ten %26,5'e yükselmiştir (20). Fakat hastalara uygulanan TMZ ve radyoterapi sonrasında, çoğu hastada altı ay içerisinde nüks meydana gelmektedir.

Günümüzde nüks eden GBM hastaları için yeni bir tedavi yaklaşımı söz konusu değildir. Tedavi seçenekleri arasında; cerrahi müdahale, yeniden ışınlama ve sistemik tedaviler yer almaktadır. Cerrahi yaklaşım lokal olarak nükseden veya ilerleyen malign gliomalar için uygulanmakta ve bu durumda hastaların ortalama sağkalım süreleri 6 ile 17 ay arasında değişmektedir. Nüks eden GBM hastalarında, ikinci basamak tedavi olarak; bevacizumab, TMZ veya lomustin kullanılmakta ve bu tedavi seçenekleriyle sağkalım süreleri 6 ile 12 ay arasında değişim göstermektedir (21). Anti-anjiyojenik bir ilaç olan bevacizumab, 2009 yılında FDA tarafından onaylanmasının ardından, nüks eden GBM hastaları için standart tedavi olarak kabul edilmektedir (17). Buna karşılık lomustin, Avrupa Birliği'nde ikinci basamak kemoterapi olarak önerilmektedir (21). Literatürde GBM hastalarıyla yapılan bir çalışmada, lomustin ve bevacizumab alan hastalar ile yalnızca lomustin alan hastalar arasında benzer bir sağkalım süresi (yaklaşık dokuz ay) olduğu gösterilmiştir (22). Faz III çalışmalarından elde edilen sonuçlar, yeni tanı almış GBM hastalarına; eş zamanlı bevacizumab ve kemo-radyoterapi uygulandığında, hastaların progresyonsuz sağkalım sürelerinde iyileşme

sağlandığını ortaya koymuştur (23). Ancak, hastaların genel sağkalımında anlamlı bir iyileşme olmadığı görülmektedir (24). 2015 yılında FDA, değişken tümör tedavi alanları üreten bir tıbbi cihaz olan Optune (Novocure, Portsmouth, ABD)'in kullanımını GBM hastalarında onaylamıştır. Bu onay, özellikle TMZ ile kombinasyon halinde uygulandığında, yeni tanı almış GBM tedavisi için verilmiştir. Bununla birlikte, bu kombine terapinin uygulanmasına rağmen; hastaların önemli bir kısmında ilk tanıyı takiben bir ile iki yıl içerisinde tümör nüksü gözlenmeye devam etmektedir (25).

Ne yazık ki, literatürdeki mevcut veriler bu tedavilerin hasta sağkalım sürelerini uzattığına dair kesin bir kanıt sunmamaktadır. Klinikte uygulanan tüm bu tedavi seçeneklerine rağmen, hastaların TMZ'ye uzun süre maruz kalması ve GBM'in mutasyona eğilimli yapısından dolayı; bu tümörlerin TMZ'ye direnç geliştirmesi oldukça yaygındır. Gelişen bu dirençle birlikte, GBM hastaları tedaviye etkili yanıt verememektedir (26). Günümüzde TMZ'ye direnç geliştiren hastaların klinikteki tedavi seçenekleri hala sınırlıdır. Buna ilaveten, MSS'deki tümör mikroçevresi ve konumu, heterojen hücre popülasyonu, glioblastoma kök hücreleri, invazif yapı, hipoksi ve kemorezistans/radyodirenç gibi çeşitli engeller de tedaviyi kısıtlamaktadır. Özellikle beyinde bulunan KBB tedaviyi kısıtlayan en büyük engeldir. İlaçların KBB'den geçerek beyine etkili şekilde penetre olamaması; GBM tedavisinde aşılması gereken önemli bir klinik engeli teşkil etmektedir.

Kan Beyin Bariyeri Yapısı ve Fonksiyonları

MSS, tüm organ ve sistemlerin aktivitelerini düzenleyen kontrol sistemlerinden biri olarak kabul görmektedir. Beyin, organizmanın bütünlüğünü ve hayatta kalmasının sağlanması amacıyla homeostazı sürdürmede diğer organ sistemleriyle iletişim kurmaktadır. Beynin hayati önemi göz önüne alındığında, onu korumak için birçok mekanizma evrimleşmiştir. Genellikle "beyin bariyeri" kavramı; KBB, kan-beyin-omurilik sıvısı (BOS) bariyeri ve BOS-beyin bariyeri olmak üzere üç temel savunma mekanizmasını kapsamaktadır. Tüm bu koruyucu mekanizmalar arasında, KBB en belirgin ve en iyi anlaşılan yapıdır (27). Besinlerin taşınımı ve vücut ile beyin arasındaki iletişimden sorumlu olan KBB, aynı zamanda yabancı maddelerin beyine ulaşmasını engelleyen kritik bir yapıdır (2). İlk kez 19. yüzyılın sonlarında tanımlanan bu bariyer, o tarihten bu yana kapsamlı bilimsel araştırmalara konu olmuştur. Paul Ehrlich, doku boyama tekniklerini kullanarak periferik dolaşıma enjekte edilen boyaların neredeyse tüm dokulara nüfuz ettiğini, ancak beyin ve BOS'a geçmediğini gözlemlemiştir (28). Ehrlich'in öğrencisi Edwin Goldmann, 1909 ve 1913 yıllarında yaptığı çalışmalarla, tripan mavisi boyasının beyin ventriküllerine enjekte edildiğinde beyin dokusunu boyadığını, ancak intravenöz yoldan uygulandığında ise beynin veya omuriliğin boyanmadığını tespit etmiştir (27).

