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The Relationship Between Depression and Domestic Violence Towards Women

Kadına Yönelik Aile İçi Şiddet ve Depresyon Arasındaki İlişki

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ABSTRACT

Objective: This study was conducted to determine the relationship between depression and domestic violence toward women.

Materials and Methods: This cross-sectional study was carried out with 464 married women. A Descriptive Information Form, the Domestic Violence Scale and Beck Depression Inventory were used as the data collection instruments. In the statistical analysis, frequencies, percentages, means, ANOVA, t-test, correlation analysis and regression analysis were utilized.

Results: It was determined that the mean score for domestic violence of women was 42,58±15,96 and the mean score for depression was 10,32±8,92. There was a positive and significant relationship between domestic violence and depression (p=0.000). Factors such as the age of the woman and her spouse, status of being subjected to violence, employment status, educational level and form of marriage were determined as factors that were effective on domestic violence and depression.

Conclusions: It was determined that the level of domestic violence and depression levels of women were mild. In line with this result, it is necessary to create social policies regarding the problem of violence, develop awareness among healthcare professionals, and healthcare professionals need to take responsibility regarding violence in their professional lives.

Keywords: Depression, domestic violence, woman

ÖZ

Amaç: Bu araştırma kadına yönelik aile içi şiddet ve depresyon arasındaki ilişkiyi saptamak amacıyla yapıldı.

Materyal ve Metot: Kesitsel tipte yapılan araştırma 464 evli kadın ile yürütüldü. Veri toplama aracı olarak kadınları tanıtıcı anket formu, aile içi şiddet ölçeği ve depresyon ölçeği kullanıldı. İstatistiksel analizde sayı, yüzde, ortalama, ANOVA, t testi, korelasyon ve regresyon analizleri kullanıldı.

Bulgular: Kadınların aile içi şiddet puan ortalamasının 42,58±15,96 depresyon puan ortalamasının ise 10,32±8,92 olduğu belirlendi. Aile içi şiddet ile depresyon arasında pozitif yönde anlamlı bir ilişki olduğu saptandı (p=0,000). Kadının ve eşinin yaşı, şiddete maruz kalma durumu, çalışma durumu, eğitim düzeyi, evlenme şekli gibi faktörlerin aile içi şiddet ve depresyon üzerinde etkili değişkenler olduğu belirlendi.

Sonuç: Kadınların aile içi şiddet düzeyi ile depresyon seviyelerinin hafif düzeyde olduğu saptandı. Bu sonuç doğrultusunda şiddet sorununa yönelik sosyal politikaların oluşturulması, sağlık profesyonellerinde farkındalık geliştirilmesi ve sağlık profesyonellerinin mesleki yaşamlarında şiddete yönelik sorumluluk alması gerektiği öngörülmektedir.

Anahtar Kelimeler: Aile içi şiddet, depresyon, kadın

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INTRODUCTION

While its forms and prevalence vary, domestic violence towards women is a universal problem experienced by women worldwide, which is an infringement of human rights and freedom.[1] In the report published by the World Health Organization (WHO), it is stated that approximately one-third of women experience physical and/or sexual violence throughout their lives.[2] According to WHO, their partner inflicts most of this violence. WHO reported that the prevalence of violence in developed countries is 23.2%, while it is approximately 37% in developing countries.[3] In Türkiye, 44% of women experience emotional, 36% experience physical and 12% experience sexual violence.[4]

It is stated that factors such as low educational level, witnessing domestic violence, and childhood exposure to abuse and violence influence women's vulnerability to higher rates of domestic violence.[2] Research in Turkey shows that domestic violence occurs in the form of male violence against women and is more common.[5] In Türkiye, which has a patriarchal social structure, the risk of women experiencing violence increases even more. It is known that patriarchal culture is a factor that legitimizes domestic violence.[6] Women between the ages of 15-49 constitute a risk group regarding many factors that threaten health. One of these factors is depression. Depression affects women twice as much as men.[7] A study conducted in our country determined that 36.4% of women between the ages of 15-49 had depression, and there was a significant relationship between exposure to violence and depression.[8] It is stated that exposure to violence leads women to experience problems that affect their physical, mental, sexual and reproductive health negatively. At the top of these problems, physical and mental behavioral changes, hopelessness, and depression, which accompany cognitive function and mood deteriorations, have a significant place.[4,9-11] Furthermore, WHO reported that women who experience violence have 2 times higher rates of depression than those who do not experience violence.[3]

Likewise, the prevalence of depression in the world has been determined as 4.4%, and it is considered that domestic violence, a stressful life event, is one of the most significant risk factors for depression.[1,12-15]

In the study, it was aimed to determine the relationship between depression and domestic violence toward women.

MATERIALS AND METHODS

Ethics Committee Approval: The ethical approval for the study was obtained. This study was conducted to determine the relationship between depression and domestic violence towards women at the Health Sciences Scientific Research and Publications Ethics Board of Inonu University (Date: 17/03/2020, decision no: 2020/549). All procedures have been carried out in accordance with the Helsinki Declaration.

Study Design and Participants: This cross-sectional study was conducted to determine the relationship between domestic violence toward women and depression.

The study was conducted with married women registered at Family Health Centers (FHC) located in the city center of a province in eastern Turkey. The population covered all women registered at the FHCs in this city center. In contrast, the sample consisted of 464 married women who agreed to participate in the study and met the inclusion criteria. In calculating the required sample size, the sample size with unknown population formula was used, and the values of the research article published by Tel et al. in 2019 (p: 0.305, q: 0.695) were taken as a reference.[16] According to this formula, the required sample size was a minimum of 326 women. The inclusion criteria were: Being a woman and married and having no communication problems.

Data Collection: A Descriptive Information Form, the Domestic Violence Scale and the Beck Depression Inventory were used in the study as the data collection instruments. This form that was prepared by the researchers based on the literature review consisted of 10 questions (age, gender, age of marriage, education level, etc.).

Domestic Violence Scale: The 30-item scale form developed by Çetiner in 2006 to determine the dimensions and conditions of violence experienced in the family was tested for validity and reliability by İdiz.[17] It is a 5-point Likert-type scale. The scale is coded as 1 ‘never,’ 2 ‘rarely,’ 3 ‘sometimes,’ 4 ‘often,’ and 5 ‘always.’ There is no reverse-scored item in the scale. The Cronbach’s alpha coefficient of the scale was reported as 0.95.[17]

Beck Depression Inventory (BDI): In the Turkish validity and reliability inventory study by Hisli in 1989, the Beck Depression Inventory consists of 21 items and four options for each item. Each item is scored in the range of 0-3, while the total score range is 0-63. The cutoff point of the Turkish form of the inventory was determined as 17.[18]

Data analysis: The statistical analyses of the data of the study were performed by using the SPSS 22.0 (Statistical Package for the Social Sciences) package software. Frequencies, percentages, and means were used as descriptive statistics, while ANOVA, t-test, correlation, and regression analyses were used to compare the scales and sociodemographic characteristics. The results were accepted as statistically significant in a confidence interval of 95% and a signif-

icance level of p<0.05.

RESULTS

Among the women who participated in the study, 25.4% were high school graduates, 70.5% were not employed, the spouses of 91.8% were employed, 60.3% had arranged marriages, the husbands of 69.8% called the shots at home, 79.5% were satisfied with their husbands, their mean age was 36.54±9.32 years, the mean age of their husbands was 40.35±9.83 years, and their mean duration of marriage was 14.91±10.90 years. The women's mean score on the Domestic Violence Scale was 42.58±15.96, while their mean Beck Depression Inventory score was 10.32±8.92 (Table 1).

As the level of domestic violence increased, the probability of depression in women increased. As the age of the woman, the period of her husband, and the duration of marriage increased, their levels of being subjected to violence and depression levels increased (p<0.05) (Table 2).

Table 1. Descriptive characteristics and mean scale scores of the women.

Characteristic		n (%)
Education level	Illiterate	32 (6.9)
	Primary school	123 (26.5)
	Secondary school	82 (17.7)
	High school	118 (25.4)
	University and higher	109 (23.5)
Employed	Yes	137 (29.5)
	No	327 (70.5)
Spouse employed	Yes	426 (91.8)
	No	38 (8.2)
Form of marriage	Arranged marriage	280 (60.3)
	Dating marriage	184 (39.7)
Exposed to violence from spouse	Yes	90 (19.4)
	No	374 (80.6)
Who calls the shots at home?	Me	140 (30.2)
	My husband	324 (69.8)
Satisfied with spouse	Yes	369 (79.5)
	No	95 (20.5)
		X±SD
Age		36.54±9.32
Age of the spouse		40.35±9.83
Years of marriage		14.91±10.90
Domestic Violence Scale		42.58±15.96
Beck Depression Inventory		10.32±8.92

Table 2. Correlation analysis between the scales and some sociodemographic characteristics.

	Age r	p	Spouse age r	p	Duration of marriage r	p	Depression r	p
Domestic Violence Scale	0.156**	0.001	0.173**	0.000	0.160**	0.001	0.582**	0.000
BDI	0.219**	0.000	0.223**	0.000	0.202**	0.000	-	-

**: p< .01; r: bivariate correlation; BDI: Beck Depression Inventory .

As the participants' education levels decreased, their domestic violence exposure levels and depression levels increased (p<0.05). Both the domestic violence exposure and depression levels of the women who were not employed were higher in comparison to the women who were employed (p<0.05). The domestic violence exposure and depression levels of the women whose husbands were not employed were higher than those whose husbands were employed (p<0.05). The domestic violence exposure and depression levels of the women who had arranged marriages were higher than those who got married after dating (p<0.05). Those who were exposed to violence from their spouses had higher scores on the Domestic Violence Scale and Beck Depression Inventory than those who were not exposed to violence (p<0.05). Those who were not satisfied with their spouses had higher scores on the Domestic Violence Scale and Beck Depression In-

ventory than those who were satisfied with their spouses ($p<0.05$) (Table 3).

As a result of simple linear regression analysis, a significant relationship ($R = 0.68$; $R^2 = 0.46$; $p<0.01$) was found between the sociodemographic characteristics of women's domestic violence. A significant relationship ($R = 0.56$; $R^2 = 0.32$; $p<0.01$) was found between the sociodemographic characteristics of the women and beck depression. It was determined that the sociodemographic characteristics of the women (age, spouse age, educational level, duration of marriage, exposure to violence from spouse, employment status, spouse employment status, who calls the shots at home, a form of marriage, wanting to marry the same person if she were born again) who participated in the study were cumulatively effective on domestic violence by 46% and depression by 32% (Table 4).

Table 3. Comparison of sociodemographic characteristics and Domestic Violence Scale and Beck Depression Inventory Scores.

		Domestic Violence			Depression		
		X±SD	Test statistic	p	X±SD	Test statistic	p
Education level	Illiterate	49.63±18.51	2.859[a]	0.023*	15.41±10.79	6.640[a]	0.000**
	Primary school	44.22±15.54			12.46±9.11		
	Secondary school	40.96±12.92			9.51±8.47		
	High school	42.47±17.37			8.86±7.53		
	University and higher	39.98±15.61			8.61±8.94		
Employed	Yes	41.26±16.99	-1.148[b]	0.251	8.88±8.03	-2.260[b]	0.024*
	No	43.13±15.51			10.93±9.21		
Spouse employed	Yes	41.72±14.84	-3.952[b]	0.000**	9.76±8.43	-4.668[b]	0.000**
	No	52.24±23.56			16.66±11.61		
Form of marriage	Arranged marriage	44.09±16.27	2.530[b]	0.012*	11.82±9.22	4.544[b]	0.000**
	Dating marriage	40.28±15.24			8.05±7.94		
Satisfied with spouse	Yes	38.29±10.25	-13.413[b]	0.000**	8.33±7.02	-10.548[b]	0.000**
	No	59.22±22.18			18.06±1109		

[a:] ANOVA; [b:] t-test; *: $p< 0.05$ **: $p<0.01$.

Table 4. Regression analysis of the sociodemographic characteristics of the women and their domestic violence and depression characteristics.

Model	Domestic Violence Scale					Beck Depression Inventory				
	Unstandardized Coefficients		Standardized Coefficients			Unstandardized Coefficients		Standardized Coefficients		
	B	Std Error	Beta	t	p	B	Std Error	Beta	t	p
(Constant)	46.817	8.198		5.711	0.000	11.321	5.164		2.192	0.029
Age	0.086	0.206	0.050	0.415	0.678	0.200	0.130	0.209	1.539	0.125
Spouse age	-0.008	0.191	-0.005	-0.042	0.966	-0.067	0.120	-0.074	-0.556	0.578
Education level	-0.013	0.622	0.001	-0.020	0.984	-0.417	0.392	-0.060	-1.065	0.287
Duration of marriage	-0.092	0.130	-0.063	-0.707	0.480	-0.122	0.082	-0.149	-1.486	0.138
Exposure to violence from spouse	-17.668	1.533	-0.438	-11.522	0.000	-7.019	0.966	-0.311	-7.266	0.000

B: unstandardized coefficients; Std Error: standard error; Beta: standardized coefficients; R^2: determination coefficient; F: Anova; $p<0.05$.

Table 4. Continue.

Model	Domestic Violence Scale					Beck Depression Inventory				
	Unstandardized Coefficients		Standardized Coefficients			Unstandardized Coefficients		Standardized Coefficients		
	B	Std Error	Beta	t	p	B	Std Error	Beta	t	p
Employment status	-1.068	1.342	-0.031	-0.796	0.427	0.143	0.845	0.007	0.170	0.865
Spouse employment status	2.489	2.104	0.043	1.183	0.238	3.121	1.325	0.096	2.355	0.019
Who calls the shots at home	4.092	1.217	0.118	3.362	0.001	0.291	0.767	0.015	0.379	0.705
Form of marriage	0.502	1.445	0.015	0.347	0.729	-1.297	0.910	-0.071	-1.424	0.155
Wanting to marry the same person if she were born again	14.739	1.481	0.373	9.952	0.000	6.614	0.933	0.299	7.090	0.000
	R=0 .68	R^2= 0.46	F=39.520		p= 0.000	R=0 .56	R^2=0 .32	F= 21.454		p=0 .000

B: unstandardized coefficients; Std Error: standard error; Beta: standardized coefficients; R^2: determination coefficient; F: Anova; $p<0.05$.

DISCUSSION AND CONCLUSION

Domestic violence is a significant societal problem that may lead to problems in social life and depression by negatively affecting individuals' mental health. Most of those exposed to violence are women. In women exposed to domestic violence, obstacles, disappointments, reductions in self-respect, and traumas are among the top factors preceding depression.[19-21] This study was conducted to determine the relationship between domestic violence toward women and depression, and the results are discussed here in light of the relevant literature.

In the literature, it has been determined that women who are inflicted violence by their partners are more at risk of psychological symptoms such as depression and anxiety.[19,21-23] It was determined that the domestic violence exposure and depression levels of the women who participated in this study were low. Some sociodemographic characteristics affected depression by 32% and domestic violence by 46%. Hsieh and Shu[24] found that demographic variables were effective on depression by 59.5%, while Cao et al.[25] determined that they were effective on violence by around 35%. Kavak et al.[26] found the levels of domestic violence among women high. While there have been differences in the rates of exposure to violence in previous studies, it is seen that women are exposed to violence, and depressive symptoms accompany this situation.[27,28] In this study, it was observed that, as domestic violence increased, depression also increased. The study has reported that depression is observed among women with a history of violence.[29] Domestic violence is an important global problem. Women who are exposed to violence distance themselves from those around them due to the phenomenon of violence, and they may adopt a more isolated lifestyle. It is thought that this situation will lead women to experience more depression. Moreover, women subjected to violence feel weak, valueless, forgotten, and unimportant. The emotions they experience against violence, like anger and helplessness, lead them to share intense depressive feelings.[11]

In this study, it was determined that both the domestic violence and depression levels of the illiterate women, those who were not employed, those whose spouses were not used, those who got arranged marriages, those who were exposed to violence, and those who were not satisfied with their spouses were higher. In the study by Akalın and Arıkan[11], it was observed that women who married with arranged marriages experienced more depressive symptoms, domestic violence was more frequent, and depressive symptom rates were higher in the groups with low education and income levels. Another study has reported that women who have arranged marriages experience more depressive symptoms.[11] It is thought that having an arranged marriage increases violence and depression rates as it is a situation that prevents the woman from deciding on one of the most important aspects of her life, makes her dependent, neglects her autonomy, and harms her confidence.[11] Higher education levels increase the coping skills of individuals and allow development of their sense of control. This way, women can find the power to fight against violence in themselves, and depression rates decrease.[9]Additionally, high education levels may increase women's opportunities of finding a job and make an economic contribution.[9,25] It was reported that a person's employment status affects their mental health and well-being.[29] Studies have determined a relationship between education levels and depression symptoms, and it has been found that depressive symptoms are higher among groups with low education levels, and as education levels increase, the rates of depressive symptoms

decrease.[11,27] Similarly, Cao et al. stated that low education levels and unemployment of family members increased the likelihood of practicing violence among family members.[25]

In conclusion, it was determined that both the domestic violence and depression scores of the participants were low, and the mean depression score of the women who were exposed to violence was higher than those who were not. Women may hide an event of violence in the family due to environmental pressure, family pressure, and economic problems. It is believed that ensuring that this violence is identified by women and increasing their awareness to a level on which they can access the required help will contribute to their healthier mental state. Essential duties fall upon healthcare professionals in this matter. It is thought that increasing the awareness of women against emotional states such as fear, lack of confidence, and helplessness and life events and arranging policies to prevent violence as a country will carry great importance in preventing violence. A limitation of the current study concerns the generalizability of results. The results can be generalized to the population and region of the study.

Ethics Committee Approval: This study was approved by the Health Sciences Scientific Research and Publications Ethics Board of Inonu University (Date: 17/03/2020, decision no: 2020/549).

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Evaluation of Healthcare Workers Infected with COVID-19: A Qualitative Research on the Disease Process and Its Effects

COVID-19 Geçiren Sağlık Çalışanlarının Değerlendirilmesi: Hastalık Süreci ve Etkileri Üzerine Nitel Bir Araştırma

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ABSTRACT

Objective: This study aimed to investigate the impacts of COVID-19 on healthcare workers and their experience of the disease.

Materials and Methods: The research was conducted using qualitative methods between December 12, 2020 and February 5, 2021 with 30 health workers who had recovered from COVID-19. The research data were collected using semi-structured interviews during which participants were asked about their socio-demographic characteristics, their experiences of the disease, and the impacts of the disease. The data obtained were grouped according to themes and sub-themes.

Results: The participants identified the emotional, cognitive, and behavioral effects of the disease, including emotional responses at different stages of the disease, attitudes towards the disease, changes in dietary style, overattention to symptoms and vital findings, and changes in prevention measures after recovery from the disease. Uncertainty, quarantine periods, fear of infecting others, stress factors such as healthcare worker identity and stigma were also identified by the participants.

Conclusions: Providing support for the protection of the mental health of healthcare workers who contract the disease is essential to allow them to continue providing quality patient care. We think it would be beneficial to conduct further research on the nature of psychological support for medical personnel who experience COVID-19.

Keywords: COVID-19 virus, medical staff, mental health, pandemic, qualitative research

ÖZ

Amaç: Bu çalışma, COVID-19'un sağlık çalışanları üzerindeki etkilerini ve hastalık deneyimlerini araştırmayı amaçlamıştır.

Materyal ve Metot: Araştırma, 12 Aralık 2020 - 5 Şubat 2021 tarihleri arasında COVID-19'dan iyileşen 30 sağlık çalışanı (23 doktor ve 7 hemşire) ile kalitatif yöntemler kullanılarak gerçekleştirilmiştir. Araştırma verileri, katılımcılara sosyo-demografik özellikleri, hastalık deneyimleri ve hastalığın etkileri hakkında sorular sorulan yarı yapılandırılmış görüşmeler kullanılarak toplanmıştır. Elde edilen veriler temalara ve alt temalara göre gruplandırılmıştır.

Bulgular: Katılımcılar; hastalığın farklı evrelerinde duygusal tepkiler, hastalığa karşı tutumlar, beslenme tarzındaki değişiklikler, semptomlara ve hayati bulgulara aşırı dikkat ve hastalıktan iyileştikten sonra hastalığı önleme tedbirlerindeki değişiklikler dahil olmak üzere hastalığın duygusal, bilişsel ve davranışsal etkilerini tanımladılar. Katılımcılar tarafından ayrıca belirsizlik, karantina süreleri, başkalarına bulaştırma korkusu, sağlık çalışanı kimliği ve damgalanma gibi stres faktörlerini de belirtildi.

Sonuç: Hastalığa yakalanan sağlık çalışanlarının ruh sağlığının korunmasına destek verilmesi, kaliteli hasta bakımı sunmaya devam edebilmeleri için esastır. COVID-19 yaşayan sağlık personeline yönelik psikolojik desteğin niteliği konusunda daha fazla araştırma yapılmasının faydalı olacağını düşünüyoruz.

Anahtar Kelimeler: COVID-19 virüsü, nitel araştırma, pandemi, ruh sağlığı, sağlık çalışanları

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INTRODUCTION

The COVID-19 pandemic has placed a heavy burden on healthcare systems around the world and has left most healthcare systems and healthcare workers under great pressure.[1,2] In the face of this pandemic, healthcare workers are under both mental and physical stress. Healthcare professionals, particularly those working on the front line with suspected or confirmed COVID-19 patients, may be vulnerable to both a high risk of infection and mental health problems. Many fears contracting the disease or transmitting the virus to their families, friends, and colleagues.[3] The previous literature reports mental health problems in healthcare workers during and after outbreaks, including symptoms of PTSD, burnout, depression, and anxiety, which are attributed to occupational factors.[4-6]

A cross-sectional study from China that included 1257 healthcare workers from 34 hospitals reported symptoms of depression in 634 participants (50.4%), anxiety in 560 (45%), insomnia in 427 (34%), and distress in 899 (72%). Moreover, all mental health symptom measures were higher in women than men, in nurses than doctors, in those working in Wuhan than other cities, and those working on the front line in the diagnosis and treatment of COVID 19 compared to other healthcare professionals.[7]

In order to improve response measures and address the psychological impact of the COVID-19 pandemic, it is necessary to examine psychosocial data from the whole population, vulnerable groups, and COVID-19 patients.[8] Several qualitative studies have been performed concerning the experiences of COVID-19 patients and their caregivers,[9-11] but there are no studies concerning the experiences of healthcare workers who contracted COVID-19.

This study aimed to investigate the experiences of healthcare workers who contracted COVID-19 and the impact of the illness through interviews and to provide new data to support future targeted interventions.

MATERIALS AND METHODS

Ethics Committee Approval: The study was carried out under the Helsinki declaration and was approved by Sakarya University Local Ethics Committee (Date: 01.12.2020, decision no:585).

Research Design: In this study, Colaizzi's phenomenological method was used to qualitatively analyze healthcare workers', who have had COVID-19, experiences and its effects. Colaizzi's phenomenological method focuses on the experience and feelings of participants. It finds shared patterns rather than individual characteristics in the research subjects.

Study Subjects: By using a purposeful sampling method, we selected 30 health workers (23 physicians and seven nurses) who had recovered from COVID-19. Inclusion criteria were as follows: (1) COVID-19 patients were diagnosed according to the "Republic of Türkiye Ministry of Health COVID-19 guide"; (2) Patients who volunteered to participate. We determined the number of required respondents by interviewing health workers who met the inclusion criteria until the data were saturated, and no new topics were generated.

Interview Outline: Semi-structured questionnaire form to be used in the interview was prepared by taking expert opinions and with preliminary tests, after the relevant literature was reviewed. Participants' age, gender, marital status, years of work experience before the interview, hospitalization was obtained at the start of the interview. The main interview questions posed to the participants were as follows:

- Can you tell me how you felt during the time you had COVID-19?
- Can you tell me about the positive and negative effects the disease had on you, if any?
- Can you tell me about the changes that occurred in your life during or after your illness?
- Can you tell me about how you coped with having the disease?
- Can you tell me about stressors that you encountered during your illness?

Data Collection: The purpose and significance of the research was explained to the participant in advance and a suitable meeting time was planned. The interviews were made by video conference method due to pandemic conditions. Researchers used techniques such as unconditional acceptance, active listening, and clarification to promote the authenticity of the data and to avoid bias. With the permission of the participants, audio recordings were obtained in all interviews. The interviews took 40-60 minutes per person. The audio recordings were transcribed verbatim by researchers within 48 hours of the interviews and reviewed by the interviewers for accuracy. During data analysis, all researchers agreed with the results and chose the highlighted quotations.

Data Analysis: Within 48 hours of each interview, the recording was transcribed and analyzed by Colaizzi's phenomenological analysis method. Three researchers independently reviewed the interview materials, summarized, and extracted meaningful statements, and formulated the themes. Conflicting opinions on the contents of a theme were discussed and resolved by a research group.

RESULTS

The research included 30 healthcare workers (16 females, 14 males) who had a history of COVID-19

infection. The average age was 34.33±7.62 years. Among the participants, 23 were physicians and 7 were nurses. The average work experience was 11.06±9.14 years. Seven were unmarried, 17 married with children, and 6 married without children. Seven subjects were hospitalized due to COVID-19.

We explored the disease process and its effects of healthcare workers with COVID-19 using phenomenological methods. Five theme categories emerged from analysis of the interviews. These themes and sub-themes are displayed in Table 1.

Table 1. Themes identified through interviews with healthcare workers **.**

Theme 1: Emotional, Cognitive, and Behavioral Responses	
Subtheme	**Quotations**
Emotional responses during different stages of the disease	*(Female, 48 years, physician): "I was very upset, scared, and pessimistic… I felt helpless… as I started to recover, I started to feel more at ease… I was so happy when my follow-up test came back negative; knowing that I was free of disease felt great."* *(Female, 33 years, physician): "… At first I felt really bad… I had severe anxiety… I was afraid I might die… as I recovered and saw that I was getting better, I started to calm down… I was very happy when my test came back negative…"*
Attitude toward the disease	*(Female, 25 years, physician): "I started to watch what I eat and tried to stay positive… I just had to accept the situation and do my part."* *(Female, 33 years, physician): "… I just faced the truth… I took the medication and waited to recover…"* *(Female, 28 years, physician): "I couldn't conceive that I had COVID-19… I just did whatever it took to get better."*
Changes in diet	*(Female, 33 years, physician): "… I made a big change to my diet… I was really careful to have regular meals… I was careful to eat more fruit… I was careful to eat fruits rich in vitamin C. I tried to eat everything that I thought was healthy."* *(Male, 43 years, physician): "… when I was sick, I forced myself to drink more water, even though I wasn't thirsty. I didn't have much appetite and I couldn't eat all that much, but I didn't wait to feel hungry to eat… when it was time to eat, I tried to eat a lot of protein."*
Excessive attention to signs and symptoms	*(Female, 25 years, physician): "I was constantly checking to see if I was feeling short of breath, if my cough was too severe… I was constantly watching my oxygen saturation… I felt cold and immediately took my temperature to see if I had a fever."* *(Female, 29 years, physician): "I was constantly checking if my cough or dyspnea got worse or better… I often measured my blood oxygen saturation."*
Changes in prevention measures	*(Male, 34 years, physician): "… I am now more careful about preventive measures… I pay closer attention…"* *(Male, 38 years, physician): "… I've become too careful about everything. I am more aware of prevention."* *(Male, 30 years, nurse): "I became more careful. I am careful to wear my mask at all times."*
Theme 2: Stress Factors	
Subtheme	**Quotations**
Uncertainty	*(Female, 32 years, physician): "… I was worried because I did not know how my prognosis would be… I was thinking 'Anything can happen'."* *(Male, 46 years, physician): "I was worried and constantly thinking about when my test would come back negative, when I would fully recover, if I could resume my normal life after the quarantine… the prognosis of the disease is uncertain, and the lack of a definitive treatment is also worrisome…"*
Quarantine	*(Female, 34 years, nurse): "… you want it to end as soon as possible so you can go back to being with other people… Friends, relatives, buddies, they can't come and visit you when you are sick. You have to stay away from your kids. It's tough. It's upsetting."* *(Female, 28 years, physician): "… you feel like you need help, but your loved ones cannot be with you."*

Table 1. Continue.

Fear of infecting others	*(Male, 32 years, physician): "I feared that I might have infected my wife and my colleagues at the hospital." (Female, 41 years, nurse): "I had just seen my father before I tested positive… I was really afraid that I infected him… I kept calling him and seeing if he was OK. It was really hard" [crying as she was speaking].*
Being a healthcare worker	*(Female, 33 years, physician): "It was definitely worse that I knew what could happen down the road… Having worked in a hospital made it tougher on me… I kept having images of patients in poor condition flash before my eyes. I always imagined the worst-case scenario." (Female, 42 years, physician): "I feel like I would not be this worried about being sick if I wasn't a healthcare worker or if I didn't know this much about the disease."*
Stigma	*(Female, 28 years, physician): "My colleagues avoided me for a while after I got better. They didn't want to be near me even with a mask." (Female, 33 years, physician): "After I went back to work, my colleagues were avoiding me. They didn't want to eat in the same room as me.*
Theme 3: Sources of Motivation	
Subtheme	**Quotations**
Social support	*(Female, 29 years, physician): "My family and friends were my pillars during my sickness… One teacher from the clinic constantly remained in contact with me and closely monitored my condition." (Female, 28 years, physician): "My family and friends always called and asked how I was doing… I got great support from people I never expected. They brought me all kinds of food so many times."*
Self-support	*(Female, 34 years, nurse): "… I kept myself busy with housework. I tried to get things done, things that I kept putting off because I had to work. When I focused on doing things, I was distracted from thinking about my illness." (Female, 41 years, nurse): "… it was really good for me to do other things… there was an online convention and attending that made me feels much better."*
Religious beliefs	*(Female, 34 years, nurse): "I believe that the disease is from Allah, and after all, no matter what, Allah is testing us, and all living things will one day die. These thoughts affected me positively." (Female, 32 years, physician): "… As I lay in bed, I constantly tried and prayed to Allah." (Female, 28 years, physician): "I prayed constantly. My belief in Allah gave me strength because I knew He was omnipotent and could heal me."*
Being a healthcare worker	*(Male, 32 years, physician): "Being acquainted with the physicians working in the hospital, having easy access to information and medical support, if needed, helped me feel more at ease. Also, it was comforting to be aware of possible prognoses and being able to take precautions accordingly." (Male, 26 years, physician): "Being a healthcare worker, I guess I was a little more at ease during my illness compared to others. I thought, even if I should end up being hospitalized, I needn't worry since my friends at the hospital will support me the best they can."*
An effective healthcare system	*(Female, 26 years, physician): "My family physician kept track of my condition by regular phone calls. Apart from that, I was followed up by two different teams whose only purpose was COVID-19 follow-up. Although I already worked in the healthcare system, it was different and reassuring to experience the process from this aspect." (Female, 50 years, nurse): "I was relieved to know that all bases were covered when it came to my well-being."*
Theme 4: Mental Growth and Empowerment	
Subtheme	**Quotations**
Meaning of life	*(Female, 46 years, nurse): "You better understand the value of your loved ones. You understand that what you take for granted is actually a blessing." (Male, 34 years, physician): "I realized that even one second is precious and we don't have any time to waste."*
Re-setting priorities	*(Female, 26 years, physician): "It gets away from us during the hustle and bustle of life, but at least this experience reminded me that the well-being of my family and loved ones is what matters the most." (Female, 41 years, nurse): "What can be more important than being in good health? When you think about it that way, all else becomes insignificant."*
Setting new goals	*(Female, 34 years, nurse): "I now try to plan more family activities and spend quality time with my family." (Female, 41 years, nurse): "I always wanted to work out regularly, but I couldn't find the time. Turns out, I can. I stopped fooling myself and started to exercise regularly. I hope it will last this time."*
Gratitude	*(Female, 28 years, physician): "I'm grateful to everyone who stayed by my side when I was sick." (Male, 26 years, physician): "I feel grateful to my family and friends who supported me during my illness."*

Table 1. Continue.

Theme 5: Effect on Work	
Subtheme	**Quotations**
Increased empathy for COVID-19 patients	*(Female, 25 years, physician): "… I don't only see my patients through the eyes of a doctor but also better understand them as I have gone through what they are going through."* *(Male, 41 years, physician): "I understand better what patients mean when they say 'I am in great pain' and what they are going through."*
Changes in approach to COVID-19 patients	*(Male, 38 years, physician): "I visit patients' rooms, I talk to them more often."* *(Female, 41 years, nurse): "I talk more to patients who are conscious. I go to patients, hold their hands… I try and help them overcome their fears."*

Theme 1: Emotional, Cognitive, and Behavioral Responses

The participants reported different emotional responses associated with the disease, including sadness, fear, and anxiety in the early stages; relief with the improvement of disease symptoms; and happiness and excitement with disease resolution. Several participants also reported loneliness due to being alone during quarantine or guilt over the thought of infecting family members. We found that healthcare workers displayed various attitudes towards the disease, such as denial, confrontation, acceptance, and cooperation with treatment and prevention measures.

Participants aimed to maintain body strength and, to recover more easily; they adopted a more balanced diet. Several participants reported taking nutritional supplements. Many constantly monitored any changes in their symptoms or the development of new symptoms and frequently measured vital signs. Participants reported both increased (changing masks more frequently, wearing a mask around non-family members, disinfecting hands more often, etc.) and decreased (not wearing a mask around non-family members, disinfecting hands less often, using less personal protective equipment in the hospital, etc.) compliance with prevention measures after the illness.

Theme 2: Stress Factors

Causes of stress during illness included the uncertain prognosis, lack of a definitive treatment, and potential sequelae. Participants also emphasized the stress associated with potentially infecting family members, close friends, colleagues, or others. Some expressed concern about the possibility of infecting others immediately after leaving quarantine due to the relatively short duration of the isolation period (10 days). Participants reported various challenging factors, including social isolation due to the quarantine, being in need of help, and being away from loved ones. Having a detailed knowledge of the disease and its potential complications and outcomes and having previously witnessed the poor prognosis of severe COVID-19 patients adversely affected our participants' mental health during their illness. They expressed concern about the potential stigma, and several participants reported experiencing prejudice after recovery.

Theme 3: Sources of Motivation

In our study, the major source of motivation was the support given by family, friends, and others during recovery. Participants reported resorting to various activities such as watching TV, cleaning, reading, cooking, trying to distract themselves, and positive thinking to cope with the disease. Religious prayer, forbearance, fate, and trust were reported to positively affect participants' mental state during the illness. Being healthcare workers, participants mentioned feeling more at ease due to having confidence in receiving the required medical support. Having access to accurate information about the disease and the medical staff as needed and, in the case of possible hospitalization, being acquainted with the hospital staffs were reported as sources of mental relief. Participants also noted that adequate medical support positively affected their mental health.

Theme 4: Mental Growth and Empowerment

The disease experience helped participants find meaning in their lives. Participants stated that they realized the importance of mental and physical well-being for themselves and their families and they expressed less concern about money and social status. They emphasized that the experience contributed to setting new goals. They indicated feeling grateful for their family members, friends, the medical staff, and the government for their support during this illness.

Theme 5: Effect on Work

Participants indicated that having personally experienced COVID-19 helped them better understand and relate to and changed their perspective on other COVID-19 patients. They reported a positive change in their behavior towards COVID-19 patients.

DISCUSSION AND CONCLUSION

The present study, healthcare workers identified the emotional, cognitive, and behavioral effects of the disease, including emotional responses at different stages of the disease, attitudes towards the disease, changes in dietary style, over-attention to symptoms and vital findings, and changes in prevention measures after recovery from the disease.

Sudden pandemic-related changes in working conditions may be associated with the prevalence of mental disorders among healthcare workers.[12] During the SARS outbreak, Reynolds et al. reported anxiety, sadness, fear, loneliness, and guilt among quarantined individuals.[13] Individuals with confirmed or suspected COVID-19 may fear the consequences of contracting a possibly fatal disease.[14] In this study, we found that healthcare workers exhibited anxiety, fear, sadness, guilt, and loneliness during COVID-19.

Sun et al. conducted a qualitative study on people with a history of COVID-19. Participants' attitudes toward the disease included denial in early stages and acceptance and confrontation during later stages. Eighty percent of the patients exhibited excessive attention to symptoms due to fear of progression of the disease or not recovering.[11] In our study, healthcare professionals with COVID-19 similarly exhibited denial, acceptance, confrontation, and cooperation. They also devoted excessive attention to new and existing symptoms due to fear of disease progression and frequently measured and monitored vital signs.

We also found that our participants tried and adopted a balanced and regular diet and took nutritional supplements to strengthen their immune systems and for a rapid recovery. Such a finding was not reported in other similar studies.

A qualitative study by Moradi et al. on COVID-19 patients found that the confusion resulting from the uncertain prognosis caused anxiety, and fear of infecting others caused mental stress.[15] We found that our participants perceived themselves as a threat to the well-being of family members (particularly children and the elderly) and others. A systematic review by James et al. documented that people who contracted the Ebola virus feared infecting others due to the ability of the virus to persist in tissues long after recovery.[16] In our study, healthcare workers who developed COVID-19 were anxious due to the uncertain prognosis of the disease and the yet unknown long-term complications and feared transmitting the virus to family members (particularly the elderly and children), coworkers, friends, and others. For most participants, this fear resumed for a while after being discharged from quarantine and they avoided close contact and public places.

A meta-analysis concluded that quarantine placed a heavy mental burden on individuals.[17] Gammon et al. reported that 33% of the participants who had undergone isolation had a poor mental health status.[18] Studies on individuals who were quarantined due to COVID-19 indicate that the main causes of stress during quarantine include being away from family members, disruption of life, and limited options for activity,[19] as well as anxiety about the illness, disruption of normal life, social isolation, and stigma.[20] Similarly, our participants mentioned feeling stressed due to being away from loved ones, social isolation, the uncertainties surrounding the disease, and stigma.

We also identified a seldom mentioned risk factor for COVID-19-related anxiety: being a healthcare professional. Our participants indicated that having detailed knowledge of the disease and its potential complications and outcomes and having previously witnessed the poor prognosis of severe COVID-19 cases adversely affected their mental health during this illness.

A study of 549 hospital personnel from 3 years after the SARS outbreak found that 8.8% of all subjects exhibited severe depressive symptoms, 60% of whom had been quarantined, compared to 14.9% of participants who exhibited low rates of depressive symptoms.[21] Thirty months after the SARS epidemic, Mak et al. found the prevalence of mental illness in people with a history of SARS infection to be 33.3%, and they reported PTSD in 25% and depression in 15.6% of the participants.[22] In this setting, timely psychological guidance is crucial.[8,17,23,24] In reference to these data, our participants are also at risk of mental illness and require mental health support.

Self-support and social support play key roles in maintaining mental health among patients with infectious diseases.[25,26] One qualitative study indicated that all participating COVID-19 patients depended on their family members for mental support.[11] Similarly, our participants derived benefit from the social support of family, friends, and others as well as self-care and support during recovery.

Gashi found that religious faith had a significant impact on COVID-19 patients' understanding of the disease and religious prayer, forbearance, worship, and submission significantly contributed to coping with the disease.[27] In our study, Muslim participants indicated that religious forbearance, worship, and prayer had positive effects during their illness.

Having accurate medical information can reduce stress, anxiety, depression, and the mental impact of a disease.[28] Participants mentioned that being a healthcare worker had a positive effect on their mental well-being. Having easy access to accurate information and acquaintances in healthcare had a positive influence. This aspect appears to be ignored by

other similar studies.

COVID-19 patients face physical and mental stress. Attitudes toward the disease and emotional responses change throughout different stages of the illness. The negative emotions in early stages gradually develop into coexisting negative and positive emotions. Identifying stress factors and providing targeted support play key roles in patients' mental health. Timely mental health interventions can prevent physical and mental damage and improve patients' moods, attitudes, and behaviors.

Healthcare workers require psychosocial support to be able to maintain adequate patient care.[12] Considering that the pandemic may last for a few more years, future experimental studies may investigate the qualities of the psychological support to be provided to healthcare professionals.

However, motivation is also vital to more easily overcome the disease, both physically and mentally. Further studies are needed to investigate the sources of motivation that emerged in our study, including social support, self-support, religious beliefs, being a healthcare worker, and an effective healthcare system.

In this study, we also found that contracting COVID -19 allowed healthcare professionals to better understand and communicate with COVID-19 patients and to give patients moral support.

As a limitation of the study, this research was performed in only one of Turkey's provinces and the results should be interpreted in this context.

Ethics Committee Approval: This study was approved by the Sakarya University Local Ethics Committee (Date: 01.12.2020, decision no:585).

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

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Ten-Year Recurrence Outcomes in Early Stage and Early Onset Colon Cancer and the Expression Profiles of MicroRNAs and Cancer Stem Cell Markers in These Tumors

Erken Evre Kolon Kanserinde On Yıllık Nüks Sonuçları ve Bu Tümörlerde MikroRNA'ların ve Kanser Kök Hücre Belirteçlerinin Ekspresyon Profilleri

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ABSTRACT

Objective: We hypothesized that microRNAs (miRNAs) might be involved in tumor development by critically regulating cancer stem cell (CSC) markers in the early stages of colon cancer (eCC). This study aimed to determine the expression profiles of miRNAs in CSC-positive eCC patients and examine their associations with recurrence.

Materials and Methods: We analyzed CD133, LGR5 and SOX2 expression profiles to determine CSC status in 30 eCC specimens. Then, using the results of RT2 miRNA PCR custom arrays, we evaluated the expression profiles of 38 miRNAs in CSC-positive eCC patients.

Results: Recurrence occurred in 5 patients within ten years after surgery. We determined down-regulation of miR-125b and up-regulation of miR-135b were significant in CSC-positive eCC patients (p=0.021, p=0.001, respectively). We found that low expression of miR-125b was associated with recurrence in eCC (p=0.0022).

Conclusions: We suggest that recurrence might be prevented by increasing the expression of miR-125b in eCC.

Keywords: Cancer stem cell, colon cancer, early-onset, early-stage, microRNA

ÖZ

Amaç: MikroRNA'ların (miRNA'lar), kolon kanserinin erken evrelerinde (eCC) kanser kök hücre (CSC) belirteçlerini kritik olarak düzenleyerek tümör gelişiminde rol oynayabileceğini varsaydık. Bu çalışmanın amacı, CSC pozitif eCC hastalarında miRNA'ların ekspresyon profillerini belirlemek ve nüks ile ilişkilerini incelemektir.

Materyal ve Metot: 30 eCC örneğinde CSC durumunu belirlemek için CD133, LGR5 ve SOX2 ekspresyon profillerini analiz ettik. Ardından, RT2 miRNA PCR özel dizilerinin sonuçlarını kullanarak, CSC pozitif eCC hastalarında 38 miRNA'nın ekspresyon profillerini değerlendirdik.

Bulgular: Ameliyattan sonraki 10 yıl içinde 5 hastada nüks meydana geldi. CSC pozitif eCC hastalarında miR-125b'nin aşağı regülasyonunun ve miR-135b'nin yukarı regülasyonunun anlamlı olduğunu belirledik (sırasıyla p=0,21, p=0,001). miR-125b'nin düşük ifadesinin eCC'de tekrarlama ile ilişkili olduğunu bulduk (p=0,0022).

Sonuç: eCC'de miR-125b ekspresyonunun arttırılmasıyla nüksün önlenebileceğini düşünüyoruz.

Anahtar Kelimeler: Erken başlangıçlı, erken evre, kanser kök hücresi, kolon kanseri, mikroRNA

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INTRODUCTION

Colon cancer (CC) is the third leading cause of cancer-related death worldwide. Recurrence following surgery is a major problem in CC. Approximately 10 –20% of early-stage CC (eCC) patients (T1-T3N0M0) develop recurrence after curative resection.[1] Recent studies suggested that CC tumors with poor prognosis originated from a unique and rare subset of cancer cells having self-renewal capacity and the potential to differentiate into several cell lineages.[2] These cancer cells with stem cell-like properties, referred to as cancer stem cells (CSCs), are responsible for cancer initiation, progression, and metastasis.[3]

Many surface proteins, such as CD44, CD133, CD24, SSEA3, LGR5 and SOX2, have been identified as CSC markers. CD133 (prominin 1, AC133) is a transmembrane and cell surface protein. Overexpression of CD133 correlated with survival and recurrence in cancer.[4] It has been shown to be a characteristic of CSCs in CC. LGR5 is a G-protein-couple receptor considered a CSC biomarker in CC. It is rarely expressed in normal tissue.[4] SOX2, involved in the induction of pluripotent stem cells, is associated with poor prognosis in cancer tissue.[8] Emerging evidence suggests that CD133, LGR5 and SOX2 may be involved in tumor maintenance, therapy resistance, tumor progression, and recurrence in colorectal cancer. Despite their potential clinical significance, how intrinsic CSC properties are regulated at the molecular level needs to be better understood.[6] Therefore, there is an urgent need to understand the molecular mechanisms controlling CSC populations and functions to develop effective therapies to eradicate recurrences, which are a real threat to complete cancer cures.

MicroRNAs (miRNAs) are small noncoding RNAs that function as negative regulators of mRNAs. Functional studies indicate that miRNAs regulate molecular pathways in cancer via targeting various oncogenes and/or tumor suppressors.[5] Recent evidence suggests that miRNAs may also be involved in tumor development by critically regulating CSCs.[9,10]

This study aimed to determine the expression profiles of miRNAs in CSC-positive and early-stage CC patients and to examine their associations with recurrence formation. The identification of patients with a high risk of dissemination in this subset may optimize the use of adjuvant therapies. In addition, there are no known molecules specific to CSCs among current cancer treatment modalities. Therefore, we aimed to contribute to developing biomarkers for miRNA-based therapies, mainly targeting CSCs.

MATERIALS AND METHODS

Ethics Committee Approval: The study was approved by Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee (Date: 13.01.2015, decision no: 2015-1/35) and was by the ethical standards of the Declaration of Helsinki.

Patient Population and Clinical Specimens: The eCC archive database of the Uludag University Medical Faculty and the Department of General Surgery was used to collect clinical information and follow-up data for these patients. Basic clinical and tumor characteristics, such as age, gender, location, and pathological stage (assessed by the tumor-node-metastases classification), were analyzed. All patients were considered sporadic cases and microsatellite stable. Only CC patients under the age of 50 were included. Any patient receiving preoperative chemotherapy and/or radiation was excluded to avoid a confounding influence on tumor composition and clinical outcome.

Total RNA Extraction and Quality Control: Colon tumors and surgical margins (normal colon tissue) from 30 patients were formalin-fixed and paraffin-embedded (FFPE). Total RNA from microdissected cells was isolated using the RNeasy FFPE Kit (Qiagen, Germantown, Maryland, USA). According to the manufacturer's protocol, miRNAs were also extracted from specimens using a miRNeasy Mini Kit (Qiagen). All RNA samples were assessed for RNA quantity and quality using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA samples with 1.8–2.0 for 260:280 ratios, >1.8 for 260:230 ratios and with a total concentration ranging from 200 to 400 ng/µl were selected for complementary DNA synthesis.

RT–PCR Assay: Complementary DNA was synthesized from 5 ng total RNA using the RT2 mRNA First Strand Kit (Qiagen), followed by analysis by Light Cycler 480II (Roche Diagnostics, Indianapolis, USA) to profile CD133, LGR5 and SOX2 expression levels in samples. Accession numbers; ACTB: NM_001101.2, SOX2: NM_003106, LGR5: NM_003667 and CD133: NM_00356.

RT-PCR-based miRNA Expression Profiling: Complementary DNA was synthesized from 5 ng small RNA-enriched total RNA using the RT2 miRNA First Strand Kit (Qiagen). Samples were analyzed for the presence and differential expression of 38 miRNAs related to CRC formation using custom RT2 miRNA PCR arrays (SABiosciences, Frederick Md, USA) according to the manufacturer's instructions using a Light Cycler 480II (Roche Diagnostics). One 384-well plate containing 48 assays in six replicates (42-miRNA panel consisting of four duplicated miRNAs and 38 individual miRNAs) was used for six samples. The accession numbers of pri-

mers are listed in Table 1. The RNA input was normalized to endogenous controls including SNORD 44, SNORD 47 and SNORD 48 for miRNAs and the TATA-binding protein for protein-encoding genes.

Statistical analysis: Significant differences among the various pathological and clinical characteristics depending on mRNA expression levels in the study group were calculated using the Chi-squared test (χ^2)

Table 1. Accession numbers of miRNA primers.

miRNA Sanger ID	miRNA accession number	miRNA Sanger ID	miRNA accession number	miRNA Sanger ID	miRNA accession number
hsa-miR-21	MIMAT0000076	hsa-miR-16	MIMAT0000069	hsa-miR-221	MIMAT0000278
hsa-miR-143	MIMAT0000435	hsa-miR-181b	MIMAT0000257	hsa-miR-223	MIMAT0000280
hsa-miR-145	MIMAT0000437	hsa-miR-200c	MIMAT0000617	hsa-miR-148a	MIMAT0000243
hsa-miR-19a	MIMAT0000073	hsa-miR-139-3p	MIMAT0004552	hsa-miR-200a*	MIMAT0001620
hsa-let-7a	MIMAT0000062	hsa-miR-26a	MIMAT0000082	hsa-miR-15b	MIMAT0000417
hsa-let-7b	MIMAT0000063	hsa-miR-27a	MIMAT0000084	hsa-miR-15a	MIMAT0000068
hsa-let-7c	MIMAT0000064	hsa-miR-30a	MIMAT0000087	hsa-miR-20a	MIMAT0000075
hsa-miR-17	MIMAT0000070	hsa-miR-34a	MIMAT0000255	hsa-miR-99a	MIMAT0000097
hsa-miR-155	MIMAT0000646	hsa-miR-96	MIMAT0000095	hsa-miR-498	MIMAT0002824
hsa-miR-29b	MIMAT0000100	hsa-miR-133b	MIMAT0000770	hsa-miR-320	MIMAT0000510
hsa-miR-106a	MIMAT0000103	hsa-miR-135b	MIMAT0000758	hsa-miR-183	MIMAT0000261
hsa-miR-139-5p	MIMAT0000250	hsa-miR-125b	MIMAT0000423	hsa-miR-124	MIMAT0000422
hsa-miR-191	MIMAT0000440	hsa-miR-137	MIMAT0000429		

and Fisher's exact test. The samples were analyzed for the presence and differential expression of a panel of 38 miRNAs, depending on LGR5 and SOX2 expression status, using RT^2 Profiler PCR Array Data Analysis (http://www.sabiosciences.com/pcr/arrayanalysis.php) to compare the PCR array analysis results and the characteristics of the tumors and patients. Progression-free survival curves were plotted using the Kaplan-Meier method. The log-rank test was used to assess the survival differences between groups. Overall survival was defined as the interval between sampling and the last follow-up. The chi-squared test (χ^2) and Fisher's exact test were performed using SPSS 16.00 software for Windows (Chicago, IL, USA), and the Kaplan-Meier analysis and log-rank test were performed using MedCalc 12.4.0 statistical software (Ostend, Belgium). The 95% confidence intervals were calculated using associated estimated standard errors. A p-value < 0.05 was considered significant.

RESULTS

The present study included 30 unrelated patients who were diagnosed with early-onset eCC. The median age at diagnosis was 36.39±1.15 years (range: 18–47 years), and the study included 16 males and 14 females. Primary tumors were localized in the right colon in 15 patients and the left in 15 patients. Recurrence was observed in 5 patients within ten years of surgery. The expression profiles of CD133, LGR5 and SOX2 were evaluated among different patients and tumor characteristics to determine prognostic predictions. The rate of positive expression of CD133, SOX2 and LGR5 in our eCC tumor samples were 57% (17/30), 24% (7/30) and 77% (23/30), respectively. Twenty-three of 30 tumors had high expression levels for at least two of the CD133, LGR5 and SOX2 genes, and they were identified as aggressive tumors due to CSC marker positivity. There was no correlation between the expression of these genes and age, gender, or stage (p>0.05). CSC marker-positive status was associated with right localization and recurrence over ten years of follow-up (p<0.05). CSC-positivity was observed in 86% of patients with recurrences. The expression profiles of LGR5 were 3.24-fold (Figure 1A), the expression profiles of CD133 were 2.17-fold (Figure 1B), and the expression level of SOX2 was 2.05-fold upregulated (Figure 1C) in recurrent CC compared with non-recurrent CC (Figure 1, p<0.05).

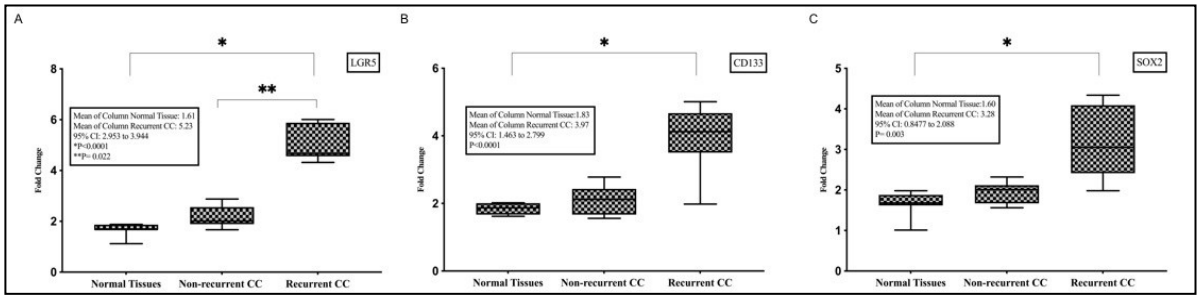

Figure 1. CSC marker status in non-recurrent, recurrent tumors and normal tissues.

Using RT^2 miRNA PCR custom arrays, we determined the expression profiles of 38 different miRNAs in the 30 colon tumor samples. The expression profiles of these tumor cells were compared with 10 normal colon mucosa samples. Among the 38 miRNAs, low expression levels of miR-125b, miR-143, miR-145, miR-125b and high expression levels of miR-106a, miR-135b were found to be significant in early-onset eCC (Table 2).

Table 2. Clinical and bio-pathological features of the CRC patients included in this study, depending on the presence of CSCs markers.

	Characteristics	Number of cases (%)	CSC markers negative (%)	CSC markers positive (%)	p value
Total number		30	7	23	
Patients Median age (year)	18-29	3 (10.00)	0 (00.00)	3 (13.04)	
	30-39	17 (56.67)	4 (57.14)	14 (60.86)	> 0.05
	40-47	10 (33.33)	3 (42.86)	6 (26.08)	
Gender	Male	16	3 (42.86)	13 (56.52)	> 0.05
	Female	14	4 (57.14)	10 (43.48)	
Tumor side	Right Colon	15 (50)	2 (28.57)	13 (56.53)	< 0.05
	Left Colon	15 (50)	5 (71.43)	10 (43.48)	
Stage (T)	T1	9 (26.67)	2 (28.57)	7 (30.43)	
	T2	7 (26.67)	2 (28.57)	5 (21.74)	> 0.05
	T3	14(46.66)	3 (42.85)	7(30.43)	
Recurrence status	No	14 (46.67)	3 (50.00)	11 (57.89)	< 0.05
	Yes	11 (36.67)	3 (50.00)	8 (42.10)	
Overall survival	Five years and over	26 (86.67)	7 (100.00)	19 (82.60)	> 0.05
	Less than five years	4 (13.33)	0 (00.00)	4 (17.39)	
Disease free survival	Five years and over	11 (36.67)	4 (57.14)	12 (52.17)	< 0.05
	Less than five years	14 (46.67)	3 (42.86)	11 (47.83)	

Then, miRNA expression differences between normal colon mucosa and the tumors with different CD133, LGR5 and SOX2 expression statuses as their CSC statuses were evaluated. When sorting the 38 miRNAs based on the fold-change of expression levels (more than 1.5-fold) between these two groups, miR-125b was the top miRNA-exhibiting expression level loss, and miR-135b was the maximum miRNA-exhibiting expression level gain in colon tumors with CSC markers compared to colon tumors without CSC markers. The expression level of miR-135b exhibited approximately 2.01-fold gain, and the expression level of miR-125b exhibited approximately 2.12-fold loss in colon tumors with CSC markers compared to colon tumors without CSC markers (Figure 2A and Figure 2B).

Expression profiles were particularly evaluated for T1-3 CC tumors to determine the true prognostic values of CSC status and characteristic features. The expression level of miR-125b was lower, and that of miR-135b was higher in colon tumors with CSC markers than in colon tumors without CSC markers. Expression levels of these miRNAs were evaluated in different subgroups of tumor tissues to clarify the role of these miRNAs in terms of clinicopathological features. There were no correlations between the

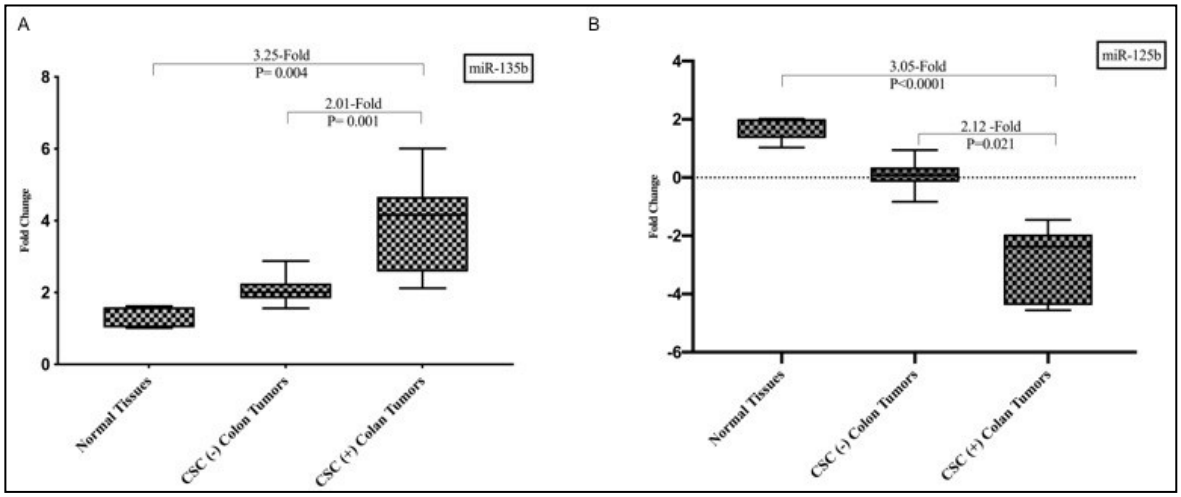

Figure 2. The differential expression of miR-135b (A) and miR-125b (B) depending on CSC marker expression status

expression of these miRNAs and tumor size, gender, or age in CSC-positive eCC tissues. However, a low expression level of miR-125b was associated with recurrence in CSCs (4.12-fold; p=0.002). There were no associations between miR-135b expression status, recurrence status and any histopathological characteristic in CSC-positive eCC tumors.

Follow-up was carried out for ten years in surviving patients. According to Kaplan-Meier analysis and log-rank test, DFS increases in patients with high expression levels of miR-135b expression were not significant (log-rank, p value >0.05). However, DFS was higher in patients with low expression levels of miR-125b than in patients with the opposite expression levels of these miRNAs (Figure 3A and Figure 3B, log-rank, p value <0.05). Therefore, these results suggest that the downregulation of miR-125b would be associated with DFS in colon tumors having CSCs if the case numbers were larger.

DISCUSSION AND CONCLUSION

CSC has been identified in almost all major cancer types, including breast cancer, leukemia and CC. Importantly, CSCs are resistant to traditional anti-cancer therapies, such as chemo-radiotherapy, and these cells also have the ability to sustain systemic/ local relapse. Thus, understanding the biology of CSC in CC is of great clinical significance.[6]

Recent findings indicate that the role of the abnormal expression of miRNAs is related to the regulation of CSC properties, such as asymmetric cell division, tumorigenicity, and drug resistance.[7,8] CSCs usually share specific stemness-related markers, such as CD44 and CD133, but they can differ depending on the cancer type. Early studies showed that miR-34a suppressed prostate CSCs and metastasis by directly repressing CD44.[10] However, there has been no study focusing on miRNAs specifically involved in the regulation of CSCs in early-onset eCC tumors. In our study, we evaluated 38 individual miRNAs in CSC-positive early-onset eCC patients who were at an early stage and had sporadic tumors.

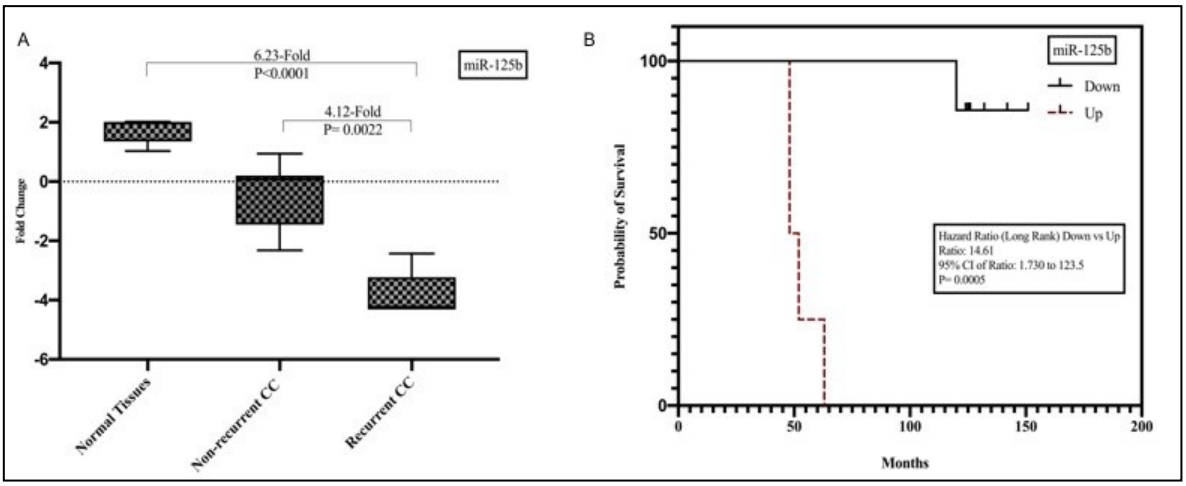

Figure 3. Low expression of miR-125b was associated with recurrence in CSC-positive CC tumors (A). Kaplan-Meier survival curves for CC patients according to miR-125b (B)

First, we determined CSC-positive tumors (n= 23), and we evaluated the association of CSC status with tumor size, stage, gender, OS and DFS to determine the predictive potential of these features for the aggressiveness of tumors. Two studies showed reduced OS in colorectal cancer patients with CD133-positivity. CSCs play a role in cancer metastasis, and the ability of CSC markers to predict disease progression and patients' survival is being intensely investigated. Recent studies reported that recorded expression of LGR5 mRNA in ~60% of circulating tumor cells in CRC patients reported a high correlation between LGR5 expression and metastasis development.[11,12] In a recent study, LGR5+ stem cells were reported to be responsible for recurrence and therapeutic resistance in Stage II-III colon cancer.[13] In our study, CD133, SOX2 and LGR5 were used to determine CSC status. High expression of at least two of them was accepted as a criterion for CSC-positive tumors. There was no significant association between gender, tumor stage and OS (p>0.05). On the other hand, CSC-positivity was associated with right colon localization. Furthermore, we found that patients with CSCs had recurrence over five years and had reduced DSF. Although Stotz et al. examined variants of CD44 in stage II and stage III with poor prognosis, there are no studies regarding the expression status of CSCs in eCC.[14]

In the present study, the down-regulation of miR-

125b and up-regulation of miR-135b were significant in CSC-positive eCC. Recent studies also found that miRNAs played an important role in regulating the expression of colon CSCs.[15-17] Zhang et al. showed that overexpression of miR-125b inhibited proliferation, promoted apoptosis in the SW480 colon cancer cell line, and was accompanied by upregulated Bax and downregulated Bcl-2 expression.[18] Yu et al. found that miRNAs, specifically miR-21 and miR-145, played important roles in regulating colon CSCs. They found the expression of miR-21 to be much higher and miR-145 to be lower in colon cancer cells that were highly enriched in CSCs, suggesting a role for these miRNAs in regulating CSCs.[19]

When we evaluated associations between prognosis and expression of miR-135b and miR-125b, there were no associations between miR-135b and any clinicopathological features in CSC-positive eCC tumors. Our results suggested that miR-135b played a role in cancer initiation, but our data have shown that this miRNA was ineffective in recurrence. Similarly, data from colorectal tissues suggested that miR-135b upregulation was an early event in tumor transformation.[20] According to the result from siRNA silencing, miR-135b regulation appeared to be independent of c-Myc activation; thus, these two factors may control collateral transcriptional programs orchestrating the tumor initiation process.[20] The miR-135b has been documented as a tumor-promoting factor and to play a role in migration and metastasis in CRC.[21,22.]

In the present study, we found that low expression levels of miR-125b were associated with recurrence in eCC. We previously determined miRNA expression profiles in early-onset colorectal cancer. In that study, the expression of miR-106a was upregulated, and levels of miR-143 and miR-125b were downregulated in different stages of colon and rectal tumor compared with normal tissues.[23] In our study, we selected only young patients and those with early-onset colon tumors. Although some reports highlighted the oncogenic aspects of miR-125b, many suggested that miR-125b worked as a tumor-suppressive microRNA in various cancers. miR-125a was significantly downregulated in breast and gastric cancer, and miR-125a substantially inhibited cancer cells' proliferation, migration, and invasion activities.[27-29] There is only one study showing an association between miR-125b and CSCs. Zhoug et al. determined that miR-125b negatively correlated with CD133 expression in hepatocellular carcinoma patients.[30]

Although this study included a homogeneous and carefully selected group of patients, its limitation was the relatively small group of patients analyzed. We evaluated only 30 patients at early onset and early stage.

In conclusion, currently, the reasons underlying the recurrence of eCC remain unclear, and the role of stem cells and microRNAs in tumor biological processes has not yet been fully elucidated. In the present study, we showed that miR-125b was significantly downregulated in early-onset eCC tumors. The expression of miR-125b increased tumor aggressiveness and recurrence potential. We suggest that our findings will inspire researchers to create new strategies for confidently diagnosing eCC patients with recurrence potential, offering promise for discovering new targets related to this mechanism, and contributing to future targeted cancer therapy studies based on this new direction. Further studies and validations are required; miR-125b may constitute a novel molecular target for CSC-positive CC treatment. Collectively, a better understanding of miRNA functions associated with CSC properties could provide new insight into cancer therapeutics.

Ethics Committee Approval: The study was approved by Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee (Date: 13.01.2015, decision no: 2015-1/35) and was by the ethical standards of the Declaration of Helsinki.

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

Author Contributions: Concept – SAA, BT; Supervision – SAA, BT, TY, OI, EO; Materials –SAA, ME, CT, BG, NU, OY; Data Collection and/or Processing – SAA, BT, ME, CT, GC, UE; Analysis and/or Interpretation – SAA, BT, TY, OI, EO.

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The Mediating Role of Germ Aversion in the Relationship between Fear of COVID-19 and Intention to Vaccinate

COVID-19 Korkusu ile Aşı Olma Niyeti Arasındaki İlişkide Mikroptan Kaçınmanın Aracılık Rolü

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ABSTRACT

Objective: In the study, it was aimed to determine the fear level of COVID-19 and to demonstrate the relationship between fear of COVID-19 and intention to vaccinate.

Materials and Methods: The study population consisted of young, middle, and elderly patients and their relatives who applied to a family medicine unit operating in the city center of Düzce. Data were collected from 530 people using the face-to-face survey technique. The researchers used IBM SPSS Statistic Base 23 V and AMOS package programs for statistical analysis.

Results: The empirical result of the study revealed that as the fear level of COVID-19 increased, the intention to get vaccinated for COVID-19 increased positively. In addition, it has been determined that germ aversion behavior has a significant indirect effect and fully mediates the relationship between fear of COVID-19 and intention to get vaccinated.

Conclusions: This research revealed that increasing COVID-19 fear level positively increases the intention to be vaccinated through high germ aversion behavior.

Keywords: COVID-19, fear, germ aversion, structural equation modeling, vaccine

ÖZ

Amaç: Çalışmada COVID-19 korku düzeyinin belirlenmesi ve COVID-19 korkusu ile aşılama niyeti arasındaki ilişkinin ortaya konulması amaçlanmıştır.

Materyal ve Metot: Araştırmanın evrenini Düzce İl merkezinde faaliyet gösteren bir aile hekimliği birimine başvuran genç, orta ve ileri yaş grubu hasta ve hasta yakınları oluşturmuştur. Veriler yüz yüze anket tekniği kullanılarak 530 kişiden toplanmıştır. Verilerin analizinde IBM SPSS 23 ve AMOS paket programları kullanılmıştır.

Bulgular: Çalışmanın ampirik sonucu COVID-19 korku düzeyi attıkça, COVID-19 aşı olma niyetinin de olumlu yönde arttığını ortaya koymuştur. Bunun yanı sıra mikroptan kaçınma davranışının, COVID-19 korkusu ile aşı olma niyeti arasındaki ilişkide önemli derecede dolaylı bir etkiye sahip olduğu ve tam bir arabuluculuk ettiği tespit edilmiştir.

Sonuç: Bu araştırma, artan COVID-19 korku düzeyinin yüksek mikroptan kaçınma davranışı yoluyla aşı olma niyetini olumlu yönde artırdığını ortaya koymuştur.

Anahtar Kelimeler: Aşı, COVID-19, korku, mikroptan kaçınma, yapısal eşitlik modellemesi

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INTRODUCTION

It has been seen that there has been more than one epidemic disease in the world throughout history, and millions of people lost their lives in these epidemics. The last epidemic disease humankind has to fight is the COVID-19 epidemic.[1] COVID-19 respiratory infectious disease, which emerged in Wuhan city of Hubei province of China in December 2019 and affected the whole world, was declared a pandemic by the World Health Organization on March 11, 2020.[2] In our country, the first case of COVID-19 was seen on March 11, 2020.[3] Although the virus differs from country to country until now, it has been seen that it has infected more than 200 million people worldwide, and it is known that it has caused more than 4 million people to die.[4]

During this extraordinary pandemic, governments worldwide have taken various initiatives to prevent the spread of the epidemic. In this direction, they have taken measures such as curfews, quarantine practices, promotion of flexible working in business life, the adoption of the distance education model by closing schools, overseas travel restrictions, mask requirements, and social distance rules.[5] However, it has been stated that the most promising solution is to protect people from the coronavirus epidemic, which infects people globally due to its highly contagious nature, and to reduce the death and disease rates caused by the epidemic.[6]

Previous research on other vaccine-preventable diseases has shown that identifiable factors can influence vaccination intentions and acceptance. For example, certain sociodemographic factors such as socioeconomic status, age, race and ethnicity, and geographic location played a role in favor of adult vaccines.[7,8] In addition, since vaccination is based on the principle of "herd immunity", it has been stated that behaviors that benefit others, such as general altruism, prosocial behavior, and sympathy, play an essential role in some vaccination decisions.[9] At the same time, theoretical models such as the health belief model stated that variables such as perceived severity and susceptibility to disease could predict behavioral intentions and behaviors.[10,11]

In this study, the mediating role of germ avoidance behavior, which is described as a psychological factor in the effect of fear of COVID-19 on the intention to be vaccinated, on this relationship was revealed. In the literature, it has been stated that people's fear levels increase more, especially in cases where the risk of death is high. This situation leads individuals to engage in preventive health behaviors.[12] Similarly, it was emphasized that individuals with a high fear of COVID-19 use personal protective equipment more often and wash their hands more often.[13] Based on those mentioned scientific theoretical evidence and the established hypothesis, the mediating role of germ avoidance behavior on vaccination intention was tested in the current study. According to the protection motivation theory, it has been stated that how a person perceives the possibility of being infected can be triggered by fear and then result in protective behavior.[14]

In the study, it was aimed to determine the fear level of COVID-19 and to demonstrate the relationship between fear of COVID-19 and intention to vaccinate.

MATERIALS AND METHODS

Ethics Committee Approval: This study was approved by Düzce University Scientific Research and Publication Ethics Committee (Date: 24.06.2021, decision no: 2021/179), and a verbal informed consent form was obtained from all patients. This study was conducted by Declaration of Helsinki.

Study Design, Participants, Instruments: This cross-sectional study was carried out on young, middle, and advanced age group patients and their relatives who applied to a family medicine unit operating in Düzce city center between July 1 and September 17, 2021.

Approximately 600 people said that they would like to participate in the survey. However, 70 people could not fill out the questionnaire due to the density. Therefore, the final sample consisted of 530 participants.

Statistical Analysis: The data were collected by the researchers themselves by face-to-face survey technique. All statistical analyzes were performed using IBM SPSS Statistic Base 23 V and AMOS programs. The COVID-19 fear level of the participants was determined using the COVID-19 Fear Scale.[15] The questionnaire consisted of 7 items measuring a general COVID-19 fear level. Germ aversion behavior was measured using an eight-item scale.[16] The questionnaire, prepared in Turkish, was evaluated using a five-point Likert scale. Intention to get vaccinated against COVID-19 was measured using a two-item scale.[17] In addition, the questionnaire, which was prepared in Turkish, was evaluated using a five-point Likert scale.

RESULTS

Table 1 presents the frequency values revealing the socio-demographic characteristics of the participants and the t-test and ANOVA analysis results revealing whether the COVID-19 fear levels, vaccination intentions and germ avoidance of the participants differ significantly according to these socio-demographic characteristics. In Table 1, frequency values revealing the socio-demographic characteris-

tics of the participants and the results of the t-test and ANOVA analysis are given.

Figure 1 shows the structural relationships between the research variables (the research model) and these relationship values. Also Figure 1 shows the confirmatory factor analysis results and model fit for the variables of fear of COVID-19, germ aversion, and intention to get vaccinated. In Figure 1, since the values of goodness of fit were within acceptable limits, significant relationships were found between the variables of the study. The fit values given for the model above show that the model fit is achieved. There is no limit to the values to look up. The reported values may vary according to the values the researcher wants to draw attention to.

Table 1. Sociodemographic characteristics of participants.

				Fear of COVID-19		**Germ Aversion**		**Intention to Get Vaccinated**	
	Variables	n	%	t Test/	p-value	t Test/	p-value	Anova (t/F) t Test/	p-value (2tailed)
Sex	Male	130	24.5	4.453[a]	0.001	6.879[a]	0.001	1.453[a]	0.147
	Female	400	75.5						
Age categories	18–25 years	48	9.1	0.610[a]	0.525	0.183[b]	0.932	3.029[a]	0.003
	26-35 years	173	32.6						
	36-45 years	200	37.8						
	46-55 years	90	17						
	>55	19	3.6						
Educational attainment	Junior college and below	174	32.8	0.083[b]	0.972	0.180[b]	0.893	6.289[b]	0.002
	College	326	61.5						
	Master's degree	25	4.7						
	Doctoral degree	5	.9						
Job	Employee	17	3.2	2.620[b]	0.083	1.830[b]	0.051	1.037[b]	0.378
	Officer	31	5.8						
	Retired	14	2.6						
	Housewife	328	61.9						
	Self-employment	13	2.5						
	Student	44	8.3						
	Unemployed	18	3.4						
	Private sector employee	57	10.8						
	Other	8	1.5						
Presence of chronic diseases	Yes	75	16.3	0.297[a]	0.771	1.832[a]	0.064	1.876[a]	0.060
	No	455	83.7						
Regular drug use	Yes	98	18.5	1.823[a]	0.087	2.165[a]	0.055	3.484[a]	0.002
	No	432	81.5						

[a:] Independent sample t-test; [b]: ANOVA test.

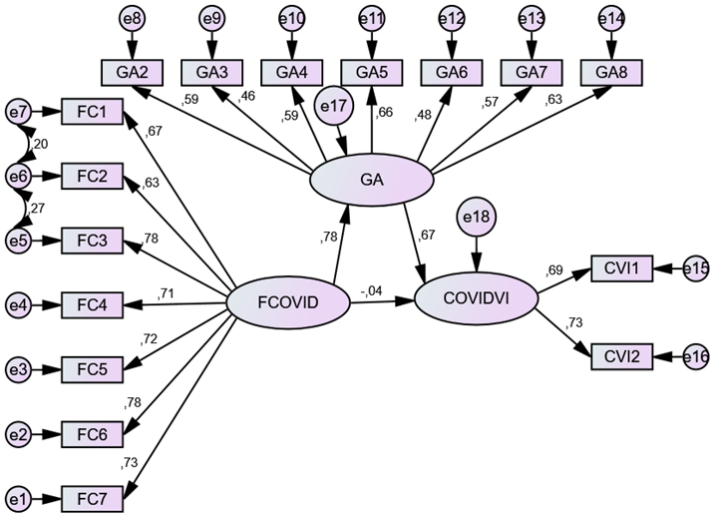

Figure 1. The results of the full model and goodness of fit values. Goodness of fit values: (CMIN/DF=4.033; GFI=0.917; CFI=0.911; AGFI=0.886; TLI=0.904; IFI=0.912; AGFI=0.837; RMSEA=0.074).

In Table 2, standardized regression coefficients, mean and standard deviation values, reliability coefficients, Average Variance Extracted (AVE) values and Critical Ratio (CR) values of the sub-variables of the study are given as a result of the path analysis. Since the calculated AVE values are greater than 0.5 and the CR coefficients are greater than 0.7, the factors have high construct reliability and thus concordance validity. The results for measuring the reliability and validity of the measurement model provide various measures, as shown in Table 2. The findings obtained from confirmatory factor analysis and path analysis show that the construct validity of the model is provided. As a result of the confirmatory factor analysis, the overall reliability coefficient was Alpha=0.918. In addition, the regression values in Table 2 show the power of the observed variables to predict latent variables, that is, factor loadings. The significant "p" values indicate that the items were loaded correctly on the factors.

Table 2. The items' estimate and the constructs' Cronbach's α, AVEs, and CRS.

Constructs	Items	Estimate	Cronbach's α	AVE	Mean ±SD	C.R.
Fear of COVID-19 (FCOVID)	FC7	0.733	0.885	0.52	4.2457±0.02978	0.90
	FC6	0.781				
	FC5	0.721				
	FC4	0.712				
	FC3	0,778				
	FC2	0.633				
	FC1	0.674				
Intention to Get COVID-19 Vaccinated (COVIDVI)	CVI1	0.686	0.663	0.50	3.7217±0.03940	0.70
	CVI2	0.730				
Germ Aversion (GA)	GA2	0.589	0.765	0.32	3.7962±0.02632	0.76
	GA3	0.458				
	GA4	0.595				
	GA5	0.656				
	GA6	0.475				
	GA7	0.569				
	GA8	0.633				

Table 3 shows the results of the structural model, the structural relationships among the variables, and the path coefficients before and after mediation. Moreover, Table 3 shows the results of the structural model, the structural relationships among the variables, and the path coefficients before and after mediation. The path coefficient shows how many units will change in the dependent variable for a one-unit change in the independent variable. Therefore, in Table 3, there is a full mediation relationship since the direct relationship between the independent and dependent variables is completely eliminated.

The indirect relationship and its properties are shown in Table 4. When the mediator variable (perceived infectability) is included in the model with the independent variable (fear of COVID-19) are included in the model, the direct effect of the independent variable (fear of COVID-19) on the

Table 3. The result of the structural model.

Hypothesis	Paths	Estimate	S.E.	C.R.	p	Result
Effect of Fear of COVID-19 on Intention to Get COVID-19 Vaccinated (Before Mediation)						
H_1	COVIDVI <--- FCOVID	0.499	0.082	8.342	0.001	H_1 supported
Effect of Fear of COVID-19 on Intention to Get COVID-19 Vaccinated (After Mediation)						
H_2	GA <---FCOVID	0.776	0.067	11.350	0.001	H_2 supported with a full mediation
	COVIDVI <--- FCOVID	-0.039	0.131	-0.386	0.699	
	COVIDVI <--- GA	0.667	0.159	5.589	0.001	

dependent variable (intention to get vaccinated) becomes insignificant and creates a whole mediation relationship. Therefore, as seen in Table 4, when the mediating variable (Germ Aversion) is included in the model together with the independent variable (Fear of COVID-19), the effect of the independent variable (Fear of COVID-19) on the dependent variable (Intention to Get COVID-19 Vaccinated) is insignificant (p =0.699).

Table 4. Indirect effect of the model.

Indirect Path	Unstandardized Estimate	Standardized Estimate	p value
FCOVID --> GA --> COVIDVI	0.623	0.479	0.699

DISCUSSION AND CONCLUSION

In this research, there was a statistically significant difference in Fear of COVID-19 score according to sex (t-test = 4.453; $p<0.05$). However, there was no significant relationship among the age of the participants, the educational level of the participants, the jobs of the participants, the presence of chronic disease in the participants, and the regular drug use of the participants. Furthermore, there was a statistically significant difference in germ aversion score according to sex (t-test = 6.879; $p<0.05$). However, there was no significant relationship among the age of the participants, the educational level of the participants, the jobs of the participants, the presence of chronic disease in the participants, and the regular drug use of the participants. Moreover, there was a statistically significant difference in intention to get vaccinated score according to age (ANOVA test = 3.029; $p<0.05$), educational attainment (ANOVA test = 6.289; $p<0.05$), and regular drug use (t-test = 3.484; $p<0.05$). However, there was no significant relationship between the sex of the participants, the job of the participants, and the presence of chronic diseases ($p>0.05$).

Based on the above-mentioned theoretical discussions and hypotheses, this study tested the mediating role of germ avoidance behavior on the intention to vaccinate. Fear of COVID-19[15] has been suggested in research as a potential precursor to vaccination intention. However, it has been stated that this level of fear can be triggered by several mediating factors[18] or through high levels of existential anxiety.[19] In addition, it is possible that while individuals are trying to cope with existential anxiety, conspiracy theories that will inevitably reduce their intention to be vaccinated may also develop.[20] As a result, on the one hand, the direct impact of fear of COVID-19 may positively influence the intention to vaccinate; On the other hand, the mediating effect of germ avoidance behavior may take this positive effect even further.

The results of our research showed the existence of a mediation model with direct and indirect effects on the intention to vaccinate. The data confirmed our hypothesis that fear of COVID-19 is positively associated with choosing to vaccinate. Therefore, the findings of this study reflect conclusions from many studies that fear of COVID-19 is related to the intention to get the COVID-19 vaccine.[21] In addition, in the present study, the mediating effect of germ avoidance behavior was revealed in the relationship between fear of COVID-19 and intention to be vaccinated. Therefore, it has been observed that when individuals experience fear, they can directly and rationally face proximal defenses to remove dangerous stimuli such as vaccination.

Recent studies have shown that the fear of COVID-19 is increasing worldwide.[22] High levels of fear of COVID-19 may be associated with anxiety, distress, and depression[23] and lead to more severe cases such as suicide.[24] According to the protection motivation theory, individuals tend to have more healthy behaviors in the presence of a health risk. Similar to the current study results, it has been found that individuals with a high fear of COVID-19 tend to use personal protective equipment more frequently, wash their hands more regularly, and prefer remote medical consultations.[13]

In addition, the current findings provide evidence showing a potential mechanism for why the fear of COVID-19 is associated with getting a COVID-19 vaccine. More specifically, fear of COVID-19 may trigger the psychological factor of an individual's germ-avoidance behavior upon receiving the COVID-19 vaccine. This psychological factor will likely contribute to individuals' intentions to get the COVID-19 vaccine. Furthermore, Pyszczynski et al.[25], awareness of a death threat can activate proximal defenses to reduce feelings of vulnerability. Thus, proximal reasons can lead people to make healthy choices, such as engaging in healthy behaviors or getting vaccinated.[26]

Also, this study revealed that germ avoidance behavior is essential in explaining individuals' intention to be vaccinated against COVID-19. Supporting the conclusion of our research, according to the protec-

tion motivation theory, it has been argued that perceived vulnerability is triggered by fear, followed by an intention to protect.[27,14]

In conclusion, current research has explored different ways of confronting the fear caused by the COVID-19 pandemic. In addition, the present findings provided potential evidence as to why the fear of COVID-19 is associated with getting a COVID-19 vaccine. More specifically, it has been observed that fear of COVID-19 can trigger the psychological factor of germ avoidance on individuals' COVID-19 vaccine intake. It has also been observed that when people focus on the real risks of COVID-19 infection, their closest defensive behavior may require rational choices, such as the intention to vaccinate as soon as possible. Therefore, the direct impact of fear of COVID-19 may positively influence the intention to vaccinate; On the other hand, the mediating effect of germ avoidance behavior may take this positive effect even further.

Ethics Committee Approval: The study was approved by Düzce University Scientific Research and Publication Ethics Committee (Date: 24/06/2021, decision no: 2021/179).

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Determining the Constipation Status and Associated Factors in the Elderly People Receiving Institutional Care

Kurumsal Bakım Alan Yaşlıların Konstipasyon Durumları ve İlişkili Faktörlerin Belirlenmesi

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ABSTRACT

Objective: The study aims to determine the constipation status and associated factors in elderly people receiving institutional care.

Materials and Methods: This descriptive and analytic research was conducted with 108 elderly people in three institutional care centers in a province in Türkiye. Data were collected with the Individual Characteristics Form, the Mini-Cog© test, and the International Physical Activity Questionnaire–Short Form. Diagnosis of constipation was made according to the Rome IV criteria.

Results: The average age of the participants was 74.16±7.51, 74.1% were female, and 46.3% were receiving institutional care for 2-4 years. It was found that 46.3% of the elderly had constipation and 72% were using laxatives. The prevalence of constipation was found to be statistically significantly higher among females, elderly people receiving institutional care for one year or less, physically inactive elderly who habitually delay defecation and elderly people who consume insufficient amounts of water ($p<0.05$).

Conclusions: The results of the study revealed that one out of the two elderly people suffers from constipation. Thus, preventive care interventions such as diet and exercise programs, and exercises for bowel habits must be applied by determining the risk factors of constipation in elderly people.

Keywords: Constipation, defecation, elderly, laxatives, old age homes

ÖZ

Amaç: Bu çalışmanın amacı uzun süreli kurumsal bakım alan yaşlıların konstipasyon durumları ve etkileyen faktörlerin belirlenmesidir.

Materyal ve Metot: Tanımlayıcı ve analitik türdeki çalışma, bir ilde bulunan üç kurumsal bakım merkezinde yaşayan 108 yaşlı ile yapıldı. Veriler Bireysel Özellikler Formu, Mini-Cog testi ve Uluslararası Fiziksel Aktivite Anketi Kısa Formu ile toplandı. Konstipasyon tanılaması Roma IV kriterlerine göre yapıldı.

Bulgular: Yaş ortalaması 74,16±7,51 olan yaşlıların % 74,1'i kadın ve %46,3'ü 2-4 yıldır kurumsal bakım almaktadır. Yaşlıların %46,3'ünde konstipasyon sorunu olduğu, %72'sinin laksatif kullandığı belirlendi. Kadınlarda, 1 yıl ve daha az süredir kurumsal bakım alanlarda, fiziksel olarak inaktif olanlarda, defekasyonu erteleme alışkanlığı olanlarda, günlük su tüketimi yetersiz olanlarda konstipasyon görülme oranının anlamlı düzeyde yüksek olduğu belirlendi ($p<0,05$).

Sonuç: Çalışmanın sonucunda kurumsal bakım alan iki yaşlının birinde konstipasyon görüldüğü belirlendi. Yaşlılarda öncelikle konstipasyon risk faktörleri belirlenerek, diyet ve egzersiz programları, tuvalet eğitimi gibi önleyici bakım girişimleri uygulanmalıdır.

Anahtar Kelimeler: Dışkılama, konstipasyon, laksatifler, yaşlı, yaşlı evleri

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INTRODUCTION

Constipation is a common health problem that negatively affects health-related quality of life due to decreased physical, mental, and social well-being. The incidence of constipation increases with age and is more common in females. While the rate of constipation for the 65-83 age group is 26% for females, and 16% for males living in society, this rate climbs to 34% for females, and 26% for males in the >84 age group.[1-3] According to studies conducted with elderly people in institutional care in Türkiye, however, the rate of constipation was 44.6%, strain during defecation was 50.9%, and severe intestinal gas complaints were 45.7%.[4,5]

Constipation is not a disease but a symptom and has many potential causes for people in old age. Age-associated conditions related to the anatomic and physiological changes in the gastrointestinal system such as normal/slow transit constipation, intestinal problems such as dyssynergic defecation, and anorectal functional changes are among the primary causes. Secondary causes include gastrointestinal disorders (tumors, biliary tract disorders, rectal prolapse, anal fissure, irritable bowel syndrome, etc.), metabolic and endocrine disorders (hyper and hypokalemia, diabetes, hyperparathyroidism, etc.), neurological disorders (Parkinson disease, stroke, dementia, etc.), congestive heart failure, and psychological disorders. Moreover, some medications (opioids, anti-inflammatory drugs, anticholinergics, etc.), dehydration, low-fiber diet, inactive lifestyle, gender, age, stress, and delaying defecation are among the secondary causes.[2,3,6,7]

Constipation adversely affects the health status of the elderly and may cause fecal impaction, fecal incontinence, hemorrhoid, anal fissure, and rectal prolapse. Extreme straining may cause fainting, cardiac ischemia, and even mortality by affecting cerebral and coronary circulation.[8,9] In addition to discomfort, the symptoms cause a decrease in the quality of life and an increase in the health care cost.[2]

It is of great importance in planning nursing care interventions to diagnose through easy-to-apply and valid tools to diagnose constipation and to prevent severe problems related to constipation. In this respect, the study aims to determine the prevalence of constipation in elderly people receiving institutional care and the associated factors.

MATERIALS AND METHODS

Ethical Considerations: Ethics approval was obtained from "Non-Clinical Research Ethical Committee" of Düzce University (Date: 05.08.2019, decision No: 2019/159). Written approval was obtained from the institutions where elderly people are receiving long-term care. The objective of the research was explained to the participants, and written consent from the volunteer participants was obtained before the interviews.

Type of Research: The study is a descriptive and analytical type of research.

Research Questions:

- What is the incidence of constipation in elderly people?
- What are the factors affecting constipation in elderly people?
- Are there any relations between the affecting factors of constipation in elderly people?

The Population and Sample: The study population consists of 189 elderly individuals living at "A", which is a retirement home (65 elderly adults), "B", which is an elderly care and rehabilitation center (87 elderly adults), and "C" which is another retirement home (37 elderly adults), which are all subsidiary of the Ministry of Family, Labor and Social Services. Without any sample selection, only the elderly people over 60 years of age who agreed to participate or whose relatives agreed to give permission were included in the study. Patients with communication difficulties declined cognitive function (Mini-Cog < 3), or patients confined to bed were excluded. Of the 108 elderly individuals in the sample, 56 had severe cognitive impairment, 12 did not want to participate in the research, and 13 elderly individuals were out of the institution during the data collection period, between September and October 2019. Of the study population, 57% were included in the study.

Data Collection Method and Tools: The Individual Characteristics Form was used to collect sociodemographic characteristics, as well as the Mini-Cog test, and International Physical Activity Questionnaire – Short Form were used for data collection. Diagnosis of constipation was made according to the Rome IV criteria. Data were collected by researchers using the face-to-face interview method. The questionnaires were completed in approximately 20–25 minutes.

Individual Characteristics Form: The form was developed by the researchers in line with the research objectives. The form includes sociodemographic information such as age, gender, marital status, education level, dietary habits, bowel habits, and stool characteristics.

Mini-Cog© Test: Mini-Cog test, developed by Borson et al.[10] is a combination of 3-item recall and clock drawing tests. In the item recall test, each word is scored one point, so a patient can get between 0 and 3 points. If the patient can recall all three words, the patient is not diagnosed with dementia. If the patient can recall one or two items, then a clock drawing test is performed. If the clock

is correct, then two points are given for a normal clock face. If it is non-normal, the patient gets zero points, indicating a sign of dementia. The total assessment is five points. A score less than three indicates the possibility of cognitive disorder and that a cognitive screening test is needed, four and five-point show that there is no sign of cognitive disorder. In their study conducted among institutionalized elderly individuals for the psychometric suitability of the test in the Turkish population, Bayram et al.[11] found that the Mini-Cog test has 90% sensitivity and 83% specificity.

Constipation Diagnosis Form: This questionnaire was developed for constipation diagnosis according to Rome IV criteria. The criteria require the presence of two or more of the symptoms listed below, which occurred at least 6 months before diagnosis, and were present during the last 3 months.

Symptoms:

1. Straining for more than 25% of defecation attempts
2. Lumpy or hard stools for at least 25% of defecation attempts
3. The sensation of incomplete defecation for at least 25% of defecation attempts
4. The sensation of anorectal obstruction/blockage for at least 25% of defecation attempts
5. Manual maneuvering to facilitate defecation for at least 25% of defecation attempts (e.g., digital evacuations, applying pressure to the pelvis)
6. Fewer than three spontaneous defecation attempts per week

In addition, loose stool must be rare without laxatives, and criteria for irritable bowel syndrome should not indicate this syndrome.[7]

International Physical Activity Questionnaire – Short Form – IPAQ: This questionnaire was developed by Craig et al.[12] to determine the physical activity level of individuals. Its validity and reliability study on the Turkish population was conducted by Öztürk in 2005.[13] The scale questions how much time an individual spent sitting, walking, and doing moderate and vigorous physical activities in the last week. Its short form was developed for survey studies and consists of seven items. While calculating the total score, Metabolic Equivalent of Task (MET) values are assigned to activities (Vigorous activity=8 METs, moderate activity=4 METs, walking=3.3 METs), and they are multiplied by the period (minutes) and their frequency (per day) to obtain weekly MET-minutes. Physical activity levels of individuals are divided into three categories "inactive", "minimally active", and "HEPA (health-enhancing physical activity) active".

Statistical Analysis: Data were converted into digital format and evaluated using the IBM SPSS 21.0 (IBM Statistical Package for Social Science) software. Constant variables were presented using mean±standard deviation, while categorical variables were given in numbers and percentages. The normal distribution of the data was assessed with the Kolmogorov-Smirnov test. While the relationships between categorical variables were evaluated using Pearson Chi-Square, Yates Chi-Square, Fisher-Freeman-Halton Exact Test, and Fisher's Exact Test, constant variables were assessed using the Mann-Whitney U test, and Student t-test. The Bonferroni Post Hoc test was used to determine the group that causes a difference in the categorical variables. Logistic regression analysis was used in multi-way regression analysis. Values with a probability of $p<0.05$ were considered statistically significant.

RESULTS

The average age of the elderly participants was 74.16±7.51, 74.1% were male, and 50.9% were elementary school graduates. Of the participants, 46.3% were receiving institutional care for 2-4 years (Table 1).

The percentage of elderly people with 3 or more

Table 1. Socio-demographic characteristics (n=108).