KBB, özelleşmiş beyin endotel hücreleri tarafından çevrelenmekte ve bu hücreler astrositler, perisitler ve perivasküler makrofajlar gibi hücrelerle birlikte "Nörovasküler Birim (NVU)" olarak adlandırılan sıkı düzenlenmiş bir yapı oluşturmaktadır (29). Bu birim, sağlıklı beyin fonksiyonlarının sürdürülebilmesi için koordineli bir şekilde çalışarak beyin çevresindeki kimyasal bileşenlerin korunmasını sağlar (30). Astrositler, endotel hücrelerinin bariyer özelliklerinin korunmasında kritik bir rol oynamaktadır. Perisitler ise endotelyumu örtmekte ve KBB'nin yapısal bütünlüğüne katkıda bulunmaktadır (29). Kapillerlerde endotelyal hücrelerin sıralanması, geniş sıkı bağlantılar oluşturarak bariyerin yapısal bütünlüğünü korumaktadır. Endotel hücreleri arasındaki bu sıkı bağlantılar, iyon kanalları ve taşıyıcı proteinler, birçok ilacın MSS'ye girişini sınırlayarak farmakolojik tedavilerin etkinliğini azaltmaktadır (31). Beyin kapillerleri, diğer damar yapılarından farklı olarak sıkı bağlantılar ve astrosit uç uzantıları ile karakterize edilmektedir. KBB'nin temel yapısal bileşenini oluşturan sıkı bağlantılar (tight junctions, TJs), endotel hücreleri arasındaki sınır bölgelerinde bulunur ve kan dolaşımındaki suda çözünen maddelerin parasellüler geçişini büyük ölçüde sınırlar. Bu mekanizma, serebral dokuların sıvı mikroortamına zararlı veya istenmeyen maddelerin girişini engellenmesinde kritik bir rol oynar. Ayrıca, kapillerlerin bazal laminasını çevreleyen, astrosit uç uzantıları (astrocytic end-feet) nöronlarla hücresel bağlantıyı sağlamaktadır.

KBB, beyin ve MSS dokularını kan dolaşımından ayıran selektif bir bariyer işlevi görmektedir. Bu bariyer, büyük moleküllerin, toksinlerin ve patojenlerin beyin dokusuna girişini engeller. Ancak, MSS hastalıklarının tedavisinde ilaçların beyin hedeflerine ulaşabilmesi için bu bariyerin aşılması gerekmektedir. Özellikle GBM gibi malign tümörlerde KBB bütünlüğü, heterojen bir şekilde korunarak ilaçların tümör dokusuna

penetrasyonunu daha da güçleştirmektedir. Bununla birlikte, GBM mikroçevresinde oluşan vasküler anormallikler, KBB'nin bazı bölgelerinde bozulmaya neden olabilmekte; bu durum, hedefe yönelik tedavilerin geliştirilmesinde önemli bir engel teşkil etmektedir (29). Bu nedenle, KBB'yi aşabilen ilaç taşıyıcı sistemler ve alternatif tedavi yöntemleri, GBM tedavisinde önemli araştırma alanları arasındadır.

Kan-Beyin Bariyerinin Glioblastoma Multiform ile İlişkisi

Beyin tümörleri arasında en agresif ve ölümcül olan GBM, beyinde bulunan KBB yapısında birtakım değişikliklere yol açmaktadır. Glioblastoma hücreleri, klinikte uygulanan tedavilere karşı direnç geliştirmektedir. Bu direncin gelişmesinde, KBB başta olmak üzere birden fazla mekanizma rol oynamaktadır. KBB, hem normal beyin dokusuna hem de tümör içine ilaç penetrasyonunu ve ilaç etkinliğini sınırlayarak tedavi direncinde önemli bir faktör olarak değerlendirilmektedir (32). Her ne kadar GBM'de KBB kısmen bozulmuş olsa da, invazif hücrelerin bulunduğu tümör mikroçevresinde bu bariyer bütünlüğünü korumaktadır. Terapötik ajanlara karşı KBB tarafından korunan bu invazif hücreler, sıklıkla tedavide direnç ve tümör nüksünün bir kaynağıdır (33). Beyin tümörlerinin infiltratif büyüme paterni, tümör mikroçevresinde anormal bir vasküler yapı oluşmasına ve bu bariyerin bozulmasına neden olmaktadır (34). Tümöral vaskülarizasyonda, perisit yoğunluğundaki azalma ve endotel hücrelerinin sıkı bağlantılarındaki disfonksiyon nedeniyle KBB'nin geçirgenliği artmaktadır. Bunun sonucunda, kan-plazma bileşenlerinin ve immün hücrelerin beyin parankimine girişinde artış gözlemlenmektedir (35). Ancak, KBB'nin bu lokalize disfonksiyonu, genellikle tümör kitlesinin yakın bölgelerinde sınırlı kalmakta ve MSS'nin diğer bölgelerinde bu bariyer bütünlüğünü korumaktadır. Bu tümörlerin infiltratif doğası ve genetik heterojenliği gibi çeşitli faktörler tedavide büyük zorluklar yaratmaktadır. GBM hastalarında tedavi sonuçlarının iyileştirilmesi için aşılması gereken en önemli engellerden biri de KBB varlığıdır (8). Tedavideki amaç, terapötik ajanların KBB'yi geçerek doğrudan tümöre ulaşmasını ve tümör hücrelerini hedef alarak bu hücrelerin yok edilmesini sağlamaktır (36).

Dolaşım sistemi ile MSS'nin ekstraselüler alanı arasında koruyucu bir sınır oluşturan KBB, özellikle beyin kapiller endotel hücrelerinin sıkı bağlantılar oluşturması, perisitlerin ve astrosit uç ayaklarının bu endotel tabakasını çevrelemesi ile karakterizedir (37). Endotel hücrelerindeki düşük pinositoz aktivitesi, özel taşıyıcı proteinlerin varlığı ve çoklu ilaç direnç proteinleri KBB'nin seçici geçirgenlik özelliklerini desteklemektedir (38). Bu yapılar hidrofobik moleküller, iyonlar ve birçok makromolekülün MSS'ye girişini sınırlandırırken; glukoz ve amino asit gibi temel maddelerin kontrollü taşınmasını sağlamaktadır (39). Literatürdeki bulgular, tümör mikroçevresindeki KBB alanında; küçük molekül yapısına sahip aday ilaçların (%98'inden fazlasının) beyin dokusuna geçişinin kısıtlı olduğunu ortaya koymaktadır. Buna ilaveten, makromoleküler yapıya sahip ajanların ise bu bariyeri aşarak terapötik doz seviyesinde tümör hücrelerine ulaşma kapasitesinin sınırlı olduğunu göstermektedir (36). KBB'nin çoğu ilaç geçişine izin vermemesi, beyin tümörlerinin tedavisinde kullanılabilecek terapötik ajanların sayısını önemli ölçüde sınırlamaktadır. Yüksek dereceli gliomalarda, tümör hücrelerinin parankimaya göç ederek infiltre olduğu peri-tümöral beyinde KBB bütünlüğünü korumakta ve bu durum ilaçların etkinliğini azaltmaktadır (40). Yapılan bir çalışma, yüksek dereceli gliomaların kontrastlı Manyetik Rezonans Görüntülemesi (MRI), bazı beyin bölgelerinde vazojenik ödemin (bozulmuş KBB ve vasküler yapı) bulunduğunu göstermektedir. Buna ilaveten, Pozitron Emisyon Tomografisi (PET) görüntüleme ve MRI ile cerrahi olarak çıkarılan tümör dokularının analiz sonuçlarına göre, gliomaların bazı bölgelerinde sağlam bir KBB'nin olduğu ortaya konmaktadır (41). Literatürde yer alan benzer bir çalışmada; U251 hücreleriyle oluşturulmuş *in-vivo* fare GBM modelinde, kontrastlı MRI'da vazojenik ödemi temsil eden belirgin bir sınır gözlemlenmiştir (42). Bu doğrultuda, GBM tedavisindeki kilit konu; ilaçların KBB'yi etkili bir şekilde geçerek, beyindeki hedeflenen bölgelere ulaşmasını sağlamaktır. Sağlam bir KBB, beyin dokusunu periferik kandan etkin bir şekilde izole ederek, beyin dokusunun iç ortam stabilitesini ve MSS'nin normal fizyolojik durumunu sürdürmede hayati bir biyolojik rol oynar. Ek olarak, beyni toksinlerden korumasına rağmen ilaçların tümörlü bölgeye geçişini engellemektedir. Sadece küçük molekül ağırlığına sahip (<400kDa) ve lipofilik karakterdeki moleküller KBB'yi aşabilmektedir.

Gliomalarda tümör progresyonu ile KBB bütünlüğü bozulmakta ve bu durum tümörün malignite derecesiyle doğrudan ilişki göstermektedir. Malignite düzeyindeki artış, artan metabolik gereksinimlerle bağlantılıdır. Ancak besin ve