Characteristics		n (%)
Retirement home	A	49 (45.4)
	B	30 (27.8)
	C	29 (26.9)
Age (Mean±Standard Deviation)		74.16±7.51
Gender	Female	28 (25.9)
	Male	80 (74.1)
Level of education	Literate	28 (25.9)
	Elementary	55 (50.9)
	Secondary and Higher	25 (23.1)
Duration of institutional care	0-1 years	24 (22.2)
	2-4 years	50 (46.3)
	5 years or more	34 (31.5)

chronic diseases was 57.4%. The three most common diseases include hypertension (60.2%), musculoskeletal system disorders (42.6%), and cardiovascular diseases (39.8%). The ratio of elderly adults who can move independently was 67.6%. Yet, 49.1% were inactive with a weekly MET score of 830.69±693.00. More than half of the sample (54.6%) were found to eat fruits and vegetables daily. The average water consumption of the elderly was 7.66±3.57 glasses. However, 4.6% of the sample had the habit of delaying defecation (Table 2). Since 46.3% of the participants had constipation according to Rome IV criteria, 72% were using laxatives. Among the elderly individuals with constipation problems, 86% had straining problems during defecation, 86% had hard stools, 72% had a sense of incomplete evacuation, and 68% had to manually facilitate defecation. The weekly defecation average of the elderly individuals, who met constipation criteria, was 4.18±2.35 (Table 3).

Table 2. Characteristics related to health and habits (n=108).

Characteristics		n (%)
Chronic diseases	None	4 (3.7)
	1-2	42 (38.9)
	3 and more	62 (57.4)
Diseases*	Cardiovascular Diseases	43 (39.8)
	Hypertension	65 (60.2)
	Type II Diabetes	27 (25.0)
	GIS-Liver Diseases	9 (8.3)
	Urinary System Diseases	32 (29.6)
	Respiratory System Diseases	23 (21.3)
	Neurological Disorders	22 (20.4)
	Thyroid Diseases	10 (9.3)
	Musculoskeletal Disorders	46 (42.6)
	Psychiatric Disorders	31 (28.7)
Mobility	Independent	73 (67.6)
	Wheelchair	9 (8.3)
	Walking stick/walker	26 (24.1)
Level of activity	Inactive	53 (49.1)
	Minimally active	50 (46.3)
	HEPA active	5 (4.6)
Level of activity MET minutes/week (Mean±Standard Deviation)		830.69±693.00
Frequency of fruit and vegetable consumption	Each meal	59 (54.6)
	3-4 meals a week	42 (38.9)
	Fewer than 3 meals a week	7 (6.5)
Water consumption glass/day (Mean±Standard Deviation)		7.66±3.57
Bowel habits	I delay it	5 (4.6)
	I do it when I get the urge	103 (95.4)

*: More than one option marked.

Table 3. Characteristics related to constipation (n=108).

Characteristics		n (%)
Constipation	None	58 (53.7)
	Diagnosed	50 (46.3)
Use of laxatives*	No	14 (28.0)
	Yes	36 (72.0)
Straining during defecation*	No	7 (14.0)
	Yes	43 (86.0)
Sensation of incomplete evacuation during defecation*	No	14 (28.0)
	Yes	36 (72.0)
Hard stool*	No	7 (14.0)
	Yes	43 (86.0)
Sensation of obstruction in anorectal area*	No	16 (32.0)
	Yes	34 (68.0)
Manually maneuver to facilitate defecation*	No	19 (38.0)
	Yes	31 (62.0)
Frequency of defecation (Mean±Standard Deviation)		4.18±2.35

*: People with constipation diagnosis (n=50).

Results on the comparison of constipation with some variables are presented in Table 4. The prevalence of constipation was statistically significantly higher in elderly people who receive institutional care for one year or less, inactive patients, and people with the habit of delaying defecation ($p<0.05$). In addition, the average water consumption of the participants with constipation problems was significantly lower than those without constipation complaints ($p<0.05$) (Table 4).

Table 4. Comparison of the prevalence of constipation with some variables.

Characteristics		Constipation None	Constipation Diagnosed	Test Statistics
		n (%)	n (%)	
Age, year		73.43±6.74**	75.00±8.30**	-1.084 ¥ p=0.281
Gender	Female	9 (32.1)	19 (67.9)	5.945 β
	Male	49 (61.3)	31 (38.8)	p=0.015
Level of education	Literate	10 (35.7)	18 (64.3)	5.154 *
	Elementary	32 (58.2)	23 (41.8)	p=0.076
	Secondary or higher	16 (64.0)	9 (36.0)	
Duration of institutional care	0-1 year (a)	8 (33.3)	16 (66.7) (b)	8.720 *
	2-4 years (b)	34 (68.0)	16 (32.0)	p=0.013
	5 years and over (c)	16 (47.1)	18 (52.9)	
Chronic diseases	None	2 (50.0)	2 (50.0)	1.863 º
	1-2	26 (61.9)	16 (38.1)	p=0.417
	3 or more	30 (48.4)	32 (51.6)	
Level of activity	Inactive	22 (41.5)	31 (58.5)	8.864 º
	Minimally active	31 (62.0)	19 (38.0)	p=0.008
	HEPA active	5 (100.0)	0 (0.0) (a)	
Level of activity MET minutes/week		1019.59±1008.76**	611.58±569.95**	-2.006 £ p=0.045
Frequency of fruit and vegetable consumption	Each meal	35 (59.3)	24 (40.7)	1.993 º
	3-4 meals a week	19 (45.2)	23 (54.8)	p=0.376
	Fewer than 3 meals a week	4 (57.1)	3 (42.9)	
Bowel habits	I delay it.	0 (0.0)	5 (100.0)	6.082 ∞
	I do it when I get the urge.	58 (56.3)	45 (43.7)	p=0.019
Water consumption glass/day		8.64±3.94**	6.52±2.71**	-2.412 £ p=0.016

*: Pearson Chi-Square; ¥: Student t test; β: Yates Chi-Square; £: Mann Whitney U; º: Fisher–Freeman–Halton Exact Test; ∞: Fisher's Exact Test; **: Mean±Standard Deviation.

According to the model created as a result of the logistic regression analysis performed for determining distinctive variables that cause constipation in elderly individuals, the total variance explained was 31%. This statistically significant explanatory variable was found to be water consumption (OR=0.851, 95% Cl=0.733-0.987) (Table 5).

Table 5. Logistic regression analysis of significant variables related to constipation.

Variables		β	Standard error	p	OR	95% Cl	
Gender	Female	Reference					
	Male	0.710	0.521	0.172	2.035	0.733	5.644
Duration of institutional care	0-1 year	Reference					
	2-4 years	0.240	0.601	0.689	1.272	0.392	4.129
	5 years or more	-0.984	0.507	0.052	0.374	0.138	1.011
Level of activity MET		0.000	0.000	0.189	1.000	0.999	1.000
Bowel habits	I delay it.	Reference					
	I do it when I get the urge.	1.440	1.169	0.218	4.219	0.427	41.736
Water consumption		-0.162	0.076	0.033	0.851	0.733	0.987

β: Regression coefficient; OR: odds ratio; Cl: Confidence Interval.

DISCUSSION AND CONCLUSION

According to the study results, one of every two elderly people (46.3%) had a constipation problem as diagnosed by the Rome IV criteria. When we look at the rate of constipation among the elderly population in social studies around the world, this rate is in the range of 18-22% in Asian countries,[14,15] 20-33% in the United States, and 9-21% in European countries.[15] However, the prevalence of constipation among elderly people who receive institutional care is higher, similar to the results of this study. The rate of constipation among the elderly who receive institutional care in Türkiye is in the range of 45-52%.[5,8] Yet, studies from different countries report a rate ranging from 23% to 80%.[16-19]

The most common symptoms experienced by these

elderly individuals include straining, hard stool, the sensation of incomplete evacuation, the sensation of anorectal obstruction, and manual maneuvers to facilitate defecation. Likewise, descriptive studies report that the most common symptoms that elderly people with constipation suffer from are straining during defecation and hard stools.[4,20]

Studies focusing on the prevalence of constipation stated that women had a higher rate of constipation issues than men.[6,14,15] This prevailing rate among females is explained by hormonal factors, the anatomic structure of the pelvic floor, innervational damage in pelvic floor muscles due to childbirth or gynecological surgery, or genital prolapses.[14] In this study, the number of women with constipation (68%) was twice the number of men (39%) with constipation, similar to the literature. Considering the relationship between the duration of institutional care and constipation, the prevalence of constipation was higher among patients who have received institutional care for less than a year. In a study conducted in Türkiye, constipation was found to be more common among people who receive institutional care for more than 11 years.[8] In studies in Taiwan and Norway, however, no statistically significant relationship was found between constipation and the duration of institutional care.[16,19]

Repressing the defecation reflex and the habit of delaying defecation may cause the rectum to enlarge by adapting to the feces. This increases the risks of fecal incontinence and impaction. In retrospective studies, chronic constipation is known to increase the occurrence of fecal incontinence 1.7 times,[21] and the risk of fecal impaction 5 to 6 times.[22] In this study, each of the elderly individuals with the habit of delaying defecation was found to suffer from constipation problems. Studies similar to the present research report that 18% of the elderly with constipation delay defecation,[20] and more than half of the elderly people lacked a regular bowel program.[23]

In the study, physically inactive people were found to suffer constipation more than physically active people. In studies conducted on elderly and middle-aged groups in the literature, it was stated that people with a higher level of dependency in their daily activities,[5] people with inactive lifestyles,[24] people without regular physical activity,[8] physically limited people, people who cannot walk without aid,[25] and people who take comparatively fewer number of steps daily[26] experience more constipation problems. While the mechanisms of physical activity affecting constipation cannot be fully explained, it is stated that higher mechanical stimulation in the intestines during exercise, lowered blood flow, and more complex compression of abdominal muscles on the intestines decrease the severity of constipation.[24,27]

We determined that the daily water consumption of elderly people with constipation problems is low and that the daily amount of water consumption has a significant explanatory value in this regard. Similar to this result, it is shown in the literature that there is a negative relationship between fluid consumption and constipation.[8,26] Although the daily fluid need of each person is different, a daily fluid intake below 1.5 liters leads to constipation as a result of the absorption of liquid from the feces, leading to hard stools and constipation.[28]

In the medical treatment of constipation, using laxatives is the first option. Among the elderly people who receive institutional care in different countries, the rate of laxative usage ranges from 59% to 68%.[5,16,26] Similar to the literature, 72% of elderly individuals with constipation were found to use laxatives. In their study, which evaluates the effectiveness of laxatives, Fosnes et al.[30] reported that while they normalize the frequency of defecation and stool types, they fail to relieve straining, a sense of incomplete evacuation, and manual evacuation symptoms.

Although the study results have an authentic value, they cannot be generalized due to the limitation of being conducted in retirement homes associated with only one institution in one city. Numerous factors play a role in constipation. Yet, this study focuses on the relationship between constipation and the factors related to the lifestyle of elder people receiving institutional care. The sample selection of the participants after evaluating their level of cognitive functions reinforced the reliability of the study results.

In conclusion, the study showed that approximately half of the elderly who received institutional care had constipation problems, and 3/4 of them were using laxatives. Gender, duration of institutional care, physical inactivity, the habit of delaying defecation, and the amount of daily water consumption were found to be effective factors in the prevalence of constipation. Although constipation in old age decreases the quality of life, it's a manageable issue. In treating constipation, the first step is evaluating risk factors for each individual, revealing the causes of constipation, proposing lifestyle changes, and supporting elderly individuals for gaining regular bowel habits. Psychological and social support may facilitate the adherence of elderly individuals to treatment, and help them change their dietary and exercise habits. In addition to appropriate pharmacological treatment for constipation, evidence-based nursing care interventions with non-pharmacological approaches can also be implemented. Moreover, it is also recommended to conduct future studies with more factors and larger samples in this regard.

Ethics Committee Approval: Ethics approval was obtained from "Non-Clinical Research Ethical Committee" of Düzce University (Date: 05.08.2019, de-

cision no: 2019/159). Written approval from the institutions where older adults are receiving long-term care was obtained. Participants have been explained the objective of the research and their written consent was obtained prior to the interview and their participation was on a voluntary basis.

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

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Secondary Bacterial Agents and Antibiotic Resistance Profiles in Respiratory Tract Specimens of Patients with COVID-19 Pneumonia

COVID-19 Pnömonili Hastaların Solunum Yolu Örneklerinde Sekonder Bakteriyel Ajanlar ve Antibiyotik Direnç Profilleri

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ABSTRACT

Objective: To assess secondary bacterial pneumonia agents and antibiotic resistance rates in patients with COVID-19 pneumonia and to compare findings with the pre-pandemic period.

Materials and Methods: Bacteria grown in endotracheal aspirate fluid and bronchoalveolar fluid samples of patients diagnosed with COVID-19 between January 2020 and December 2020, and antibiotic resistance rates were retrospectively compared with samples of the year before the pandemic. Isolates were identified at the species level with an automated system (VITEK 2, bioMérieux, France), and antimicrobial susceptibility was determined according to EUCAST criteria.

Results: A total of 900 culture results were examined in 2019. Acinetobacter baumannii was detected in 36%, Klebsiella pneumoniae in 23%, Pseudomonas aeruginosa in 14%, and Staphylococcus aureus in 8%. In 2020, 660 culture results were examined, and the same bacteria were detected in 43%, 23%, 16%, and 5%, respectively. K. pneumoniae's resistance to third-generation cephalosporins, and A. baumannii's resistance to gentamycin and tobramycin, were found to have increased significantly during the pandemic period.

Conclusions: The growth of multidrug-resistant Gram-negative bacteria was frequently detected in respiratory secretions obtained during the COVID-19 pandemic. Regional bacterial agents and antibiotic resistance profiles should be clarified, and empirical therapy should be selected accordingly in COVID-19.

Keywords: Antibiotic resistance, bacterial superinfection, COVID-19, intensive care unit, pneumoniae

ÖZ

Amaç: COVID-19 pnömonisi olan hastalardaki sekonder bakteriyel pnömoni etkenleri ve antibiyotik direnç oranlarının değerlendirilmesi ve sonuçların pandemi öncesi dönemle karşılaştırılmasıdır.

Materyal ve Metot: Ocak 2020-Aralık 2020 arasında COVID-19 tanısı konan hastaların endotrakeal aspirat sıvı ve bronkoalveolar sıvı örneklerinde üreyen bakteriler ve antibiyotik direnç oranları, pandemiden önceki yıl gelen hasta örnekleriyle retrospektif olarak karşılaştırılmıştır. Kültürde üremesi olan örnekler, otomatize sistemle (VITEK 2, bioMérieux, France) tür düzeyinde tanımlanmış ve antimikrobiyal duyarlılıkları EUCAST kriterlerine göre değerlendirilmiştir.

Bulgular: 2019 da mikrobiyolojik kültürlerinde üreme saptanan 900 hasta örneği incelendi. Acinetobacter baumannii %36, Klebsiella pneumoniae %23, Pseudomonas aeruginosa %14, Staphylococcus aureus ise %8 oranında saptandı. 2020 de ise 660 hasta örneği incelendi ve sırasıyla aynı bakteriler %43, %23, %16 ve %5 oranında saptandı. K. pneumoniae'nin 3. kuşak sefalosporin direncinde ve A. baumannii'nin gentamisin ve tobramisin direncinde pandemi öncesine göre anlamlı bir artış görüldü.

Sonuç: COVID-19 pandemisinde solunum sekresyonlarında çoğunlukla çok ilaca dirençli Gram negatif bakterilerin ürediği görüldü. COVID-19 hastalığı olanlarda, bölgesel bakteriyel etkenler ve antibiyotik direnç profilleri bilinip, uygun ampirik tedavi seçilmelidir.

Anahtar Kelimeler: Antibiyotik direnci, bakteriyel süperenfeksiyon, COVID-19, pnömoni, yoğun bakım ünitesi

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INTRODUCTION

COVID-19, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has spread rapidly worldwide since 2019. It can lead to findings ranging from mild flu-like symptoms to severe pneumonia and multi-organ failure.[1] It is evident that only a portion of the population is vaccinated, and available vaccines also appear to have little effect on new variants. There is still no effective treatment for the disease, and patients may require prolonged hospitalization or treatment in intensive care units (ICUs); thus, these individuals with COVID-19 face the risk of secondary infection.[2]

There are many reasons for the development of secondary bacterial pneumonia in COVID-19. One of these is that the disease causes acute respiratory failure, leading to the need for mechanical ventilation.[3,4] Another reason is that SARS-CoV-2 can destroy the respiratory tract epithelium or can alter the normal respiratory tract microbiota due to microbial migration. These effects disrupt the flora and predispose patients to be secondary bacterial and/or fungal co-infections associated with high mortality.[5,6] According to recent reports, approximately 15-50% of deaths in COVID-19 are related to secondary infections.[7]

Antibiotic therapy has been frequently used during the COVID-19 pandemic. The rate of bacterial superinfection due to COVID-19 is between 3-15%, but up to 70% of hospitalized COVID-19 patients are believed to have received antibiotic therapy. Even mild pneumonia has often been treated with antibiotics, which can increase antibiotic resistance.[8,9]

Since the clinical management of severe COVID-19 is difficult, empirical initiation of anti-bacterial therapy was recommended by the WHO. It was included in the COVID-19 treatment guidelines of the Turkish Ministry of Health.[10,11] Empirical antibiotic use creates problems including side effects and antibiotic resistance.[1,12] To reduce these problems, it is important to identify the incidence and epidemiology of bacterial infections in such patients.

We aimed to determine which bacteria cause secondary infections in patients diagnosed with COVID -19, to assess the resistance rates of these bacteria to antibiotics, and to compare these results with pre-pandemic data.

MATERIALS AND METHODS

Ethical Statement: COVID-19 study approval was obtained from the Ministry of Health. on November 15, 2021, with Decision Number 2021/11-00-36. Ethics committee approval for the study was obtained from the Ethics Committee of Karatay University Faculty of Medicine (Date: 19/11/2021, decision no: 2021/005). The study was carried out according to the Helsinki Declaration.

Study Design and Data Collection*:* This is a retrospective study in which the types and antibiotic resistance profiles of bacteria grown in endotracheal aspirate fluid (ETA) or bronchoalveolar fluid (BAL) samples from adult clinical wards and ICUs of our hospital were compared before and during the pandemic.

Pre-pandemic data were from samples received between January 2019 and December 2019. Pandemic data were from samples obtained between January 2020 and December 2020. All data were collected from electronic hospital records and included patient demographics, laboratory findings, and microbiology data (including ETA and BAL cultures and antimicrobial susceptibility). The ages of the patients were between 20-80 years (median age 56). Specimens were taken at least three days after the patients were hospitalized and included in the study. Pre-pandemic data were obtained from 880 ETA and 20 BAL samples. Pandemic data were obtained from 650 ETA and 10 BAL samples. Respiratory specimens without growth in culture were excluded from the study. Our hospital was a "pandemic center", so all samples obtained during 2020 were from patients with a proven diagnosis of COVID-19.

Laboratory Procedures: All analyses were performed with a standardized routine methodology. Briefly, samples were seeded semi-quantitatively on 5% sheep blood agar, eosin methylene blue (EMB) agar, and chocolate agar, with incubations conducted at 37°C for 24-48 hours. If there were >25 leukocytes and <10 epithelial cells in the gram staining of ETA samples, the bacteria grown were considered secondary infection agents, and these samples were included in the study. If there was growth only in the first quadrant of the medium, the result was semi-quantitatively evaluated as "few". In the presence of growth in the second quadrant, it was assessed as "moderate". Whereas growth detection in the third quadrant was classified as "many". BAL samples were included in the study if there was $>10^4$ CFU/mL growth in the medium.

Specimens with growth in culture were identified at the species level by conventional methods, such as gram staining and colony morphology, using an automated system (VITEK2 automated system, bioMérieux, France). Antimicrobial susceptibility was determined with the same system according to EUCAST criteria.

Susceptibility to levofloxacin and ceftazidime for *S. maltophilia* was evaluated according to Clinical Laboratory Standards Institute (CLSI) criteria because these particular susceptibilities are not included in EUCAST criteria. The tigecycline MIC results for *A.*

Baumannii were evaluated according to the Food and Drug Administration (FDA) recommendations (> 8 µg/mL, resistant). Resistant strains were confirmed by the agar gradient test (bioMérieux, France) method. Over two years, the bacteria growth in BAL and ETA samples and the data containing the antibiotic resistance rates of these bacteria were recorded in a Microsoft Excel worksheet. Data before and after the pandemic were compared.

Statistical Analysis: The data obtained during the study were assessed via the SPSS v21 software (SPSS Inc., Chicago, IL, USA) with a predetermined significance threshold of $p< 0.05$. Categorical descriptive data were given with frequency (number and percentage). Between-group categorical distribution analyses were conducted via Pearson chi-square or Fisher's exact tests.

RESULTS

A total of 1560 BAL and ETA culture samples with bacterial growth were examined. In 2019, a total of 900 culture results with growth were examined: *Acinetobacter baumannii* was yielded in 328 (36%), *Klebsiella pneumoniae* in 205 (23%), *Pseudomonas aeruginosa* in 125 (14%), *Staphylococcus aureus* in 76 (8%), *Escherichia coli* in 47 (5%), *Streptococcus pneumoniae* in 27 (3%), *Enterobacter cloacae* in 15 (2%), *Serratia marcescens* in 18 (2%), *Stenotrophomonas maltophilia* in 10 (1%), *Haemophilus influenzae* in 10 (1%), and other bacteria in 39 (4%) cases. In 2020, 660 culture results with growth were examined: *A. baumannii* was reproduced in 286 (43%), *K. pneumoniae* in 150 (23%), *P. aeruginosa* in 105 (16%), *S. aureus* in 30 (5%), *E. coli* in 15 (2%), *S. pneumoniae* in 17 (2.5%), *E. cloaca* in 3 (0.5%), *S. marcescens* in 27 (4%), *S. maltophilia* in 10 (1.5%), *H. influenzae* in 8 (1.2%), and other bacteria were detected in 9 (1.3%) cases. The most common bacteria before the pandemic were *A. baumannii* (36%), *K. pneumoniae* (23%), and *P. aeruginosa* (14%). Although the order did not change during the pandemic, the positivity rate for *A. baumannii* increased, and this increase was significant ($p= 0.006$) (Figure 1).

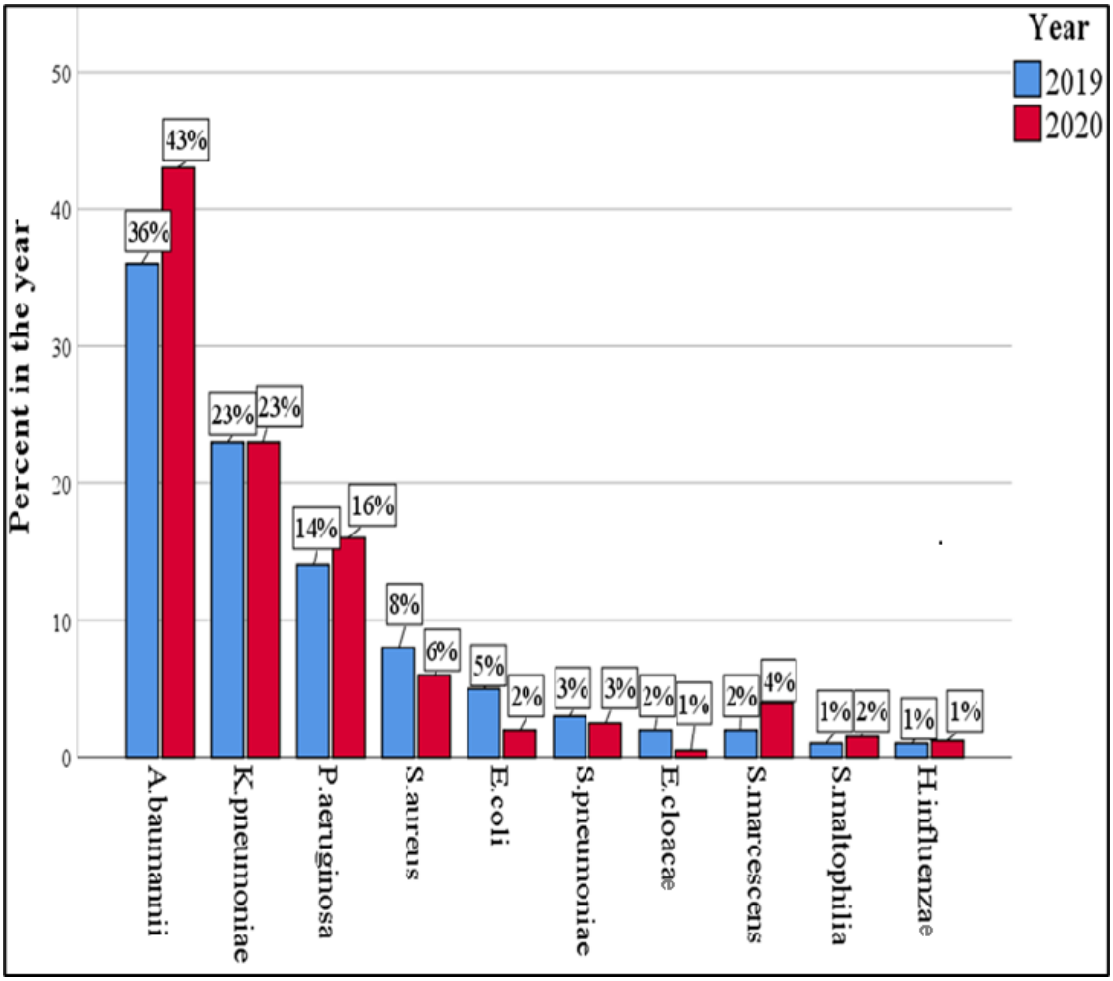

Figure 1. Percentage of bacteria detected with regard to years.

The positive rate for *K. pneumoniae* remained at 23%. Meropenem resistance for *K. pneumoniae* increased from 64% in 2019 to 70% in 2020. There was no significant increase in carbapenem resistance. Resistance to third-generation cephalosporins increased from 83% to 94%. Compared to 2019, *K. pneumoniae* strains from 2020 were found to be significantly more resistant to amoxicillin-clavulanate (p= 0.002), ceftazidime (p< 0.001), cefotaxime (p= 0.002), ceftriaxone (p= 0.002), ciprofloxacin (p< 0.001), cefepime (p< 0.001), ertapenem (p< 0.001), and gentamycin (p< 0.001) (Table 1). The positivity rates of *E. coli* decreased during the pandemic (p= 0.003), but extended-spectrum beta-lactamase (ESBL) frequency increased from 45% in 2019 to 60% in 2020. The frequency of *S. marcescens* increased from 2% to 4%, and this increase was significant (p= 0.015) (Table 1).

Table 1. Positive Rates of Fermentative Gram-Negative Bacteria and Antibiotic Resistance Rates in BAL and ETA Samples Before and During the Pandemic.

	K. pneumoniae			**E. coli**			**E. cloacae**			**S. marcescens**		
	2019 Number n (%)	**2020 Number n (%)**	**p**	**2019 Number n (%)**	**2020 Number n (%)**	**p**	**2019 Number n (%)**	**2020 Number n (%)**	**p**	**2019 Number n (%)**	**2020 Number n (%)**	**p**
Ampicillin	-	-	-	37 (7)	15 (100)	N/A	-	-	-	-	-	-
Amoxicilin-clavulanicacid	172 (84)	142 (95)	**0.002**	33 (70)	12 (80)	0.459	-	-	-	-	-	-
Amikacin	135 (66)	102 (68)	0.672	4 (9)	1 (7)	0.819	2 (14)	0 (0)	N/A	2 (12)	1 (4)	0.952
Ceftazidime	170 (83)	142 (95)	**0.001**	16 (34)	10 (67)	**0.026**	2 (14)	1 (34)	0.396	4 (22)	10 (37)	0.293
Cefotaxime	170 (83)	141 (94)	**0.002**	23 (49)	10 (67)	0.231	2 (14)	1 (34)	0.396	13 (72)	11 (40)	**0.038**
Ceftriaxone	170 (83)	141 (94)	**0.002**	21 (45)	10 (67)	0.138	2 (14)	1 (34)	0.396	5 (28)	11 (40)	0.373
Ciprofloxacin	168 (82)	142 (95)	**0.001**	19 (40)	10 (67)	0.076	2 (14)	1 (34)	0.396	0 (0)	2 (7)	N/A
Cefepime	164 (80)	140 (93)	**0.001**	13 (28)	6 (40)	0.367	2 (14)	1 (34)	0.396	2 (12)	3 (11)	1
Ertapenem	157 (77)	138 (92)	**0.001**	2 (4)	1 (7)	0.704	2 (14)	1 (34)	0.396	0 (0)	2 (7)	N/A
Gentamycin	137 (67)	148 (99)	**0.001**	10 (21)	5 (34)	0.342	2 (14)	1 (34)	0.396	2 (12)	2 (7)	0.669
Meropenem	131 (64)	105 (70)	0.229	3 (6)	1 (7)	0.968	2 (14)	1 (34)	0.396	0 (0)	0 (0)	NA
Piperacillin-tazobactam	166 (81)	141 (94)	**0.001**	16 (34)	4 (27)	0.595	2 (14)	1 (34)	0.396	4 (22)	4 (15)	0.524
Trimethoprim-sulfamethoxazole	166 (81)	130 (87)	0.155	12 (25)	7 (47)	0.122	0 (0)	1 (34)	N/A	0 (0)	2 (7)	N/A
TOTAL PATIENTS	205 (23)	150 (23)	0.981	47 (5)	15 (2)	**0.003**	15 (2)	3 (0.5)	0.057	18 (2)	27 (4)	**0.015**

Total number of patients with growth in respiratory secretions in 2019: 900
Total number of patients with growth in respiratory secretions in 2020: 660

N/A: not applicable.

Tigecycline resistance (strains with MIK> 8) for *A. baumannii* increased during the pandemic, and this increase was significant (p< 0.001). Compared to 2019, the resistance of *A. baumannii* strains to gentamycin (p= 0.005), tobramycin (p= 0.012), and tigecycline (p< 0.001) antibiotics were significantly higher in 2020. Ceftazidime resistance for *P. aeruginosa* increased during the pandemic, and this increase was also significant (p= 0.028) (Table 2).
The positivity rates for *S. aureus* decreased during the pandemic (p= 0.003). The frequency of methicillin-resistant *S. aureus* (MRSA) remained unchanged during the pandemic. (Table 3)

Table 2. Positive rates of gram-negative non-fermentative bacteria and antibiotic resistance rates in BAL and ETA samples before and during the pandemic.

Antibiotics	A. baumannii			P. aeruginosa			S. maltophilia		
	2019 Number n (%)	2020 Number n (%)	p	2019 Number n (%)	2019 Number n (%)	p	2019 Number n (%)	2019 Number n (%)	p
Ceftazidime	-	-	-	33 (27)	42 (40)	**0.028**	6 (60)	6 (60)	1.0
Cefepime	-	-	-	45 (36)	38 (36)	0.976	-	-	-
Piperacillin	-	-	-	57 (46)	42 (40)	0.393	-	-	-
Piperacillin-tazobactam	-	-	-	53 (43)	38 (36)	0.337	-	-	-
Amikacin	216 (66)	188 (66)	0.975	5 (4)	4 (4)	0.941	-	-	-
Gentamycin	288 (88)	270 (94)	**0.005**	-	-		-	-	-
Tobramycin	291 (89)	270 (94)	**0.012**	6 (5)	5 (5)	0.989	-	-	-
Ciprofloxacin	325 (99)	286 (100)	0.104	30 (24)	30 (28)	0.432	-	-	-
Levofloxacin	325 (99)	286 (100)	0.104	53 (43)	40 (38)	0.507	6 (60)	6 (60)	1.0
Imipenem	325 (99)	284 (99)	0.767	58 (47)	50 (47)	0.854	-	-	-
Meropenem	325 (99)	284 (99)	0.767	42 (33)	37 (35)	0.794	-	-	-
Tigecycline (MIK >8)	19 (6)	72 (25)	**0.001**	-	-	-	-	-	-
Trimethoprim-sulfa-methoxazole	-	-	-	-	-	-	5 (50)	5 (50)	1.0
Total Patients	328 (36)	286 (43)	**0.006**	125 (14)	105 (16)	0.266	10 (1)	10 (1.5)	0.483
Total number of patients with growth in respiratory secretions in 2019: 900									
Total number of patients with growth in respiratory secretions in 2020: 660									

Table 3. Positive rates of gram-positive bacteria and antibiotic resistance rates in BAL and ETA samples before and during the pandemic.

Antibiotics	S. aureus			S. pneumoniae		
	2019 Number n (%)	2020 Number n (%)	p	2019 Number n (%)	2020 Number (%)	p
Penicillin	60 (79)	25 (83)	0.609	3 (11)	0	0.155
Cefoxitin	27 (36)	11 (36)	0.912	-	-	-
Erythromycin	16 (21)	6 (20)	0.904	10 (37)	6 (38)	0.906
Clindamycin	5 (7)	2 (7)	0.987	12 (45)	7 (42)	0.831
İnd. Clindamycin resistance	9 (12)	3 (10)	0.787	-	-	-
Ciprofloxacin	3 (4)	0	0.270	-	-	-
Levofloxacin	2 (3)	0	0.370	7 (26)	4 (24)	0.858
Cefotaxime	-	-	-	10 (37)	6 (35)	0.907
Ceftriaxone	-	-	-	10 (37)	6 (35)	0.907
Vancomycin	0	0	-	0	0	-
Teicoplanin	0	0	-	0	0	-
Linezolid	0	0	-	0	0	-
TOTAL	76 (8)	30 (6)	**0.003**	27 (3)	17 (2.5)	0.617
Total number of patients with growth in respiratory secretions in 2019: 900						
Total number of patients with growth in respiratory secretions in 2020: 660						

DISCUSSION AND CONCLUSION

Many studies have explored factors affecting the prognosis of COVID-19, and it has been observed that co-morbid diseases and advanced age are the leading factors.[13] If respiratory failure develops in a patient with COVID-19 infection, a need for intubation and mechanical ventilation may arise. Hospital-associated or ventilator-associated pneumonia may develop due to these invasive procedures.[14] Data on causative agents and antibiotic susceptibility in these pneumonia cases secondary to COVID-19 are still insufficient, but they are usually caused by multi-drug-resistant bacteria and have very high mortality rates.

In our study, culture analyses were used. Before the pandemic, the most common bacteria in BAL and ETA samples were *A. baumannii* (36%), *K. pneumoniae* (23%), and *P. aeruginosa*. Although the ranking did not change during the pandemic, the positivity rate of *A. baumannii* increased significantly. We attributed this increase to long-term hospitalization and a higher frequency of steroid use in COVID-19 patients. Of note, *E. coli, E. cloacae*, and *S. aureus* frequency decreased during the pandemic, while *S. marcescens* and *S. maltophilia* frequencies increased slightly. Although the number of patients admitted to the hospital decreased due to the COVID -19 pandemic, findings related to the types of bacteria causing hospital infections and their antibiotic resistance profiles appeared to be unchanged. This was attributed to the fact that healthcare personnel contacted many patients due to high workloads.

It is well established that 20-30% of influenza infections are accompanied by secondary bacterial pneumonia with the most common causative bacteria identified as *S. pneumoniae* and *S. aureus.*[15] The association between COVID-19 and bacterial pneumonia is less apparent, and the most common bacteria appear to be gram-negative and antibiotic-resistant bacteria, such as *A. baumannii* and *K. pneumoniae.*[13] Around 7% of patients with a diagnosis of COVID-19 were hospitalized in wards, and 14% of those hospitalized in ICUs are suggested to have secondary bacterial pneumonia.[16,17]

Various studies have examined the respiratory tract samples of patients with COVID-19 using multiplex PCR and culture methods. In France, Camelana et al. detected bacterial infection in 35% of BAL samples and found *P. aeruginosa* and *S. aureus* to be the most common bacteria.[18] In a study conducted in Korea using multiplex PCR and culture methods together, *A. baumannii* was found to be present in 33% and *P. aeruginosa* in 30% as secondary bacterial pneumonia agents in COVID-19 patients.[19] A similar study from the USA reported that *Enterobactericea* and *P. aeruginosa* were the most common bacteria.[20] Rapid and reliable multiplex PCR assays have been recommended for the assessment of respiratory tract samples during the pandemic. In cases where bacterial agents could not be detected, discontinuation of antibiotic treatment was recommended, particularly when the radiological imaging findings were in favor of viral pneumonia.[1,21]

The frequencies of resistant bacteria in clinical services and ICUs differ between countries and hospitals. In a study from China, bacterial pneumonia was observed in 7% of patients with COVID-19, and 75% of them were determined to have *A. baumannii* and carbapenem-resistant *K. pneumoniae*. It has been emphasized that bacterial pneumonia caused by these factors is the most important cause of death in patients with COVID-19.[14] In our study, the increase in carbapenem resistance for *K. pneumoniae* was not significant, but the increase in resistance to third-generation cephalosporins was found to be significant. We only included samples with growth among COVID-19 patients, which may explain these relatively high rates.

Empirical antibiotic use is life-saving due to the prevention of these infections, and national and international guidelines already recommend this approach.[22] But antimicrobial therapy has undesirable effects including toxicity, diarrhea, and the spread of antimicrobial resistance. Therefore, appropriate empirical antibiotic selection should be kept in mind during the management of such patients. Antibiotic therapy should be rapidly terminated after bacterial pneumonia is excluded.[23]

The reason for the development of secondary bacterial pneumonia in COVID-19 is likely to be associated with prolonged hospitalization, the use of steroids, or the need for mechanical ventilation.[6,22] Many antibiotics have been used in COVID-19 to prevent the development of secondary bacterial pneumonia and to treat bacterial co-infections. Antibiotics were selected for empirical treatment based on data from cases of bacterial pneumonia developing in pre-COVID-19 viral infections.[24] However, since COVID-19 cases are hospitalized for a long time, and the disease is often very severe, more antibiotics have been used compared to previous viral pneumonia cases, causing increased antibiotic resistance.

In this study, COVID-19 patients with suspected secondary bacterial pneumonia were assessed, and all samples that the laboratory received were evaluated and included in the study. However, we did not have access to the clinical data of the patients or other clinical information concerning the presence/ absence of secondary infection. This is a limitation of our study.

Additionally, due to COVID-19-related lockdowns, the number of patients admitted decreased during the pandemic; therefore, the frequency of infectious

bacteria and antibiotic resistance may have been overestimated. The study may become more meaningful if it is combined with data from other hospitals, and the number of samples increases.

In conclusion, in hospitalized patients with COVID-19, secondary bacterial pneumonia agents are mostly Gram-negative bacteria with high pathogenicity and mortality, demonstrating resistance to most drugs. Unfortunately, antibiotic resistance rates were found to have increased during the pandemic. To prevent this increase, laboratory parameters that can aid physicians in distinguishing between bacterial and viral infections should be used. Appropriately empirical treatment should be selected by accurately describing local/regional bacterial agent types and antibiotic resistance profiles.

Ethics Committee Approval: Ethics committee approval for the study was obtained from the Ethics Committee of Karatay University Faculty of Medicine (Date: 19/11/2021, decision no: 2021/005). The study was carried out according to the Helsinki Declaration.

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

Author Contributions: Concept- OA developed the concept; Supervision- OA, ARU, HO, FA, MOG; Materials- OA, AR; Data collection and/or processing- OA, ARU, HO, FA; Analysis and/or interpretation- OA, ARU, HO, FA; Writing- OA.

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Investigation of Pain, Function, Anxiety, and Depression Levels in Patients with Total Knee Arthroplasty Before Discharge

Total Diz Artroplastisi Yapılan Hastaların Taburculuk Öncesi Ağrı, Fonksiyonel Durum, Anksiyete ve Depresyon Düzeylerinin İncelenmesi

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ABSTRACT

Objective: We aimed to investigate the pain, function, and anxiety-depression levels in patients who had undergone total knee arthroplasty (TKA) in the early postoperative period.

Materials and Methods: Fifty-eight patients between the ages of 60-85 with TKA were included. From the follow-up files of the patients, data on physical characteristics, pain intensity, function, and anxiety-depression levels were used.

Results: When the measurement scores were compared according to gender, no statistically significant difference was found in pain (p:0.49) and physical function (p:0.20). When anxiety-depression levels were compared, it was found that females got higher scores than males (p:0.00). While there was no significant relationship between the pain and knee scores; it was found that there was a moderate positive correlation between pain and anxiety-depression levels (r1:0.520, r2:0.514; p<0.01). There was a statistically significant moderate negative correlation between knee scores and anxiety-depression levels (r1:-0.469, r2:-0.482; p<0.01).

Conclusions: This study showed that the physical and psychological states are interrelated. Evaluation in the postoperative period by considering both parameters; will provide a successful disease management process by playing an active role in the follow-up of patients.

Keywords: Dysfunction, knee prosthesis, osteoarthritis

ÖZ

Amaç: Çalışmamızın amacı; total diz artroplastisi yapılan postoperatif erken dönemdeki hastaların; taburculuk öncesi ağrı, fonksiyonel durum, anksiyete-depresyon düzeylerini incelemekti.

Materyal ve Metot: Retrospektif çalışmamıza 60-85 yaş aralığındaki 58 hasta katıldı. Hastaların takip dosyalarından; fiziksel özelliklerine, ağrı şiddetlerine, fonksiyonel durumlarına ve anksiyete-depresyon düzeylerine ilişkin verileri kullanıldı.

Bulgular: Olguların cinsiyetlerine göre ölçüm skorları karşılaştırıldığında ağrı ve fiziksel fonksiyon parametrelerinde istatistiksel olarak anlamlı fark bulunmadı (p:0.49; p:0,20). Hastane anksiyete ve depresyon düzeyleri karşılaştırıldığında, kadınların erkeklere göre daha yüksek skor aldığı saptandı (p:0,00). Olguların ağrı şiddetleriyle diz skoru arasında anlamlı ilişki bulunmazken; anksiyete-depresyon düzeyleriyle arasında pozitif yönlü-orta düzeyde ilişki olduğu görüldü (r_1:0,520, r_2:0,514; p<0,01). Diz skoruyla anksiyete-depresyon düzeyleri arasındaysa negatif yönlü-orta düzeyde bir ilişki olduğu bulundu (r_1:-0,469, r_2: -0,482; p<0,01).

Sonuç: Çalışmamızın sonuçları, olguların fiziksel ve psikolojik durumlarının ilişkili olduğunu gösterdi. Hastaların taburculuk öncesi postoperatif erken dönemde, her iki parametrenin de göz önünde bulundurularak değerlendirilmesi; hastaların taburculuk sonrası takiplerinde etkin rol oynayarak başarılı bir hastalık yönetim süreci sağlayacaktır.

Anahtar Kelimeler: Disfonksiyon, diz protezi, osteoartrit

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INTRODUCTION

Osteoarthritis (OA), one of the world's most common chronic degenerative diseases, occurs primarily in load-bearing joints.[1,2] OA, which causes functional loss in the knee joint, reduces the quality of life of individuals by negatively affecting their daily living activities and may cause physical and psychosocial deficiencies by limiting their social activities.[1,3] In addition, the most common symptom in these patients is pain. Pain may lead to limitations in even simple daily living activities as the disease progresses. This may lead to impairment in the patient's psychological health and social functions.[4,5] Functional status of patients with OA is affected by factors such as immobility due to pain, limitation of joint movement, decrease in muscle strength, etc. Concerning these, walking difficulties may also occur in the advanced stages of OA.[3]

Total knee arthroplasty (TKA) is a frequently preferred surgical intervention in advanced OA cases where medical treatment and physiotherapy do not benefit.[3] It is stated that factors such as depression and anxiety may play a role in the pain intensity and functions of patients with OA. Therefore, the functional disorder, pain, and social isolation caused by the disease play a role in the development of both anxiety and depression in individuals.[6]

It's been reported that the most common psychological disorders along with physical illnesses are anxiety and depression, and these are frequently seen together.[7] States of depression and/or anxiety, which occur before surgery and increase in the early postoperative period, may lead to the development of chronic pain and negatively affect the functions of patients.[8,9]

The occurrence of a psychological disorder accompanying the physical illness; can adversely affect the patient's response to treatment, compliance with the treatment and rehabilitation program, quality of life, and the effectiveness of the treatment applied.[10] For this reason, it is essential to determine the severity of these conditions in the early postoperative period and to treat them in time. Considering the impact of emotional states on the worsening of OA symptoms, this study aimed to examine the pain, function, and anxiety-depression levels of TKA patients in the early postoperative period.

MATERIALS AND METHODS

Ethical Status: This study was approved by the Human Research Ethics Committee of Muğla Sıtkı Koçman University (Date: 22.09.2020, decision no:200002/3), and institutional permission was obtained. It has been conducted according to the ethical rules in the Declaration of Helsinki.

This descriptive study was conducted on patients who had undergone TKA surgery and were hospitalized at the Department of Orthopedics and Traumatology in Muğla Sıtkı Koçman University Training and Research Hospital between January 2019-March 2020. Patients aged 60-85 years, who received physiotherapy and rehabilitation (PTR) care in the early postoperative period during hospitalization in the service, were taken as the sample group. Exclusion criteria were as follows: (1) being a note in the patient's file regarding hearing, vision, and mental problems that may affect the results, (2) having a history of surgery other than TKA in the last 6 months, and (3) missing data. The study was completed by retrospectively examining the PTR records of patients kept by physiotherapists and data obtained from the records of 58 patients who met the criteria.

Outcome Measures

Visual Analogue Scale (VAS): This scale was used to determine the intensity of pain. The patients were asked to mark their pain intensity on a 10 cm line, considering that the starting point is 0 means no pain and the ending point is 10 means unbearable pain. The intensity of pain was determined by measuring the distance between the starting and the patient's marked points.[11]

Hospital for Special Surgery (HSS) Knee Score: The Turkish version of the HSS Knee Score was used to evaluate the functional status.[12] This scale, which is widely used in the clinic by physiotherapists and orthopedic surgeons, was developed by Insall et al. and includes subjective (52%) and objective (48%) examination criteria. The maximum score that can be obtained (pain (30 points), function (22 points), range of motion (18 points), muscle strength (10 points), deformity (10 points), and instability (10 points)) is 100 points. The function is evaluated as 'excellent' if the score is >85, 'good' if it is between 70-84, 'moderate' if it is between 60-69, and bad if it is <60.[13]

The Hospital Anxiety and Depression (HAD) Scale: The Turkish version of HAD Scale was used to determine the anxiety-depression levels of the patients.[14] This instrument was developed by Zigmond and Snaith[15] and contains 14 items (7 for anxiety and 7 for depression symptoms). The questions are scored between 0-3, and the cut-off score for each subscale is ≥ 8.[7,14]

Statistical Analysis: G-Power (Windows version 3.1.9.4) was used to determine the sample size (power:0.80, alpha:0.05, and effect size:0.3 were considered), and it requires a total sample size of 64 participants. Data were analyzed using the Statistical Package for the Social Science (Version 24.0). Skewness-Kurtosis tests were used and it was seen that the data were not suitable for normal distribu-

tion. The Mann-Whitney U test was used to analyze the mean rank differences between categories of different variables, and the relationship between categorical variables was analyzed by Spearman correlation. Statistical significance was set at a 95% confidence interval and $p<0.05$.