oksijen yetersizliği, lokal hipoksiye yol açarak anormal anjiyogenez ve KBB disfonksiyonunu tetiklemektedir. KBB bütünlüğündeki bu değişkenliğe rağmen, lokal bozulmaların tümör dokularındaki ilaç konsantrasyonlarını belirgin şekilde etkilemediği görülmektedir (35). Bu bariyerin asıl görevi, MSS'nin homeostazını sağlamak ve zararlı maddelerin beyin dokusuna girişini engellemektir. Ancak, GBM durumunda zayıf bir yapıya sahip olan KBB'nin, artırılmış taşıyıcı protein ekspresyonu ve azalmış sıkı bağlantı proteinleri nedeniyle geçirgenliği artabilmektedir (43). Bununla birlikte, KBB'nin bozulması, tümör genelinde homojen bir şekilde gerçekleşmez; bazı bölgelerde kan damarlarının geçirgenliği daha yüksekken, bazı bölgelerde ise damarlar daha sağlam yapıda olabilir. Sonuç olarak, GBM dolaşımdaki ilaçlara karşı heterojen bir geçirgenlik sergilemektedir. Yapılan bir çalışmada, glioma hücreleriyle (GBM8401) intrakraniyal olarak oluşturulmuş in vivo fare GBM modelinde, doksorubisin içeren lipozomların tümör içindeki dağılımının, çevreleyen beyin dokusuna kıyasla daha yüksek olduğu gözlemlenmiştir. Bununla birlikte, odaklanmış ultrason uygulamasıyla ilaç birikimini artırarak KBB'nin bir alt kümesi olan kan-tümör bariyerinin (BTB) hasar görmüş olduğu gösterilmiştir (44). Literatürde yer alan farklı bir çalışmada ise GL261 hücreleriyle ortotopik olarak oluşturulmuş fare GBM modeli; kontrastlı MRI, Evans blue boyaması ve histopatolojik incelemeler ile değerlendirilmiştir. Oluşturulan fare GBM modelinde, tümör ilerledikçe KBB'de belirgin bir bozulma gözlemlenirken; Hs683 insan oligodendroglioma modelinde ise daha sınırlı bir bozulma olduğu tespit edilmiştir (45). Yapılan bir başka çalışmada, kontrastlı MRI ve immünohistokimya analizleri (GFAP, AQ4, CD31) yapılmış ve düşük/yüksek dereceli gliomalarda sağlam KBB bölgelerinin mevcut olduğu gösterilmiştir (46). Kemoterapötik bir ilaç, tümör dokusuna ekstravaze olmayı başarsa dahi; GBM hücreleri tarafından etkinlik kazanan eflüks pompalarının yukarı regülasyonu nedeniyle bu hücrelerde terapötik seviyelere ulaşması genellikle mümkün olmaz (47). Yapılan araştırmalar doğrultusunda, GBM'in önemli bir kısmında, bölgesel olarak sağlam KBB ve artmış eflüks pompa varlığının devam ettiği bilinmektedir (9). KBB, beyin tümörleri ve MSS hastalıkları dahil olmak üzere, beyin parankimine ilaç geçişi açısından araştırılması gereken konulardan biridir. Bu doğrultuda, patolojik beyin koşullarında ilaç biyoyararlanımını artırmayı hedefleyen, KBB'nin

aşılması veya bu bariyerin geçirgenliğinin modüle edilmesine yönelik çeşitli stratejiler geliştirilmelidir (29). Bu durum, hedefe yönelik tedavi yaklaşımlarının geliştirilmesinde dikkate alınması gereken öncül bir konudur.

TARTIŞMA

Yetişkinlerde en sık görülen primer beyin tümörü olan GBM, tüm MSS malignitelerinin yaklaşık %45'ini oluşturmaktadır. Tanı konulan hastaların ortalama sağkalım süresi iki yıldan kısadır ve hastaların yaklaşık yarısı, ilk yıl içerisinde yaşamını yitirmektedir (48). Günümüzde, hastalara GBM tanısı konulduktan sonra ilk olarak cerrahi rezeksiyon, ardından eş zamanlı kemoterapi ve radyoterapi tedavisi uygulanmaktadır. Yüksek dereceli beyin tümörlerinde, farmakolojik ve cerrahi tedavilerdeki önemli ilerlemelere rağmen kötü prognoz önemli bir sorun olmaya devam etmektedir. Beyinde bir arayüz olarak bulunan KBB, bu prognozun iyileştirilmesinde karşılaşılan temel engellerden biri olarak öne çıkmaktadır (49). Bu bariyer, MSS ile sistemik dolaşım arasında seçici bir bariyer oluşturarak MSS'nin homeostazını koruyan dinamik ve kompleks bir yapıdır. Anatomik ve moleküler özellikleri sayesinde, KBB bir yandan MSS'yi dış çevreden gelen potansiyel zararlı moleküllere karşı korurken, diğer yandan beyin için gerekli olan besin madde ve metabolitlerin kontrollü bir şekilde taşınmasını sağlamaktadır. Bununla birlikte, KBB'nin bu seçici geçirgenliği, terapötik moleküllerin MSS'ye etkin bir şekilde ulaşmasını büyük ölçüde sınırlamaktadır. İlaçların KBB'yi geçebilmesi için genellikle moleküler ağırlıklarının <400kDa boyutunda ve lipofilik karakterde olmaları gerekmektedir. Bu özellikler, birçok farmakolojik ajanın MSS'ye erişimini kısıtlamakta ve beyin tümörlerinin tedavisinde karşılaşılan zorlukları artırmaktadır. Bu bariyerin koruyucu niteliği, küçük moleküllü ilaçların %98'inden fazlasının yanı sıra makromoleküler ajanların büyük bir kısmının MSS'ye ulaşımını da ciddi şekilde kısıtlamaktadır. Bu durum, beyin tümörleri tedavisinde etkin farmakolojik ajanların uygulanmasını zorlaştıran temel bir engel olarak öne çıkmaktadır (29).

Glioblastomalarda kemoterapi etkinliği, tümörün lokalizasyonuna ve KBB durumuna bağlı olarak değişkenlik göstermektedir. Tümör merkezinde KBB'nin bozulması, bu bölgeye ilaçların ve kemoterapötik ajanların daha yüksek dozlarda ulaşmasını sağlarken; tümör mikroçevresinde

KBB'nin sağlam kalması ise ilaçların etkin bir şekilde dağılmasını engellemektedir. Cerrahi rezeksiyon ile çıkarılan primer kitlenin bulunduğu bölgede kalan hücreler genellikle tedaviye daha iyi yanıt verirken, KBB'nin korunduğu çevre dokularda kalan invazif glioblastoma hücreleri yeterli ilaca maruz kalamadığı için tedaviye direnç geliştirebilmektedir. Bu durum, GBM'deki doğal tedavi direncinin önemli bir nedeni olarak KBB'nin durumunu ve tümörün beyindeki lokalizasyonunu öne çıkarmaktadır.

SONUÇ

Glial tümörlerin tedavisinde karşılaşılan en büyük zorluklardan biri, KBB yapısının tam olarak anlaşılamaması ve bu bariyeri aşabilen etkili ilaçların sınırlı olmasıdır. Moleküler ağırlığı 400kDa'nın altında olan ve KBB'yi geçebilen ilaçlar, MSS tümörlerinin tedavisinde kritik bir öneme sahiptir. Bu ilaçların geliştirilmesinde, KBB'nin fizikokimyasal özellikleri ile taşıma mekanizmalarının dikkate alınması gereklidir. Buna ilaveten, KBB'yi aşmayı kolaylaştıran yenilikçi teknolojiler, MSS tümörlerine yönelik daha etkili farmakolojik yaklaşımların geliştirilmesine önemli katkılar sağlayabilir.

Çıkar çatışması: Bu makaleyle bağlantılı olarak herhangi bir çıkar çatışması yoktur. Yazarların tümü, derlemenin tasarımına, yürütülmesine ve analizine katkıda bulunduklarını ve son halini onayladıklarını beyan etmektedir.

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Araştırma Makalesi, yeni bilgiler içeren ve güncel konularda yapılmış olan orijinal çalışmaları tanımlar. Bu çalışmalar randomize kontrollü, gözlemsel, tanımlayıcı, teşhis veya tedavi doğrulayıcı, klinik, deneysel veya deney hayvanları ile yapılmış olabilirler. Kaynaklar, Öz-Abstract bölümleri ve Tablo/Şekil açıklamaları hariç, ana metin 3000 sözcük sayısını aşmamalıdır.

Olgu Sunumu, okuyucular için önemli olabilecek yeni bir bulgu veya nadir ve ilginç vaka veya durumları, tanı veya tedavi ile ilgili bir yaklaşımı içermelidir. En fazla beş yazar, Kaynaklar listesi hariç, 1000 sözcük ve 10 kaynak ile sınırlıdır. Sadece bir tablo ya da bir şekil ile desteklenebilir.

Klinik Görüntü, eğitsel önemi olduğu düşünülen, orijinal, ilginç ve yüksek kaliteli görüntü içermelidir. En fazla beş yazar, beş kaynak ve bir şekil (fotoğraf, görüntü, çizim, grafik vb.) içerebilir. Kaynaklar listesi hariç 500 kelimeyi geçmemeli, şekil alt yazısı 100 kelimeyi aşmamalıdır.

Teknik Not, eğitim, araştırma, tanı veya tedavi amaçlı gerçekleştirilmiş olan yeni ve orijinal bir uygulamayı, tekniği, alet veya cihazı tarif etmelidir. En fazla beş yazar, beş kaynak ve bir şekil (fotoğraf, görüntü, çizim, grafik vb.) veya tablo içerebilir. Kaynaklar listesi hariç 500 kelimeyi geçmemeli, şekil (varsa) alt yazısı veya tablo (varsa) açıklaması 50 kelimeyi aşmamalıdır.

Editöre Mektup, yayımlanan metinlerle veya mesleki konularla ilgili olarak 500 sözcüğü aşmayan ve beş kaynak ile bir tablo veya şekil içerecek şekilde yazılabilir. Ayrıca daha önce dergide yayınlanmış metinlerle ilişkili mektuplara cevap hakkı verilir.

Davetli Derleme Yazıları, Yayın Kurulunun daveti üzerine, tıpta özellikli konuların kapsamlı değerlendirmelerini içeren, konusunda deneyimli ve yetkin yazarların yazdığı derlemelerdir. Derleme yazıları da derginin değerlendirme sürecinden geçirilir. Kaynaklar, tablo ve şekil alt yazıları hariç 5000 kelimeyi geçmemelidir. En fazla beş yazar ve 80 kaynak ile sınırlıdır. Davetli yazılar dışında derleme yazıları kabul edilmez.

YAZININ HAZIRLANMASI

Ege Tıp Dergisine gönderilen tüm yazılar aşağıdaki kurallara uygun olarak hazırlanmalıdır.