RESULTS

The data of 62 patients were analyzed and 58 patients (38 females and 20 males) were included in this study according to inclusion-exclusion criteria. The mean age of the patients was 69.93±6.24 years. When the pain intensity of the patients was questioned, the median (min-max) values were 5.7 (3.7-8.7) and 5.6 (2.2-8.9), respectively. Functional status was examined, and it was found that the median (min-max) HSS knee score was 47 (25-71). Depression levels of the patients were within normal limits, and the median (min-max) was 7 (0-18), while anxiety levels were borderline, and the median (min-max) was 8 (0-19). The relationship between these was analyzed and it was found a statistically significant strong positive correlation (r:0.825; $p<0.01$).
The comparison of pain intensity, function, and hospital anxiety-depression levels by gender is shown in Table 1.

Table 1. Comparison of pain intensity, function, and hospital anxiety-depression levels of patients by gender.

	Gender	Median (min-max)	U	p
VAS	Female Male	5.7 (3.7-8.7) 5.6 (2.2-8.9)	338.00	0.49
HSS Knee Score	Female Male	45 (32-71) 53 (25-63)	302.00	0.20
HAL	Female Male	10 (1-19) 4 (0-15)	164.00	**0.00***
HDL	Female Male	9 (0-18) 2.5 (0-8)	124.00	**0.00***

VAS: Visual Analogue Scale; HAL: Hospital Anxiety Level; HDL: Hospital Depression Level; *p<0.05.

Table 2. The relationship between the pain intensity, function, and hospital anxiety-depression levels of patients.

r	VAS	HSS Knee Score	HAL	HDL
VAS	1	-0.246	0.520*	0.514*
HSS Knee Score	-0.246	1	-0.469*	-0.482*
HAL	0.520*	-0.469*	1	0.825*
HDL	0.514*	-0.482*	0.825*	1

VAS: Visual Analogue Scale; HSS: Hospital Special Surgery; HAL: Hospital Anxiety Level; HDL: Hospital Depression Level; r: Spearman correlation coefficient; *p<0,05.

The relationship between all parameters was determined by Spearman correlation analysis and is shown in Table 2.

DISCUSSION AND CONCLUSION

Our study was completed with the data obtained from the records of 58 patients aged between 60-85 years. Our findings indicated no statistically significant difference between genders in pain and physical function scores. When we compared the anxiety-depression levels, we found that females got higher scores than males. While there was no significant relationship between the pain and knee scores, it was found that there was a moderate positive correlation between pain and anxiety-depression levels. Also, there was a statistically significant moderate negative correlation between knee scores and anxiety-depression levels.
Pain and functional status after TKA depend on a wide range of factors, including gender. Many studies reported females having higher pain intensity and/or lower function than males. Nandi et al. found

that female patients had higher pain intensity compared to males. Also, they reported function scores in favor of male subjects in this study with 100 individuals.[16] However, there are also studies in the literature showing that there is no difference by gender. Sanchez-Santos et al. reported that there was no difference by gender in outcome scores, including pain and function in the patients (aged 60-80 years).[17] Similarly, we found no statistical difference between the genders with regard to HSS knee scores. The mean HSS knee scores were 46.16 for females and 48.6 for males, which were classified as "poor". In our study, the pain intensity of females was found to be higher than males, but there was no statistically significant difference. It was thought that this situation might be due to the small sample size of the current study.

When the Hospital Anxiety Level (HAL) and Hospital Depression Level (HDL) results in our study were compared by gender, it was seen that females got higher scores than males. It was reported in many studies that mental disorders such as depression-anxiety are more common in females. In a study conducted by Stundner et al. on TKA patients, it was reported that anxiety-depression levels of females were higher than males.[18] Similarly, Nandi et al. found a similar conclusion.[16] Considering the results obtained, our findings were supported by the literature. When the relationship between these two parameters was examined, we determined a strong positive correlation between anxiety and depression levels. The reason may be that these psychiatric conditions are frequently seen together in the clinic and the commonality in their diagnostic symptoms.[7]

When the studies in the literature are reviewed, although most of the pain and function evaluations were made in the chronic postoperative period after discharge, there are also acute period studies. As a matter of fact, if the pain is high in the early period, it aggravates the knee functions; similarly, the decrease in the chronic period positively affects the knee scores. Many studies reported that high-intensity pain affects function negatively.[19,20] However, Wylde et al. reported that there was insufficient evidence to draw firm conclusions about the association between any postoperative patient-related factors, such as function and chronic pain after TKA.[21] Similarly, our results showed that there was no statistically significant relationship between the VAS and the HSS knee scores.

It is known that the psychological status of patients influences their perception of pain after TKA by focusing on the roles of depression and anxiety. Also, pain catastrophizing may be a consistent predictor of poor outcomes after TKA.[22] According to the findings of our study, it was seen that the pain intensity and hospital anxiety-depression levels of patients were correlated. Similarly, many studies are reporting a statistically significant relationship between pain intensity and anxiety-depression levels. Lots of studies reported that pain has an inevitable relationship with anxiety-depression and patients with anxiety/depression symptoms have higher pain scores.[16]

When we examine the current publications, it is seen that there are many studies resulting that there is a relationship between functional status and anxiety and depression. Xu et al. reported a significant correlation between the postoperative functional status of patients who had undergone TKA and their anxiety and depression levels.[23] In the study conducted by Kılınç, as the physical activity levels of individuals in the postoperative period who had TKA increased, it was found that their pain decreased, their functionality increased, and their anxiety and depression levels decreased.[24] A previously published research by Scopaz et al. has shown that high levels of anxiety and depression affect function negatively.[6] In our study, it was found that there was a moderate negative correlation between the function and anxiety-depression levels of the patients. It can be said that the increase in the depression-anxiety levels of the patients is related to the decrease in their functions. These results showed that although we consider the physical and psychological states of the patients separately, they are indeed related to each other. It is known that the presence of physical illness increases the risk of any psychiatric disorder and is mostly seen together with depression and anxiety.[7] From a clinical perspective, research on these psychological factors is necessary because it helps us to identify factors warranting our attention when designing interventions to improve outcomes after TKA. According to a definition by World Health Organization, health is "a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity".[25] As you can see, the holistic health approach includes many different components. If these components have any problem, the general health status may also be adversely affected.[26] The most significant limitation of our study is the small sample size and the lack of evaluations during the preoperative period.

Most studies identified that a higher baseline anxiety and depression level predicted a poorer outcome. Also, it is stated that higher pre-operative knee pain and poor function were consistent predictors of worse outcomes after surgery.[27,28] A systematic review by Sorel et al. showed that perioperative interventions targeting psychological distress for TKA patients seem to positively affect postoperative outcomes such as pain, function, and quality of life.[29] Based on these, it can be thought that education about the operation, physiotherapy, and psychologi-

cal support in the preoperative period may have positive effects on the management of the disease in the postoperative period.

In conclusion, it is very important to determine the severity of symptoms such as pain, physical dysfunction, and anxiety-depression in TKA patients in the early postoperative period and to treat in time to prevent them from worsening the current situation by affecting each other. The success of TKA surgery is related to the effective postoperative treatment approaches, and the patient's compliance and willingness to this treatment program should be kept in mind.

Ethics Committee Approval: Our study was approved by the Ethical Committee of Muğla Sıtkı Koçman University (Date:09.22.2020, decision no:200002/3). This study was conducted in accordance with the declaration of Helsinki.

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

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Relation between Health Quality Perceptions and Patient Safety Cultures of Nurses Working in Surgical Clinics

Cerrahi Kliniklerde Çalışan Hemşirelerin Sağlık Kalitesi Algıları ile Hasta Güvenliği Kültürleri Arasındaki İlişki

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ABSTRACT

Objective: The study was carried out to determine the effect of health quality perceptions of nurses working in surgical clinics on patient safety culture.

Materials and Methods: The research is descriptive type and correlational. The sample of the research consisted of 237 nurses. Personal Information Form created by the researchers, Patient Safety Culture Scale (PSCS), and Quality Perception Scale (QPS) were used as data collection tools in the study. Data were collected between January 2021 and February 2022.

Results: The total mean score obtained from PSCS was found to be 3.36±0.21 in the study. In the study, it was found that the total mean score obtained from the QPS was 82.15±6.80. A positive, moderate correlation was found between the total score of PSCS and the total score of QPS (r= 0.407, p<0.01).

Conclusions: It was determined that the increase in the quality perceptions of the nurses working in the surgical clinics increased the patient safety culture. It was observed that nurses who care for surgical patients attach importance to patient safety culture, especially in terms of patient benefit. It is recommended to organize in-service training programs to increase both quality perceptions and patient safety culture of healthcare professionals.

Keywords: Patient safety, surgical nursing, quality of healthcare

ÖZ

Amaç: Araştırma cerrahi kliniklerde çalışan hemşirelerin sağlık kalitesi algılarının hasta güvenliği kültürüne etkisini belirlemek amacıyla yapılmıştır.

Materyal ve Metot: Araştırma tanımlayıcı ve ilişki arayıcı tiptedir. Araştırma örneklemini 237 hemşire oluşturmuştur. Araştırmada veri toplama aracı olarak Kişisel Bilgi Formu, Hasta Güvenliği Kültürü Ölçeği (HGKÖ) ve araştırmacılar tarafından oluşturulan Kalite Algı Ölçeği (KAÖ) kullanılmıştır. Veriler Ocak 2021 ile Şubat 2022 arasında toplanmıştır.

Bulgular: Çalışmada HGKÖ'den elde edilen toplam puan ortalaması 3,36±0,21 olarak bulundu. Çalışmada KAÖ'den alınan toplam puan ortalamasının 82,15±6,80 olduğu bulunmuştur. HGKÖ toplam puanı ile KAÖ toplam puanı arasında pozitif, orta düzeyde bir ilişki bulundu (r=0,407, p<0,01).

Sonuç: Cerrahi kliniklerde çalışan hemşirelerin kalite algılarındaki artışın hasta güvenliği kültürünü arttırdığı saptandı. Cerrahi hastalarına bakım veren hemşirelerin özellikle hasta yararı açısından hasta güvenliği kültürüne önem verdikleri görüldü. Sağlık çalışanlarının hem kalite algılarını hemde hasta güvenliği kültürünü arttırmaya yönelik hizmet içi eğitim programları düzenlenmesi önerilmektedir.

Anahtar Kelimeler: Cerrahi hemşireliği, hasta güvenliği, sağlık hizmetinin kalitesi

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INTRODUCTION

Quality perception in health can generally be defined as ensuring the safety of patients and employees, increasing satisfaction, providing effective and accurate healthcare service delivery. However, situations that are not predictable due to the complex organizational structure of health institutions cause many medical errors.[1] Medical errors that compromise patient safety are among priority quality problems because errors are irreversible.[2]

Attitude and behavior of healthcare professionals, past experiences and expectations of service areas constitute a personal sense of quality.[3-5] It is important to increase the perception and awareness of healthcare professionals in order to create a culture on patient safety.[6] One of the key objectives within the concept of patient safety is to ensure safe surgical steps. The safe surgical process, which includes the hospitalization of the patient, pre-operative, post-operative and even discharge processes, aims to provide treatment and care services safely. Due to the presence of complex medical devices and equipment in surgical clinics, the risk of undesirable events such as injury or accident is higher than in other clinics.[6-7] For this reason, nurses working in surgical clinics have the responsibility to provide safe care for safe surgical processes, which reduces the risk of damage to patients before and after surgery.[8] The concept of patient safety is an important concept that depends on the characteristics of nurses, clinics and patients in the surgical field.[6] In a study examining the obstacles to the formation of a patient safety culture, which is one of the perceptions of quality in health in nurses, it is stated that the event notifications are insufficient.[9] Similarly, in a different study, it was reported that nurses were generally reluctant to report event reports and.[10]

The nurses, who are the largest workforce in their healthcare presentation, spend almost all their time caring for patients.[11,12] However, the working conditions of nurses are very difficult due to excessive workload, high hours of work, patient-induced emotional stress, inadequate nurse, lack of materials, and irregular working hours.[13-15] These adverse working conditions increase the risk of unintended exposure to problems related to patient safety for nurses.[8]

In this study, the relationship between health quality perceptions and patient safety cultures of nurses working in surgical clinics was investigated.

MATERIALS AND METHODS

Ethics Committee Approval: Necessary permissions were obtained from the hospital where the study was conducted and the İnönü University Health Sciences Non-Invasive Clinical Research Ethics Committee (Date: 24.12.2019, decision no: 2019/138). The nurses were informed by the researchers and the volunteer information form was presented to the nurses together with the questionnaire in line with the Helsinki Declaration.

Research Design and Sampling: The descriptive and correlational study was carried out between January 2021 and February 2022 with nurses working in the surgical clinics of a university hospital in Turkey.

The research population consisted of 298 surgical nurses working in the surgical clinics of the university hospital. It was aimed to reach the entire population without using any sampling method. The population consisted of nurses who met the inclusion criteria and volunteered to participate in the research (N=237). 79.5% of the research population has been reached. Since 19 of the nurses took unpaid leave, 28 of the nurses refused to participate in the study, and 14 of the nurses filled in the study questionnaire incompletely, they were not included in the sample. Criteria for inclusion in research: Working in surgical clinics/surgical intensive care units for at least six months, accepting participation in the study. The data were collected by the researchers in the surgical clinics of the relevant university hospital using a face-to-face survey method. It took an average of 7-8 minutes to fill out each questionnaire.

Data Collection Tools: "Personal Information Form" consisting of 14 questions, "Patient Safety Culture Scale" (PSCS), and "Quality Perception Scale" (QPS) were used as data collection tools in the research. Information about PSCS and QPS is presented below:

Patient Safety Culture Scale (*PSCS*): This scale, which was developed by Turkmen et al., consists of 51 items in total and includes five sub-dimensions.[16] The scale is a four-point Likert scale. The total PSCS score is obtained by adding the mean score of the 5 sub-dimensions of the scale and dividing the result by 5. Interpretation of scale scores between 1 and 4 points: A mean score of 1 indicates the presence of a negative patient safety culture, and an increase of 4 indicates the presence of a positive patient safety culture. Türkmen et al.[16] reported the Cronbach's alpha value of the PSCS as 0.97. In this study, the Cronbach's alpha value of the scale was 0.88.

Quality Perception Scale (QPS): The "Quality Perception Scale" developed by Bayer and Baykal and brought to the literature is divided into 7 sub-dimensions and includes 70 questions.[17] The scale is of a 5-point Likert type and the lowest score that can be obtained in QPS is 20, and the highest score is 100. A high total score from the QPS and its sub-dimensions indicates that the perception of quality is positive, while a low score indicates that the individ-

ual's perception of quality is negative.[17] Bayer and Baykal found the Cronbach's alpha value of the QPS to be 0.94. In this study, the Cronbach's alpha value of the scale was 0.93.

Analysis of Data: After the data obtained in our study was transferred to the computer environment, the Statistical Package for Social Sciences (SPSS) version 25.0 for the Windows computer program was used for statistical analysis. Descriptive statistical methods such as frequency, mean and standard deviation were used to evaluate the study data. Pearson correlation analyzes were used to determine the relationship between the scales. In the interpretation of the Pearson correlation coefficient, $0.00 \leq r \leq 0.49$ is considered a weak relationship, $0.50 \leq r \leq 0.69$ an intermediate relationship, and $0.70 \leq r \leq 1.00$ a high-level relationship.[18] Cronbach's alpha coefficients of the scales were checked for validity and reliability analysis. The data of our study was accepted as a confidence interval of 95 and a statistical significance level of "$p<0.05$".

RESULTS

When some sociodemographic data of the nurses shown in Table 1 were examined, it was determined that the mean age was 32.90±5.69. It was observed that 67.5% of the participants were women, 89.5% of the participants had a bachelor's degree, and 50.2% of the participants worked in surgical clinics. It was observed that 38.4% of the participants had been nursing for 6-11 years, 63.3% of the participants worked 160 hours or more per month, and 87.3% of the participants worked in shifts. It was determined that 54.9% of the participants chose the profession voluntarily, 54.9% of the participants did not receive patient safety training and 67.5% received a training about quality (Table 1).

Table 1. Socio-demographic characteristics of nurses.

Socio-demographic characteristics		n (%); X±SD (Min-Max)
Age		32.90±5.69 (23 - 46)
Gender	Female	160 (67.5)
	Male	77 (32.5)
Marital status	Married	144 (60.8)
	Single	93 (39.2)
Education level	Associate degree	11 (4.6)
	Licence	212 (89.5)
	Graduate	14 (5.9)
Economical situation	Income less than expenses	33 (13.9)
	Income equals expenses	126 (53.2)
	Income more than expenses	78 (32.9)
Surgical unit	Surgical services	119 (50.2)
	Surgical Intensive Care Units	116 (49.8)
How many years have you been nursing?	0-5 years	71 (30.0)
	6-11 years	91 (38.4)
	12 years and above	75 (31.6)
How many years have you been working in the clinic?	0-5 years	115 (48.5)
	6-11 years	91 (38.4)
	12 years and above	31 (13.1)
Monthly working period	Under 159 hours	2 (0.8)
	160 hours	85 (35.9)
	Above 161 hours	150 (63.3)
Working Shift	All day long	30 (12.7)
	Day/Night shift	207 (87.3)
Did you choose nursing willingly?	Yes	105 (45.1)
	No	132 (54.9)
Have you received training on patient safety culture?	Yes	107 (45.1)
	No	130 (54.9)
Have you received training on quality?	Yes	160 (67.5)
	No	77 (32.5)

Min-max: Minimum-Maximum; X±SD: Mean±Standard deviation.

The lowest and highest scores, mean and standard deviation values obtained according to the PSCS and its five sub-dimensions used in this study are shown in Table 2. In the study, it was determined that the total mean score from PSCS was 3.36±0.21. A PSCS total score close to 4 indicates a positive patient safety culture.[16] Among the sub-dimensions of PSCS, the highest score was 3.48±0.28 for the employee behavior sub-dimension, and the lowest score was 3.06±0.35 for the care environment sub-dimension (Table 2).

Table 2. The mean score of the patient safety culture and quality perception scale and its sub-dimensions.

Scale		X±SD	Min-Max
Scale and sub-dimensions of patient safety culture	Management and Leadership	3.37±0.27	1.29 (4.00)
	Employee Behavior	3.48±0.28	1.86 (4.00)
	Unexpected Event and Error Reporting	3.46±0.39	2.00 (4.00)
	Employee Training	3.40±0.30	2.00 (4.00)
	Maintenance Environment	3.06±0.35	1.63 (4.00)
Total		3.36±0.21	2.06 (3.95)
Quality perception scale and its sub-dimensions	Management and Leadership	82.66±9.38	38.33 (100.0)
	Human Resources Use	72.45±11.11	34.29 (94.29)
	Quality Education	84.09±8.12	56.67 (100.0)
	Measurament and evaluation	83.23±0.40	43.33 (100.0)
	Institutional Benefit	86.59±8.68	42.00 (98.00)
	Employee Benefit	90.50±8.75	47.50 (100.0)
	Patient Benefit	82.19±5.71	45.71 (92.86)
Total		82.15±6.80	43.14 (94.29)

Min-max: Minimum-Maximum; X±SD: Mean±Standard deviation.

The lowest and highest scores, mean and standard deviation values obtained according to the QPS and seven sub-dimensions used in this study are shown in Table 2. In the study, the total mean score obtained from the QPS was found to be 82.15±6.80. Among the sub-dimensions of the QPS, the highest score was 90.50±8.75 for the employee benefit sub-dimension, and the lowest score was 72.45±11.11 for the human resources use sub-dimension (Table 2).

Table 3 shows correlation analyzes between PSCS and its sub-dimensions and QPS and its sub-dimensions. A strong positive correlation was determined between the PSCS total score and its sub-dimension, employee behaviors ($r=0.753$, $p<0.01$). There was a moderate positive correlation between PSCS total score and QPS total score ($r=0.407$, $p<0.01$). However, a strong positive correlation was found between PSCS and QPS patient benefit sub-dimension ($r=0.706$, $p<0.01$).

Table 3. Correlation analysis between patient safety culture and quality perception scale.

Correlation Matrix***							
Variables	**X±SD**	**Patient Safety culture (Total)**	**Management and Leadership**	**Employee Behavior**	**Unexpected Event and Error Reporting**	**Employee Training**	**Maintenance Environment**
Patient Safety culture (Total)	3.36± 0.21	1					
Management and Leadership	3.37±0.27	0.718**	1				
Employee Behavior	3.48±0.28	0.753**	0.638**	1			
Unexpected Event and Error Reporting	3.46±0.39	0.665**	0.294**	0.354**	1		
Employee Training	3.40±0.30	0.560**	0.304**	0.197**	0.223**	1	
Maintenance Environment	3.06±0.35	0.650**	0.305**	0.423**	0.195**	0.206**	1
Quality Perception (Total)	82.15±6.80	0.407**	0.448**	0.398**	0.312**	0.081	0.149*
Management and Leadership	82.66±9.38	0.337**	0.374**	0.350**	0.299**	0.040	0.083
Use of Human Resources	72.45±11.11	0.179**	0.164*	0.184**	0.179**	0.036	0.038
Quality Training	84.09±8.12	0.322**	0.365**	0.251**	0.191**	0.096	0.197**
Measurament and Evaluation	83.23±10.40	0.365**	0.361**	0.345**	0.138*	0.148*	0.270**
Institutional Benefit	86.59±8.68	0.197**	0.286**	0.198**	0.238**	-0.025	-0.029
Employee Benefit	90.50±8.75	0.344**	0.423**	0.332**	0.326**	0.043	0.048
Patient Benefit	82.19±5.71	0.706**	0.642**	0.591**	0.321**	0.174*	0.359**

*: $p<0.05$; **: $p<0.01$; ***: Pearson Correlation Co-efficient..

DISCUSSION AND CONCLUSION

It has been reported that employees' perceptions and attitudes related to patient safety must be measured and evaluated continuously in order to place the patient safety culture in their institution's policies.[16] In this direction, the aim of the research is to examine the relationship between the health quality perceptions of surgical nurses and patient safety cultures. It has been reported that as the experience in nursing increases, the skills of truth-seeking, self-confidence, open-mindedness and inquisitiveness increase, and critical thinking skills are higher.[19] It can be said that the experiences of nurses affect their perceptions of quality and thus increase their perceptions of patient safety. When the nurses participating in the study were evaluated in terms of patient safety cultures, it was determined that the average score was 3.36±0.21. In another study examining the factors affecting the patient safety culture of nurses working in a university hospital, it was reported that the mean total score of the scale was 2.64±0.43.[20] In a similar study on nurses, the average score of the patient safety culture was 2.81±0.40.[21] The highest sub-dimension score was determined as 3.48±0.28 in the "Employee Behavior" sub-dimension. When the studies in the literature were examined, it was seen that the highest score of the nurses was "Employee Behavior" 2.80±0.58.[21] In another study, the "Employee Education" sub-dimension was reported as 3.04±0.60.[22] The total score of nurses' patient safety in an accredited hospital was found to be 3.23±0.37. Similar to our study, the highest score in the sub-dimension scores of the scale was found to be the "behavior of the employees" sub-dimension. The lowest score was in the "unexpected event and error reporting" sub-size.[23] However, our research found that the "maintenance environment" is the lowest sub-size. It has been reported that there has been an increase in reporting unexpected incidents and errors in institutions with a perception of patient safety culture. The results of the research show relative similarity with the literature. It can be suggested that improvement activities can be conducted to increase the patient safety culture to higher levels.

It was determined that the majority of the nurses participating in the study did not receive any training on patient safety culture but received training on quality. This situation can be considered as a negative situation in terms of patient safety. When the educational status of the nurses participating in the study was examined, it was determined that the majority of the participants were undergraduate graduates. In a study, it was reported that there is a relationship between the education level of nurses and the quality of patient care, and that the decreasing interventions as the education level decreases endanger patient safety.[23] In another study, it was reported that senior nurses and training nurses had a better perception of patient safety culture.[24] In order to promote patient safety culture in nurses, it is believed that updating the undergraduate education curriculum and in-service training programs provided in the working life will contribute to patient safety.

When the quality perceptions of the nurses attending the study were examined, it was determined that the total score averages were 82.15±6.80, and that the "employee benefit" sub-dimension was found as the highest score with 90.50±8.75 points. In a study, it was reported that the quality work carried out in the institution and the quality perceptions of the employees were greatly affected and that these studies were beneficial to the institution and positively impacted the quality perceptions of the employees.[25] In this respect, training programs or various informational meetings that can increase the quality perceptions of nurses should be organized to provide positive behaviors.

In conclusion, it was seen that the increase in quality perception increased the patient safety culture of surgical nurses. It was determined that nurses who care for surgical patients attach importance to patient safety culture in terms of patient benefit. The physical environments in which nurses work in relation to the care environment should be arranged in a way that supports the effective maintenance of patient and employee safety. Institutions should develop approaches to develop a safety culture, including management attitudes that try to find system-related problems and improve quality in unexpected events, as well as arrangements for adequate material supply and appropriate working hours, and encouraging nurses to report unexpected events and errors. The limitation of the study is that the study was conducted in a university hospital located in the east of Turkey and only on surgical nurses.

Ethics Committee Approval: Necessary permissions were obtained from the hospital where the study was conducted and the İnönü University Health Sciences Non-Invasive Clinical Research Ethics Committee (Date: 24.12.2019, decision no: 2019/138). The nurses were informed by the researchers and the volunteer information form was presented to the nurses together with the questionnaire in line with the Helsinki Declaration.

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Author Contributions: Concept – GK, YÇ, EHY; Supervision-EHY, GK; Materials – YC; Data collection and/or processing- GK, YÇ; Analysis and/or interpretation –GK, YÇ, EHY; Written by – GK, YÇ, EHY.

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Relationship between Loneliness, Social Support and Readiness for Hygienic Care of the Newborn in Pregnant Women

Gebelerde Yalnızlık, Sosyal Destek ve Yenidoğanın Hijyenik Bakımına Hazır Oluşluk Arasındaki İlişkinin İncelenmesi

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ABSTRACT

Objective: In the study, we aim to examine the relationship between loneliness, social support, and readiness to hygienic care for newborns in pregnant women.

Materials and Methods: The descriptive, cross-sectional and correlational study was conducted on primigravida pregnant women (n=254). The data were collected by using Questionnaire Form, the UCLA Loneliness Scale (UCLA), the Multidimensional Scale of Perceived Social Support (MSPSS), and the Scale for Readiness of Pregnant Women to Hygienic Care of the Newborn (SRPWHCN) were evaluated with Pearson correlation analysis.

Results: Median NO2 levels for all countries decreased between 1-The mean age of the pregnant women was 25.75±4.64. A significant relationship was found between various descriptive characteristics of the pregnant women and the scales. It was determined that there was a significant negative relationship between MSPSS and some subdimensions and UCLA of the pregnant women ($p<0.05$). There was a negative relationship between the mean scores of the UCLA and SRPWHCN of the pregnant women and a positive relationship between the mean scores of the MSPSS and SRPWHCN.

Conclusions: It was observed that the risk of loneliness increased as social support decreased in pregnant women and their readiness for hygienic care of their newborns decreased as their level of loneliness increased.

Keywords: Hygienic care, loneliness, newborn, pregnancy, social support

ÖZ

Amaç: Bu çalışmada gebelerde yalnızlık, sosyal destek ve yenidoğanın hijyenik bakımına hazır oluşluğu arasındaki ilişkinin incelenmesi amaçlanmıştır.

Materyal ve Metot: Tanımlayıcı, kesitsel ve ilişki arayıcı desende tasarlanan çalışma, primigravida gebeler (n=254) ile yürütülmüştür. Soru Formu, UCLA Yalnızlık Ölçeği (UCLA), Çok Boyutlu Algılanan Sosyal Destek Ölçeği (ÇBASDÖ) ve Gebelerin Yenidoğanın Hijyenik Bakımına Hazır Oluş Ölçeği (YHBHÖ) kullanılarak toplanan veriler Pearson korelasyon analizi ile değerlendirilmiştir.

Bulgular: Çalışmaya katılan annelerin yaş ortalaması 25,75±4,64 idi. Araştırmaya katılan gebelerin çeşitli tanıtıcı özellikleri ile ölçekler arasında anlamlı ilişki olduğu saptanmıştır. Araştırmaya katılan gebelerin ÇBASDÖ ve aile, arkadaş alt boyutları ile UCLA arasında negatif yönde anlamlı bir ilişki olduğu belirlenmiştir ($p<0,05$). Araştırmaya katılan gebelerin UCLA ile YHBHÖ puan ortalamaları arasında negatif yönde ve ÇBASDÖ ile YHBHÖ puan ortalamaları arasında pozitif yönde anlamlı derecede bir ilişki bulunmaktadır.

Sonuç: Gebelerde sosyal destek azaldıkça yalnızlık riskinin arttığı, yalnızlık düzeyleri arttıkça doğacak bebeklerinin hijyenik bakımlarına hazır oluşluklarının azaldığı söylenebilir.

Anahtar Kelimeler: Gebelik, hijyenik bakım, sosyal destek, yalnızlık, yenidoğan

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INTRODUCTION

Loneliness is the social, physical, and emotional support that an individual perceives as inadequate.[1] In the studies on loneliness and social isolation examining the effects of loneliness in different dimensions throughout life, perceived insufficiency in personal networks (e.g., friends, family members, coworkers, etc.) was associated with wide-ranging physical and mental effects.[2,3] According to age groups, increased risk of mortality compared to smoking up to 15 cigarettes per day,[4] impaired sleep hygiene,[5] and increased physiological deterioration can be listed among these morbidity factors.[6] Therefore, the presence of an environment that meets the physiological, psychological, emotional, and spiritual needs of the fetus in the intrauterine period is a compulsory and primary requirement.[7]

A woman who receives sufficient social support can ask for help from those around her for a healthy pregnancy and can reduce pregnancy complications by increasing beneficial health practices and behaviors. In a study, it was observed that due to the social support that women get from their circle, they had a more positive pregnancy, adapted to the role of mother faster, and had fewer postpartum problems.[8] The periods of quarantine may have resulted in negative consequences on pregnant women by affecting the continuity of psychological well-being and a common source of distress due to lack of social support. Therefore, it is important to understand how a possible decrease in perceived social support affects the psychological health and loneliness levels of women in the perinatal period during quarantine.[7]

While the process of pregnancy and birth is stressful, it also provides expectant mothers with an opportunity to prepare for the transition to parenthood. When the literature is reviewed, it is observed that especially women experiencing motherhood for the first time have anxiety about the care of their newborns, need professional and social support, and have anxiety about their self-efficacy.[9] Insufficient perceived loneliness and social support of expectant mothers during pregnancy may affect the readiness to hygienic care for the newborn, and the lack of knowledge, experience, and skills of postpartum mothers and the feeling of inadequacy may affect their adaptation to the new role by decreasing their self-confidence.[10]

In line with all this information in the study, we aim to examine the relationship between loneliness, social support, and readiness for hygienic care of the newborn in pregnant women.

MATERIALS AND METHODS

Ethical Dimension of the Study: Ethics committee approval of the Sakarya University Faculty of Medicine Ethics Committee (Date:02.03.2021, decision no: E-71522473-050.01.04-14801-81). The necessary permissions of the managers of the relevant training and research hospital were obtained before the study was conducted. Participants were informed about the study, where/how the data would be used, and the confidentiality of the answers; pregnant with written consent were included in the sample.

Study Design: The research is a descriptive, cross-sectional and correlational study.

Place and Time of the Study: The study was conducted with pregnant women who applied to a hospital in Sakarya in March-June 2021.

Population and Sample of the Study: While pregnant women who applied to the hospital constituted the population of the study, primigravida pregnant women who voluntarily agreed to participate in the study, were 19 years of age and older, had no previously diagnosed chronic or psychiatric disorders, and were in the third trimester of their pregnancy (n=254) constituted the sample.

Power analysis was conducted using the G*Power (3.1.9.2) program to determine the sample size. The sample size was determined as 215 pregnant women according to the effect size value of 0.19 and when Type 1 error probability (α) was accepted as 0.05 (at the 95% confidence level). Type 2 error probability (β) was accepted as 0.20 (at the 80% power level) for the determination of the relationship between the Multidimensional Scale of Perceived Social Support and the UCLA Loneliness Scale obtained from the publication titled “The Relationship of Loneliness with Social Support, Breastfeeding, and Maternal Attachment”.11 While calculating the sample size, it is stated that the sufficient sample size can be increased by 10-20% depending on the nature of the study, considering possible case losses.12 70 participants who filled out the questionnaire form incompletely were excluded from the study. The data from 254 pregnant women were included in the study.

Data Collection Tools: In the study, the data were collected using the Questionnaire Form prepared by the researchers, the UCLA LS3, the MSPSS, and the SRPWHCN. The data collection forms were given to the participants by the researchers, and they were asked to fill out them. The data collection lasted for approximately 15 minutes.

Questionnaire Form: This section involves 16 questions developed by the researchers on the literature, including the socio-demographic characteristics of pregnant women.[7,10,11,13] These questions consisted of questions about age, educational background, employment status, economic level, and receiving support in infant care.

UCLA Loneliness Scale (Version 3) (UCLA LS3): It was developed by Russell et al. to determine the

level of loneliness perceived by the individual. [14] The scale, validity and reliability study conducted by Durak and Senol-Durak, is a self-report scale consisting of 20 items. [15] High scores on the scale indicate a high level of loneliness. The internal consistency coefficient (Cronbach Alpha) of the scale varies between .89 and .94 in different samples (university students, nurses, teachers, and elderly). In the elderly sample, the test-retest coefficient is known as .73 for one year. The Cronbach's alpha reliability coefficient of the scale for this study was 0.82.

Multidimensional Scale of Perceived Social Support (MSPSS): MSPSS is a 7-point Likert-type scale consisting of a total of 12 items. The validity and reliability of the form, which was developed by Zimet et al. [16] and revised by Eker et al., [17] was performed. The scale has a total of three sub-dimensions including family, friends, and significant other. The lowest score and the highest score obtained from the sub-dimensions of the scale are 4 and 28, respectively. It is indicated that the higher the total score obtained from the scale is, the higher the perceived social support in that dimension is. The Cronbach's alpha reliability coefficient of the scale for this study was 0.84.

***Scale for Readiness of Pregnant Women to Hygienic Care of the Newborn (SRPWHCN)*:** SRPWHCN is a 7-point Likert-type scale consisting of 10 items. It was developed by Çaka and Çınar to evaluate pregnant women's readiness for hygienic newborn care.[13] The lowest score and the highest score obtained from the scale are 10 and 70, respectively. The scale is calculated over the total score, and the high scores indicate that the readiness of the pregnant woman is also high. The Cronbach's alpha reliability coefficient of the scale for this study was 0.93.

Statistical Analysis: In the study, the data of 254 participants were evaluated in the IBM SPSS Statistics 23 program in the computer environment. The descriptive characteristics of the participants were analyzed using frequency (n, %) for categorical variables and mean-standard deviation for continuous variables. Independent sample t-test and One-Way ANOVA test were used to examine the difference between groups. The Levene test was first performed to determine the homogeneity of variance, and then, Bonferonni or Tamhane's T2 test was performed to observe the difference. Pearson correlation analysis was used to investigate the relationship between the scales.

RESULTS

The mean age of the pregnant women was 25.75±4.64. The data on the descriptive characteristics of 254 pregnant women are presented in Table Among the pregnant women who were included in the study, 46.5% were between 25-24 years of age, 35.0% were university graduates, 80.3% were housewives, 86.6% have a nuclear family, and 46.5% were married for 2 years and above. When obstetric and fetal characteristics of women were evaluated, it was determined that 76.0% of them had a planned pregnancy and 55.9% of the infants were expected to be male (Table 1).

Table 1. Demographic and clinical characteristics of pregnant women (N: 254).

Variables		**n (%)**
Age	19-24	121 (47.6)
	25-34	118(46.5)
	35 and above	15(5.9)
Education	Primary/ Secondary	76 (29.9)
	High school	89 (35.0)
	University	89 (35.0)
Perceived economic level	Income more than an expense	28 (11.0)
	Income equivalent to an expense	181 (71.3)
	Income less than an expense	45 (17.7)
Type of residence	Village	45 (17.7)
	Town	137 (53.9)
	City	72 (28.3)
Family type	Nuclear family	220 (86.6)
	Extended family	34 (13.4)
Leght of marriage	1 year and below	136 (53.5)
	2 years and above	118 (46.5)
Employment	Working	50 (19.7)
	Housewife	204 (80.3)
Pregnancy planning status	Planned	193 (76.0)
	Unplanned	61 (24.0)
Gender of newborn	Female	112 (44.1)
	Male	142 (55.9)

There was no significant difference between the age groups, economic levels, marriage duration, employment status, planned pregnancy, and the sex of the infant of the pregnant women who participated in the study, and the scales. While there was no significant difference between the educational level of the pregnant women and the MSPSS scale, there was a statistically significant difference between the UCLA and SRPWHCN ($p<0.05$). Accordingly, the mean scores of UCLA and SRPWHCN of those with primary school education were significantly higher than those with university education. When the relationship between the settlements of the pregnant women and the scales was examined, there was no significant relationship between MSPSS and SRPWHCN. However, there was a statistically significant difference between UCLA and the settlements of the pregnant women ($p<0.05$). Accordingly, it was determined that the participants who expressed their settlement as a village had higher mean scores of the UCLA compared to the province and district. While there was no significant relationship between the family types of the pregnant women and the MSPSS and SRPWHCN, there was a statistically significant difference between them and the UCLA ($p<0.05$). Accordingly, it was determined that the mean score of UCLA of pregnant women with nuclear family type was significantly lower than those with extended family. While there was no significant difference between the pregnant women's employment status and planned pregnancy and the MSPSS and SRPWHCN scales, there was a statistically significant difference between them and the UCLA ($p<0.05$). Accordingly, it was determined that those who were housewives and those with unplanned pregnancies had a significantly higher mean score at UCLA compared to the other group (Table 2).

Table 2. Comparison of the mean scores of the scales and some descriptive characteristics (N: 254).

Variables		UCLA Mean ± SD	MSPSS Mean ± SD	Family Mean ± SD	Friends Mean ± SD	Significant Other Mean ± SD	SRPWHCN Mean ± SD
Age	19-24	38.32 ± 8.54	70.47 ± 15.63	24.83 ± 5.39	23.18± 6.04	22.45±6.75	62.17±8.84
	25-34	38.18 ± 8.82	71.92 ± 13.00	25.29 ± 4.36	23.75± 5.29	22.87±5.77	59.39±12.13
	35 and above	37.20 ± 9.51	70.33 ± 18.89	25.26 ± 5.36	24.20± 6.21	20.86±9.34	61.80±16.17
	F/p	0.110/0.896	0.316/0.729	0.273/0.761	0.416/0.660	0.661/.0.517	1.966/0.142
Education	Primary/ Secondary (1)	40.21±8.15	68.25±15.48	24.43±5.25	22.52±6.55	21.28±7.31	63.25±9.45
	High school (2)	38.73±9.34	71.03±14.85	24.86±5.05	23.67±5.09	22.49±6.41	58.39±13.17
	University(3)	35.93±8.05	73.70±13.32	25.83±4.43	24.17±5.46	23.69±5.63	61.29±9.40
	F/p	5.395/.005 *, a	2.893/0.057	1.784/0.170	1.789/.0.169	2.866/0.059	4.186/0.016*,a
Perceived economic level	Income more than an expense	37.28±8.96	75.85±10.67	26.42±2.76	24.75±6.07	24.67±4.09	63.57±10.95
	Income equivalent to an expense	38.11±8.73	70.49±15.47	24.95±5.15	23.13±5.88	22.40±6.86	60.21±11.62
	Income less than an expense	39.06±8.53	70.77±12.92	24.71±4.97	24.22±4.59	21.84±6.00	61.77±8.07
	F/p	0.384/0.681	1.649/0.194	1.235/0.293	1.400/0.249	1.829/0.163	1.316/0.270
Type of residence	Village (1)	42.11±8.26	70.13±12.24	24.95±4.19	23.44±4.50	21.73±6.07	59.33±14.10
	Town (2)	37.01±8.45	70.59±15.25	24.81±5.24	23.24±6.12	22.53±6.57	61.49±10.02
	City (3)	37.98±8.84	72.79±14.87	25.63±4.74	24.04±5.58	23.11±6.61	60.61±10.71
	F/p	6.070/0.003 **, a,b	0.656/0.520	0.670/0.512	0.458/0.633	0.624/0.537	0.677/0.509
Family type	Nuclear family	37.60±8.68	71.56±14.61	25.15±4.95	23.59±5.77	22.80±6.35	60.76±11.28
	Extended family	41.97±7.96	68.38±14.76	24.52±4.74	22.94±5.26	20.91±7.19	61.47±9.26
	t/p	-2.755/0.006**	1.180/0.239	0.693/0.489	0.621/0.535	21.591/0.113	-0.345/0.730
Leght of marriage	1 year and below	38.73±8.66	70.55±14.90	24.86±5.25	23.29±5.98	22.38±6.64	60.62±11.13
	2 years and above	37.56±8.74	71.81±14.37	25.31±4.53	23.75±5.381	22.74±6.33	61.13±10.92
	t/p	1.067/0.287	-0.684/0.494	-0.719/0.473	-0.640/0.523	-0.435/0.664	-0.368/0.714
Employment	Working	38.26±8.44	72.76±12.15	25.70±3.50	23.70±4.82	23.36±5.56	59.60±12.90
	Housewife	38.17±8.78	70.74±15.19	24.92±5.21	23.46±5.91	22.35±6.69	61.17±10.52
	t/p	-2.581/0.011*	1.500/.136	.976/.330	1.145/.254	1.500/.136	-.169/.866
Pregnancy planning status	Planned	38.259±8.88	70.94±14.87	25.07±4.94	23.42±5.60	22.44±6.52	61.16±10.32
	Unplanned	37.98±8.17	71.75±13.97	25.08±4.90	23.77±6.05	22.90±6.41	59.90±13.04
	t/p	0.215/0.830	-0.376/0.707	-0.013/0.990	-0.412/0.681	-0.478/0.633	-0.780/0.436
Gender of newborn	Female	38.42±8.83	72.34±12.97	25.59±4.03	23.91±5.30	22.83±6.32	62.12±9.80
	Male	38.00±8.62	70.18±15.81	24.66±5.50	23.18±6.00	22.33±6.63	59.86±11.83
	t/p	0.383/0.702	1.171/0.243	1.508/0.133	1.021/0.308	0.599/0.549	1.627/0.105

UCLA: UCLA Loneliness Scale; MSPSS: Multidimensional Scale of Perceived Social Support; SRPWHCN: Scale for Readiness of Pregnant Women to Hygienic Care of the Newborn; F: One-way ANOVA test; t: Independent sample t-test; a: 1-3; b: 1-2; *: $p<0.05$; **: $p<0.001$.

It was determined that there was a significant negative relationship between the MSPSS and family and friend sub-dimensions and the UCLA of the pregnant women ($p<0.05$). Accordingly, the risk of loneliness increased as social support decreased. There was a significant negative relationship between the mean scores of the UCLA and SRPWHCN of the pregnant women ($p<0.05$). Accordingly, it can be said that pregnant women's readiness for hygienic care of their newborns decreased as their level of loneliness increased. There was a significant positive relationship between the MSPS, its sub-dimensions,s and the SRPWHCN ($p<0.05$). Considering this result, it can be said that pregnant women's readiness for hygienic care of their newborns increased as social support increased (Table 3).

Table 3. Relationship Between Scales and Sub-Dimensions.

		UCLA	MSPSS	Family	Friends	Significant Other	SRPWHCN
UCLA	r	1	-0.146	-0.182	-0.126	-0.081	-0.158
	p		0.020*	0.004**	0.045*	0.197	0.011*
MSPSS	r		1	0.844	0.840	0.876	0.209
	p			0.000**	0.000**	0.000**	0.000**
Family	r			1	0.589	0.628	0.235
	p				0.000**	0.000**	0.000**
Friends	r				1	0.569	0.196
	p					0.000**	0.002**
Significant Other	r					1	0.122
	p						0.052
SRPWHCN	r						1
	p						

UCLA: UCLA Loneliness Scale; MSPSS: Multidimensional Scale of Perceived Social Support; SRPWHCN: Scale for Readiness of Pregnant Women to Hygienic Care of the Newborn; r: Pearson correlation analysis; *:p<0.05; **: p<0.001.

DISCUSSION AND CONCLUSION

In recent years, attention has been drawn to the importance of social support during pregnancy. It has been emphasized that supporting pregnant women during this period will positively affect postpartum outcomes.[18] Women who do not receive adequate social support during this period may feel lonely. According to the results obtained, it was determined that pregnant had a moderate level of loneliness, that the risk of loneliness increased as social support decreased, and that their readiness for hygienic care of their newborns decreased.

Although some studies focused on perinatal loneliness, some evidence indicates that these relationships also exist in the antenatal and postnatal periods.[19,20] Those with lower levels of perceived social support may have an increased level of loneliness, leading to negative social and cognitive biases and reinforcing or supporting negative emotions and behaviors associated with depression and anxiety.[6] However, as far as we know, there is no data on the extent to which women experienced loneliness during the perinatal period during the pandemic and whether their loneliness levels mediated their levels of perceived social support during this period. It may be related to the fact that pregnant women experience loneliness by negatively affecting their social relationships outside their own family due to the restrictions imposed to control the pandemic in this study group. This result reinforces the qualitative evidence that family and peer support are the most valuable form of social support among women with perinatal loneliness.[8] In this study, it was observed that the mean scores of UCLA of those who had primary school education, lived in rural areas, had an extended family, were housewives and those with unplanned pregnancies were significantly higher compared to the other groups. Similar to our study, it was observed in a study that the mean score of loneliness was higher among those who lived in rural areas and had an extended family.[21] In this study, the relationship between family support, one of the MSPSS sub-dimensions, and loneliness were found to be significantly high probably because it was the most accessible form of support available during the pandemic. Nevertheless, the exclusion of husbands from prenatal screenings or appointments may have had a negative effect on their perception of support. From another perspective, the husbands' stay at home the pandemic may have increased social support. Since studies have revealed that husband's support is significantly associated with the mother's prenatal and postnatal mental health.[22]

The pregnancy is one period during which women mostly need care for themselves and their babies. As it is seen in the studies, primiparous mothers without sufficient knowledge and skills in the care of their newborns inevitably have high levels of anxiety and worry.[23,24] In the study, it was observed that pregnant women's readiness for hygienic care of their newborns decreased as their loneliness level increased and social support decreased. Social support

positively affects the process of adaptation of women to the role of motherhood in the pregnancy and postpartum period and increases her sensitivity toward her baby.[8] Mothers make efforts to adapt to the changes and to meet their care and needs of their babies in the postpartum period. In fact, providing mothers with adequate social support during the pregnancy period will positively affect their physical and mental health by reducing their sense of loneliness.[25] Furthermore, the woman's readiness for the changes in the postpartum period from the pregnancy period is important for accelerating the role of motherhood and skill development. When the literature was reviewed, no study was found on the effects of social support and loneliness on the readiness to hygienic care for newborns. This study will contribute to the relevant literature.

In this study, it was found that the mean scores of SRPWHCN of those with a primary school education were significantly higher compared to those with a university education (Table 2). There are many studies examining the relationship between perinatal, neonatal, and/or infant mortality and maternal education level.[26-29] In these studies, it was observed that there was lower neonatal mortality if the mother had a higher education level. In our study, the reason why the mean scale scores of pregnant women with primary/secondary school education were found to be higher compared to university graduates can be associated with the fact that previous childcare experiences of pregnant women were not investigated, although they had no children, which is a limitation of this study. Although there are many studies on newborn care for mothers, there are limited studies examining the readiness to care for the newborn during pregnancy. There is a need for other large randomized controlled studies in which the factors affecting the readiness to care for newborn are evaluated.

In conclusion, it is inevitable for mothers to experience anxiety and stress while adapting to new roles and responsibilities in the postpartum period. Health professionals need to determine the loneliness and social support factors that may increase anxiety and stress in the mother's proper care of her baby in the postpartum period. Therefore, it should be kept in mind that mothers may need help, and they should be followed up from the pregnancy period to provide family support or professional support when needed.

Ethics Committee Approval: This study was approved by the Sakarya University Faculty of Medicine Ethics Committee (Date: 02.03.2021, decision no: E -71522473-050.01.04-14801-81).

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

Author Contributions: Concept – NÇ, SYÇ; Supervision -NÇ; Data Collection and/or Processing – HUY, SP; Analysis and/or Interpretation –SYÇ; Writing – NÇ, HUY, SYÇ, SP.

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Evaluation of Receptor Relationships of Some Drugs Used in the Treatment of COVID-19 by Modeling Studies

COVID-19 Tedavisinde Kullanılan Bazı İlaçların Reseptör İlişkilerinin Modelleme Çalışmaları ile Değerlendirilmesi

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ABSTRACT

Objective: It is important to investigate the interactions of drugs used in the treatment process of COVID-19 with cellular mechanisms. In this study, the aim was to investigate the interactions of Dexamethasone, Favipiravir, and Hydroxychloroquine drugs used in the treatment of COVID-19 with the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2).

Materials and Methods: Within the scope of the study, firstly, 3-dimensional structures of receptors and drug molecules were formed. Then the interactions of each of the receptor and drug molecules at the binding site were examined by molecular docking studies, which is a computer-aided drug design method, and their binding affinities were evaluated.

Results: As a result of the analyses, it was determined that the drug named Hydroxychloroquine has the highest and the drug called Dexamethasone has the lowest binding affinity for all three receptors. In addition, it has been determined that Dexamethasone develops inappropriate interactions with ER and HER2 receptor active site amino acids.

Conclusions: In this study, preliminary data on how receptor interactions can occur when normal individuals and breast cancer patients use Dexamethasone, Favipiravir, and Hydroxychloroquine are presented.

Keywords: Breast cancer, COVID-19 drugs, molecular docking, receptor

ÖZ

Amaç: COVID-19 tedavi sürecinde kullanılan ilaçların hücresel mekanizmalarla etkileşimlerinin araştırılması önemlidir. Bu çalışmada, COVID-19 tedavisinde kullanılan Deksametazon, Favipiravir ve Hidroksiklorokin adlı ilaçların östrojen reseptörü (ER), progesteron reseptörü (PR) ve insan epidermal büyüme faktörü reseptörü-2 (HER2) ile etkileşimlerinin belirlenmesi hedeflenmiştir.

Materyal ve Metot: Çalışma kapsamında, reseptörler ve ilaç moleküllerinin ilk olarak 3-boyutlu yapıları oluşturulmuş, ardından reseptör ve ilaç moleküllerinin her birinin bağlanma bölgesindeki etkileşimleri bilgisayar destekli ilaç tasarım yöntemi olan moleküler kenetlenme çalışmaları ile incelenmiş ve bağlanma afiniteleri değerlendirilmiştir.

Bulgular: Yapılan analizler sonucunda, Hidroksiklorokin adlı ilacın her üç reseptöre de en yüksek bağlanma afinitesi gösteren ilaç olduğu ve Deksametazon adlı ilacın ise reseptörlere en düşük afinite ile bağlandığı belirlenmiştir. Ayrıca, Deksametazonun ER ve HER2 reseptör aktif bölge aminoasitleri ile uygun olmayan interaksiyonlar geliştirdiği tespit edilmiştir.

Sonuç: Bu çalışmada, normal bireyler ve meme kanseri hastalarının Deksametazon, Favipiravir ve Hidroksiklorokin adlı ilaçları kullanması durumunda reseptör etkileşimlerinin nasıl olabileceğine ilişkin ön veriler sunulmuştur.

Anahtar Kelimeler: COVID-19 ilaçları, meme kanseri, moleküler kenetlenme, reseptör

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INTRODUCTION

COVID-19 which is regarded as a pandemic and a global threat to public health, emerged in Wuhan, China in December 2019 and has spread all over the world as of March 2020.[1] As a result of understanding SARS-CoV-2 virology, current and effective pharmacological treatments are being investigated against COVID-19.[2] Dexamethasone (USA, PA), Favipiravir (Toyama Chemical, Japan) and Hydroxychloroquine (USA, Salisbury) are among the potential therapeutic agents used in COVID-19 treatment.[3]

Dexamethasone is a synthetic glucocorticoid used in conditions with inflammation such as lupus, rheumatoid arthritis, acute gouty arthritis and also used in allergic conditions such as allergic rhinitis, bronchial asthma, contact and atopic dermatitis.[4] In addition to these therapeutic indications, it is also recommended for the treatment of nausea and vomiting that may occur in the post-surgical period. Furthermore, it is a drug that is effective in the post-surgical period due to its effect on accelerating the wound healing process and consequently shortening the duration of hospital stay.[5]

Favipiravir is an antiviral drug that is approved in Japan.[6] Favipiravir triphosphate is a purine analog which is a competitive inhibitor of RNA-dependent RNA polymerase.[7] It has been used in many countries to treat new viral infections, including Ebola and Lassa. As an antiviral drug, Favipravir is authorized for use in the treatment of COVID-19 under emergency use recommendations in several countries, including Japan, Russia, and India.[8]

Hydroxychloroquine has been shown that SARS-CoV-2 has affinity for the protease enzyme and Hydroxychloroquine inhibits SARS-CoV-2 more effectively than Chloroquine.[9] It also considered that it accumulates in acidic environments such as lysosome and inflamed tissues, making the content basic and inhibiting viral replication.[10] It has also been predicted that Hydroxychloroquine may reduce the release of IL-1, IL -6, TNF and IFN-γ from mononuclear cells.[10,11]

Estrogen receptor (ER) and progesterone receptor (PR) are hormone receptors which are found in breast cells and receive hormone signals that result in cell growth. Similarly, positive status of breast carcinoma for human epidermal growth factor receptor-2 (HER2) indicates that the *HER2* gene produces too much HER2 protein that acts as a receptor on the cell surface, helping the cells grow and divide. Receptor studies such as ER, PR, and HER2 are routinely performed in breast cancer. This not only helps in the prognosis of the tumor, but also helps to determine the treatment strategy.[12]

This study was carried out to understand in advance how the receptor pathway may be affected *in vitro* if normal individuals and breast cancer patients have COVID-19 and use these drugs.

MATERIALS AND METHODS

Ethics Committee Approval: Since this article is a bioinformatics analysis, it does not contain any studies with human or animal subjects performed by any of the authors, there is no need for ethics committee approval.

***Preparation of Target Receptors*:** In the molecular docking study, protein database (PDB) data were used in the process of obtaining the required ER (Estrogen receptor), PR (Progesterone receptor) and HER2 (Human epidermal growth factor receptor 2) receptors. Obtained proteins, PDB IDs, resolutions and 3D structures are shown in Table 1. Water molecules in the receptors were removed, polar hydrogens were added, and the receptors were made ready for molecular docking using the Discovery Studio (Germany, 2021) program.

***Preparation of Ligands*:** The drug molecules to be docked were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Table 1). For the energy optimization of the molecules, the number of updates in 50 steps, MMFF94 (Merck Molecular Force Field 94)[13] and the steepest descent algorithm[14] were selected in the Avogadro (version 1.2.0) software and energy optimizations of the molecules were made so that the ligands were ready for molecular docking.

Molecular Docking: For the docking simulations of drug molecules to ER, PR and HER2 receptors, 3-way multi-readout was performed with Lamarc Genetic Algorithm[15] using AutoDock Vina (Version 1.1.2; USA California) software. As insertion sites, the ligands were removed from the receptors which are obtained with the ligands in the PDB data, and insertion simulations were made on the active sites. Images were created using Discovery Studio Visualizer (Germany, 2021) software.

RESULTS

Dexamethasone, Favipiravir and Hydroxychloroquine molecules, whose molecular docking was investigated, have developed interactions with the active sites of ER, PR and HER2 receptors. In these interactions, conventional hydrogen bonds, carbon hydrogen bonds, halogen bonds, van der Waals interactions, pi-sigma, pi-alkyl, and alkyl interactions were found. Aside from these positive interactions, it was also shown that inappropriate binding occurs between the ligand and active site of receptor. Amino acids with interactions, interaction types and bond distances are shown in Table 2.

Dexamethasone molecule, which improperly binds to ALA-350 amino acid with low affinity (4.6 kcal/mol) in the estrogen receptor (ER) active site, forms a conventional hydrogen bond with CYS-530 amino acid, while developing van der Waals interaction

Table 1. 3D structures and PDB database information of receptors, ligands and their properties.

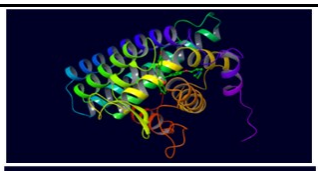
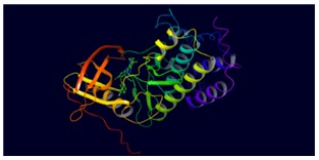
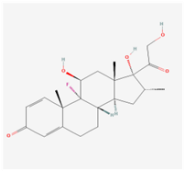
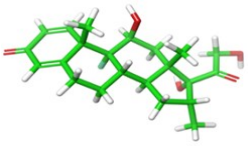
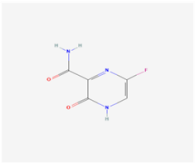
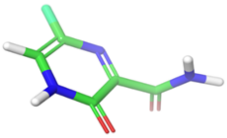
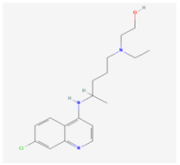
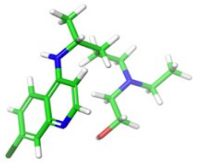
Receptor Name	PDBID	Resolution (Å*)	Method	3D**
ER	1XPC#	1,60 Å	X-Ray Diffraction	
PR	1A28#	1,80 Å	X-Ray Diffraction	
HER2	3PP0#	2,25 Å	X-Ray Diffraction	
No	Ligand	PubChem CID	Molecular Weight (g.mol-1)	2D** 3D***
1	Dexame-thasone	5743##	392.5 g/mol	 
2	Favipira-vir	492405##	157.1 g/mol	 
3	Hy-droxychl oroquine	3652##	335.9 g/mol	 

Table 2. Results of docking of drugs to receptor active sites.

Receptor	Ligands	Binding Energy (kcal/mol)	Number of Hydrogen Bonds	Amino Acid Residue
ER	Dexamethasone	4.6	1	ALA 350 - CYS 530
ER	Favipiravir	-4.9	0	LEU 346 - LEU 387 - ALA 350
ER	Hydroxychloroquine	-6.4	1	MET 343 - LEU 346 - MET 388 - PHE 404 - MET 421 - LEU 428 - HIS 524 - LEU 525
PR	Dexamethasone	-1.0	1	ASN 719 - VAL 760
PR	Favipiravir	-5.2	1	LEU 718 - PHE 778
PR	Hydroxychloroquine	-6.3	3	LEU 718 - MET 759 - MET 909
HER2	Dexamethasone	-0.6	0	VAL 734 - LEU 852
HER2	Favipiravir	-6.1	5	VAL 734 - ALA 751- LYS 753 - LEU 796 - THR 798 - THR 862 - ASP 863
HER2	Hydroxychloroquine	-5.1	0	VAL 734 - ALA 751 - LEU 852

with MET-388 amino acid. Favipiravir molecule binds to estrogen receptor (ER) active site with higher affinity (-4.9 kcal/mol) and more interaction compared to Dexamethasone molecule. Favipiravir molecule which made van der Waals interactions with five amino acids, also performed pi-sigma, pi-alkyl and halogen interactions. The hydroxychloroquine molecule also binds to the estrogen receptor (ER) active site with higher affinity (-6.4 kcal/mol) and more interaction compared to Dexamethasone molecule (Figure 1).

Dexamethasone molecule, which binds to the progesterone receptor (PR) active site with low affinity (-1.0 kcal/mol) compared to other molecules, made conventional hydrogen bond with ASN-719 and carbon hydrogen bond with VAL-760 amino acid. In addition, the Dexamethasone molecule carried out numerous van der Waals interactions in the PR active site. Favipiravir molecule binds to the PR active site with higher affinity (-5.2 kcal/mol) and with different interactions than Dexamethasone molecule. Among these interactions, there are conventional

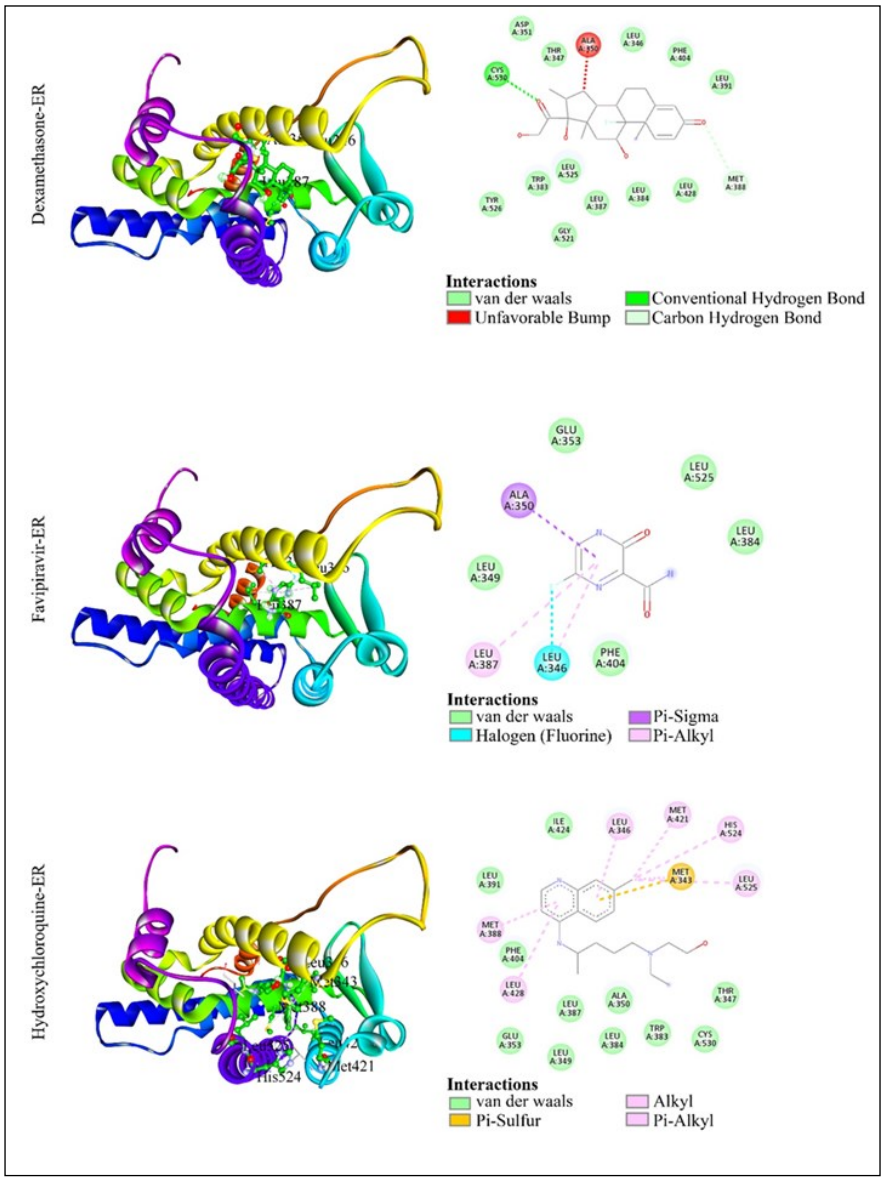


Figure 1. 3D and 2D images of the interactions of Dexamethasone, Favipiravir and Hydroxychloroquine molecules with ER, respectively.

hydrogen bonds, van der Waals interactions, and pi-pi interactions. Favipiravir molecule and Hydroxychloroquine molecule, which binds to the PR receptor active site with higher affinity (-6.3 kcal/mol) than Dexamethasone molecule, have developed van der Waals and pi-pi interactions (Figure 2).

Of these inappropriate bonds, the binding affinity of the Dexamethasone molecule (-0.6 kcal/mol), which interacts only with the HER2 receptor, was found to be lower than the other molecules, and that it showed inappropriate interactions with the amino acids VAL-734 and LEU-852. The Favipiravir molecule, which interacts with HER2 receptor, developed more proper interactions compared to Dexamethasone molecule, and binds to the receptor active site with higher affinity (-6.1 kcal/mol). Favipiravir molecule developed conventional hydrogen bonds with 3 amino acids at the HER2 receptor active site and showed van der Waals interaction with 5 amino acids. Also, the Hydroxychloroquine molecule was bound to HER2 receptor active site with proper interactions and higher affinity (-5.1 kcal/mol) compared to Dexamethasone molecule (Figure 3).

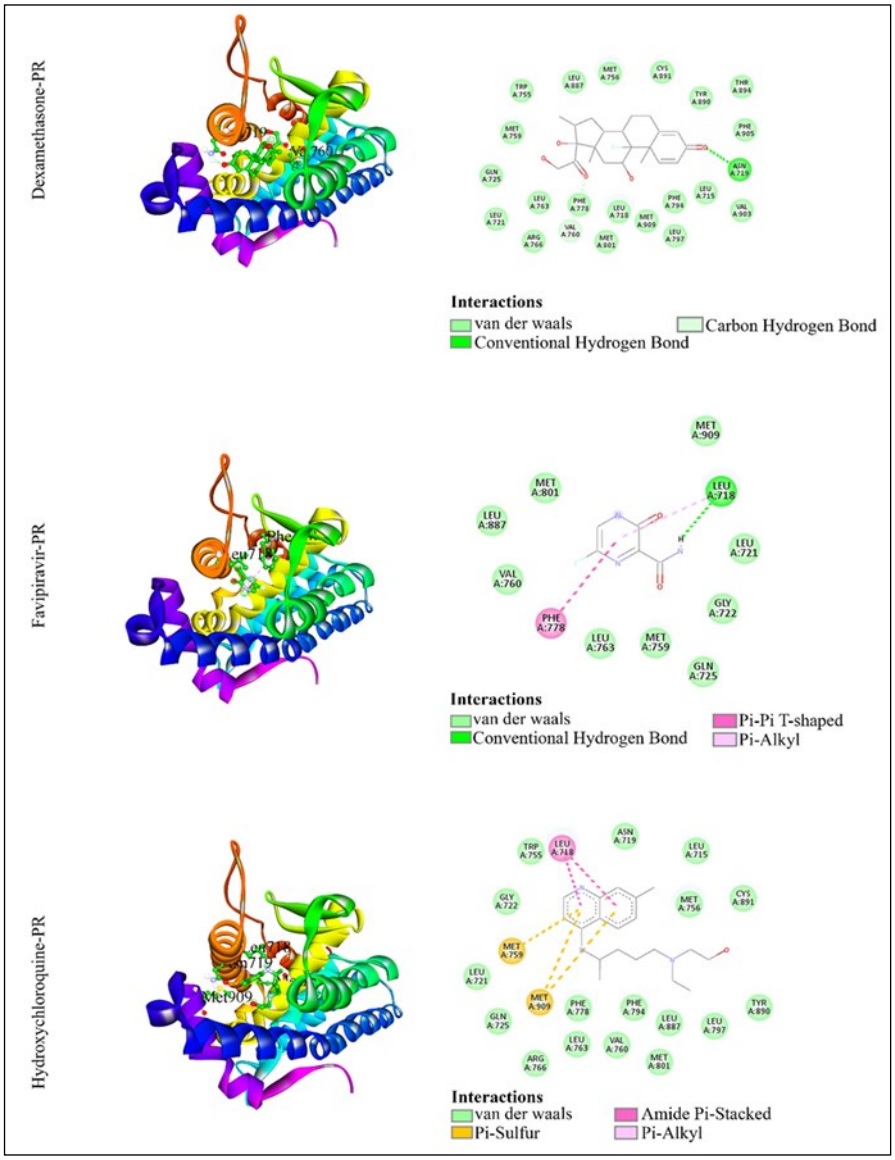


Figure 2. 3D and 2D images of the interactions of Dexamethasone, Favipiravir and Hydroxychloroquine molecules with PR, respectively.

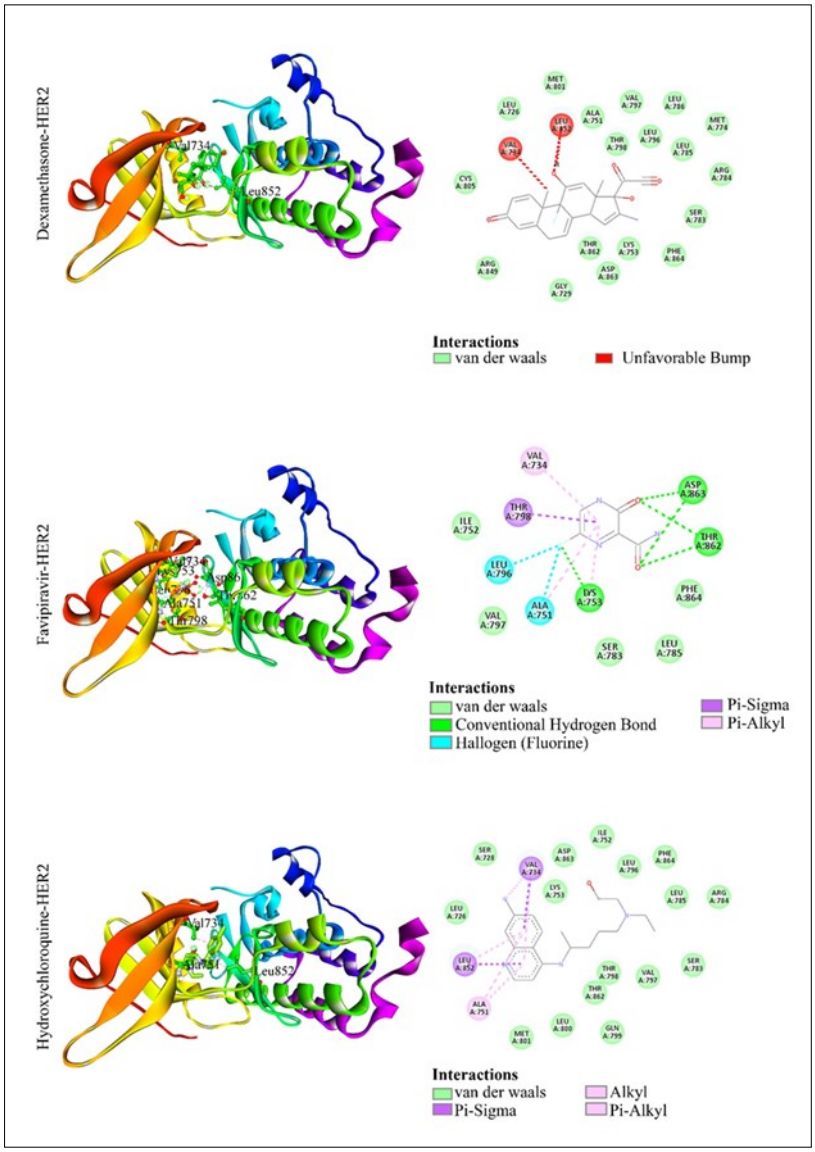


Figure 3. 3D and 2D images of the interactions of Dexamethasone, Favipiravir and Hydroxychloroquine molecules with HER, respectively.

DISCUSSION AND CONCLUSION

The studies to determine the most effective drugs in COVID-19 treatment process are still ongoing, and the effectiveness of these drugs are being investigated with computer-aided drug design methods which have been progressing along with developing computer technologies .[16,17] Molecular docking is one of the main constituents of these techniques and widely used to understand the interactions between ligands and target proteins, allowing us to predict the structure of the ligand-receptor complex.[17–19] In this sudy, molecular docking studies were performed to analyze the interactions of drugs that are Dexamethasone, Favipiravir and Hydroxychloroquine which are used in the treatment of COVID-19, with ER, PR and HER2 receptors. According to the results, hydroxychloroquine binds to all three of these receptors with the highest affinity, while Dexamethasone binds to the active sites of ER and HER2 receptors with the lowest affinity among these three drugs.

Molecular docking studies using either of these drugs in previous studies in the literature have generally investigated the binding of drugs to a SARS-CoV-2 protein.[18,20-22] Celik et al. analyzed the interactions of both hydroxychloroquine and chloroquine with proteins of SARS-CoV-2 such as SARS-CoV-2 RNA polymerase, main protease and spike proteins which play an important role in the structure, survival, reproduction, attachment and survival of the SARS-CoV-2 virus.[20] They showed that neither hydroxychloroquine nor chloroquine do not act on SARS-CoV-2 proteins however both molecules prevent the binding of SARS CoV-2 spike protein to angiotensin-converting enzyme 2 (ACE2) by inter-

acting with the allosteric site.[20] In another study, Wang et al. investigated the binding affinities of the favipiravir to SARS-CoV-2 and human coronavirus NL63 RNA-dependent RNA polymerase (RdRp) and indicated that favipiravir has similar binding affinities to these proteins.[22] In the present study, we show for the first time the interactions of these drugs with the ER, PR and HER2 receptors and evaluate their binding affinities. Thus, it will help to understand how ER, PR, and HER2 receptor pathways can be affected in cellular mechanisms in vitro when these drugs are used by normal individuals and breast cancer patients who have COVID-19.

Defects in the expression of ER, PR and HER2 and increased mutations play a role in the development of different types of cancer, primarily breast cancer.[23,24] Inhibition of these receptors, which play a role in the course of this disease, plays an important role in the treatment process.[25] During the process of cancer treatment, glucocorticoids are routinely used as adjuvant therapy.[26] Dexamethasone is a member of the glucocorticoid family and is used to mitigate the undesirable side effects of chemotherapy as well as to treat COVID-19.[3,26] Favipiravir, an antiviral drug, is an RNA-dependent RNA polymerase inhibitor and there are not enough resources in the literature showing its potential effects on breast cancer.[7] Hydroxychloroquine, a chloroquine analogue, has been shown to increase the effects of chemotherapy and radiotherapy on tumors.[23] It has been shown that the hydroxychloroquine molecule increases the anti-estrogen response, possibly via the autophagy pathway.[27] In addition, there are not enough studies in the literature showing the fate of the hydroxychloroquine molecule on HER2 and PR receptors. According to the results of molecular docking in this study, Hydroxychloroquine was found to be the drug molecule that binds to ER, PR and HER2 receptors with the highest affinity when compared to Dexamethasone and Favipiravir molecules. These three drug molecules show high and low affinities and different molecular interactions at the active sites of the three receptors. On the other hand, Dexamethasone molecule bound to the receptor active sites with the lowest affinity and also developed improper interactions with the active site amino acids.[28]

In conclusion, within the scope of this study, molecular docking studies were carried out to examine the interactions of drugs that are Dexamethasone, Favipiravir and Hydroxychloroquine which are used in the treatment of COVID-19, with ER, PR and HER2 receptors, and their binding affinities were evaluated. It was observed that hydroxychloroquine binds to all three receptors with the highest affinity, while Dexamethasone binds to the active sites of ER and HER2 receptors with the lowest affinity among the three drugs. In addition, it has been determined that Dexamethasone develops inappropriate interactions with receptor active site amino acids. Therefore, this is a preliminary clinical study conducted to understand how receptor interactions can occur when normal individuals and breast cancer patients are treated for COVID-19 using Dexamethasone, Favipiravir, and Hydroxychloroquine. However, computer simulations are the initial step in drug research and development based on predictions and algorithms; more research and clinical testing is needed.

Ethics Committee Approval: Since this article is a bioinformatics analysis, it does not contain any studies with human or animal subjects performed by any of the authors, there is no need for ethics committee approval.

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

Author Contributions: Concept – TK; Supervision – TK, MK; Materials – TK, MK; Data Collection and/or Processing –TK, MK, MOT; Analysis and / or Interpretation – TK, MK, MOT; Writing –TK, MK, MOT.

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The Effect of Pupil Dilation on Ocular Biometry Measurements in the Senile Cataractous Eyes

Senil Kataraktlı Gözlerde Pupil Dilatasyonunun Oküler Biyometri Ölçümlerine Etkisi

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ABSTRACT

Objective: The study aimed to evaluate the effect of cycloplegia on the ocular biometric parameters and intraocular lens (IOL) power calculated by Sanders–Retzlaff–Kraff/Theoretical (SRK/T) formula in the cataractous eyes.

Materials and Methods: This cross-sectional study included 68 senile cataractous eyes of 68 patients scheduled to undergo cataract surgery. Measurements of anterior chamber depth (ACD), axial length (AL), white-to-white (WtW) diameter, keratometry (K1, K2, Kmean), central corneal thickness (CCT), and pupil size (PS) were obtained with AL-Scan (Nidek Co., Ltd, Gamagori, Japan) before and after cycloplegia. The SRK/T formula was used to calculate IOL power with target refraction of 0 D. Cycloplegia induced by cyclopentolate hydrochloride %1.

Results: The mean age of the patients was 70.60±4.07 years (range 65 to 80). A significant increase was observed in ACD after cycloplegia ($p< 0.001$). There was no statistically significant difference between pre-dilation and post-dilation AL, WtW, CCT, K1, K2, Kmean, and IOL power readings ($p> 0.05$). Two cases observed a decrease above 0.5 D of IOL power after cycloplegia.

Conclusions: Cycloplegia induced by cyclopentolate hydrochloride %1 does not affect the measurement of the AL, WtW, CCT, keratometry, and SRK/T calculated IOL power except ACD in senile cataractous eyes.

Keywords: AL-Scan, axial length, cycloplegia, IOL power, senile cataract

ÖZ

Amaç: Çalışmada, kataraktlı gözlerde siklopleji̇nin oküler biyometrik parametrelere ve Sanders-Retzlaff-Kraff/teorik (SRK/T) formülü ile hesaplanan göz içi lens (GİL) gücüne etkisini değerlendirmek amaçlanmıştır.

Materyal ve Metot: Bu kesitsel çalışmaya, katarakt cerrahisi planlanan 68 senil kataraktlı hastanın 68 gözü dahil edildi. Siklopleji öncesi ve sonrası AL-Scan (Nidek Co. Ltd, Gamagori, Japonya) ile ön kamara derinliği (ÖKD), aksiyel uzunluk (AU), beyazdan beyaza (BB) çap, keratometri (K1, K2, K ortalama), merkezi kornea kalınlığı (MKK) ve pupil çapı (PÇ) ölçüldü. Göz içi lensi gücü, hedef refraksiyon 0 olacak şekilde SRK/T formülüne göre hesaplandı. Siklopleji için %1'lik siklopentolat hidroklorür kullanıldı.

Bulgular: Hastaların yaş ortalaması 70,60±4,07 yıl (65-80) idi. Siklopleji sonrası ÖKD'de anlamlı bir artış izlendi ($p< 0,001$). Dilatasyon öncesi ve sonrası AU, BB, MKK, K1, K2, Kortalama ve GİL gücü değerleri arasında istatistiksel olarak anlamlı fark yoktu ($p> 0,05$). Siklopleji sonrası iki olguda GİL gücünde 0,5 D'nin üzerinde bir azalma gözlemlendi.

Sonuç: Senil kataraktlı gözlerde %1'lik siklopentolat hidroklorürün neden olduğu siklopleji SRK/T formülüyle hesaplanan ortalama GİL gücünü etkilememektedir.

Anahtar Kelimeler: Aksiyel uzunluk, AL-Scan, GİL gücü, senil katarakt, siklopleji

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INTRODUCTION

Cataract surgery is one of the most common ophthalmic surgeries worldwide and is considered one of the refractive surgeries. Postoperative visual and refractive expectations of the patients have increased due to developments of small incision surgical techniques and intraocular lens (IOL) technology. Accurate ocular biometry and precise calculation of targeted postoperative results are required to obtain satisfying results in cataract surgery. The rates of errors in IOL power calculation are due to axial length (AL) of 36%, anterior chamber depth (ACD) of 42%, and keratometry (K) values of 22%.[1]

The measurement of AL with optical biometry has been shown to produce significantly more precise IOL power, thereby avoiding possible compression of the eye with applanation A-scan ultrasound and difficulty with immersion A-scan ultrasound in AL measurements.[2] Optical biometry is currently considered as the gold standard for AL measurement except in uncooperative patients, posterior subcapsular, dense cataract, or fixation instability such as macular degeneration.[3,4]

Cyclopentolate is a widely used synthetic anticholinergic mydriatic with the advantage of rapid onset and successful mydriasis. The pharmacological effect of cyclopentolate is due to the competitive antagonism of muscarinic acetylcholine receptors, causing a mydriatic and cycloplegic effect. Clinically, it induces the relaxation of the circular muscle of the iris (mydriasis) and prevents the radial ciliary muscle's contraction, relaxing the suspensory ligaments and, therefore, the lens capsule (cycloplegia).[5]

The present study aimed to evaluate the effect of cycloplegia induced by cyclopentolate hydrochloride %1 on AL-Scan readings and IOL power in eyes with senile cataracts.

MATERIALS AND METHODS

Ethical Status: The study followed the tenets of the Declaration of Helsinki. Ethical board approval was obtained from the Ethical Committee of Kafkas University Faculty of Medicine, Kars, Turkey (Date: 31.01.2018, decision no: 28) before the study was initiated. Written informed consents were obtained from all participants.

Studying Group: Sixty-eight eyes of 68 participants over 65 years of age with various degrees of senile cataract were included in the study. Cases with previous intraocular or corneal surgery, penetrating eye trauma, chronic eye disease, unhealthy corneas, any macular pathology, complicated cataracts (secondary to chronic uveitis, trauma, glaucoma, and silicone oil), and eyes with insufficient signaling were excluded.

Study and Evaluations: After evaluating the best corrected visual acuity, the presence of cataracts was roughly confirmed by examining the anterior segment with a slit lamp biomicroscopy. Then, biometric parameters were measured before dilation with the Al-Scan. Phenylephrine, tropicamide, and cyclopentolate are frequently used mydriatic agents in clinical practice. The strong mydriatic effect of cyclopentolate can last for 24 hours. The long-lasting mydriatic effect of cyclopentolate allows ocular examination and cataract surgery to be performed on the same day. Cycloplegia was achieved by three drops of an eye solution containing cyclopentolate hydrochloride 1% every 5 minutes. A complete ophthalmologic examination was performed after dilation. Intraocular pressure was measured with an air-puff tonometer. Biometric measurements were repeated approximately 45 minutes after the first instillation of cyclopentolate hydrochloride 1%. The same experienced examiner (MBÜ) performed all measurements under the same scotopic light condition. Participants were asked to blink before every measurement to create an optically smooth tear film.

Optical Biometry and IOL Power: AL-Scan optical biometer was used in this study. AL-Scan (Nidek CO., Gamagori, Japan) optical biometer uses a non-contact technique and has a 3D automated eye tracking system. Six values, including AL, ACD, K, central corneal thickness (CCT), pupil size (PS), and white-to-white (WtW) distance, may be obtained in ten seconds. The AL-Scan relies on an optical low-coherence interferometry technique through low-coherence superposition of light waves emitted from an 830 nm super luminescent diode to measure AL. It measures K using double mire rings reflected onto the cornea at 2.4 mm and 3.3 mm diameter zones. The Scheimpflug principle obtains ACD and CCT. The WtW is measured from a captured image of the anterior segment. The PS, AL, ACD, CCT, WtW, K1, K2, Kmean, and IOL power were recorded before and after cycloplegia. The IOL power required for emmetropia with a target refraction of 0 D was calculated through the Sanders–Retzlaff–Kraff/ theoretical (SRK/T) formula with an optical constant of 118.4. Pre-cycloplegic parameters were compared with post-cycloplegic parameters.

Statistical Analysis: Statistical analysis was performed with SPSS for Windows (version 20.0, SPSS, Inc., Chicago, Illinois, USA). All results are presented as mean ± SD. Paired t-test was used to compare variables between the pre and post-cycloplegia. Bland–Altman plot was used for ocular biometry confirmation testing with limits of agreement (LoA) at 95%. A value of $p< 0.05$ was accepted to be statistically significant.

RESULTS

The mean age of the patients was 70.60±4.07 years (range; 65 to 80 years), including 30 (44%) females and 38 (56%) males. The results of the AL-Scan measurements pre and post-cycloplegia and the differences are shown in Table 1. Pre- and post-cycloplegia was not statistically significant for mean AL, K1, K2, Kmean, CCT, WtW, and IOL power measurements (p> 0.05). Only two patients in the study demonstrated changes in IOL power higher than 0.5 D (post-cycloplegia, a decrease of 0.63 D and 0.85 D). After cycloplegia, the ACD significantly increased by 0.08±0.05 mm (p< 0.001).

Table 1. Demographic information, and AL-Scan readings before and after cycloplegia.

		Pre-cycloplegia Mean±SD (min-max)	Post-cycloplegia Mean±SD (min-max)	Mean difference between pre-and post-cycloplegia	Limits of agreement at 95%	p
K1 (D)		43.29±1.36 (40.18-46.75)	43.28±1.35 (40.27-46.68)	0.0088±0.2297	-0.0468 to 0.0644	0.752
K2 (D)		44.20±1.49 (40.71-47.87)	44.24±1.51 (40.91-48.08)	-0.0362±0.2526	-0.0973 to 0.0250	0.242
Kmean (D)		43.74±1.40 (40.62-47.24)	43.76±1.40 (40.69-47.28)	-0.0127±0.1984	-0.0607 to 0.0354	0.601
WtW (mm)		11.79±0.54 (10.7-12.9)	11.79±0.54 (10.7-12.9)	0.000±0.846	-0.021 to 0.021	0.99
CCT (µm)		511.10±30.58 (446-573)	511.03±30.52 (445-572)	0.074±1.097	-0.192 to 0.339	0.582
ACD (mm)		3.14±0.22 (2.64-3.62)	3.22±0.23 (2.70-3.75)	-0.0821±0.0489	-0.0939 to -0.0702	**0.001**
AL (mm)		23.42±0.69 (21.96-24.77)	23.42±0.68 (21.98-24.80)	-0.0047±0.0208	-0.0098 to 0.0003	0.067
IOL power (D)		20.60±1.67 (16.08-24.36)	20.58±1.67 (16.01-24.21)	0.0250±0.2134	-0.0267 to 0.0767	0.338
PS (mm)		4.53±0.97 (2.9-6.7)	6.55±0.93 (4.3-8.3)	-2.012±0.809	-2.208 to -1.816	**0.001**
Gender Totally (n/%)	Male			38 (%56)		
	Female			30 (%44)		
Age Totally (mean±SD)				70.60±4.07		

K: Keratometry; D: Diopter; WtW; White-to-White; CCT: Central corneal thickness; ACD: Anterior chamber depth; AL: Axial length; IOL: Intraocular lens; PS: Pupil size; SD: Standart deviation; Min: Minimum; Max: Maximum.

The Bland-Altman plots illustrate post-cycloplegia measurement differences in mean AL, IOL power, ACD, CCT, K1, and K2 (Figure 1). The 95% LoA were -0.0098 to 0.0003 mm for AL, -0.0267 to 0.0767 D for IOL power, -0.0939 to -0.0702 mm for ACD, -0.192 to 0.339 µm for CCT, -0.0468 to 0.0644 D for K1 and -0.0973 to 0.0250 D for K2.

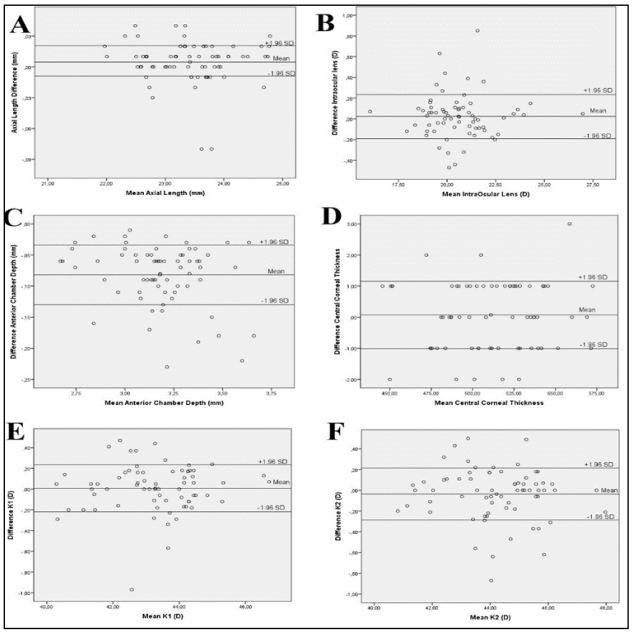

Figure 1. Bland-Altman plots of measurements before and after cycloplegia with cyclopentolate hydrochloride %1. A) Axial length; B) Intraocular lens power; C) Anterior chamber depth; D) Central corneal thickness; E) Keratometry 1; F) Keratometry 2.

DISCUSSION AND CONCLUSION

Tropicamide is the primary pharmacological agent for pupil dilation because of its rapid onset cycloplegic effect and shorter peak effect duration. However, cyclopentolate is more potent than tropicamide or phenylephrine in terms of effect. Pre-operative assessment and complete ophthalmologic evaluation including dilated fundus examination, biometry, and IOL calculations are done on the same day for patients with cataracts using cyclopentolate or tropicamide.

Our study observed no significant change in the mean CCT reading after dilation (p= 0.582). Ozyol et al. reported no significant difference in presbyopics with cyclopentolate by IOL Master 700; Momeni -Moghaddam et al. did not report any significant changes in individuals between 23 and 58 years of age with tropicamide 1% by IOL Master 700, and Hashemi et al. reported similar changes in children with the different refractive status after cyclopentolate using Allegro Biograph.[6-8] Ozcaliskan et al. and Tuncer et al. reported statistically a significant but clinically insignificant increase in the CCT after cyclopentolate, respectively, with 3 µm and 1.10 µm.[9,10] Autrata et al. detected a significant thickness increase with an average of 6 µm with 1% tropicamide+10% phenylephrine instillation in cataract patients with Lenstar LS900.[11] Zeng et al. hypothesized that Mydrin (0.5% Tropicamide+0.5% phenylephrine) destroys the integrity of the intercellular junctions among epithelial cells, which eventually causes corneal edema that mediate increase in CCT.[12] On the contrary, Palamar et al. reported a significant thinning in the mean CCT after cyclopentolate in children, and assumed that cyclopentolate, an atropine-like muscarinic receptor antagonist, probably reduced tear film thickness and caused decreased CCT measurements similar to systemic atropine.[13]

Optical biometry measures the AL from the corneal epithelium to the Bruch membrane. The results herein have shown that cycloplegia does not affect AL readings, as in many studies using tropicamide, tropicamide+phenylephrine, or cyclopentolate.[6,7,9,11,14,15] However, some studies have reported a significant increase in AL of 10 µm following cyclopentolate and 13 µm following tropicamide 0.4%.[10,16] Cheng and Hsieh hypothesized that a posterior shift of the lens-ciliary body diaphragm might produce a compression force pushing toward the vitreous cavity which may cause temporary elongation of the AL or AL change may be related to the sagittal corneal depth after cycloplegia.[16] Cycloplegic agents can affect the choroidal thickness.[17,18] A decrease in subfoveal choroidal thickness, an increase in CCT, and an elongation of 9 µm in AL were observed after cyclopentolate in myopic children.[18] Cyclopentolate mediate vasoconstriction of the choroidal perivascular plexuses and contraction of nonvascular smooth muscle cells, thus causing choroidal thinning. Therefore, elongation in AL after cycloplegia may develop secondary to choroidal thinning. The increase in the AL shown in the studies above was clinically insignificant. Because an error of 0.01 mm (10 µm) in the AL is equivalent to an error of about ±0.027 D in the spectacle plane.[1]

The WtW corneal diameter is important for anterior chamber IOL/phakic IOL implantation, the size of capsular tension ring, and IOL calculation. The WtW is correlated with the lens diameter.[19] In the present study, no significant difference was observed between the pre-and post-dilation WtW values. Some studies have reported a significant increase in WtW after dilation.[6,10] Huang et al. suggested that the image analysis system distinguishes the difference in the light and shade in the region between the iris and the sclera, and fits the best circle to the detected edge. Iris bundling increases tissue darkness and may therefore affect edge detection. Pupil dilation with iris bunching may make the difference between the iris and sclera more obvious. The image analysis system detects the edge closer to the iris sclera interface. This can make WtW measurements after pupil dilation larger than those obtained before dilation.[15]

Some studies have reported no significant change in keratometry readings following cycloplegia.[7,9,11,14] However, the pupil dilation could affect the keratometric readings, although differences were not clinically significant. Corneal flattening or steeping has been reported after cycloplegia.[10,16,20] Bakbak et al. found a significant steeping of 0.04 mm in the K1 reading with Lenstar LS 900 in cataractous eyes. The authors speculated that tropicamide 1% may have caused corneal epithelial changes, thus affecting the reproducibility of the K values.[20] In contrast, another study reported a significant flattening of 0.054 D in the mean keratometric reading with AL-Scan in presbyopics.[10] During miosis, the contractive force of ciliary muscles (CMs) acts on the sclera spur where the ciliary body is attached, and the centripetal force of the peripheral cornea steepens the cornea. Cycloplegia may release the contractile force of the CMs and flatten the cornea.[16] In the current study, no significant change was observed in the K1, K2, or Kmean after cycloplegia.

The ACD significantly increased after pupil dilation in this study (–0.0821±0.0489 mm) ($p<0.001$). Tuncer et al. found that the ACD was significantly increased after cyclopentolate in all age groups, and the most significant increase was observed in the age group of 10-20 years.[10] Ozyol et al. reported significant deepening of the ACD in both pre-presbyopics and presbyopics and significant thinning in the LT

after cyclopentolate. The deepening of the ACD and thinning in the LT were higher in pre-presbyopic patients.[6] Similarly, Teshigawara et al. reported significant changes in ACD and LT in eyes with cataracts after tropicamide±phenylephrine.[22] The anterior portion of the CMs becomes thicker throughout life, and there is no significant decrease in the contractile ability of the muscle, even in eyes with established presbyopia.[23] The contraction ability of the CMs may explain the deepening of the ACD by pulling the lens backward after cycloplegia in presbyopic and cataractous eyes, as in the current study.

The SRK/T is one of the third-generation formulas representing a linear regression method combination on a theoretical eye model. The formula only requires the AL and K, and estimates the effective lens position. The manufacturer provides the ACD constant for SRK/T. This study observed no significant change in the mean IOL power calculated by the SRK/T after cycloplegia. Can et al. reported that cyclopentolate did not affect the IOL power using SRK/T in healthy subjects, except for an increase of power of more than 0.50 D in 2 cases.[14] The IOL power was calculated with the SRK/T, which uses two different predictions (ideal IOL power for emmetropia, lowest myopic sphere equivalent residual refraction) was not affected after tropicamide+phenylephrine.[24,25] In our study, IOL power decreased by higher than 0.5 D (0.63 D and 0.85 D) after cycloplegia in only 2 patients. When 2 cases were evaluated, it was seen that the decrease in the IOL power was caused by the increase in the keratometric values, although the AL was almost the same (Case 1 pre-cycloplegia Kmean: 43.58 D; post-cycloplegia Kmean:44.18 D, and case 2 pre-cycloplegia Kmean:42.65 D; post-cycloplegia Kmean:43.41 D). A 0.1-mm error in the corneal radius is equivalent to an error of about 0.57 D, and a 0.1 D error in the IOL power is equal to an error of about 0.067 D in the spectacle plane by assuming normal eye dimension, accuracy within 0.1 mm is necessary.[1] In some cases, a difference of 0.067 D in the refractive prediction may cause a shift in the closest refractive prediction value to the target refraction that could result in a 0.5 D difference in the IOL power determined.[6] The ACD was the only factor that changed statistically in the present study. An error of 0.1 mm in the ACD may result in a 0.1 D error in the post-operative refraction. According to the results of the present study, it may result in an error of approximately 0.08 D, although the ACD may deepen after cycloplegia.