Genel biçim

a- Metin iki satır aralıklı olarak Arial 10 punto ile yazılmalıdır,

b- Sayfa kenar boşlukları 2,5 cm olmalıdır,

c- Sayfalar başlık sayfasından başlamak üzere, sağ üst köşesinden numaralandırılmalı ve satır numaraları eklenmelidir (Microsoft Office Word™ - Düzen - Satır numaraları - Sürekli)

d- Kısaltmalar, metinde ilk olarak açık şekliyle yazılmış olanı takiben, yuvarlak parantez içinde yazılmalı ve tüm metin boyunca kısaltma aynı şekilde kullanılmalıdır. Başlık ve Öz bölümünde kısaltma kullanmaktan kaçınılmalı, metin içinde de gereksiz kısaltma kullanılmamasına özen gösterilmelidir. Cümleler kısaltma ile başlatılmamalıdır.

e- Ana metin içerisinde belirtilen ürün (ilaç, cihaz, donanım veya yazılım vb.), ürünün adını takiben, üretici şirketin adı, şehri ve ülkesi parantez içinde yazılmalıdır. Örnek: Discovery St PET / CT tarayıcı (General Electric, Milwaukee, WI, ABD).

f- Tüm ölçümlerin birimleri metrik sisteme (Uluslararası Birimler Sistemi, SI) göre yazılmalıdır. Örnek: mg/kg, µg/kg, mL/min, µL/h, mmHg, vb. Ölçümler ve istatistiksel veriler, cümle başında olmadıkları sürece rakamla belirtilmelidir.

g- Eğer varsa, uygulanan istatistiksel yöntem, Gereç ve Yöntem bölümünde belirtilmelidir.

h- Herhangi bir birimi ifade etmeyen ve 10'dan küçük sayılar ile cümle başında yer verilen sayılar yazı ile yazılmalıdır. Ondalık sayılar tam sayıdan Türkçe metinlerde virgül ile, İngilizce metinlerde nokta ile ayrılmalıdır.

i- İlgili yazı, yazı türüne göre tarif edilmiş olan bölümler şeklinde hazırlanmış olmalıdır.

Ön Yazı

Editöre hitaben yazının başlığı, yazı türü, ilgili yazının neden Ege Tıp Dergisinde yayımlanması gerektiğini özetleyen kısa bir açıklama ile sorumlu yazar belirtilerek tüm yazarların adı-soyadı, ORCID numarası, kurum ve iletişim bilgileri (telefon, e-posta ve posta adresleri) yazılmalıdır. Yazının daha önce başka bir yerde yayımlanmadığına veya yayımlanmak üzere gönderilmediğine dair yazılı ifade içermelidir. Ege Tıp Dergisi başka bir dilde dahi olsa daha önce yayımlanmış, kabul edilmiş veya değerlendirme aşamasında olan hiçbir yazıyı yayımlamayı kabul etmemektedir. Yazı yazar(lar)ın daha

önce yayımlanmış bir yazısındaki konuların bir kısmını içeriyorsa, bu durumun da ön yazıda belirtilmelidir.

Daha önce bilimsel bir toplantıda sözlü veya poster bildiri şeklinde sunulmuş olan yazılar, sunumun gerçekleştirildiği toplantı ile ilgili bilgiler (tarih, yer, toplantının ismi) olacak şekilde Ön Yazıda belirtilmeli, Öz bölümünün sonuna da not olarak yazılmalıdır.

Ana Metin

Sisteme yüklenen Microsoft Office Word™ formatındaki ana metin dosyasında yazarlara ait isim ve kurum bilgileri <u>yer almamalıdır</u>. Ana metin yazı türüne göre aşağıdaki bölümlerden oluşmalıdır:

- Araştırma Makalesi: Türkçe başlık, Öz ve Anahtar Sözcükler / İngilizce başlık, *Abstract* ve *Keywords* / Giriş / Gereç ve Yöntem / Bulgular / Tartışma / Sonuç / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Tablolar (başlıkları ve açıklamalarıyla beraber) / Şekil Alt Yazıları.

- Olgu Sunumu: Türkçe başlık, Öz ve Anahtar Sözcükler / İngilizce başlık, *Abstract* ve *Keywords* / Giriş / Olgu Sunumu / Tartışma / Sonuç / Çıkar Çatışması / Kaynaklar / Tablo (başlıkları ve açıklamalarıyla beraber) / Şekil Alt Yazısı.

- Klinik Görüntü: Türkçe başlık / İngilizce başlık / Olgu / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Şekil Alt Yazısı.

- Teknik Not: Türkçe başlık / İngilizce başlık / Teknik not / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Tablo (başlıkları ve açıklamalarıyla beraber) (varsa) / Şekil Alt Yazısı (varsa).

Yazının Başlığı

Kısa, kolay anlaşılır ve yazının içeriğini tanımlar özellikte, kısaltma içermeyecek şekilde Türkçe ve İngilizce olarak yazılmalıdır.

Özler

Türkçe (Öz) ve İngilizce (*Abstract*) başlığı altında yazılmalıdır. Araştırma Makalelerinde Amaç, Gereç ve Yöntem, Bulgular ve Sonuç (*Aim, Materials and Methods, Results, Conclusion*) olmak üzere dört bölümden oluşmalı, en fazla 250 sözcük içermelidir. Araştırmanın amacı, yapılan işlemler, gözlemsel ve analitik yöntemler, temel bulgular ve ana sonuçlar belirtilmelidir. Öz metninde kaynak numarası ve mümkün olduğunca kısaltma kullanılmamalıdır. Olgu Sunumlarında bölümlere ayrılmamalı ve 200 sözcüğü aşmamalıdır. Klinik Görüntü, Teknik Not ve Editöre Mektup için öz gerekmemektedir.