Unlike the SRK/T, fourth-generation formulas use four predictions (AL, K, ACD, and LT). The mean absolute change in predicted postoperative refraction (PPR) between pre- and post-dilation was significantly higher for fourth-generation formulas compared with third-generation. The fourth-generation formulas show a positive correlation between the change in PPR and the change in ACD.[26] Another new generation formula, Barrett Universal II, is one of the most reliable formulas using five variables (K, AL, ACD, LT, and WtW).[27,28] Unlike the SRK/T, IOL power is affected by cycloplegia with the Barrett Universal 2. In a study, the recommended IOL power changed in 23.3% of cases after cycloplegia when using Barrett Universal II, while SRK/T showed no change.[22]

This study had some limitations. All types of cataracts were included in the study, and no grading system was used to classify the lens opacities. Another area for improvement was the lack of repeatability of the results. However, this was a minor limitation, as the high repeatability and reproducibility of the AL-Scan were confirmed previously.[14] The study population consisted of elderly cataract patients who had low vision. This could lead to fixation difficulties. In this study, the required IOL power for emmetropia was calculated according to a target refraction of 0 D. However, stock IOLs are manufactured in 0.5 D intervals.

In conclusion, the present findings indicate that pupil dilation with cyclopentolate hydrochloride 1% in senile cataractous eyes does not cause significant changes in the mean AL, WtW and K values. However, it causes a significant increase in ACD. Pupil dilation did not significantly affect the mean IOL power calculated by the SRK/T formula. Although, the IOL power measurement with the SRK/T formula could be performed immediately after the examination while the patient is dilated, surgeons should be careful about the effect of pupil dilation on IOL power prediction. It should be kept in mind that in a few cases, deviations in IOL power may occur due to keratometry after dilation.

Ethics Committee Approval: Our study was approved by the Ethics Committee of Kafkas University (Date: 31.01.2018, decision no: 28). The study was carried out in accordance with the international declaration, guidelines, etc

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

Author Contributions: Concept – MBÜ; Supervision – MBÜ, EB, HG; Materials – MBÜ, EB; Data Collection and/or Processing – MBU, EB; Analysis and/ or Interpretation – MBÜ, EB, HG; Writing – MBÜ.

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Association and Haplotype Analysis of the PON1, ITGB3 and CYP3A4 Genes, Strong Candidates for Familial Coronary Artery Disease Susceptibility

Ailesel Koroner Arter Hastalığına Yatkınlıkta Güçlü Adaylar PON1, ITGB3 ve CYP3A4 Genlerinin Hastalıkla İlişkisi ve Haplotip Analizi

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ABSTRACT

Objective: Genetic predisposition is very common among the patients with coronary artery disease (CAD), a complex and multifactorial disease. Our objective was to determine the possible association between the most remarkable functional variants in the paraoxonase 1(PON1), cytochrome P450 3A4 (CYP3A4), integrin subunit beta 3 (ITGB3) genes and familial CAD.

Materials and Methods: We included 117 patients diagnosed with familial CAD and 99 healthy subjects with no family history of CAD. PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P single nucleotide polymorphisms were genotyped using the Sequenom MassARRAY system.

Results: Comparison of genotype and allele frequencies in inheritance models of polymorphisms between the patient and control groups did not reveal any significant findings related to CAD. Stratified analysis by gender did also not display any association both in females and males. There was no significant difference in the frequencies of the haplotypes of the PON1 Q192R and L55M polymorphisms between the groups.

Conclusions: Our findings confirmed previous studies that did not consider PON1, CYP3A4 and ITGB3 genes as risk loci. The fact that our study was conducted only in patients with familial CAD shows the originality and importance of our results.

Keywords: CYP3A4, familial coronary artery disease, PON1, ITGB3

ÖZ

Amaç: Kompleks ve multifaktöriyel bir hastalık olan koroner arter hastalığında (KAH) genetik yatkınlık çok yaygındır. Amacımız; paraoksonaz 1(PON1), sitokrom P450 3A4 (CYP3A4), integrin subunit beta 3 (ITGB3) genlerindeki en dikkat çekici fonksiyonel varyantlar ile ailesel KAH arasındaki olası ilişkiyi belirlemekti.

Materyal ve Metot: Çalışmamıza ailesel KAH tanısı almış 117 hasta ile ailesinde KAH öyküsü olmayan 99 sağlıklı bireyi dahil ettik. PON1 Q192R, PON1 L55M, CYP3A4*1G ve ITGB3 L33P tek nükleotid polimorfizmleri Sequenom MassARRAY sistemi kullanılarak genotiplendirildi.

Bulgular: Polimorfizmlerin kalıtım modellerindeki genotip ve allel frekanslarının hasta ve kontrol grupları arasında karşılaştırılması KAH ile ilişkili anlamlı bir bulgu ortaya çıkarmadı. Cinsiyete göre tabakalı analiz yöntemi de hem kadınlarda hem de erkeklerde herhangi bir ilişki göstermedi. PON1 Q192R ve L55M polimorfizmlerinin haplotip frekansları hasta ve kontrol grupları arasında analiz edildiğinde ise yine anlamlı bir fark yoktu.

Sonuç: Bulgularımız, PON1, CYP3A4 ve ITGB3 genlerini risk lokusları olarak kabul etmeyen önceki çalışmaları doğrulamış oldu. Çalışmamızın sadece ailesel KAH hastalarında yapılmış olması, sonuçlarımızın özgünlüğünü ve önemini göstermektedir.

Anahtar Kelimeler: Ailesel koroner arter hastalığı, CYP3A4, PON1, ITGB3

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INTRODUCTION

Myocardial infarction, the most serious complication of coronary artery disease (CAD), is the leading cause of death in the world.[1] With the use of microarray technology enabling the large scale analysis of functional variants, a growing number of genetic risk factors for CAD were identified.[2]

Paraoxonase 1 (PON1) has a key role in the prevention of the lipoprotein oxidation through the hydrolyzation of the lipid peroxides in oxidized LDL (oxLDL), plays an important role in the initiation and progression of atherosclerosis. Amino acid substitution of glutamine (Q) to arginine (A) at codon 192 (Q192R, c.575A>G, rs662) and the amino acid substitution of leucine (L) to methionine (M) at codon 55 (L55M, c.163T>A, rs854560) are the most common polymorphisms, which affect the PON1 enzyme level.[3]

Several cytochrome P450 (CYP) enzymes were found in the heart, endothelium and smooth muscle cells of the blood vessels and it was demonstrated that they participated in the catalyzation of the metabolites, which played a role in the protection of the cardiovascular health.[4] CYP3A protein group constitutes of the CYP3A4 and CYP3A5 enzymes. Minamiyama et al. discovered that endothelial cells expressed the CYP3A4 enzyme in the endocardium and coronary vessels.[5] In addition, He et al. with a high sample size study determined that the G to A substitution in intron 10 of the CYP3A4 (CYP3A4*1G, rs2242480) gene was related to the coronary artery disease.[6]

Glycoprotein IIb/IIIa (GpIIb/IIIa) complex serves as a receptor for the ligands like fibrinogen, von Willebrand factor and vitronectin, which enable the aggregation and binding of the platelets to the extracellular matrix found in the walls of the blood vessels. This aggregation leads to thrombus formation in atherosclerosis. It was suggested that a single polymorphism (Leu33Pro, c.176T>C, rs5918), which is emerging in the gene encoding the GpIIIa subunit of the receptor, increases the platelet adhesion and aggregation.[7]

Until today, several case-control studies based on the opinion that the variants in the genes encoding the proteins regarding the pathophysiology of CAD might be genetic risk factors, has resulted in conflicting findings. Therefore, we believed that it would be more proper to investigate the variants, which might be genetic risk factors for the multifactorial CAD, in the patients with a positive familial medical history. Our objective was to investigate the association of the functional PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P gene polymorphisms with CAD in the patients with familial history.

MATERIALS AND METHODS

Ethical Statement: The study protocol was approved by the Eskişehir Osmangazi University Clinical Trials Ethics Committee (Date: 28/02/2011, decision no: 2011/17). All the participants were informed about the content of the study and written consent form was taken from all of them. The study protocol was designed in accordance with the Helsinki Ethical Principles and Declaration of Good Clinical Practices and carried out in accordance with these standards.

Subjects and Study Design*:* This study was conducted with the individuals who admitted to the Cardiology Department of the Training and Research Hospital in the Eskişehir Osmangazi University between February 2014 and January 2016. 177 patients (age interval: 18-80 years) diagnosed with familial CAD and had no familial relationship with each other and 99 healthy individuals were included in the study. CAD was defined as the presence of a 50% stenosis at least in one epicardial coronary artery. Stenosis was confirmed by angiography for all patients. Familial CAD was defined as evidence of coronary artery disease in a parent or sibling before 60 years of age.[8] Healthy volunteers were included in the control group, if they and their family had no cardiovascular disease, diabetes, hyperlipidemia and hypertension. Control subjects underwent a physical examination and clinical screening for the confirmation of their health. Exclusion criteria included acute coronary syndromes, congenital heart disease, renal dysfunction, neurological or hematological disorders, morbid obesity, alcohol and drug abuse and pregnancy or lactation.

Genotyping: DNA samples were isolated from 200 µl of peripheral blood using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The amount and purity of the DNA samples was optimized using the Thermo Scientific NanoDrop™ 1000 (Thermo Fisher Scientific, Wilmington, DE, USA) spectrophotometer. Primer sequences for the amplification and extension PCR reactions are designed in the Sequenom Assay Designer 3.1 (Sequenom, San Diego, CA, USA) software. PON1 Q192R (rs662), PON1 L55M (rs854560), CYP3A4*1G (rs2242480) and ITGB3 L33P (rs5918) polymorphisms are then genotyped by single base extension reactions (iPLEX, Sequenom Inc., San Diego, CA, USA) using the MassARRAY system (Sequenom Inc., San Diego, CA, USA). MALDI-TOF mass spectrometry is used to analyze the amplicons in this system. The MassARRAY System with high levels of accuracy is widely used for fine mapping and validation of GWAS studies.

Statistical Analysis: Statistical analyses were performed using the IBM SPSS (Statistical Package for the Social Sciences) Statistics 21.O (IBM Corporation, NY, USA) software package. The difference in the average age of the CAD and control groups was analyzed using an independent-sample *t*-test. Pearson Chi-square analysis was used for the categorical variables between the groups. Hardy-Weinberg equilibrium (HWE) was assessed for each polymorphism using a chi-square analysis. It was performed a haplotype-based case-control analysis based on the genotype data of the PON1 Q192R and L55M polymorphisms using the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). Haplotypes with a frequency of <0.03 were excluded. The statistical significance level was accepted as being $p<0.05$.

RESULTS

When compared between the control and CAD group in terms of gender ratio, there was no statistically significant difference (p=0.263). The mean age was 55.6±9.03 years in the 117 CAD patients and 42.53±6.12 years in the control group. There was a significant difference between the mean ages of the groups (p<0.001). On the other hand, smoking, which is the most important risk factor for CAD was similarly distributed between the patient and control group (p=0.969).

The genotype and allele frequencies of the polymor-

Table 1. Genotype and allele frequencies of the PON1 Q192R (rs662, c.575A>G) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Total Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	AA	49 (41.9)	49 (49.5)		28 (41.2)	24 (48)		21 (42.9)	25 (51)	
Genotyping	AG	57 (48.7)	36 (36.4)	0.756	32 (47.1)	15 (30)	0.800	25 (51)	21 (42.9)	0.503
	GG	11 (9.4)	14 (14.1)		8 (11.8)	11 (22)		3 (6.1)	3 (6.1)	
Recessive model	GG	11 (9.4)	14 (14.1)	0.592	8 (11.8)	11 (22)	0.381	3 (6.1)	3 (6.1)	0.840
	AA+AG	106 (90.6)	85 (85.9)		60 (88.3)	39 (78)		46 (93.9)	46 (93.9)	
Dominant model	AA	49 (41.9)	49 (49.5)	0.262	28 (41.2)	24 (48)	0.460	21 (42.9)	25 (51)	0.418
	AG+GG	68 (58.1)	50 (50.5)		40 (58.9)	26 (52)		28 (57.1)	24 (49)	
Additive model	AG	57 (48.7)	36 (36.4)	0.067	32 (47.1)	15 (30)	0.061	25 (51)	21 (42.9)	0.418
	AA+GG	60 (51.3)	63 (63.6)		36 (53)	35 (70)		24 (49)	28 (57.1)	
Allele	A	155 (66.2)	134 (67.7)	0.751	88 (64.7)	63 (63)	0.787	67 (68.3)	71 (72.4)	0.531
	G	79 (33.8)	64 (32.3)		48 (35.3)	37 (37)		31 (31.7)	27 (27.6)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

Table 2. Genotype and allele frequencies of the PON1 L55M (rs854560, c.163T>A) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	TT	19 (16.2)	11 (11.1)		10 (14.7)	7 (14)		9 (18.4)	4 (8.2)	
Genotyping	TA	44 (37.6)	36 (36.4)	0.237	24 (35.3)	16 (32)	0.728	20 (40.8)	20 (40.8)	0.149
	AA	54 (46.2)	52 (52.5)		34 (50)	27 (54)		20 (40.8)	25 (51)	
Recessive model	AA	54 (46.2)	52 (52.5)	0.229	34 (50)	27 (54)	0.820	20 (40.8)	25 (51)	0.115
	TT+TA	63 (53.8)	47 (47.5)		34 (50)	23 (46)		29 (59.2)	24 (49)	
Dominant model	TT	19 (16.2)	11(11.1)	0.277	10 (14.7)	7 (14)	0.914	9 (18.4)	4 (8.2)	0.136
	TA+AA	98 (83.8)	88 (88.9)		58 (85.3)	43 (86)		40 (81.6)	45 (91.8)	
Additive model	TA	44 (37.6)	36 (36.4)	0.850	24 (35.3)	16 (32)	0.708	20 (40.8)	20 (40.8)	1.000
	TT+AA	73 (62.4)	63 (63.6)		44 (64.7)	34 (68)		29 (59.2)	29 (59.2)	
Allele	T	92 (37.7)	58 (29.3)	0.203	44 (32.4)	30 (30)	0.700	38 (38.8)	28 (28.6)	0.130
	A	152 (62.3)	140 (70.7)		92 (67.6)	70 (70)		60 (61.2)	70 (71.4)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

Table 3. Genotype and allele frequencies of the CYP3A4*1G (rs2242480, IVS10+12G>A) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Total Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	GG	78 (66.7)	68 (68.7)		46 (67.6)	32 (64)		32 (65.3)	36 (73.5)	
Genotyping	GA	37 (31.6)	29 (29.3)	0.807	22 (32.4)	17 (34)	0.541	15 (30.6)	12 (24.5)	0.346
	AA	2 (1.7)	2 (2)		0 (0)	1 (2)		2 (4.1)	1 (2)	
Recessive model	AA	2 (1.7)	2 (2)	0.892	0 (0)	1 (2)	0.234	2 (4.1)	1 (2)	0.505
	GG+GA	115 (98.3)	97 (98)		68 (100)	49 (98)		47 (95.9)	48 (98)	
Dominant model	GG	78 (66.7)	68 (68.7)	0.751	46 (67.6)	32 (64)	0.679	32 (65.3)	36 (73.5)	0.380
	GA+AA	39 (33.3)	31 (31.3)		22 (32.4)	18 (36)		17 (34.7)	13 (26.5)	
Additive model	GA	37 (31.6)	29 (29.3)	0.710	22 (32.4)	17 (34)	0.850	15 (30.6)	12 (24.5)	0.497
	GG+AA	80 (68.4)	70 (70.7)		46 (67.6)	33 (66)		34 (69.4)	37 (75.5)	
Allele	G	193 (82.5)	165 (83.3)	0.814	114 (83.8)	81 (81)	0.571	79 (80.6)	84 (85.7)	0.339
	A	41(17.5)	33 (16.7)		22 (16.2)	19 (19)		19 (19.4)	14 (14.3)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

Table 4. Genotype and allele frequencies of the ITGB3 Leu33Pro (rs5918, c.176T>C) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Total Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	TT	87 (77.7)	72 (72.7)		55 (80.9)	37 (74)		36 (73.5)	35 (71.4)	
Genotyping	TC	23 (20.5)	25 (25.3)	0.437	12 (17.6)	13 (26)	0.511	12 (24.5)	12 (24.5)	0.701
	CC	2 (1.8)	2 (2)		1 (1.5)	0 (0)		1 (2)	2 (4.1)	
Recessive model	CC	2 (1.8)	2 (2)	0.851	1 (1.5)	0 (0)	0.413	1 (2)	2 (4.1)	0.556
	TT+TC	110 (98.2)	97 (98)		67 (98.5)	50 (100)		48 (98)	47 (95.9)	
Dominant model	TT	87 (77.7)	72 (72.7)	0.404	55 (80.9)	37 (74)	0.372	36 (73.5)	35 (71.4)	0.821
	TC+CC	25 (22.3)	27 (27.3)		13 (19.1)	13 (26)		13 (26.5)	14 (28.6)	
Additive model	TC	23 (20.5)	25 (25.3)	0.414	12 (17.6)	13 (26)	0.272	12 (24.5)	12 (24.5)	1.000
	TT+CC	89 (79.5)	74 (74.7)		56 (82.4)	37 (74)		37 (75.5)	37 (75.5)	
Allele	T	197 (87.9)	169 (85.4)	0.433	122 (89.7)	87 (87)	0.518	84 (85.7)	82 (83.7)	0.691
	C	27 (12.1)	29 (14.6)		14 (10.3)	13 (13)		14 (14.3)	16 (16.3)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

phisms and their genotype distributions in the recessive, dominant and additive models were analyzed both in overall and when grouped by gender. The genotype frequencies of the PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P polymorphisms did not show any significant difference between the CAD and control group in respect of the volunteers in total, females and males (Table 1, Table 2, Table 3 and Table 4, respectively). Additionally, there was also no statistical difference in the genotype distribution of the recessive, dominant and additive models of the polymorphism between the CAD and control group. Similarly, allele frequencies of the polymorphisms did not show a statistical difference between the groups.

Table 5. Haplotype analysis in patients with CAD and control subjects for the PON1 Q192R (rs662) and L55M (rs854560) polymorphisms.

Haplotypes*	Frequency in Control Group	Frequency in CAD Group	p value	OR (95% Confidence Interval)
QL	0.293	0.338	0.274	1.256 (0.834-1.892)
QM	0.384	0.320	0.196	0.770 (0.517-1.145)
RM	0.323	0.330	0.818	1.048 (0.700-1.571)

*Order of the polymorphisms is PON1 Q192R, PON1 L55M, Haplotypes with frequencies 0.03 were analyzed using the SHEsis software. p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; rs, the accession number of the variant in the National Center for Biotechnology Information; OR, odds ratio.

Case-control analysis depending on the haplotypes was carried out for the PON1 Q192R and L55M polymorphisms. Haplotypes were generated with the usage of all combinations, which could be formed for the polymorphism alleles. Only frequencies of the RL haplotype were <0.03 in our study group, so this haplotype was excluded from the statistical assessment. The comparison of the haplotype frequencies between the CAD and control group did not reveal any significant difference (Table 5).

DISCUSSION AND CONCLUSION

Atherosclerosis underlying CAD is a complex and chronic pathophysiological process involves endothelial dysfunction, vascular remodeling, plaque formation, inflammation, leukocyte adhesion, platelet aggregation and thrombus formation.[9] Hypertension, hypercholesterolemia, diabetes, obesity and smoking are conventional risk factors, which might have the potential to contribute to these pathophysiological stages.[6] There are several case-control studies focused on the variants in the genes, which encode the proteins known for their role at the onset and progress of atherosclerosis.[1,10] However, these studies were designed without respecting the homogenization of the groups regarding the several environmental risk factors and the CAD family history of the subjects. A molecular genetics research, especially in the patients with a family history of CAD, will enable a reliable and correct determination of the genetic risk factors. Therefore, we conducted this study only on CAD patients with family history and healthy individuals without a family history of CAD. Besides, the healthy volunteers did not have hypertension, hypercholesterolemia, diabetes or obesity. The genetic variants contributing to the onset and progress of these risk factors were indirectly our target in the study.

PON1 Q192R and L55M are functional polymorphisms, which were commonly encountered in populations and investigated as the genetic predisposing factor in many diseases, especially in CAD. Association studies of these polymorphisms with CAD revealed several conflicting findings.[11] The strongest association was found in the PON1 Q192R polymorphism. The preventive role of the allele 192Q and the damaging effect of the allele 192R were demonstrated.[12-14] However, there are a remarkable number of studies in the literature, which did not confirm this finding.[15,16] Similarly, there are studies showing the protective effect of the allele PON1 55M against CAD,[17] but also studies resulted with the contrary findings.[15,18] The first researchers to evaluate the relationship between PON1 Q192R polymorphism and CAD in the Turkish population were Aynacıoğlu et al. Although Aynacıoğlu et al. suggested that there were no significant association,[19] Özkök et al. showed that PON1 Q192R and L55M polymorphism was correlated to CAD.[20] In a different study with Turkish subjects, it was claimed that PON1 L55M plays an important role in the progression of CAD.[21] We intended to contribute to this conflicting topic with our proposal of a new point of view in respect of a study focused on patients with a familial CAD history. We did not find any significant association between the PON1 Q192R and L55M polymorphisms and CAD regarding both the genotype and allele frequencies and dominant, recessive and additive models. The haplotype analysis of these polymorphisms revealed the same result. In a recently published study supporting our findings, no association was found between PON1 Q192R and L55M polymorphisms and coronary artery disease. Paszek et al. achieved this result in a study they conducted in 367 patients and 660 healthy individuals.[22] If the important role of the PON1 activity in the prevention of the lipoprotein oxidation is taken into the consideration, these results in our study bring the studies to mind, which demonstrated that feeding habits might affect this activity. A newly published meta-analysis study also revealed an interesting result, reporting that only the PON1 Q192R polymorphism is associated with CAD. When they performed the same analysis for the PON1 L55M polymorphism, they could not obtain a significant finding.[23]

There are only very few findings in the literature, which demonstrated the association between CYP3A4 and CAD. However, the important role of the CYP enzymes in the protection of the cardiovas-

cular health and in the disorders was well documented.[9] The importance of CYP3A4*1G polymorphism in vitamin D metabolism, which is an important protective agent in the treatment of cardiovascular diseases, has been demonstrated by studies.[24] In addition, a recently published microarray analysis study demonstrated that downregulation of the CYP3A4 gene, which is central to fatty acid metabolism, is associated with the pathogenesis of CAD.[25] He et al. suggested that CYP3A4*1G polymorphism increases the risk of CAD in their cohort study with 322 CAD patients and 306 healthy subjects.[6] Consideration of the sample size and the evaluation of the effect of CYP3A4*1G polymorphism on the enzyme function in their study, proves to be a remarkable finding. We intended to clarify this finding in a different patient population namely in the patients with a family history of CAD. Detailed genotype and allele frequency comparisons in our study revealed that the CYP3A4*1G polymorphism did not have any association with CAD.

Although, some remarkable preliminary studies were published displaying the relation between the ITGB3 Leu33Pro polymorphism and CAD, the subsequent studies did not confirm these findings.[7,26] In a study comparing angiographic findings with polymorphism genotypes, it was suggested that ITGB3 Leu33Pro polymorphism is not a risk factor for coronary atherosclerosis.[27] However, in a large-scale meta-analysis study that included 57 studies, it was suggested that it may be a significant risk factor for the development of acute coronary events in young people.[28] Our analysis of genotype and allele frequencies in overall and inheritance models did not reveal any significant difference between the ITGB3 Leu33Pro polymorphism and CAD. In a recently published study supporting our findings, ITGB3 Leu33Pro polymorphism was compared in Sudanese patients with atherosclerotic plaque and healthy individuals, and no significant results were obtained.[29] A newly published study found similar results to our findings when they analyzed the association of the same polymorphism in Iranian coronary artery patients.[30]

Although there are findings in the literature, which show that PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P polymorphisms might be risk factors for CAD, our detailed statistical analysis did not display any significant association. Thus, our findings confirmed the GWAS studies, in which large-scale genome screening was carried out with the microarray technology and did not show PON1, CYP3A4 and ITGB3 genes as risk loci. The inclusion of only the patients with CAD history and control subject with no family history of CAD in our study is important in respect of our study's reliability. The present study was limited by the relatively small sample size and incompatible mean ages of the control and patient groups.

Ethics Committee Approval: The study protocol was approved by the Eskişehir Osmangazi University Clinical Trials Ethics Committee (Date: 28/02/2011, decision no: 2011/17). . All the participants were informed about the content of the study and written consent form was taken from all of them. The study protocol was designed in accordance with the Helsinki Ethical Principles and Declaration of Good Clinical Practices and carried out in accordance with these standards.

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Evaluation of Root Canal Configuration of Permanent Maxillary Molar Teeth in a Turkish Subpopulation: A Cone-Beam Computed Tomographic Study

Bir Türk Alt Popülasyonunda Kalıcı Üst Azı Dişlerinin Kök Kanal Konfigürasyonunun Değerlendirilmesi: Konik Işınlı Bilgisayarlı Tomografi Çalışması

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ABSTRACT

Objective: Maxillary molars may be challenging for root canal treatment due to their complex canal anatomy and additional root canals, especially in the mesiobuccal root. The current study aimed to investigate the prevalence of root and root canal numbers of maxillary molar in a selected Turkish population.

Materials and Methods: A total of 905 first and second maxillary molars were evaluated using cone-beam computed tomography (CBCT) images. The number of roots and canals was recorded and the mesiobuccal canal was further evaluated with the Vertucci classification.

Results: A total of 394 teeth had a second mesiobuccal (MB2) canal (43.5%). While 90.4% of all maxillary molars had three roots, 44% had four root canals. The most common root canal anatomy of mesiobuccal root canals was Type II (42.6%) followed by Type IV (31.5%) and Type III (22.1%).

Conclusions: It is clear that the second mesial root canal in permanent maxillary molars should be carefully searched for the long-term success of root canal treatments. It is seen that CBCT sections will be beneficial in diagnosis and treatment in better understanding the anatomical structure of the teeth and determining possible anatomical deviations.

Keywords: Cone-beam CT, root canal morphology, maxillary molar

ÖZ

Amaç: Üst çene azı dişleri, karmaşık kök kanal anatomisi ve özellikle mezial kökteki ek kök kanal sayıları nedeniyle endodontik tedaviyi zorlaştırabilir. Bu nedenle başarılı bir kanal tedavisi için üst çene azı dişlerinin anatomik özelliklerinin analiz edilmesi gerekmektedir. Bu çalışmanın amacı, seçilmiş bir Türk nüfusunda üst çene azı dişlerinin kök ve kök kanal sayılarının prevalansını araştırmaktır.

Materyal ve Metot: Konik ışınlı bilgisayarlı tomografi (KIBT) kullanılarak toplam 905 birinci ve ikinci kalıcı üst azı dişi değerlendirildi. Kök ve kök kanal sayıları kaydedilerek mezyobukkal meziobukkal kök kanalı Vertucci sınıflamasına göre değerlendirildi.

Bulgular: Toplam 394 üst azıda ikinci bir mezial (MB2) kök kanalı (% 43,5) vardı. Tüm üst büyük azı dişlerinin % 90,4' ünde üç adet kök varken, %44'ünde ise 4 kök kanalı vardı. Mezial kök kanallarının en yaygın kök kanal anatomisi Tip II (% 42,6) iken, bunu Tip IV (% 31,5) ve Tip III (% 22,1) takip etti.

Sonuç: Üst daimi azı dişlerindeki ikinci mezial kök kanalının, kanal tedavilerinin uzun dönem başarısı için dikkatlice aranmasında büyük fayda olduğu açıktır. Dişlerinin anatomik yapısının daha iyi anlaşılmasında ve olası anatomik sapmaların belirlenmesinde KIBT kesitlerinin teşhis ve tedaviye oldukça yardımcı olacağı görülmektedir.

Anahtar Kelimeler: Konik ışınlı BT, kök kanal morfolojisi, üst azı dişi

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INTRODUCTION

Complete shaping, proper irrigation, and hermetic root canal filling are essential to promote successful root canal therapy. On the other hand, untouched dentin surfaces in the root canal may cause endodontic failure.[1] All root canals must be optimally shaped, irrigated, and obturated hermetically for a successful long-term prognosis of root canal treatment.[2]

Endodontic failures occur mainly in permanent maxillary molars due to their complex root and canal anatomy.[1-3] The most common reason for endodontic failure is missing root canals, especially the second mesiobuccal root canal (MB2).[4] An missing MB2 canal may contain residual microorganisms resulting in post-operative pain and re-infection of the periapical area.[5,6] According to previous studies, permanent maxillary molars generally show the formation of three roots and four canals.[7] Many researchers have also shown different morphological root canal types.[7-11]

Studies evaluating the root canal complex of teeth have used various techniques, such as canal staining and clearing, radiographic examination, and physical sectioning.[12-18] Although clearing and physical sectioning techniques are the best way to analyze root canal anatomy, they are invasive modalities indicated only for extracted teeth. According to position statements of the American Academy of Oral and Maxillofacial Radiology and the American Association of Endodontics, an intraoral radiograph is accepted as a standard pattern of the imaging modality of endodontic diagnosis.[19] Nevertheless, superimpositions observed on two-dimensional images cannot fully reflect the root canal complex, especially in the presence of complex anatomy as expected in molar teeth.[8]

Axial, coronal, and sagittal CBCT sections can clarify the enamel, dentin, and alveolar bone, so the clinicians realize the unique anatomy of the root canal complex without superimposition.[5,6] CBCT has been considered a unique device in detecting the presence of an MB2 canal in the mesial root of maxillary molars.[20] It has also been confirmed that the accuracy of the detection of MB2 with CBCT imaging is more specific than with conventional radiographs and dental magnification devices.[21-23]

Our study aims to help clinicians in the long-term success of root canal treatment by examining the root canal anatomy of permanent maxillary molars, which were reviewed on pre-existing recorded CBCT images in the university clinic, and to create references for academics interested in the subject. Our findings also will compare with the same studies in the literature.

MATERIALS AND METHODS

Ethics Committee Approval: The present study was planned following the principles of the Declaration of Helsinki at all stages and approved by the Ethical Board of the university committee. (Date: 11/01/2022, decision no: E-10840098-772.02-193).

Patient Selection: CBCT images were obtained from the patients for diagnostic purposes by reading and signing the informed consent form. Nine hundred-five maxillary molars were examined from the patients' CBCT images taken between 2000 and 2017 in the Faculty of Dentistry.

Study Protocol: CBCT was taken using the i-CAT17–19 (Imaging Sciences Int., Inc., USA) imaging device following a scanning protocol in line with the company's recommendations with a voxel size of 0.25 mm. All volumes were acquired at 120 kVp

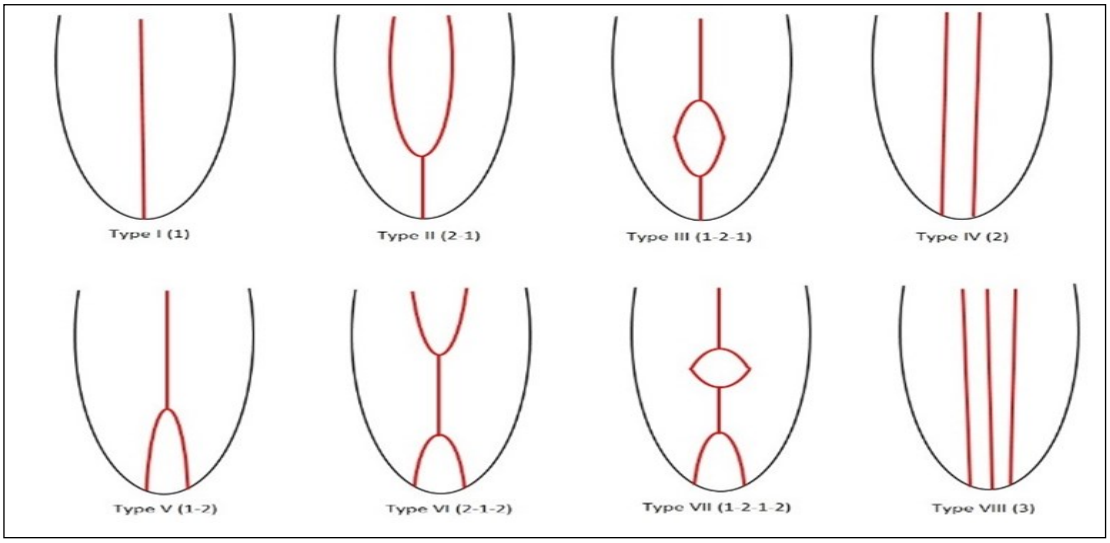

Figure 1: Vertucci's classification of canal morphology.

and 20.27 mAs using a 16 cm × 11 cm field of view.

Data Collection: The images of a routine dental procedure were taken for the preoperative planning of surgical operations and endodontic diagnosis. The age and gender of the patients were also recorded.

Study Design: The inclusion criteria of the study were complete root formation of all teeth without dental restoration and no periapical lesions. In addition, the images that could not be evaluated due to digital image errors were not included in the investigation.

The root canals were studied by an oral radiologist and two endodontists with at least ten years of experience. To calibrate the observers, 10 of the obtained data were randomly selected and examined by two endodontists twice at 10-day intervals. In disagreements, an oral radiology specialist made the final decision. The root canal anatomy of the mesial root was classified due to Vertucci's classification (Figure 1):

Statistical Analysis: The data obtained from the study were analyzed by IBM SPSS Statistics v. 22 software (IBM SPSS, Türkiye). In comparing qualitative data, the chi-square and Fisher-Freeman-Halton exact tests were used with descriptive statistical methods (mean, standard deviation, frequency) and were evaluated at $p < 0.05$. Cohen's kappa coefficient for the interobserver agreement was determined as 0.75.

RESULTS

The study was completed with 905 teeth (339 male, 566 female) with an age range of 16-81 (37 ± 13.88) years. The number of maxillary first molars was 406 (44.9%), and that of second molars was 499 (55.1%). Three hundred ninety-four maxillary molars had an MB2 canal (43.5%). The majority of the teeth (90.4%) had three roots. In addition, 52.3% of the teeth had three root canals, and 44% had four (Table 1).

Table 1: The presence of MB2, and the number of root and root canals.

Maxillary Molar Teeth		**n (%)**
Gender	Male	339 (37.4)
	Female	566 (62.6)
Age	First molar	406 (44.9)
	Second molar	499 (55.1)
	≤20	66 (0.72)
	21 – 30	281 (31.04)
	31 - 40	252 (27.62)
	41 - 50	140 (15.46)
	51 - 60	96 (10.6)
	≥61	70 (7.73)
Presence of MB2	Yes	394 (43.5)
	No	511 (56.5)
Number of Roots	1	29 (3.2)
	2	46 (5.08)
	3	818 (90.3)
	4	12 (1.32)
Number of Root Canals	1	11 (1.21)
	2	23 (2.54)
	3	473 (52.26)
	4	398 (43.97)

Table 2: The prevalence of MB2 canals in maxillary first and second molars by gender.

MB2 Root Canal		**Present n (%)**	**Absent n (%)**	**p**
Gender	Male	167 (49.3)	172 (50.7)	0.007*
	Female	227 (40.1)	339 (59.9)	

Table 3: Association of the presence of MB2 canals in maxillary molars by gender and age groups.

MB2 Root Canal			Present n (%)	Absent n (%)	p
First Molar	Age	≤20	28 (82.4)	6 (17.6)	0.043*
		21-30	73 (57.5)	54 (42.5)	
		31-40	80 (74.1)	28 (25.9)	
		41-50	38 (66.7)	19 (33.3)	
		51-60	27 (64.3)	15 (35.7)	
		≥61	26 (68.4)	12 (31.6)	
Second Molar	Gender	Male	64 (33.3)	128 (66.7)	0.000*
		Female	58 (18.9)	249 (81.1)	

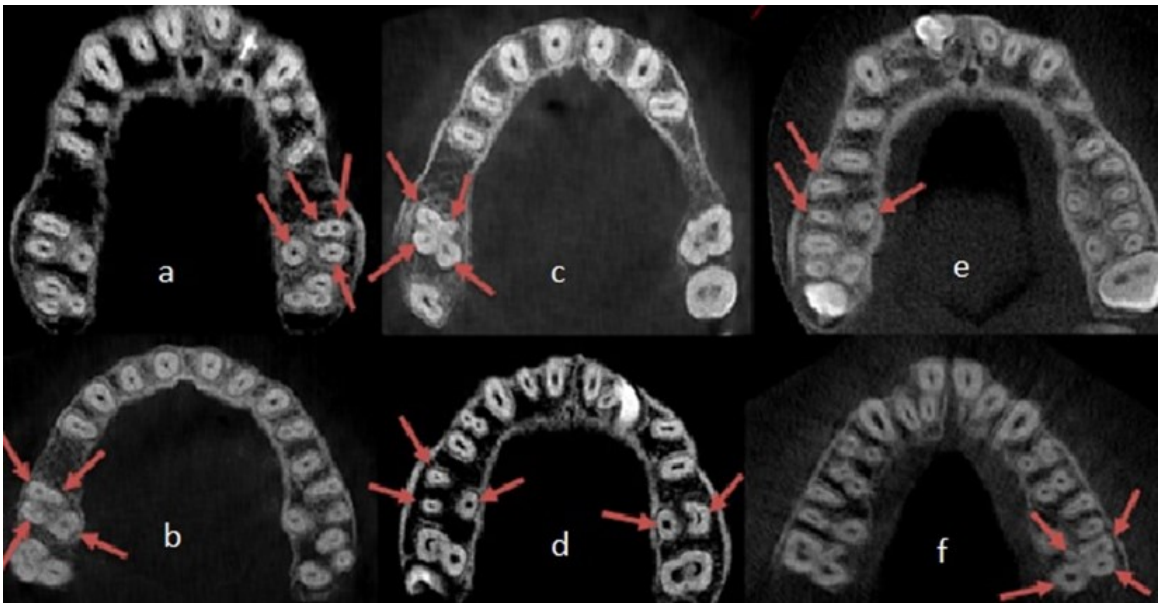

Figure 2: CBCT images of different root canal system morphologies on maxillary molars. a,b,c – four root canals formation, d,e – three and two root canals formation, f – four root canals with additional root formation of permanent maxillary molars.

The prevalence of MB2 canals in males (49.3%) was significantly higher than in females (40.1%) (Table 2), both maxillary first and second molars.

The presence of MB2 also was found to be higher in patients under 20 years (82.4%) compared to the other age groups at maxillary firsts. In contrast, there was no difference in maxillary second molars (Table 3).

When the mesial root canals were evaluated, the most common classification was Type II (42.6%), followed by Type IV (31.5%) and Type III (22.1%). There was a statistical difference between the number of roots of the first and second maxillary molar teeth. While 97.8% of the first molar teeth had three roots, 84.4% of the second molar teeth had three roots. In addition, 7.8% of the second molar teeth had two roots, and 5.6% had one root. Concerning the number of root canals, 66.5% of the first maxillary molar teeth had four root canals, and 68.3% of the second molars had three root canals (Figure 2).

DISCUSSION AND CONCLUSION

Knowledge of the unique root canal anatomy and the MB2 canal is considered a challenge for successfully treating maxillary molars. Locating and cleaning MB2 is essential for a better long-term prognosis.[4,24-27] The presence of MB2 in our study was 43.5% among all maxillary molar teeth. The results of previous studies on this subject vary considerably.

The highest incidences of MB2 were reported by Martins et al.[16] (95.2%), Pèrez-Heredia et al.[24] (86.2%), and Zhang et al.[6] (85.4%). The high percentage findings of a researcher also include Khademi et al.[21] (79.2%). In addition, our findings were similar to Razumova et al.[18] (59.8%). A study reported the lowest incidence rate as 36.3%, when examined worldwide.[27] In another study from Turkey, the same rate was reported by Aydın[25] as 65.27%.

The differences between results may be caused not only by the racial/ethnic diversity of populations but

also by methodological differences in the studies, such as the diagnostic criteria and techniques used, sample size, evaluation methods, and age range. The different CBCT devices with various exposure parameters may also affect the results. Our findings showed that the incidence of MB2 was higher in young patients, especially those under 20 years. Zhang et al.[6] stated that the calcification of root canals and secondary or tertiary dentin formation decreased the detection of MB2 canal access. Therefore, we strongly recommend that clinicians carefully detect MB2 canals, especially in young patients.

In the study, most molar teeth had three roots (90.4%), while 5.1% had two, 3.2% had one, and 1.3% had four. Our results also were similar to other studies regarding the number of roots. The literature also shows that molars have three root canals in more than 90% of individuals.[16-18,21,25,-27]

Regarding the maxillary molars' root canal number, the three-rooted configuration had the highest frequency (52.3%), while 44% had four canals, 2.5% had two, and 1.2% had one root. When the upper molars were evaluated separately, 66.5% of the first molars had four root canals, and 68.3% of the second molars had three. However, 4.2% of the second molars had two root canals. Al Shalabi et al.[28] evaluating an Irish population, reported that 78% of the maxillary first molars had four roots, the highest reported in the literature. To objectively evaluate the differences in these results, we strongly recommend conducting large-scale global studies to evaluate the effect of geographical and ethnic conditions.[26,27]

Many studies in vitro and in vivo investigate the presence of additional root canals at mesiobuccal root in maxillary molars. Results vary considerably, with a higher incidence of MB2 detected in those using in vitro analyses, in which techniques such as clearing technique and sectioning have been utilized to visualize the root canal system.[8] Although microcomputed tomography (μCT) is now accepted as a perfect way to study root canal morphology, and it can only be performed on extracted teeth.[27] CBCT is a powerful tool that can dissect the root canal system in slices of any desired thickness without damaging the tooth.[6] However, despite being the most reliable tool to evaluate the canal anatomy of molars in in-vivo studies, it should be kept in mind that CBCT should only be undertaken in cases where conventional imaging systems do not yield adequate information on root canal anatomy.

According to the findings of our study, the incidence of the MB2 canals was 43.2%, which was significantly higher in males. Three root canals were observed in 66.5% of the maxillary first molars, and 15.7% of the second maxillary molars. When the mesial roots were evaluated according to the Vertucci classification, Type II (42.6%), Type IV (31.5%), and Type III (22.1%), canal formations were significantly more common. There was a difference between the root numbers of the first and second maxillary molars statistically ($p < 0.05$). While 97.8% of the first molars had three roots, 84.4% of the second molars had three. Four-rooted maxillary molars were scarce among the first (0.2%) and second (2.2%).

CBCT is currently used in diagnosing and treating tooth resorption, pathological lesions, detection of perforations, and treatment planning. In addition, CBCT imaging with small voxel sizes before the root canal treatment is strongly recommended if it's possible for an excellent long-term prognosis and treatment quality. Because high-resolution images to be taken at different voxel intervals are more valuable for endodontic diagnosis. However, the method is more expensive than conventional radiographs, and patients receive more radiation.

In conclusion, our results concerning the root anatomy provide comprehensive information about the root canal complex of the Turkish subpopulation and present data that can be compared to other populations.

Ethics Committee Approval: The current study was affirmed by the Ethical Board of the university (Date: 11/01/2022, Decision No: E-10840098-772.02-193). International declarations, guidelines, etc., completed the present study.

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

Author Contributions: Concept – EE, KO, TFE; Supervision – EŞ, MG; Materials, Data Collection and Processing – EE, KO, MG; Analysis and Interpretation, and Writing – EE, EŞ, MG.

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Interatrial Block and Ventricular Repolarization Parameters in Young Patients with Mild Acute Myocarditis

Hafif Akut Miyokarditli Genç Hastalarda İnteratriyal Blok ve Ventriküler Repolarizasyon Parametreleri

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ABSTRACT

Objective: To evaluate ECG findings regarding interatrial block and ventricular repolarization and certain biochemical parameters in young patients diagnosed as acute myocarditis with normal ventricular functions.

Materials and Methods: 405 patients under the age of 35 who underwent diagnostic coronary angiography between January 2014 and January 2020 were retrospectively analyzed. Patients whose ECG records could not be accessed or quality were not suitable for evaluation, with sudden cardiac death, cardiomyopathy, ejection fraction <50%, diabetes mellitus, hypertension, chronic kidney and liver failure were excluded. Patients who underwent diagnostic angiography for the differential diagnosis of acute myocarditis were assigned to myocarditis group (n: 35), and age– and sex–matched subjects with normal coronary circulation and underwent coronary angiography for any other reason were assigned to control group (n: 35).

Results: Heart rate, P wave duration, P wave peak time, PR interval, QRS, QT, and Tp–e interval, QTc, Tp–e/QT and Tp–e/QTc ratios did not differ from each other. Whereas troponin (p<0.001), glucose (p=0.004), LDL (p=0.015), AST (p<0.001), ALT (p<0.026), CRP (p<0.001) levels and neutrophil count (p=0.003) were markedly higher in myocarditis group, HDL was lower (p<0.001).

Conclusions: Although biochemical parameters display differences in myocarditis group, ECG findings did not differ.

Keywords: ECG, myocarditis, Tp–e interval, troponin, ventricular repolarization

ÖZ

Amaç: Ventrikül fonksiyonları normal olan akut miyokardit tanısı almış genç hastalarda interatriyal blok ve ventriküler repolarizasyon ile ilgili EKG bulgularını ve bazı biyokimyasal parametreleri değerlendirmektir.

Materyal ve Metot: Ocak 2014 ile Ocak 2020 arasında tanısal koroner anjiyografi yapılan 35 yaş altı 405 hastanın verileri geriye dönük olarak incelendi. EKG kayıtlarına ulaşılamayan veya kayıt kalitesi değerlendirmeye uygun olmayan, ani kardiyak ölüm, kardiyomiyopati, ejeksiyon fraksiyonu <%50, diabetes mellitus, hipertansiyon, kronik böbrek ve karaciğer yetmezliği olan hastalar çalışma dışı bırakıldı. Akut miyokardit ayırıcı tanısı için tanısal anjiyografi yapılan hastalar miyokardit grubuna (n: 35) ve yaş ve cinsiyet açısından uyumlu, koroner dolaşımı normal olan ve başka bir sebepten ötürü anjiografi yapılan hastalar kontrol grubuna dahil edildi (n: 35).

Bulgular: Kalp hızı, P dalgası süresi, P dalgası tepe zamanı, PR aralığı, QRS, QT ve Tp–e aralığı, QTc, Tp–e/QT ve Tp–e/QTc oranları gruplar arasında farklılık göstermedi. Troponin (p<0,001), glukoz (p=0,004), LDL (p=0,015), AST (p<0,001), ALT (p<0,026), CRP (p<0,001) düzeyleri ve nötrofil sayısı (p=0,003) miyokardit grubunda belirgin olarak yüksek iken, HDL daha düşüktü (p<0,001).

Sonuç: Biyokimyasal parametreler miyokardit grubunda farklılık göstermsine rağmen, EKG bulguları farklılık göstermedi.

Anahtar Kelimeler: EKG, miyokardit, Tp–e aralığı, troponin, ventriküler repolarizasyon

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INTRODUCTION

Myocarditis is a kind of myocardial damage that occurs either by various microorganisms or a primary inflammatory event. Viral myocarditis is a widely accepted cause of arrythmias and sudden cardiac death (SCD).

Myocarditis might progress to dilated cardiomyopathy (DCM) in approximately 30%.[1] Patients often present with chest pain mimicking myocardial ischemia, palpitations, myalgia, arthralgia, fever, rash, fatigue, signs of heart failure (HF), or arrhythmic complications, most of which begin a few days after respiratory or gastrointestinal tract viral infection. Clinical findings and course of the disease vary according to the etiologic agent, the degree of inflammation, the presence and severity of complications such as HF and arrhythmia.[2] Acute myocarditis in young adults very typically might be presented with chest pain suggestive of pericarditis or myocardial infarction, commonly accompanied by elevated serum troponin levels.[3]

Abnormal QRS complex and left bundle branch block findings are found to be associated with more advanced stage and poor prognosis.[4] The most common ECG finding in myocarditis is supraventricular tachycardia, and are the others nonspecific ST segment and T wave changes.[5] Interatrial block (IAB) and atrial fibrillation (AF) expose DCM patients to an increased risk of life–threatening arrhythmias (LTA).[6]

In this study, existence of IAB along with certain ventricular repolarization parameters on ECG recording, such as QT interval, QTc (corrected value according to heart rate extremes), Tp–e (time between the peak and end of the T wave), and Tp–e/QT and biochemical parameters were analyzed in young acute myocarditis patients.

MATERIALS AND METHODS

Ethics Committee Approval: Ethical approval was provided by Adıyaman University Non–interventional Clinical Research Ethical Committee (Date: 20.10.2020, decision no: 2020/9–17). The whole process was carried out in accordance with the Declaration of Helsinki. All study–related data are available upon reasonable request.

Subjects: 405 patients under the age of 35 who underwent diagnostic coronary angiography between January 2014 and January 2020 were retrospectively analyzed. Patients whose ECG records could not be accessed or whose recording quality were not suitable for evaluation, those with SDC, cardiomyopathy, ejection fraction <50%, diabetes mellitus, hypertension, chronic kidney and liver failure were excluded. We calculated a sample size of 27 in each group with an alpha error of 5% and power of 95. Patients who underwent diagnostic angiography for the differential diagnosis of acute myocarditis and diagnosed as acute myocarditis in conformity with criteria determined by working group of European Society on Myocardial and Pericardial Diseases were assigned to the myocarditis group (n: 35), and age– and sex–matched subjects with normal coronary circulation, but also underwent an angiography procedure for any other reason were assigned to the control group (n: 35).[1]

Analyses: ECG recordings taken at the first admission were scanned, and then analyzed using ImageJ (imagej.nih.gov/ij) and CardioCaliper programs at 300% magnification. Heart rate, P wave duration, P wave peak time and PR interval were measured from the inferior leads. QRS, QT, and Tp–e intervals were measured from chest leads, then QTc (calculated by Bazett's formula) and Tp–e/QTc ratio was calculated. IAB was defined according to the diagnostic criteria as a P–wave ≥120 ms. A notched P wave in the inferior leads and P wave duration ≥120 ms in lead DII was considered as partial IAB. Advanced IAB was considered to be present when a P–wave ≥120 ms and a negative terminal part was detected in one of the inferior leads II, III or aVF (biphasic or positive–negative).[7]

Statistical Analyses: Data was appraised with the SPSS–24. Kolmogorov–Smirnov test was used for normality testing. When the descriptive statistics of groups had a normal distribution, variables were expressed as mean ± standard deviation (SD). Independent student's t test was used to make comparisons between quantitative variables of groups. A two –sided p value of <0.05 was accepted as the level of significance.

RESULTS

Neither parameters such as mean age, male ratio, body mass index (BMI), smoking rate, blood pressure values, creatinine, total cholesterol, triglyceride, albumin, Na+ level, hemoglobin, lymphocyte count and neutrophil/lymphocyte ratio (Table 1) nor ECG measurements which were heart rate, P wave duration in DII, P wave peak duration in DII, PR interval, QRS interval, QT interval, QTc interval, Tp–e interval, Tp–e/QT and Tp–e/QTc ratios differed significantly from each other between groups (Table 2). Pericarditis was detected in 5% of group 1. Troponin levels were significantly higher in group 1. Actually, troponin was not found to be elevated at all in group 2 patients. Whereas glucose level, low–density lipoprotein (LDL), C–reactive protein (CRP), white blood cell count (WBC), neutrophil count, CRP/Albumin ratio, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were markedly higher, high–density lipoprotein (HDL) was signifi-

Table 1. Demographic characteristics and laboratory parameters of the patients.

	Group 1 (Acute myocarditis) (n: 35)	Group 2 (Control) (n: 35)	p value
Age, years	26.61 ± 4.91	26.93 ± 2.42	0.688
***Male n (%)**	32 (91.4%)	32 (91.4%)	1.0
***Pericarditis (%. n)**	14.28 %. 5	0. 0	-
BMI (kg/m2)	26.41 ± 2.12	26.73 ± 1.83	0.541
***Smoking (%. n)**	37.14 %. 13	34.28 %. 12	0.803
Systolic blood pressure (mmHg)	115.60 ± 7.35	116.70 ± 9.74	0.345
Diastolic blood pressure (mmHg)	77.20 ± 3.45	77.80 ± 2.82	0.473
Troponin (µg/mL)	1.85 ± 2.22	0	0.000
Glucose (mg/dL)	104 ± 18	94 ± 16	0.004
Creatinine (mg/dL)	0.90 ± 0.20	0.80 ± 0.10	0.076
Total cholesterol (mg/dL)	163.50 ± 29.32	166.40 ± 52.37	0.920
Triglyceride (mg/dL)	151.10 ± 52.32	136.70 ± 62.90	0.056
HDL (mg/dL)	32.60 ± 7.20	39.40 ± 10	0.001
LDL (mg/dL)	96.70 ± 14.10	89.70 ± 17	0.015
Albumin (g/dL)	3.90 ± 0.30	4.10 ± 0.10	0.184
AST U/L	49.70 ± 22.50	25.50 ± 11.20	0.000
ALT U/L	31.80 ± 12.80	25 ± 10.70	0.026
Na (mEq/L)	138.70 ± 2	139.10 ± 2.40	0.477
K (mEq/L)	4 ± 0.50	4.30 ± 0.30	0.005
Ca (mg/dL)	9.20 ± 0.14	9.29 ± 0.10	0.001
C–reactive protein (CRP) mg/dL	4.20 ± 1.50	0.10 ± 0.10	0.000
Wight blood cell × 103/µL	9.80 ± 2.70	8.60 ± 1.70	0.001
HGb (mg/dL)	15.51 ± 0.90	15.61 ± 1.10	0.562
Platelet × 10^3/µL	235.70 ± 65	240.20 ± 59.38	0.765
Neutrophil (N) × 10^3/µL	6.52 ± 2.54	4.96 ± 1.55	0.003
Lymphocyte (L) × 10^3/µL	2.34 ± 0.79	2.57 ± 0.62	0.190
N/L ratio	2.71 ± 1.43	2.12 ± 0.84	0.090
CRP/Albumin	1 ± 0.40	0.01 ± 0.01	0.000

Continuous variables are represented as mean ± SD, with the exception of percentage of male, subjects who smoke and pericarditis cases in groups.

Table 2. Comparison ECG measurements of acute myocarditis and control groups.

	Group 1 (Acute myocarditis; n: 35) (mean ± SD)	Group 2 (Control; n: 35) (mean ± SD)	p value
Heart rate (beats/min)	75 ± 13.20	76 ± 15.40	0.892
DII P wave duration (ms)	98 ± 17.20	96 ± 14.30	0.778
DII P wave peak time (ms)	54 ± 15.80	50 ± 12.70	0.240
PR interval (ms)	147 ± 26.10	135 ± 27.50	0.066
QRS interval (ms)	93 ± 13.70	91.80 ± 15	0.817
QT interval (ms)	372 ± 46.50	371 ± 42.50	0.985
QTc interval (ms)	410 ± 42.60	413 ± 33.60	0.198
Tp-e interval (ms)	91 ± 16.90	92 ± 24.40	0.754
Tp-e/QT ratio	0.25 ± 0.05	0.25 ± 0.05	0.923
Tp-e/QTc ratio	0.22 ± 0.05	0.22 ± 0.06	1.0

Tp-e: Tpeak–Tend interval, time between the peak and end of the T wave; c = Corrected value according to heart rate extremes.

cantly lower, in group 1. As for electrolytes, K^+ and Ca^{++} levels were markedly higher in group 2 (Table 1). IAB was not encountered at all in both groups.

DISCUSSION AND CONCLUSION

It seems that because of the differences occurred in determination of the control groups make the findings of our and the previous studies not to confirm each other. In one of previous studies, it was observed that the prognosis of patients with fulminant myocarditis who had needed intensive hemodynamic support, but most of them not requiring mechanical support, was better during the course of the disease than patients with acute non–fulminant myocarditis in long–term follow–up.[8] So, the question, "How do we determine the prognosis?" stands still to be elucidated.

IAB predisposes to AF. This relationship gets stronger as the duration of the P wave gets longer. On the other hand, AF give rise to a vicious cycle of LTA and SCD. It is revealed that AF is associated with LTA and SCD independently of other factors. In case of additional considerations of patient, like advanced age, heart failure, vascular diseases, arrythmias or structural heart diseases, IAB even much vigorously predisposes to atrial fibrillation/flutter.

The importance of IAB and AF comes from the emergence of a thrombogenic sequence due to delayed left atrial excitation. Particularly, advanced IAB is a secure predictor of AF, as this is the case for Chaga's cardiomyopathy.[9–11] Uçar et al. declare that patients with clinically diagnosed as acute myocarditis display significantly higher Tp–e interval and Tp–e/QT and Tp–e/QTc ratios. The Tp-e interval is considered to be an indicator of transmural dispersion, although it is somewhat controversial.[12,13] An increase or prolongation in the Tp–e interval or Tp–e/QT ratio is considered an index of arrhythmogenesis in all cases of long, normal, or short QT intervals, or in acquired or congenital channelopathies.[14] Although Uçar et al. found the Tp –e interval or Tp–e/QT ratio to be significantly higher in the group of acute myocarditis patients compared to the control group, it should be noted that the control group in their study is consisted of completely healthy volunteers. The normal value of the Tp–e/QT ratio is around 0.192 ± 0.35.[13-15] Since our study did not involve completely healthy controls, we cannot say whether the mean value found is statistically different from the healthy control values. Güneş et al. also declare that QT interval, Tp–e interval, Tp–e/QT ratio and Tp–e/QTc ratio values are found to be markedly prolonged in myocarditis group when compared to control group.[16]

In patients with fulminant myocarditis, a decreasing trend in high–sensitivity cardiac troponin I in the first 24 hours is found to be associated with a lower incidence of in–hospital mortality.[17] As myocarditis is an inflammatory disease, CRP, WBC and neutrophil count were higher in myocarditis group in our study, as expected. High CRP level and erythrocyte sedimentation rate are among the most important parameters that allow us to accurately monitor the course of the disease and response to treatment. Although lymphocytosis often accompanies leukocytosis in myocarditis, only the neutrophil count was significantly higher in the myocarditis group in our study.[1,18]

High HDL and triglyceride levels are found to be associated with lower mortality in the group of HF patients, the majority of whom had idiopathic dilated, ischemic, or hypertensive cardiomyopathy, and Chagas' disease.[18] This is somewhat consistent with our study because we encountered significantly lower HDL and higher LDL levels in the myocarditis group. Since cholesterol is a substance that cannot be broken down and only converted, body cells have various efflux mechanisms that are responsible for removing cholesterol. It is known that increased cholesterol induces apoptosis in a myriad of cells, such as smooth muscle cells, pancreatic β–cells and macrophages.[20–22] An important component of acute myocarditis is apoptosis seen in cardiomyocytes, which is considered to be the main pathology leading to DCM or HF.[23] In an experimental autoimmune myocarditis study, it is observed that the cholesterol efflux mechanisms of cardiomyocytes are impaired. The inflammatory process is observed to be regressed and cardiac functions improved, following draining of cholesterol out of the cardiomyocytes by a cholesterol depleting agent, called methyl –β–cyclodextrin.[20] In a previous study, it is observed that troponin I, ALT and AST, which are found to be high during an acute myocarditis attack, decrease significantly after clinical remission is achieved.[24] In our study, ALT and AST levels were markedly higher in the myocarditis group compared to the control.

In conclusion, novel studies, which will be conducted by stratifying myocarditis patients according to the severity and even etiology of the disease, are required to be able to specify biomarkers or short–long term predictors in myocarditis.

Ethics Committee Approval: Ethical approval was provided by Adıyaman University Non–interventional Clinical Research Ethical Committee (Date: 20.10.2020, decision no: 2020/9–17).

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Evaluation of the Relationships between ER, PR, c-erbB2, Ki67, E-Cadherin Expressions, Nottingham Histological Grade and some Clinical Parameters in Breast Carcinomas

Meme Karsinomlarında ER, PR, c-erbB2, Ki67, E-Cadherin Ekspresyonları, Nottingham Histolojik Grade ve Bazı Klinik Parametreler Arasındaki İlişkilerin Değerlendirilmesi

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ABSTRACT

Objective: In this study, it was aimed to evaluate the relationships between Estrogen receptor (ER), Progesterone receptor (PR), c-erbB2 (HER2), Ki67, E-Cadherin expressions, Nottingham histological grade and some clinical parameters in breast carcinomas.

Materials and Methods: A total of 74 patients diagnosed with breast carcinoma (CA) in our pathology department between 2018-2019 were included in this study. Immunohistochemical preparations stained with ER, PR, HER2, Ki67 and E-Cadherin were evaluated and analyzed retrospectively. For ER and PR, ≥1% expression was considered as positive staining, and <1% was considered as negative staining. HER2 expression was scored as 0, 1, 2 and 3. Ki67 proliferation index was considered as low (<10%), intermediate (10-20%) and high risk (>20%). The data were analyzed with chi-square test.

Results: HER2 score showed a statistically significant change according to ER status (p=0.010). HER2 score also showed a statistically significant change according to PR status (p=0.004). There was a significant correlation between Ki67 and histological stage (p<0.001).

Conclusions: Detection of high Ki67 index in breast carcinomas is poor prognostic. Detection of ER and PR expression and no expression of HER2 are good prognostic indicators. Preanalytical and analytical processes should be followed meticulously by pathologists.

Keywords: Breast Carcinoma, E-Cadherin, HER2, Hormone Receptors, Ki67

ÖZ

Amaç: Bu çalışmada meme karsinomlarında Östrojen (ER), Progesteron (PR), c-erbB2 (HER2), Ki67, E-Cadherin ekspresyonları, Nottingham histolojik grade ve bazı klinik parametreler arasındaki ilişkilerin değerlendirilmesi amaçlanmıştır.

Materyal ve Metot: Bu çalışmaya 2018-2019 yıllarında patoloji bölümümüzde meme karsinom tanısı alan toplam 74 hasta dahil edildi. İmmünohistokimyasal olarak çalışılan ER, PR, HER2, Ki67, E-Cadherin boyalı preparatlar retrospektif olarak değerlendirilip incelendi. ER ve PR için ≥%1 ekspresyon pozitif boyanma, <%1 ise negatif boyanma olarak kabul edildi. HER2 skor 0, 1, 2 veya 3 olarak değerlendirildi. Ki67 proliferasyon indeksi için <%10, % 10-20, >%20 sırasıyla düşük, orta ve yüksek riskli olarak kabul edildi. Çalışmada elde edilen veriler ki-kare testi ile değerlendirildi.

Bulgular: HER2 skoru ER durumuna göre istatistiksel olarak anlamlı değişim gösterdi (p=0,010). HER2 skoru PR durumuna göre de istatistiksel olarak anlamlı değişim gösterdi (p=0,004). Ki67 ile histolojik evre arasında anlamlı ilişki vardı (p<0.001).

Sonuç: Meme karsinomlarında Ki67 indeksinin yüksek olması kötü prognostik göstergelerdendir. ER, PR ekspresyonunun saptanması ve HER2 ekspresyonunun saptanmaması ise iyi prognostik göstergelerdendir. Preanalitik ve analitik süreçler patologlar tarafından titizlikle takip edilmelidir.

Anahtar Kelimeler: E-Cadherin, HER2, hormon reseptörleri, Ki67, meme karsinomu

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INTRODUCTION

Mortality of breast cancer has decreased significantly in recent years with the development of new treatment options in terms of early diagnosis, surgery and oncology.[1] Grading has independent prognostic significance in breast cancer, and the Nottingham histological grade method has also been found to improve interobserver agreement compared to other grading systems.[2,3]

Histological findings and immunohistochemical (IHC) evaluations are very important in the diagnosis of breast carcinomas. IHC is a technique currently used to measure the level of Estrogen (ER) and Progesterone (PR) biomarker expression in breast cancer tissues and to evaluate the cancer response to endocrine therapy.[4,5] About 75% of breast cancers are ER-positive. The predictive and prognostic roles of PR alone in breast cancer are unclear. The ER-negative and PR-positive subgroups have been reported as 1-5% of all breast cancers. In other words, the ER-negative PR-positive cases are rare.[6,7] There are significant differences in how different laboratories perform ER and PR tests and interpret the results.[8] The c-erbB2 (HER2) is overexpressed and/or amplified in almost 15% of breast cancers. HER2-positive status is an unfavorable prognostic factor.[9,10]

Ki-67 is a proliferation marker evaluated immunohistochemically.[11] The mitotic rate is routinely estimated by the Ki67 value.[12] Ki67 provides independent predictive and prognostic benefits in chemotherapy response in adjuvant and neoadjuvant settings.[13]

Loss of tumor suppressive properties of E-cadherin is thought to be associated with invasion and carcinogenesis.[14,15] Loss of E-cadherin expression is generally used to determine lobular morphology, which accounts for 10-20% of all breast cancers.[16,17]

In our study, the evaluation of the relationships between ER, PR, HER2, Ki67, E-Cadherin expressions, Nottingham histological grade and some clinical parameters in breast carcinomas were examined.

MATERIALS AND METHODS

Ethics Committee Approval: Before starting the study, permission was obtained from the Clinical Research Ethics Committee (Date:2020; decision no:169). The study was performed according to the Declaration of Helsinki.

Study Design: The study was planned as a retrospective cross-sectional study and was conducted between 2018 and 2020.

Data Collection: This study included 74 patients diagnosed with breast carcinoma between 2018-2019 in the Department of Pathology, Faculty of Medicine, Ordu University. Pathology preparations were obtained from the archive for evaluation.

Histological Analysis and Evaluation: Cases with breast carcinoma were evaluated and analyzed retrospectively according to their ER, PR, HER2, Ki67, E-Cadherin expressions and Nottingham histological grades. In addition, some clinical parameters such as age and gender were also evaluated in these cases.

Immunohistochemically, ≥1% nuclear expression was accepted as positive staining for ER and PR, and <1% nuclear expression was considered as negative staining.[18]

HER2 score was evaluated as 0, 1, 2, and 3. (score 0: no staining or incomplete pale staining ≤ 10% in invasive tumor cells, score 1: incomplete pale membrane staining in more than 10% of invasive tumor cells, score 2: weak to moderate complete membrane staining in more than 10% of invasive tumor cells, score 3: complete, intense circumferential membranous staining (strong positive staining) in more than 10% of invasive tumor cells).[9]

Ki67 proliferation index was considered as low (<10%), intermediate (10-20%) and high risk (>20%).[19]

For E-Cadherin, membranous staining in the neoplasm was considered as positive staining.[20] The Nottingham Histologic grading system was used to grade the tumors. Tumors were graded as grade 1, 2, or 3 according to tubule or gland formation, nuclear pleomorphism, and the number of mitoses. Grade 1 was specified as having a good prognosis, Grade 2 was an intermediate prognosis and Grade 3 was the worst prognosis.[2]

Statistical Analysis: Categorical data were expressed as frequency (n) and percentage (%). Pearson's chi-square test was used to determine the relationship between the categorical variables. A continuous variable is defined as mean±standard deviation (SD). In the chi-square test, when the expected cell frequencies fall below 5, a Likelihood ratio test statistic value was calculated instead of Pearson's test statistic value. Kendall's tau correlation coefficient was calculated to measure the ordinal association. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS v28 (IBM Inc., Chicago, IL, USA) statistical software.

RESULTS

There were 74 cases diagnosed with breast carcinoma in the study. There were 98.6% female and 1.6% male patients, and the mean age was 56.42±14.42 years. Half of the patients were 55 years or older, 10.8% of the remaining patients were <40 years, and 39.2% were between 40-54 years (Table 1). Invasive ductal carcinoma was found to be the most common histological type (82.4%). In our study, Tru-cut bi-

opsy material was the most common sampling method (67.7%). The localizations of malignant neoplasms were determined as 50% right, 48.6% left, and 1.4% right+ left breast localization (Table 1).

HER2 score showed a statistically significant change according to ER status (p=0.010). While the rate of HER2 score 3 was higher in ER-negative patients (72.2%), the rate of HER2 score 0 in ER-positive patients was higher (34.5%). HER2 score also showed a statistically significant change according to PR status (p=0.004). Likewise, while the rate of HER2 score 3 was higher in PR-negative patients (69.6%), the rate of HER2 score 0 in PR-positive patients was higher (36.0%) (Table 2).

Table 1. Main characteristics of patients.

		n (%)
Gender	Female	73 (98.6)
	Male	1 (1.4)
Age (Mean±SD: 6.42±14.42)	<40	8 (10.8)
	40-54	29 (39.2)
	≥55	37 (50.0)
Tumor Localization	Right	37 (50.0)
	Left	36 (48.6)
	Right&Left	1 (1.4)
Diagnosis	Ductal carcinoma in situ	1 (1.4)
	Encapsular papillary and solid papillary carcinoma	1 (1.4)
	Intraductal papillary carcinoma	1 (1.4)
	Invasive ductal carcinoma	61 (82.4)
	Invasive lobular carcinoma	4 (5.4)
	Medullary invasive breast carcinoma	1 (1.4)
	Metaplastic carcinoma	1 (1.4)
	Mixed breast carcinoma (invasive lobular, invasive ductal carcinoma)	3 (4.1)
	Solid papillary carcinoma	1 (1.4)
HER2 score	0	20 (27)
	1	13 (17.6)
	2	10 (13.5)
	3	30 (40.5)
	Not calculated	1 (1.4)
Ki67 Status	Low risk	9 (12.2)
	Moderate risk	32 (43.2)
	High risk	32 (43.2)
	Not calculated	1 (1.4)
E-Cadherin	IHK not studied	39 (52.7)
	Negative	4 (5.4)
	Positive	31 (41.9)
Histological grade	Grade 1	12 (16.2)
	Grade 2	52 (70.3)
	Grade 3	7 (9.5)
	Not graded	3 (4.1)
ER	Negative (<1%)	18 (24.3)
	Positive (≥1%)	55 (74.3)
	Not calculated	1 (1.4)
PR	Negative (<1%)	23 (31.1)
	Positive (≥1%)	50 (67.6)
	Not calculated	1 (1.4)

Ki-67: Proliferation marker; HER2: Human epidermal growth factor receptor 2; ER: Estrogen; PR: Progesterone.

Table 2. Relationship between HER2 score and Hormone receptors.

		HER2 score				Total n (%)	p, χ^2
		0 n (%)	1 n (%)	2 n (%)	3 n (%)		
ER Status	Negative	1 (5.6)	2 (11.1)	2 (11.1)	13 (72.2)	18 (100.0)	p=0.010 χ^2=11.382
	Positive	19 (34.5)	11 (20.0)	8 (14.5)	17 (30.9)	55 (100.0)	
	Total	20 (27.4)	13 (17.8)	10 (13.7)	30 (41.1)	73 (100.0)	
PR Status	Negative	2 (8.7)	2 (8.7)	3 (13.0)	16 (69.6)	23 (100.0)	p=0.004 χ^2=13.134
	Positive	18 (36.0)	11 (22.0)	7 (14.0)	14 (28.0)	50 (100.0)	
	Total	20 (27.4)	13 (17.8)	10 (13.7)	30 (41.1)	73 (100.0)	

HER2: Human epidermal growth factor receptor 2; ER: Estrogen; PR: Progesterone; p: < 0.05 significant value; χ^2: Likelihood ratio chi-square test.

Ki67 was found to be high risk at a rate of 72.2% in ER receptor-negative patients (p=0.005) and 65.2% in PR receptor-negative patients (p=0.033) (Table 3).

Ki67 did not differ significantly according to age groups (p=0.342) and HER2 scores (p=0.389). There was a statistically significant correlation between Ki67 and the histological stage (p<0.001). As Ki67 increased, the histological stage was increased (r=0.349) (Table 4).

Table 3. Relationship between Ki67 and hormone receptors.

		Ki67 Status			Total n (%)	p, χ^2
		Low n (%)	Moderate n (%)	High n (%)		
ER Status	Negative	0 (0.0)	5 (27.8)	13 (72.2)	18 (100.0)	p=0.005 χ^2=10.580
	Positive	9 (16.4)	27 (49.1)	19 (34.5)	55 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	
PR Status	Negative	1 (4.3)	7 (30.4)	15 (65.2)	23 (100.0)	p=0.033 χ^2=6.836
	Positive	8 (16.0)	25 (50.0)	17 (34.0)	50 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	

Ki-67: Proliferation marker; ER: Estrogen; PR: Progesterone; p: < 0.05 significant value; χ^2:Likelihood ratio chi-square test.

Table 4. Comparison of Ki67 with age, HER2 score, histological grade.

		Ki67 Status			Total n (%)	p, χ2 and r
		Low n (%)	Moderate n (%)	High n (%)		
Age group	<40	0 (0.0)	4 (50.0)	4 (50.0)	8 (100.0)	p=0.342 χ^2=4.504
	40-54	2 (7.1)	12 (42.9)	14 (50.0)	28 (100.0)	
	>=55	7 (18.9)	16 (43.2)	14 (37.8)	37 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	
HER2 score	0	5 (25.0)	8 (40.0)	7 (35.0)	20 (100.0)	p=0.389 χ^2=6.312
	1	0 (0.0)	6 (46.2)	7 (53.8)	13 (100.0)	
	2	1 (10.0)	4 (40.0)	5 (50.0)	10 (100.0)	
	3	3 (10.0)	14 (46.7)	13 (43.3)	30 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	
Histological Grade	Grade 1	1 (8.3)	10 (83.3)	1 (8.3)	12 (100.0)	p<0.001 χ^2=20.794 r:0.349
	Grade 2	8 (15.4)	21 (40.4)	23 (44.2)	52 (100.0)	
	Grade 3	0 (0.0)	0 (0.0)	7 (100.0)	7 (100.0)	
	Total	9 (12.7)	31 (43.7)	31 (43.7)	71 (100.0)	

Ki-67: Proliferation marker; HER2: Human epidermal growth factor receptor 2; ER: Estrogen; PR: Progesterone; p: < 0.05 significant value; χ^2:Likelihood ratio chi-square test; r: Kendalltau-b correlation coefficient.

DISCUSSION AND CONCLUSION

Among the histological types of breast carcinomas, the most common type is invasive ductal carcinoma with approximately 85%.[17] In our study, invasive ductal carcinoma was the most common histological type (82.4%), and invasive lobular carcinoma was the second most common (5.4%).

In our study, the most common histological grade was grade 2 (70.3%) and the second most common was grade 1 (16.2%).

High interobserver reproducibility of the Nottingham combined histological grade was demonstrated in the study of Rakha et al., the proportion of different grades in their series was found to be almost the same as in the study of Elston et al. (grade 1, 17%; grade 2, 37%, and grade 3, 46%).[2, 21]

There may be interobserver variability in the determination of histological grade. Differences between centers are usually due to differences in tissue preparation quality. Suboptimal tissue fixation negatively affects the mitotic rate. Therefore cases that grade 3 can be reported as grade 2.[21]

In a study, the independent prognostic importance of grading in breast cancer was emphasized, and it has also been reported that the Nottingham histological grade method improves interobserver agreement compared to other grading systems.[3]

In our study, the most common histological grade was found to be grade 2, and the difference in interpretation between observers can explain this situation. Ki67 showed a statistically significant change according to histological stages in our study (p<0.001). Histological grade 2 was detected as 44.2% and grade 3 as 100% in the high-risk Ki67 group.

According to Ersöz et al., a significant relationship was found as a result of comparing Ki67 with histological grade and mitotic activity.[22] According to another study, the histological grade was shown to be associated with other well-defined prognostic variables and outcomes of patients.[21] In neoadjuvant and adjuvant applications, Ki67 is an independent prognostic and predictive useful proliferation marker

for chemotherapy response.[13] High-risk detection of Ki67 in cases with grade 3 and grade 2 in our study was consistent with the literature. Determination of appropriate breast cancer treatments and prognostic outcomes depends on accurate histological classification and measurement of two major groups of biomarkers: hormone receptors and HER2.[4,10]

In most studies, ER-positive and/or PR-positive rates were found to be higher in tru-cut biopsies compared to surgical specimens. This may be due to better fixation of the tru-cut biopsy material compared to the surgical excision specimen.[23] In our study, the majority of specimens were tru-cut biopsies (67.7%); but since the evaluations were made on all specimens (mastectomy, lumpectomy, tru-cut biopsy), no comment could be made on immunohistochemical expression differences in tru-cut biopsy and other specimens. In our study, ER-negative cases were found to be 24.3%, and PR-negative cases were found to be 31.1%. False-negative results in ER and/or PR expressions are more common in IHC studies. Therefore, IHC studies should be performed with appropriate internal and external controls.

Major causes of false negative results include tissue fixation problems, tumor heterogeneity, and interpretation of positive cases at the lower end of the spectrum. Sensitive consideration is required to avoid false negative results from preanalytical issues. False-negative results may cause patients to be deprived of treatment.[24] Our ER and PR expression rates were followed in line with the literature.

In our study, HER2 score showed a statistically significant change according to ER status (p:0.010). While the rate of HER2 score 3 in ER-negative patients was high (72.2%), the rate of HER2 score 0 in ER-positive patients was higher (34.5%).

HER2 score showed a statistically significant change according to PR status (p=0.004). While the rate of HER2 score 3 in PR-negative patients was high (69.6%), the rate of HER2 score 0 in PR-positive patients was higher (36%).

Han et al. reported that HER2-negative tumors do not require retesting. It has been reported that HER2 -negative tumors include low-grade carcinomas with ER-positive and infiltrative ductal or lobular histology.[24] Our findings were also found to support the literature.

In our study, Ki67 was found to be high risk at a rate of 72.2% in ER receptor-negative patients and 65.2% in PR receptor-negative patients. In our study, Ki67 showed a statistically significant change according to histological grades.

There are studies in the literature reporting that Ki-67 expression is inversely proportional to ER, PR receptors, and directly proportional to histological grade, tumor size, axillary lymph node involvement, and vascular invasion.[25]

High-risk detection of Ki67 in ER and PR-negative cases supports the literature findings. Ki67 did not show significant change according to age groups and HER2 scores.

In our data, E-Cadherin was not studied immunohistochemically in some tumors (52.7%),

However, negative staining was observed in all invasive lobular carcinomas (4 cases) studied with E-Cadherin (5.4%), it was studied in 31 patients (41.9%) diagnosed with invasive ductal carcinoma and positive staining was found in them. According to Wasif et al., loss of E-cadherin expression is widely used to determine lobular histology, which accounts for 10-20% of all breast cancers.[17] E-Cadherin expression findings in our study support the literature.

Breast cancer is a heterogeneous disease that requires clinical skills and a multidisciplinary approach to diagnosis and treatment.[26] Reproductive factors such as parity, duration of breastfeeding and period of lifetime menstrual may be more likely to predict the risk of hormone receptor-positive disease, but may not be valid for all types of breast cancer.[27]

Interpretation of immunohistochemical staining in the presence of histological findings is important for diagnosis and treatment.[28,29] IHC plays a vital role in predicting prognosis and response to therapy. Nowadays, traditional techniques such as IHC in breast cancer are still indispensable.[28]

The relationships between the molecular status of breast cancer and the biological and clinical course of the tumor are very important. Breast cancer profiling studies will better understand the importance of the genetic structure of breast tumors.[30]

In conclusion, today, in many centers, ER, PR, HER2 and Ki67 are used as a combination of four markers to provide better predictive and prognostic value in breast cancer.

Grading has independent prognostic significance in breast cancer. Pathologists may have different interpretations in terms of evaluating immunohistochemical staining and histological grading. In order to reduce this, preanalytical and analytical processes should be followed meticulously by pathologists. Compared to other grading systems, the Nottingham histological grade method is known to improve the interobserver agreement.

It should be kept in mind that immunohistochemical technical artifacts significantly affect the treatment strategy of the patient.

In this manuscript, we wanted to mention the differences in interpretation between pathologists and the importance of immunohistochemical technical artifacts.

False-negative results may fail to administer effective treatment. False-positive results can also lead to costly, ineffective, and overtreatment. Therefore,

high-quality and reliable receptor evaluations are very important. In conclusion, in addition to histological type, many clinicopathological parameters such as tumor grade, hormone receptors and HER2 status, Ki67 proliferation index are of great importance in terms of determining the prognosis and determining the correct treatment strategy for breast cancer.

Ethics Committee Approval: Before starting the study, permission was obtained from the Clinical Research Ethics Committee (Date:2020; decision:169).

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

Author Contributions: Concept – MAÇ; Supervision – MAÇ; Materials – MAÇ; Data Collection and/or Processing – MAÇ; Analysis and/ or Interpretation – YKA; Writing – MAÇ, YKA.

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Suppression of some IncRNAs and Invasion by Boric Acid Treatment in Human Pancreatic Cancer

İnsan Pankreas Kanserinde Borik Asit Uygulaması ile Bazı IncRNA'ların ve İnvazyonun Baskılanması

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ABSTRACT

Objective: It was aimed to investigate the effects of boric acid on some lncRNAs and invasion in PANC-1 and MIA PaCa-2 pancreatic cancer cells.

Materials and Methods: The effects of boric acid on cell viability and invasion were investigated using XTT test and invasion chambers, respectively. lncRNA H19 and UCA1 expressions were evaluated in pancreatic cancer using TCGA data. Its effects on expressions of these lncRNAs and invasion genes were determined by qRT-PCR analysis.

Results: The IC_{50} doses of boric acid were calculated as 14.25 mM in PANC-1 cells and 15.71 mM in MIA PaCa-2. TCGA data showed that H19 and UCA1 expressions were elevated in pancreatic cancer. H19 and UCA1 lncRNA levels were decreased with boric acid treatment. In addition, boric acid increased CDH1 and TIMP1 in both cell lines. However, it suppressed CDH2 expression. Boric acid increased TIMP2 in PANC-1 cells and TIMP3 expression in MIA PaCa-2 cells. In the invasion test, boric acid significantly suppressed invasion in both cells.

Conclusions: Boric acid suppressed H19 and UCA1 expressions, which were found to be high in pancreatic cancer. In addition, it showed an anti-invasive effect by changing the expressions of genes that are important in invasion.

Keywords: Boric acid, invasion, lncRNA-H19, lncRNA-UCA1, pancreatic cancer

ÖZ

Amaç: Borik asidin bazı lncRNA'lar ve invazyon üzerine etkisinin PANC-1 ve MIA PaCa-2 pankreas kanseri hücrelerinde araştırılması amaçlanmıştır.

Materyal ve Metot: Borik asitin hücre canlılığı ve invazyon üzerine etkileri sırası ile XTT testi ve invazyon kuyucukları kullanılarak araştırılmıştır. TCGA verileri kullanılarak pankreas kanserinde lncRNA H19 ve UCA1 ifadeleri değerlendirilmiştir. Bu lncRNA'ların ve invazyon genlerinin ifadeleri üzerine etkileri qRT-PZR ile belirlenmiştir.

Bulgular: PANC-1 hücrelerinde borik asitin IC50 dozu 14.25 mM, MIA PaCa-2 de ise 15.71 mM olarak hesaplanmıştır. TCGA verileri H19 ve UCA1 ifadelerinin pankreas kanserinde yüksek olduğunu göstermiştir. Borik asit muamelesi ile H19 ve UCA1 lncRNA seviyeleri azalmıştır. Ayrıca borik asit iki hücre hattında CDH1 ve TIMP1 ifadesini arttırmıştır. CDH2 ifadesini baskılamıştır. Borik asit PANC-1 hücrelerinde TIMP2, MIA PaCa-2 hücrelerinde TIMP3 ifadesini arttırmıştır. İnvazyon testinde borik asit her iki hücrede invazyonu anlamlı derecede baskılanmıştır.

Sonuç: Borik asit pankreas kanserinde ifadesinin yüksek olduğu görülen H19 ve UCA1 ifadelerini baskılamıştır. Ayrıca invazyonda önemli olan genlerin ifadelerini değiştirerek anti-invaziv etki göstermiştir.

Anahtar Kelimeler: Borik asit, invazyon, lncRNA-H19, lncRNA-UCA1, pankreas kanseri

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INTRODUCTION

Pancreatic cancer has the seventh-highest death rate.[1] Because of limitations in treating pancreatic cancer, it is important to discover a new target-specific agent. Smear samples were taken from women living in relatively boron-poor (587) and -rich (472) regions. No cytopathological findings were found in boron-rich regions. However, cytopathological findings of cervical cancer were detected in 15 women living in boron-poor regions.[2] Boric acid has antibacterial,[3] anticarcinogenic,[4] and anti-inflammatory[5] properties. High boric acid concentrations (12.5, 25, and 50 mM) exhibited antiproliferative and apoptotic effects in SK-MEL28 human skin melanoma cells.[6] Boric acid also inhibits histone deacetylases.[7] Hung et al.[8] investigated the retention status of boric acid in tumor regions during boron neutron capture therapy for hepatoma. Boric acid was localized in both hepatoma cells and tumor blood vessels. This study demonstrated that boric acid-mediated boron neutron capture therapy would specifically target the hepatoma. Boric acid-induced apoptosis by TNF signaling pathway in SW-480 human colon cancer cells[9] and boron supplementation decreased prostate tumor growth, IGF-1 expression, and serum prostate-specific antigen levels.[10]

Although 90% of the human genome is transcribed, only ~2% of the genomic sequence-includes protein-coding genes. The remaining genome consists of non-coding RNAs. Long non-coding RNAs (lncRNAs) are one of the two main classes of noncoding RNAs. lncRNAs have important functions in gene regulation and controlling many processes including apoptosis, epithelial-mesenchymal transition (EMT), invasion and metastasis.[11] lncRNA -H19 is located on 11p15.5 but is maternally expressed.[12] H19 increased EMT and metastasis through miR-675.[13] H19 is also overexpressed in pancreatic cancer and correlates with TNM stages, poor survival and metastasis.[14] lncRNA-UCA1 has been mapped at 19p13.12 and was first discovered in bladder carcinoma.[15] UCA1 expression was reported to be elevated in pancreatic cancer and correlated with tumor size, invasion and TNM stage.[16]

Cell migration is one of the most important features of solid metastatic tumors. Therefore, suppression of invasion is important in local therapies. During metastasis, cells must lose epithelial properties but acquire mesenchymal properties. In this process, the expressions of decreased CDH1 (E-cadherin) and increased CDH2 (N-cadherin) are important.[17] Decreased matrix metalloprotease inhibitors (TIMPs) are another important factor in acquiring mesenchymal features and the invasion process. Cell motility is present when matrix metalloproteinases are not inhibited.[18] Targeting the invasion cascade seems to be an important therapeutic strategy to prevent invasion. In this study, the effects of boric acid on lncRNA-H19, lncRNA-UCA1 and invasion features were investigated in pancreatic cancer cells.

MATERIALS AND METHODS

Ethical Status: This study was approved by N.E.U. (Date: 09.09.2022, decision no:3950). This study was carried out by international declarations, guidelines, etc.

Cell Culture and Chemicals: PANC-1 and MIA PaCa-2 human pancreatic cancer cells were purchased from ATCC. These cells were cultured with DMEM containing 1% penicillin/streptomycin and 5% FBS in an incubator with humidified 95% air and 5% CO_2. The boric acid obtained from Etimaden and was dissolved in the DMEM. QIAzol was purchased from Qiagen. cDNA synthesis kit was obtained from Bio-Rad. Eva Green Supermix was purchased from Solis BioDyne.

Cell Proliferation Test: The cytotoxic effects of boric acid on human pancreatic cancer PANC-1 and MIA PaCa-2 cells were evaluated using XTT assay as described elsewhere.[19] These cells were treated with eight different boric acid doses between 0.01 and 25 mM, and a previously described protocol was utilised.[20] IC_{50} doses were calculated with CompuSyn Version 1.0 software and used in further analyzes.

lncRNA Expressions: Different expression patterns of H19 and UCA1 lncRNAs were observed between pancreatic cancer tissue (179 cases) and normal tissue (171 cases) using data from the TCGA database (http://gepia.cancer-pku.cn/). The effects of boric acid on the expression of H19 and UCA1 lncRNAs were investigated. Total RNA isolation was performed from both control and boric acid-treated cells. cDNAs were synthesized by the manufacturer's instructions. H19, UCA1 and U6 (as reference gene) primers (Table 1) were designed using an online program (https://eu.idtdna.com/site). qRT-PCR analysis was performed using a previously described protocol.[21]

Invasion-related Gene Expressions: The effects of boric acid on CDH1, CDH2, TIMP1, TIMP2, and TIMP3 genes, which play an important role in invasion, were evaluated. For this purpose, cDNA synthesis was performed after total RNA isolation from control and dose groups. qRT-PCR analyses were conducted using previously designed target and ACTB reference gene primers.[21]

Invasion Assay: Invasion capacities of PANC1 and MIA PaCa-2 pancreatic cancer cells were determined using invasion chambers (Corning, USA). Control and dose group cells were seeded using the basal medium in the upper chamber. The medium

Table 1. Primer sequence of H19, UCA1 and U6 genes used in this study.

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	PCR product size (bp)
H19	CGTGACAAGCAGGACATGA	TCCGTGGAGGAAGTAAAGAAAC	131
UCA1	GAGGATTCCCAGCCATATGAAG	CGGCAGTTGGTGTGCTATAA	127
U6	TTGGAACGATACAGAGAAGATTAGC	CACGAATTTGCGTGTCATCC	60

completed with 10% FBS was placed in the lower well. After 22 h of incubation, invading cells were fixed and stained using crystal violet (0.5%). The experiments were performed in triplicates. The invasive cells were counted and photographed in five random fields.

Statistical Analysis: IC_{50} doses of boric acid were calculated with CompuSyn Version 1.0 software. qRT-PCR analysis was performed with the $2^{(-\Delta Ct)}$ method by normalizing the Ct values of target genes with reference genes. The independent sample *t*-test in the SPSS 26.0 program was–used to compare groups. $p<0.05$ was considered as statistically significant.

RESULTS

The IC_{50} doses of boric acid for PANC-1 and MIA PaCa-2 cells were calculated as 14.25 mM and 15.71 mM for 48 h, respectively as described previously.[19] These doses were treated to these cells in further experiments.

The expression levels of lncRNA H19 and UCA1 in human pancreatic cancer were determined using an online tool in the TCGA database (http://gepia2.cancer-pku.cn). The data showed that expressions of these lncRNAs were upregulated in pancreatic cancer tissues compared with normal tissue (Figure 1; A and B). Furthermore, the GEPIA database showed that H19 and UCA1 expressions were positively associated with poor survival in pancreatic cancer (Figure 1; C and D).

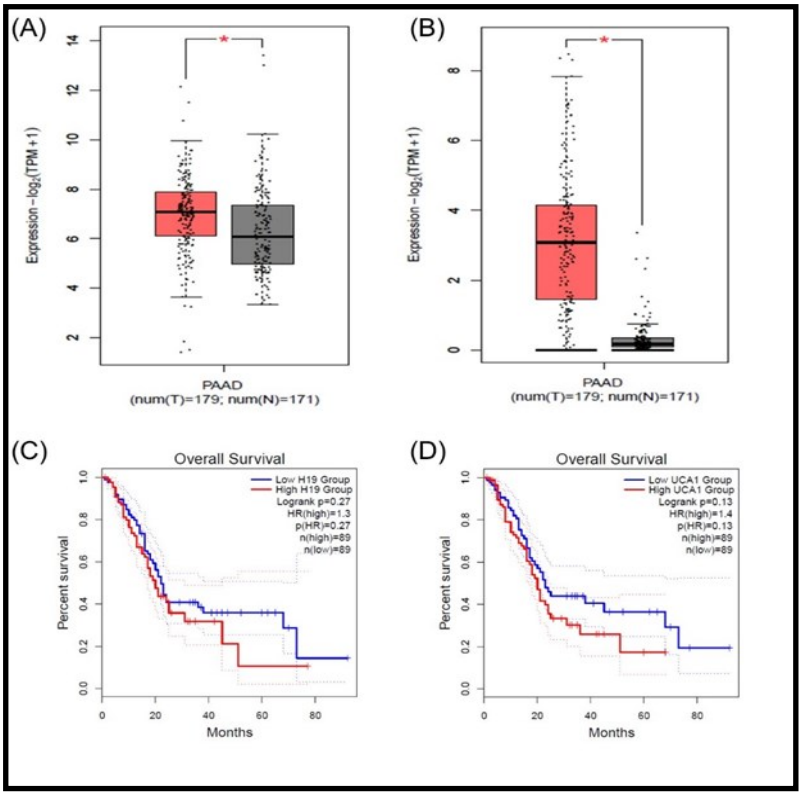

Figure 1. The expression levels of lncRNA-H19 and lncRNA-UCA1 in pancreatic cancer.
A: H19 was upregulated in pancreatic cancer tissue. B: UCA1 was upregulated in pancreatic cancer tissue. C, D: GEPIA database showed that H19 and UCA1 expressions were positively correlated with poor survival in pancreatic cancer. *: $p<0.05$. PAAD: pancreatic adenocarcinoma.

The effect of boric acid on lncRNA H19 and UCA1 expressions in PANC-1 and MIA PaCa-2 cells were determined by qRT-PCR analysis. qRT-PCR analysis results showed that H19 and UCA1 expressions in both cell lines were downregulated by boric acid treatment (Figure 2; A and B).

Results of qRT-PCR analysis indicated that boric acid caused significantly increased CDH1 and TIMP1 expressions and decreased CDH2 expression in both cell lines. In addition, boric acid caused an increase in TIMP2 expression in PANC-1 cells and TIMP3 expression in MIA PaCa-2 cells (Figure 3).