Anahtar Sözcükler

Öz (*Abstract)* bölümünün sonunda, Anahtar Sözcükler (*Keywords*) başlığı altında, bilimsel yazının ana başlıklarını yakalayan, *Index Medicus Medical Subject Headings (MeSH)*'e uygun olarak yazılmış en az üç, en fazla beş anahtar sözcük olmalıdır. Türkçe anahtar sözcüklerin, Türkiye Bilim Terimlerinden (*www.bilimterimleri.com*) seçilmesine özen gösterilmelidir.

Metin

Yazı metni, yazının türüne göre yukarıda tanımlanan bölümlerden oluşmalıdır.

Kaynaklar

Ege Tıp Dergisi, ulusal kaynaklardan yararlanmaya özel önem verdiğini belirtir ve yazarların bu konuda duyarlı olmasını bekler.

Kaynaklar metinde, tablo açıklamaları ve şekil alt yazılarında yer aldıkları sırayla, cümle içinde atıfta bulunulan ad ya da cümle bitiminde, noktadan önce yuvarlak parantez "()" içinde, Arabik rakamlarla numaralandırılmalıdır. Birden fazla kaynak numarasının belirtilmesi durumunda rakamlar birbirlerinden virgül ve bir boşluk bırakılarak ayrılmalı ardışık ikiden fazla rakam olması durumunda en küçük ve en büyük rakamlar arasına tire işareti konarak yazılmalıdır. Örnekler: (2, 5, 7); (3-7).

Dergi isimleri, *Index Medicus (PUBMED)*'de kullanıldığı şekilde kısaltılmalıdır. Kısaltılmış yazar ve dergi adlarından sonra nokta olmamalıdır. Yazar sayısı altı veya daha az olan kaynaklarda tüm

yazarların adı yazılmalı, yedi veya daha fazla olan kaynaklarda ise üç yazar adından sonra "*et al.*" veya "*ve ark.*" yazılmalıdır. Kaynak gösterilen derginin sayı ve cilt numarası mutlaka yazılmalıdır. Sayfa numaraları yazılırken başlangıç ve bitiş sayfa sayılarının sadece değişen basamakları yazılmalıdır. Örnekler: 45-48 yerine 45-8, 219-222 yerine 219-22.

Kaynaklar, yazının alındığı dilde ve aşağıdaki örneklerde görüldüğü şekilde düzenlenmelidir:

Dergilerdeki yazılar

Tkacova R, Toth S, Sin DD. Inhaled corticosteroids and survival in COPD patients receiving long-term home oxygen therapy. Respir Med 2006;100(3):385-92.

Ek sayı (Supplement)

Solca M. Acute pain management: Unmet needs and new advances in pain management. Eur J Anaesthesiol 2002;19(Suppl 25):3-10.

Erken görünümde (E-pub) makale

Butterly SJ, Pillans P, Horn B, Miles R, Sturtevant J. Off-label use of rituximab in a tertiary Queensland hospital. Intern Med J doi: 10.1111/j.1445-5994.2009.01988.x

Kitap

Bilgehan H. Klinik Mikrobiyoloji. 2. Baskı. İzmir: Bilgehan Basımevi; 1986:137-40.

Kitap bölümü

McEwen WK, Goodner IK. Secretion of tears and blinking. In: Davson H (ed). The Eye. Vol. 3, 2nd ed. New York: Academic Press; 1969:34-78.

İnternet makalesi

Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]. Available from: *www.nursingworld.org/AJN/2002/june/wawatch.htm*

Web sitesi

Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 July 2002]. Available from: *www.cancer-pain.org*

Tablolar

Tablolar metni tamamlayıcı olmalı, metin içerisinde tekrarlanan bilgiler içermemelidir. Metinde yer alma sıralarına göre Arabik sayılarla numaralandırılıp isimlendirilmelidir (örnek: Tablo-1). Tablonun üstüne tablo ismini takip eden kısa ve açıklayıcı bir başlık yazılmalıdır. Tabloda yer alan kısaltmalar, tablonun hemen altında açıklanmalıdır. Dipnotlarda sırasıyla şu semboller kullanılabilir: *, †, ‡, §, ¶.

Şekiller

Çizim, resim, grafik ve fotoğrafların tümü "Şekil" olarak adlandırılmalı ve ayrı birer dosya olarak (.jpg, .png, .tif vb., en az 300 dpi çözünürlükte) sisteme eklenmelidir. Şekil dosyaları yüksek çözünürlükte ve iyi kalitede olmalıdır. Şekiller metin içinde kullanım sıralarına göre parantez içinde Arabik rakamla numaralandırılmalıdır (örnek: Şekil-1).

Şekil Alt Yazıları

Şekil alt yazıları, şekillere karşılık gelen Arabik rakamlarla çift aralıklı olarak yazılmalıdır. Şeklin belirli bölümlerini işaret eden sembol, ok veya harfler kullanıldığında bunlar alt yazıda açıklanmalıdır. Başka yerde yayınlanmış olan şekiller kullanıldığında, yazarın bu konuda izin almış olması, bunu belgelemesi ve alt yazıda belirtmesi gerekir.

Ölçümler ve Kısaltmalar

Yazının hazırlanması bölümünde "Genel biçim" başlığı altında açıklanmıştır.

Başvuruda Yüklenecek Belgeler

- **Ön Yazı**
- **Ana Metin**
- **Yayın Hakkı Devir Formu**
- **Yazar Katkı Formu**
- **Çıkar Çatışması Formu**
- **Şekil(ler)**

REVİZYONLAR

Yazarlar makalelerinin revizyon dosyalarını gönderirken ana metin üzerindeki değişiklikleri işaretlemeli, ek olarak hakemler tarafından belirtilen önerilerle ilgili notlarını "Hakemlere Yanıt" dosyasından göndermelidir. Bu dosyada her hakemin yorumunun ardından yazarın yanıtı gelmeli ve makalede değişikliklerin yapıldığı yer de belirtilmelidir. Revize makaleler karar yazısını takip eden 21 gün içinde dergiye gönderilmelidir.

Editör Yazışmaları

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Original Articles comprise original research reporting new information on contemporary issues. These studies might be randomized controlled, observational, descriptive, methodological, clinical, experimental or animal studies. They should not exceed 3000 words excluding the abstracts and table/figure captions.

Case Reports should present an important finding, or rare, interesting case or condition or a novel approach to diagnosis or treatment. They should have a maximum of five authors and should not exceed 1000 words excluding the references and have at most 10 references. It may be accompanied by one figure or one table.

Clinical Image should include a high quality original and interesting image considered as valuable for education. It should have at most five authors, five references and a figure (photography, image,

drawing, graphic, etc.). It should not exceed 500 words excluding the references and the figure caption should not exceed 100 words.

Technical Note should describe a new and original application, technique, tool or device developed for educational, research, diagnostic or curative purposes. It should have at most five authors, five references and a figure (photography, image, drawing, graphic, etc.) or a table. It should not exceed 500 words excluding the references and if present, the figure or table caption should not exceed 50 words.

Letter to the Editor should not exceed 500 words. Short relevant comments on published articles, medical and scientific issues, particularly controversies, having no more than five references and one table or figure are encouraged. Where letters refer to an earlier published paper, authors will be offered the right to reply.

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a- The text should be doubled-spaced and typed in Arial 10 points,

b- Page margin width should be 2.5 cm,

c- All pages should be numbered consecutively in the top right-hand corner and line numbers must be added beginning with the title page.

d- Abbreviations should first be stated openly, followed by the abbreviation in () brackets and the same abbreviation should be used throughout the text. Abbreviations should be avoided in the Title and Abstract and care should be given to prevent unnecessary abbreviations. Sentences should not start with abbreviations.

e- Products (drug, device, hardware or software etc.) mentioned in the main text should be presented as product name followed by manufacturer, city and country in brackets. E.g. Discovery St PET / CT scanner (General Electric, Milwaukee, WI, ABD).

f- All measure units should be according to the metric system (International System of Units, SI). E.g. mg/kg, µg/kg, mL/min, µL/h, mmHg etc. Measures and statistical data should be presented with numbers unless at the beginning of the sentence.

g- If applied, the statistical methods should be stated in the Materials and Methods section.

h- All numbers smaller than 10 not representing a unit should be written as words. Decimals should be separated by points in English texts and by commas in Turkish texts.

i- The text should be organized under the headings described for the relevant types of manuscript.

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The title page of the main text (comprising only the Turkish and English titles of the manuscript) submitted in Microsoft Office Word™ format should not include the names and institutions of the authors. The main text should have the following sections, according to the type of the manuscript:

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- Case Reports: Turkish title, abstract and keywords / English title, abstract and keywords / Introduction / Case Report / Discussion / Conclusion / Conflict of Interest / (if present) Acknowledgements / References / Tables (with captions and notes) / Figure captions.

- Clinical Image: Turkish title / English title / Case / Conflict of Interest / References / Figure captions.

- Technical Note: Turkish title / English title / Technical note / Conflict of Interest / (if present) Acknowledgements / References / Tables (with captions and notes) / Figure captions.

Title

The title should be short, easy to understand and must define the contents of the article and should be written both in Turkish and English.

Abstracts

Abstract should be in both English and Turkish and should consist "Aim, Materials and Methods, Results and Conclusion" in original articles. The purpose of the study, the setting for the study, the subjects, the treatment or intervention, principal outcomes measured, the type of statistical analysis and the outcome of the study should be stated in this section (up to 250 words). Abstract should not include reference. In Case Reports abstracts should not be divided into sections and do not exceed 200 words. Clinical Image, Technical Note and Letter to the Editor are not required abstracts.

Keywords

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Online article not yet published in an issue

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Book

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Chapter in a book

McEwen WK, Goodner IK. Secretion of tears and blinking. In: Davson H (ed). The Eye. Vol. 3, 2nd ed. New York: Academic Press; 1969:34-78.

Journal article on the Internet

Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]. Available from: *www.nursingworld.org/AJN/2002/june/wawatch.htm*

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Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 Jul 2002]. Available from: *www.cancer-pain.org*

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The source of financial grants and the contribution of colleagues or institutions should be acknowledged.

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