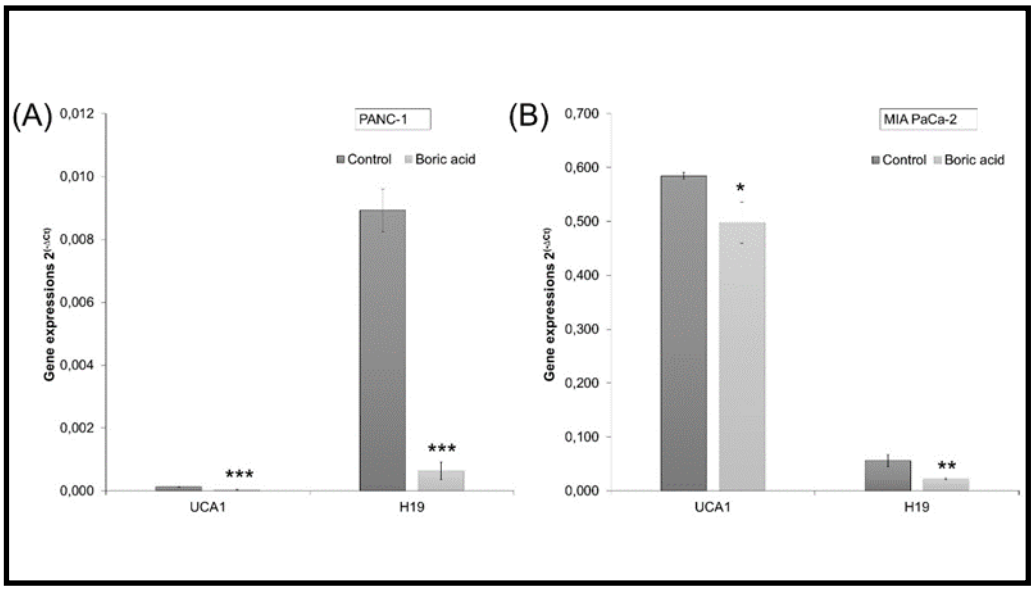

Figure 2. Effects of boric acid on lncRNA UCA1 and H19 in PANC-1(A) and MIA PaCa-2 (B) cells.
*: p<0.05; **: p<0.01; ***: p<0.001.

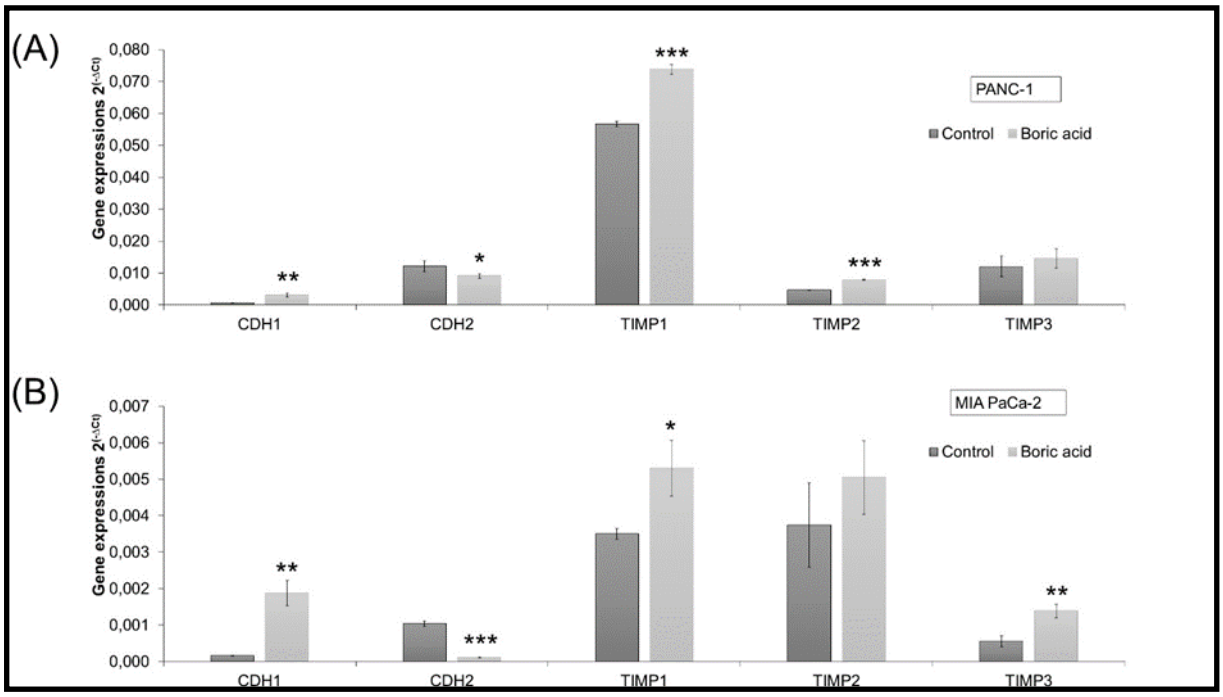

Figure 3. Effects of boric acid on the invasion-related genes in (A) PANC-1 and (B) MIA PaCa-2 cells.
*: p<0.05; **: p<0.01; ***: p<0.001.

The effect of boric acid on cell invasion was determined by the invasion test. The number of invasive cells in PANC-1 cells was counted as 448±44 in control and 334±32 in dose groups. In MIA PaCa-2 cells, control and dose groups were determined as 539±78 and 379±53, respectively (Figure 4).

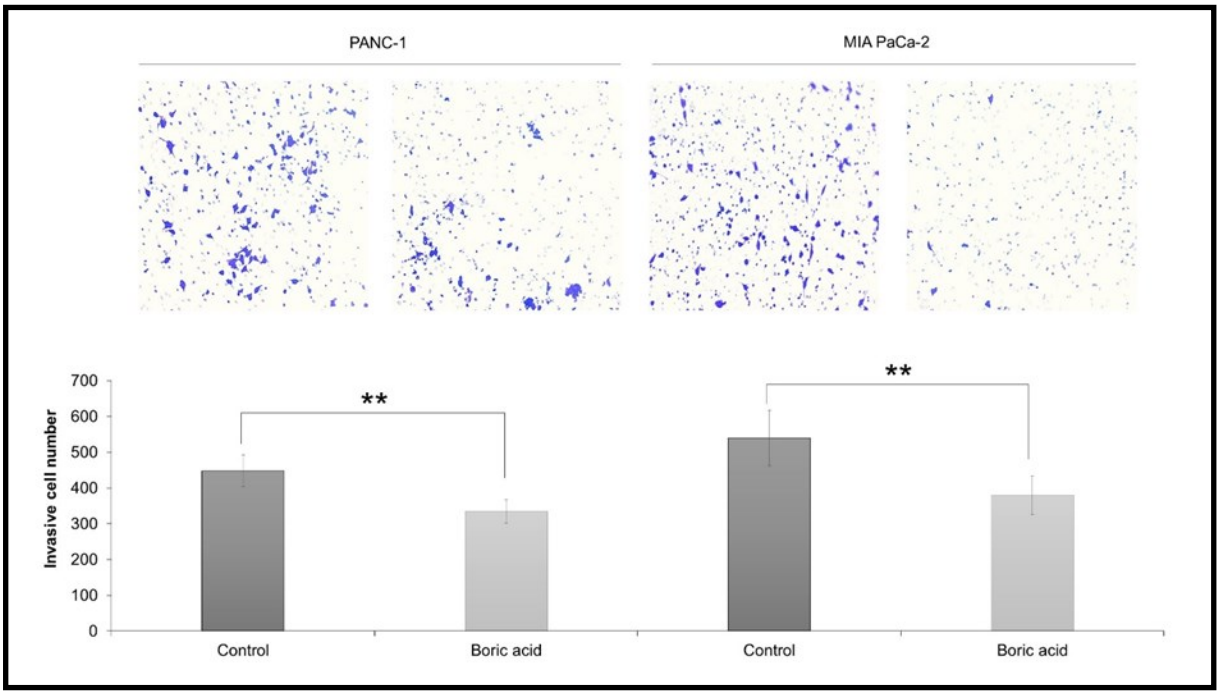

Figure 4. Effects of boric acid on the cell invasion in PANC-1 and MIA PaCa-2 cells. **: p<0.01.

DISCUSSION AND CONCLUSION

Investigating the effects of natural compounds in cancer therapy has gained importance because natural compounds have anticancer properties. Boric acid is a compound of boron. In a previous study, the EC_{50} concentration for boric acid in DMS-114 small-cell lung cancer cells was determined as 1000 µg/mL for 72 h.[22] Boric acid was shown to cause a concentration-dependent decrease in U-87MG human glioma cell viability. The LC_{50} value for boric acid in these cells was reported to be 123.45 µg/ml. Moreover, it has been reported that boric acid has shown an anticancer effect through PI3K/AKT, BRAF/MAPK and PTEN signaling pathways.[23] In a study, the IC_{50} dose of boric acid was shown to be 50 mM for 48 h in CCl-233 human colon adenocarcinoma cells using CCK-8 proliferation assay.[24] El-Hefny et al.[25] reported that the IC_{50} value of boric acid in HepG2 human hepatocellular carcinoma cells using MTT cell viability assay was 64.27 µg/ml. In a previous study conducted in our lab, the anticancer effect of boric acid was investigated in human pancreatic cancer MIA PaCa-2 and PANC-1 cells, and IC50 values of boric acid were calculated as 14.25 mM and 15.71 mM at 48 h for these cell lines, respectively. It was illustrated that boric acid also decreased colony formation and altered expression levels of some genes in apoptosis and endoplasmic reticulum stress pathways in MIA PaCa-2 and PANC-1 cells.[19] The same doses thereby were also used here.

Boric acid (250 and 1000 µM for 8 days) reduced invasion, migration and adhesion of DU-145 human prostate cancer cells with F-actin changes.[26] In the present study, boric acid increased CDH1 and TIMP1 gene expressions and decreased CDH2 expression in PANC-1 and MIA PaCa-2 pancreatic cancer cells. Moreover, boric acid treatment upregulated TIMP2 expression in PANC-1 and TIMP3 expression in MIA PaCa-2 cells (Figure 3). In addition, boric acid caused a decrease in the invasion properties of both cell lines in this study.

As is known, most RNAs are not translated into proteins. One of these classes of non-coding RNAs is called as lncRNAs. Previous literature has shown that expression of lncRNAs is altered in many malignancies including pancreatic cancer.[27] It has been reported that H19 expression is high in pancreatic cancer tissues. It has also been shown that H19 expression is higher in metastasized tumors. Knock-

down of H19 suppressed invasion and metastasis of pancreatic cancer cells. It has been stated that H19 overexpression triggers EMT in pancreatic cancer.[28] Another lncRNA that has been shown to be highly expressed in pancreatic cancer is UCA1.[29] UCA1 suppressed apoptosis and increased cell proliferation, migration and invasion in pancreatic cancer cells.[30] Consistently, the TCGA database (http://gepia2.cancer-pku.cn) suggested that expressions of lncRNA H19 and UCA1 were upregulated in pancreatic cancer tissues compared with normal tissue and correlated with poor survival of pancreatic cancer patients (Figure 1). In the present study, H19 and UCA1 expression were downregulated in both PANC-1 and MIA PaCa-2 cells by boric acid treatment (Figure 2).

In conclusion, boric acid caused a decrease in the expression of H19 and UCA1, which are known to have higher levels in pancreatic cancer. These lncRNAs are associated with poor survival and increased invasion features. In addition, boric acid showed an anti-invasive effect by changing the expression of genes that are important in invasion process. It is thought that boric acid can be a potential anti-invasive compound in pancreatic cancer. Further analyses are required for boric acid's therapeutic use in pancreatic cancer.

Ethics Committee Approval: This study was approved by N.E.U. Non-drug and Non-Medical Device Research Ethics Committee (Date: 09.09.2022, decision no:3950). The study was carried out by international declarations, guidelines, etc.

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

Author Contributions: Concept, Analysis and Interpretation, Writing- CEG.

Peer-review: Externally peer-reviewed.

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Cytotoxic Effects of Cisplatin and Carboplatin Loaded Albumin Nanoparticles on Breast Cancer Cells

Sisplatin ve Karboplatin Yüklü Albumin Nanopartiküllerin Meme Kanseri Hücreleri Üzerindeki Sitotoksik Etkileri

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ABSTRACT

Objective: This study aims to investigate and compare the anticancer effects of carboplatin and cisplatin, frequently used in cancer treatment, by loading them on albumin nanocarrier.

Materials and Methods: Carboplatin (CP) and Cisplatin (Cis) loaded albumin nanoparticles were synthesized using ultrasonication as CP-NPs and Cis-NPs, respectively. Nanoparticle size and distribution were evaluated by Dynamic light scattering (DLS). Cytotoxicities of NPs were evaluated in MDA-MB-231 and MCF-7 breast cancer cells and HUVEC using MTT test and their morphological images were compared.

Results: While the average size of CP-NPs was around 2-3 nm, Cis-NPs was 7-8 nm. It was observed that both NPs groups were homogeneously dispersed. According to the cytotoxicity results, both CP-NPs and Cis-NPs were more cytotoxic on MCF-7 breast cancer cells. In addition, CP-NPs and Cis-NPs showed significant cytotoxicity on MCF -7, MDA-MB-231 breast cancer cells, while low cytotoxicity was detected in normal HUVEC cells. The NPs treated MCF-7 was compared with the untreated MCF-7 and statistical significance was calculated as $P<0.01$ for CP-NPs and Cis-NPs.

Conclusions: Abumin-based CP-NPs and Cis-NPs showed high cytotoxicity in breast cancer cells, they have low cytotoxicity in healthy cells, making them promising for breast cancer treatment.

Keywords: Cytotoxicity, breast cancer, MCF-7, MDA-MB-231, nano drug

ÖZ

Amaç: Bu çalışmanın amacı kanser tedavisinde sıklıkla kullanılan karboplatin ve sisplatin ilaçlarının albümin nanotaşıyıcıya yüklenerek antikanser etkilerinin araştırılması ve karşılaştırılmasıdır.

Materyal ve Metot: Karboplatin (CP) ve Sisplatin (Cis) yüklenmiş albümin nanopartiküller, sırasıyla CP-NPs ve Cis-NPs olarak ultrasonikasyon kullanarak sentezlendi. Nanopartikül boyutu ve dağılımın homojenitesi Dinamik ışık saçılımı (DLS) ile değerlendirildi. Nanopartiküllerin sitotoksik aktiviteleri MDA-MB-231 ve MCF-7 meme kanseri hücrelerinde ve HUVEC hücrelerinde, MTT testi kullanılarak değerlendirildi ve morfolojik görüntüleri karşılaştırıldı.

Bulgular: CP-NPs'lerin boyutu ortalama 2-3 nm civarında iken, Cis-NPs'lerin 7-8 nm idi. Her iki nanopartikül grubunun da homojen bir şekilde dağıldığı görüldü. Sitotoksisite sonuçlarına göre CP-NPs ve Cis-NPs, MCF-7 meme kanseri hücrelerinde daha sitotoksikti. Ayrıca CP-NPs ve Cis-NPs'ler MDA-MB-231 meme kanseri hücrelerinde önemli sitotoksisite gösterirken, normal HUVEC hücrelerinde düşük sitotoksisite tespit edildi. CP-NPs ve Cis-NPs ile tedavi edilen MCF-7, tedavi edilmeyen MCF-7 ile karşılaştırıldı ve NPs'ler için istatistiksel anlamlılık $P<0,01$ olarak hesaplandı.

Sonuç: Meme kanseri hücrelerinde yüksek sitotoksisite gözlenirken, sağlıklı hücrelerde belirgin sitotoksisite gözlenmemiş olup albümine bağlı CP-NPs ve Cis-NPs kanser tedavisinde umut vadedici bir tedavi seçeneği olabilir.

Anahtar Kelimeler: MCF-7, MDA-MB-231, meme kanseri, nano ilaç, sitotoksisite

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INTRODUCTION

Breast cancer is the most frequent malignant tumor in women and one of the top causes of cancer death in many nations.[1] In particular, chemotherapy is widely used in treating breast cancer, but the frequency of side effects and drug reactions limits the efficiency of treatment.[2] Consequently, new and selective anticancer drugs with fewer side effects and ways to reduce side effects are needed.[3]

There has been a surge of interest in using nanotechnology in cancer treatment in recent years.[4,5] Nanodrug delivery systems offer considerable advantages in terms of enhanced permeability and retention (EPR), particularly when targeting tumors, and can lessen the negative effects of anticancer medications.[6] Nanoparticles smaller than 100 nm, in particular, are more effective in passively targeting tumors and are ideal carriers for inducing the drug's therapeutic potential while minimizing its side effects. Furthermore, nanocarriers may increase medication half-life in therapy, enhance pharmacokinetic characteristics, and improve patient compliance.[7]

In particular, protein-based nanocarriers are preferred over other nanomaterials as they have many favourable properties and are safe to use in biological environments.[8]Albumin is a multifunctional protein used as a medicine carrier because it is nontoxic, non-immunogenic, biocompatible, and biodegradable. As a result, it is an excellent material for use as a drug delivery platform.[9]

The principal effect of albumin-based drug delivery systems is dependent on tumor targeting and nanoparticle accumulation within the tumor. Higher drug accumulation within the tumor is caused by increased absorption, which is passively mediated by the increased permeability and retention effect.[9] Albumin can bind to certain receptors overexpressed in cancer cells, increasing nanoparticle binding and absorption. The 60 kDa glycoprotein (gp60) receptor[10] is overexpressed in a variety of malignancies, as is the acidic and cysteine-rich SPARC protein.[11] Albumin can bind selectively to gp60 and SPARC, hence actively increasing nanoparticle absorption. This mechanism prevents albumin-based nanoparticles from ejecting the medication from tumor cells.[12,13]

Although many studies on nanocarriers have been conducted in recent years, there is still a need for further inquiry regarding their effects in the field of cancer. In this study, cisplatin (Cis) and carboplatin (CP) nanoparticles were loaded independently into albumin and their anticancer effects on breast cancer cells were investigated. The cytotoxic effects of these medications, which we manufactured on the nanotechnological platform, were evaluated and compared on MCF-7, MDA-MB-231 breast cancer cells, and normal HUVEC cells.

MATERIALS AND METHODS

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

Materials: MCF-7, MDA-MB-231, and HUVEC cell lines were purchased from The American Type Culture Collection (ATCC). Analytical purity bovine serum albumin (BSA) was purchased from Sigma (USA) while pharmaceuticals CP and Cis from Koçak Farma (Turkey). Chemicals used in cell culture are from Dulbecco's Modified Eagle's Medium (DMEM), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit, Dimethylsulfoxide (DMSO), Sigma (USA) and Fetal Bovine Serum (FBS), Penicillin, streptomycin, and trypsin were obtained from Gibco (UK).

Preparation of Nanoparticles: First, stock solutions were prepared at a concentration of 10 mg/mL of albumin and 4.5 mg/mL of CP and Cis separately in distilled water (CP and Cis were previously dissolved at 10 mg/mL separately in DMSO).[14] The albumin and stock solutions were separated into Eppendorf tubes and sonicated for 30 seconds in the ultrasonication apparatus. Following that, the produced solutions were stored separately in a sterile condition for 1 hour under a 30 W (Philips brand) UV-C lamp (200-280 nm). Compounds loaded on albumin were encoded as Cis-Nps and CP-NPs.

Distribution of Nanoparticles / Size Measurements: Using a 4 mW He-Ne laser operating at ambient temperature, a wavelength of 633 nm, and a detection angle of 173°, dynamic light scattering (DLS) measurements using Zetasizer Nano ZS were used to assess the size distribution of the compounds. As the reference liquid, distilled water was utilized.

Cell Culture and Cell Lines: MDA-MB-231, MCF-7 breast cancer, and normal HUVEC cells were employed in cell culture research. They were grown in DMEM with fetal calf serum at 37°C in a humidified oven environment with 95% O_2, and 5% CO_2. Cells were checked daily and subcultured every day or every 2-3 days, depending on their condition.

Evaluation of Cell Viability by Trypan Blue Method: The trypan blue staining technique was used to measure cell viability. For this, the cells were stained with trypan blue dye, and cell viability was measured using a Neubauer slide under a light microscope. Dead cells stained with trypan blue showed blue, whereas healthy cells did not. It was observed that cells that had not been colored were alive.

Determination of Cytotoxic Activity on Cells by MTT Measurement Based on Mitochondrial Activity: The MTT test is a quantitative colorimetric

method used to assess cytotoxicity in vitro based on metabolic viability.[15] Albumin-bound Cisplatin-loaded nanoparticles (Cis-NPs) and Carboplatin-loaded nanoparticles (CP-NPs) were produced at various doses (50, 25, 12.5, 6.25, 3.12 g/mL) and incubated for 72 hours with MDA-MB-231, MCF-7, and HUVEC cells. After 72 hours, the cells were exposed to the MTT test, and the optical densities of the cells in the plate were measured with an ELISA equipment at 540 nm. All experiments were done three times, and the average was calculated.

Statistical analysis: Using the IBM SPSS 15.0 (SPSS Inc., Chicago, IL, USA) package application, the data was examined. In order to compare the variations between the control groups and CP-NPs and Cis-NPs, the Student's t-test was performed. P values less than 0.05 were considered significant. The 72-hour MTT test was used to determine cytotoxicity. Each test was conducted three times, and the average was calculated. The data are presented as mean SD.

RESULTS

CP and Cis anticancer medicines attach to albumin nanocarriers at the nanoscale, as shown in Figure 1. The nanosizes of the cisplatin-loaded albumin nanoparticles (Cis-NPs) are in the range of 5-11 nm, the nanoparticles are homogeneously disseminated, and the average Cis-NPs size is around 6-7 nm, as shown in Figure 1a. Furthermore, Figure 1b shows that the CP-loaded albumin-bound nanoparticles (CP-NPs) are homogeneously distributed in the 2-5 nm range, with the average CP-NPs size being around 2-3 nm. Furthermore, although Cis NPs had a PDI value of 0.218, CP-NPs had a PDI value of 0.481.

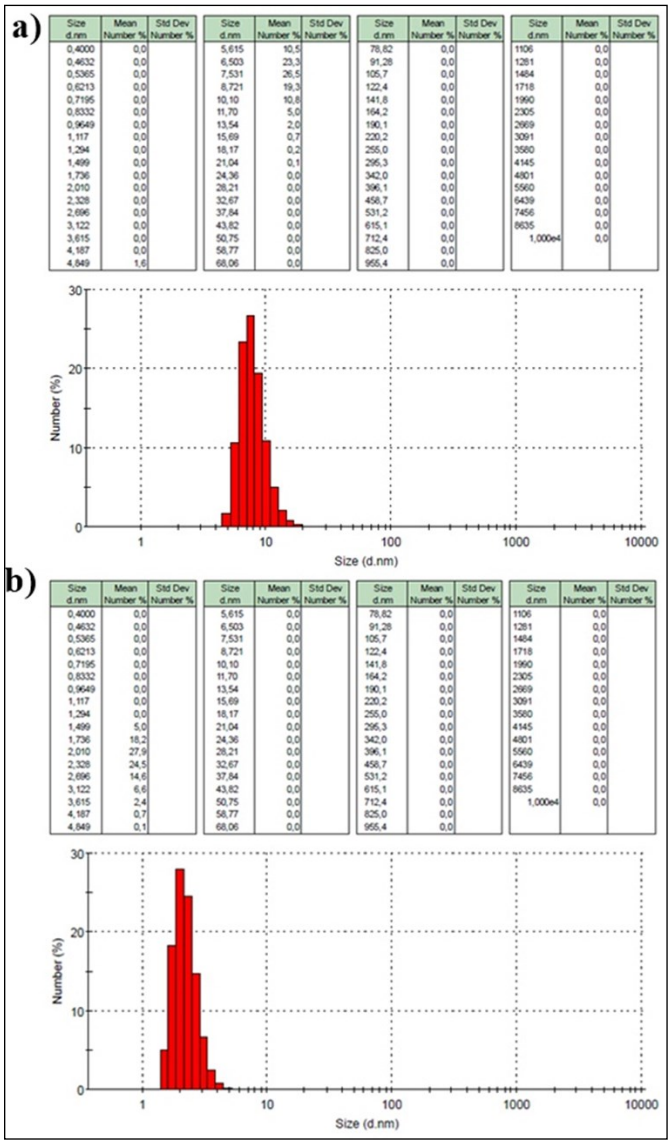

Figure 1. DLS Analysis of Albumin-based Carboplatin NPs (a) and Cisplatin NPs (b).

Figure 2 shows the cytotoxic effects of CP-NPs and Cis-NPs on MDA-MB-231, MCF-7 breast cancer cells, and normal HUVEC cells. According to our findings, both CP-NPs and Cis-NPs appear to be more cytotoxic in MCF-7 breast cancer cells. MDA-MB-231 triple-negative breast cancer cells seem to be more resistant to treatment than MCF-7. It is also noteworthy that CP-NPs and Cis-NPs samples are less cytotoxic in HUVEC cells, which are the normal cell group. Table 2 shows the IC_{50} values of Cis-NPs and CP-NPs on MCF-7, MDA-MB-231, and HUVEC cells. The IC_{50} values of Cis-NPs are 29 µg/mL in MCF-7 cells, 46 µg/mL in MDA-MB-231 cells, while the IC_{50} values of CP-NPs are 22 µg/mL in MCF-7 cells, MDA-MB-231 for 41 µg/mL (Figure 2, Table 1).

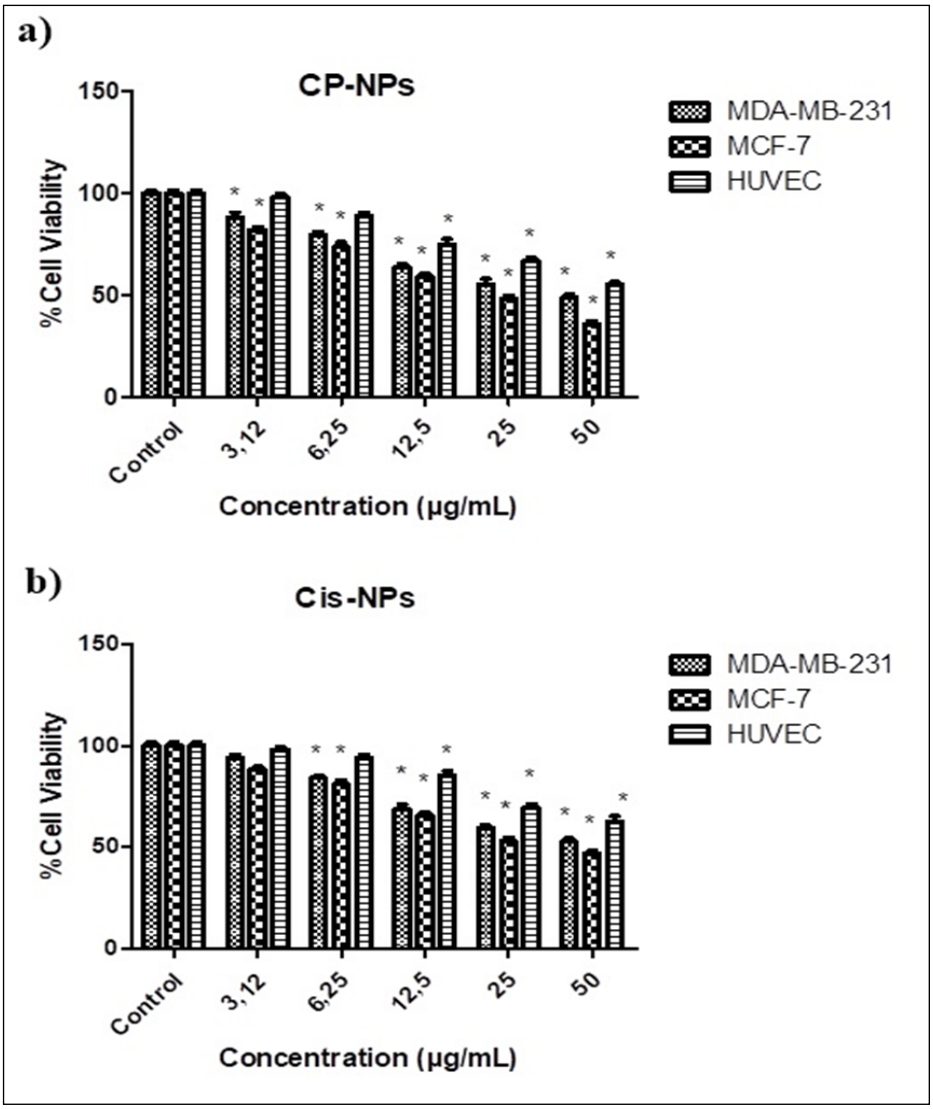

Figure 2. Cytotoxic effects of CP-NPs (a) and Cis-NPs (b) at different concentrations on MDA-MB-231, MCF -7 cancer cells and HUVEC normal cells (Concentrations are calculated based on the amount of Cis or CP loaded into the nanoparticles). Significant comparisons have a "p" value of less than 0.05. In each group, each concentration was compared with the control and the mean *: $p<0.01$ was calculated.
CP: Carboplatin, Cis: Cisplatin.

Table 1. IC_{50} concentrations of Cis-NPs and CP-NPs on MCF-7, MDA-MB-231 and HUVEC cells. Cytotoxicity was evaluated by MTT test after 72 hours.

	***IC_{50} Concentration (µg/mL)**	
Cell Line	Cis-NPs	CP-NPs
MCF-7	29	22
MDA-MB-231	46	41
HUVEC	>50	>50

IC_{50}: The concentration at which cell growth is inhibited by 50%.

Figure 3 compares the morphological changes in MDA-MB -231, MCF -7, and HUVEC cells that were not treated and in MDA-MB -231, MCF -7, and HUVEC cells treated with CP -NPs and Cis-NPs. The untreated control cells were compact, spindle-shaped, cross-linked and adherent to the surface in all cell groups. In contrast, the number of MDA-MB-231 and MCF-7 cells treated with CP-NPs, and Cis-NPs appeared to be drastically reduced. Some cells treated with the nano-drug were shrunken and round, and there was a significant decrease in cell quantity and intercellular connections. Figure 3 shows images of cells treated with CP -NPs, and Cis -NPs at IC_{50} values. According to the findings of this study, the morphological changes are consistent with cytotoxicity.

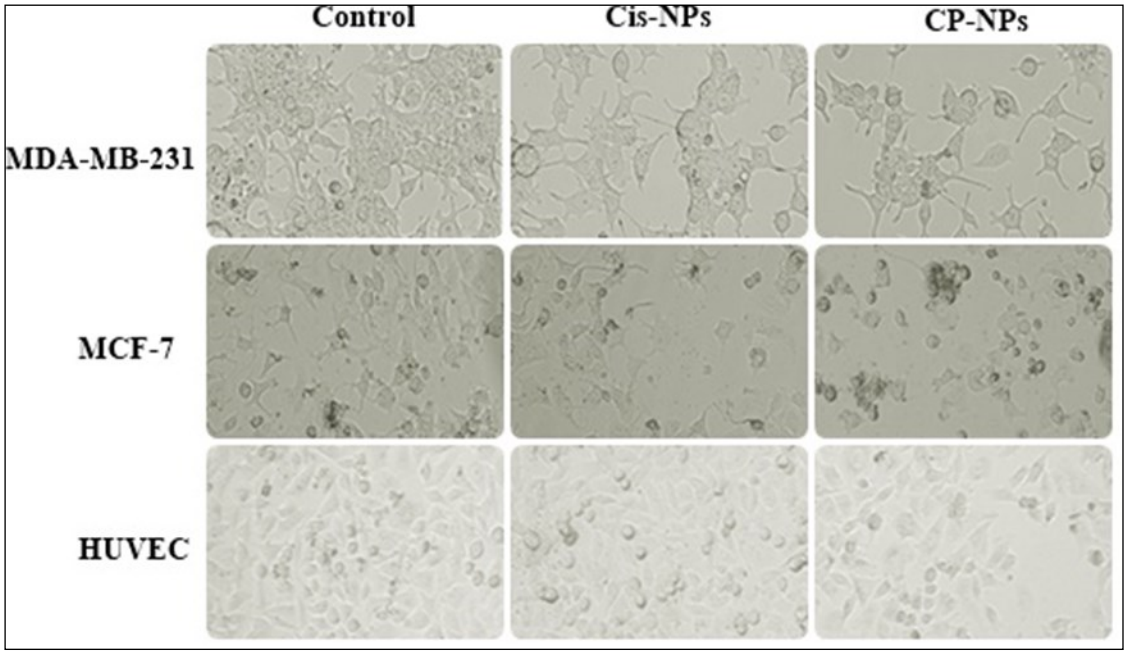

Figure 3. The morphological changes in MDA-MB-231, MCF-7, and HUVEC cells under light microscopy. Magnification 20X.

DISCUSSION AND CONCLUSION

In this study, the platinum group anticancer medicines CP and Cis were loaded on albumin nanocarrier to create two distinct nano-drugs, Cis-NPs and CP-NPs, and their cytotoxic effects on MCF-7, MDA-MB-231 and HUVEC cells were examined.

A growing body of evidence suggests that nanoparticle-based drug delivery show promise for treating cancer. Nanocarriers can effectively deliver drugs to diseased areas in a controlled and targeted manner compared to conventional drug therapy.[16] Nanocarriers may also improve pharmacokinetic characteristics, lengthen medication half-life in therapy, and improve patient compliance. Many nanoparticles have been studied for this purpose, including different polymeric nanoparticles, albumin-bound nanoparticles, lipid-based nanoparticles, inorganic nanoparticles, carbon-based nanotubes, polymer-based conjugates, and nanocrystals.[7,13] Among them, we chose albumin nanocarriers for the medications Cis and CP because it is possible to overcome the challenges posed by disease-treating drugs, such as limited solubility, surface adsorption, and high systemic toxicity, in part because of albumin nanocarriers.[8]

The literature includes synthesized nano-drug delivery technologies of various sizes.[17–19] However, passively targeting tumors with nanocarrier systems that use particles smaller than 40 nm is more effective at inducing therapeutic potential and minimizing adverse pharmacological effects.[7,13] In this study, NPs below 40 nm were obtained and their cytotoxic effects on both cancer cells and normal cells were evaluated. Albumin-bound CP-NPs and Cis-NPs with sizes of 7-8 nm and 2-3 nm, respectively (Fig.1), were created and proved to be very effective against breast cancer cells. Different sizes of gold nanoparticles (3,5,6,8,10,17 nm) were created in a study by Viyajakumar et al., and the effects of their cytotoxicity were examined. It was shown that the 3 nm nanoparticles were more cytotoxic than the others.[20]

In our study, Cis-NPs and CP-NPs were found to be highly cytotoxic in both MCF-7 and MDA-MB-231 breast cancer cell groups, but they were more effective on MCF-7 cells and had the least cytotoxicity on HUVEC normal cells (Fig. 2, Fig. 3, Table 1).

According to our results, it is expected that MDA-MB-231 cells are more resistant to treatment than MCF-7. Because MCF-7 and MDA-MB-231 breast cancer cells have different phenotypic and genotypic characteristics even though they are both invasive ductal breast cancer cells. MCF-7 cells are estrogen receptor (ER) positive and progesterone receptor (PR) positive and have low metastatic potential, while MDA-MB-231 cells known as triple-negative breast cancer (TNBC) lack ER, PR and human epidermal growth factor receptors. It is known for its lack of expression of the 2 (HER2) gene and is considered to be the most aggressive breast cancer cells that can lead to early metastasis.[21,22] For this reason, TNBC breast cancer with MDA-MB-231 cancer cells is a type of breast cancer that is difficult to treat, and it is very important that the nano-drugs we synthesize are also effective in this cell group.

Many of the drawbacks of conventional drug delivery systems can be solved by nanocarrier-dependent drug delivery applications. For instance, chemotherapeutic agents used in the treatment of cancer are traditionally non-specifically distributed systemically, causing damage to both healthy cells and cancer cells, often with low efficacy and high toxicity.[23] Nano-drug delivery systems can increase drug concentration in cancer cells by directing chemotherapeutics to the tumor site and prevent toxicity in normal cells.[24–26]

In conclusion, CP and Cis loaded on albumin nanocarriers exhibited high cytotoxicity in breast cancer cells but low cytotoxicity in healthy cells, which suggested that they are promising for the treatment of breast cancer.

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

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

Author Contributions: Concept – FDK, AK; Materials – FDK, AK; Data Collection and/or Processing – FDK, AK; Analysis and/or Interpretation – FDK; Writing –FDK, AK. Supervision – FDK, AK.

Peer-review: Externally peer-reviewed.

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The Effect of Thiol-Disulfide Homeostasis on Prognosis in Patients with Early-Stage Non-Small Cell Lung Cancer

Erken Evre Küçük Hücreli Dışı Akciğer Kanserli Hastalarda Tiyol-Disülfid Homeostazisin Prognoz Üzerine Etkisi

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ABSTRACT

Objective: Lung cancer has a poor prognosis. Thiol groups with high antioxidant capacity are converted to disulfide bonds through biochemical reactions that neutralize different oxidant compounds. The thiol-disulfide (SH-SS) homeostasis has significant effects on cell mechanisms, transcription, and apoptosis. Here we present the prognostic role of dynamic SH-SS homeostasis in patients operated for NSCLC.

Materials and Methods: Patients operating for early-stage NSCLC were prospectively analyzed. SH-SS homeostasis tests were measured using the automated spectrophotometric method.

Results: This study enrolled 138 subjects, including 77 patients and 61 healthy controls. Native thiol and total thiol levels were significantly lower in the patient group. The disulfide-to-native thiol ratio, which is an indicator of oxidative stress in SH-SS homeostasis, also reached a level of statistical significance in the patient group ($p<0.001$). According to the cut-off values (305 and 326.3), the median overall survival rate was significantly shorter in patients with low native thiol and total thiol levels ($p<0.001$).

Conclusions: This study demonstrated decreased native thiol and total thiol levels as well as decreased disulfide levels and SS/SH ratio in early-stage NSCLC. Impaired SH-SS homeostasis may contribute to lung cancer pathogenesis and poor prognosis because of enhanced oxidative stress.

Keywords: Disulfide, early-stage lung cancer, NSCLC, oxidative stress, thiol

ÖZ

Amaç: Akciğer kanseri kötü prognoza sahiptir. Antioksidan kapasitesi yüksek olan tiyol grupları, farklı oksidan bileşikleri nötralize eden biyokimyasal reaksiyonlarla disülfid (SS) gruplarına dönüşürler. Tiyol/Disülfid homeostazı (TDH) hücre mekanizmaları, transkripsiyon ve apoptoz üzerinde önemli etkilere sahiptir. KHDAK nedeniyle ameliyat edilen hastalarda dinamik TDH'nin prognozunun rolünü sunuyoruz.

Materyal ve Metot: KHDAK ile erken evre opere edilen hastalarda prospektif olarak analiz edildi. TDH testleri otomatik spektrofotometrik yöntemle ölçüldü.

Bulgular: Bu çalışmaya 77 hasta, 61 sağlıklı olmak üzere toplam 138 kişi katıldı. Native tiyol (NT) ve total tiyol (TT) düzeyleri hasta grubunda anlamlı olarak düşüktü. TDH'nin oksidatif stres göstergesi olan SS/NT oranı da hastalarda istatistiksel olarak anlamlı bulunmuştur ($p<0,001$). Medyan OS, NT ve TT düzeyi düşük olan hastalarda cut-off değerine göre (305 ve 326,3) anlamlı olarak daha kısaydı ($p<0,001$).

Sonuç: Bu sonuçlar erken evre KHDAK'de; doğal tiyol ve toplam tiyol seviyeleri azaldığını, disülfid seviyeleri ve disülfid/NT oranının da bozulduğunu göstermektedir. Bozulmuş tiyol/disülfid homeostazı, artan oksidatif stresin bir sonucu olarak akciğer kanseri patogenezine ve kötü prognoza katkıda bulunabilir.

Anahtar Kelimeler: Disülfid, erken evre akciğer kanseri, KHDAK, oksidatif stres, tiyol

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INTRODUCTION

Lung cancer is the leading cause of cancer deaths.[1] Non-small-cell lung cancer (NSCLC) accounts for most lung cancer cases. Despite the availability of new genetic technologies and advancements in surgical techniques, the overall 5-year survival rate for lung cancer remains at 15.6%. Genetic and local tissue modifications are also involved in lung carcinogenesis. Exposure to carcinogens modifies normal cells before clinically detectable malignant tumors occur.[2]

There are limited data about clear antioxidant information on the antioxidant levels in lung cancer. Reduction and oxidation stabilities depend on the action between the antioxidant systems. Thiols contribute to the total antioxidants present in the body and play a vital role in the defense against ROS. Total thiols consist of thiols either in the free form or reduced glutathione (GSH).[3]

The serum level of thiols indirectly reflects the antioxidant defense system. Disulfide bonds can be reconverted to thiols, consequently resulting in dynamic thiol-disulfide (SH-SS) homeostasis, which is the reversal of thiol oxidation. Dynamic SH-SS homeostasis plays a critical role in antioxidant protection.[4,5]

Abnormal SH-SS homeostasis status is involved in the pathogenesis of various diseases. Erel and Neselioglu developed a novel automated colorimetric method to be able to separately measure the two elements for the serum value of thiol. [4]

This prospective case-control study evaluated the relationship between dynamic SH-SS homeostasis and early-stage NSCLC.

MATERIALS AND METHODS

Ethical Statement: This study was approved by the Ethics Committee of Yıldırım Beyazıt University Faculty of Medicine (Date: 15.06.2020, decision no: 185). All participants provided informed consent. The study adhered to the principles of the Declaration of Helsinki and was conducted by the international declaration guidelines.

Design, Participants, and Setting: This was a prospective, non-randomized, and case-control study. Patients who were operated on early-stage NSCLC between 2014 and 2019 were analyzed. A total of 138 individuals, including 77 patients and 61 health controls, participated in this study. Seventy-seven patients (54 male and 23 female, with a mean age of 58 years) included in this study were newly diagnosed with and operated on stage I and II NSCLC. Serum samples of patients were collected at the time of diagnosis and before surgery. The American Joint Committee on Cancer staging system was used to classify lung cancer. Positron Emission Tomography (PET) and contrast-enhanced brain Magnetic Resonance Imaging (MRI) were performed for cancer staging. The control group consisted of demographically-matched volunteers who presented for health screening. The patients did not receive any anticancer treatment at diagnosis. The overall survival was calculated from the date of diagnosis to the last follow-up or death from any cause. Subjects with an active infection, chronic inflammatory, or autoimmune disease, both in the patient and healthy control groups, were excluded from the study. The status of SH-SS homeostasis was evaluated in both groups.

Measurement of SH-SS Homeostasis Parameters

Samples: Blood samples were obtained from a phlebotomist. Five ml of blood was collected from each subject into serum-separating tubes to measure the SH-SS homeostasis parameters. To measure the SH-SS homeostasis parameters, serum-separating tubes were allowed to clot for 15 min and then centrifuged. Serum samples were stored at -80°C. All samples were thawed on the same day of measurement, and laboratory analyses were carried out using an automated analyzer (Cobas c501, Roche-Hitachi, Mannheim, Germany).

Determination of SH-SS Homeostasis Parameters: Serum samples were used to determine the SH-SS homeostasis parameters using the method described by Erel and Neselioglu.[5]

This method involved two parallel analyses. Briefly, native SH contents of the sample were first measured with Ellman's reagent [5,5′-dithiobis (2-nitrobenzoic acid), DTNB]. In a parallel analysis, dynamic disulfide bridges were reduced to thiol groups by sodium borohydride. After the reduction procedure, excess unused sodium borohydride was removed with formaldehyde. After this reduction and removal process, the total thiol contents (2 thiols from each disulfide and one thiol from each native thiol of samples) of the sample were measured using DTNB. Disulfide levels were calculated as half the difference between serum levels of total and native thiol. Total thiol (SH+SS), native thiol (SH), and disulfide (SS) were expressed as μmol/L. Moreover, the percentage ratios of disulfide-to-native thiol (SS/SH), disulfide-to-total thiol (SS/SH+SS), and native thiol-to-total thiol (SH/SH+SS) were calculated.

Statistical Analysis: Statistical analysis was conducted using the IBM SPSS Statistics (Version 20) computer program (IBM, Armonk, NY, USA, 2011). Normal assumptions of variables were analyzed with the Kolmogrov-Smirnov test. Descriptive were expressed as median (1^{st}-3^{rd} quartile value) or mean±standart deviation for non-parametrically or parametrically distributed variables, respectively. Comparison of continuous variables was performed with the Mann-Whitney U test or Student's t-test for

non-parametrically or parametrically distributed variables, respectively. Categorical variables were shown as numbers (%) and compared with the Pearson Chi-square test or Fisher's exact test, which was appropriate. Correlation analysis was conducted with the Spearman test. Cut-off points were determined by receiver operating characteristic (ROC) curve analysis based on survival status. Sensitivity and specificity were calculated according to these cut-off points, and the patients were divided into two groups according to this cut-off point. Kaplan-Meier survival analysis was performed to evaluate the effects of the determined cut-off point on survival. A p -value<0.05 was accepted as statistically significant.

RESULTS

This study enrolled 77 patients with NSCLC (mean age of 58.36±9.16 years) and 61 health controls (mean age of 60.56±7.92 years). There was no significant difference between the patient and the control groups regarding age or gender (p>0.05 for both). The native thiol (SH) and total thiol (SH+SS) levels were lower in the patient group. The disulfide level and the percentage ratios of SS/SH, SS/SH+SS, and SH/SH+SS were statistically higher in the patients (p<0.001). All patients were classified by mortality and survival rates. There was no difference in mortality by age, gender, and histopathological types. Native thiol and total thiol levels were lower in those who died during the follow-up period. However, the percentage ratios of SS/SH, SS/SH+SS%, and SH/SH+SS were not correlated with mortality (Table 1).

In this study, the cut-off value for the native thiol level to predict mortality was determined using ROC analysis. The sensitivity of the native thiol level and the specificity of the disulfide level were 91.3% and 50%, respectively (p=0.0002) (Figure 1).

The sensitivity and specificity of the total thiol level were 65.2% and 70.4%, respectively (Figure 2).

Table 1. Thiol/disulfide homeostasis parameters and histopathological types of mortality and survival groups.

	Control Group (n=61)	Patient Group (n=77)	p-value
Age, years	58.36±9.16	60.56±7.92	0.140
Gender (female/male)	9/52	8/69	0.438
Native thiol (SH), µmol/L	430.41±48.46	288.41±62.07	0.001
Total thiol (SH+SS), µmol/L	470.4±49.41	339.95±70.26	0.001
Disulfide (SS), µmol/L	19.99±6.61	25.6±11.68	0.001
SS/SH%	4.71±1.66	9.27±4.78	0.001
SS/SH+SS%	4.27±1.38	9.77±13.41	0.001
SH/SH+SS%	91.47±2.76	84.86±6.10	0.001
Patients	Mortality Group (n=23)	Survival Group (n=54)	p-value
Age, years	59(54-66)	62(55.75-65.25)	0.978
Female/Male	1/22	7/47	0.423
Squamous cell ca/Adenocarcinoma	13/10	22/32	0.203
Survival, months	13(7-19)	34.5 (27-40)	0.001
Native thiol (SH); µmol/L	252.87±58.4	303.55±57.68	0.001
Total thiol (SH+SS); µmol/L	303.36±71.14	355.54±64.41	0.002
Disulfide (SS); µmol/	20.88(15.12-36.4)	25.9 (16.93-34.13)	0.361
SS/SH%	8.55(6.49-12.64)	7.91 (5.88-11.91)	0.701
SS/SH+SS%	7.35(6.03-10.33)	7.2 (5.26-9.64)	0.407
SH/SH+SS%	85.39(79.82-88.5)	86.72 (80.76-89.48)	0.567

Numerical data are shown as the median ± standard deviation.

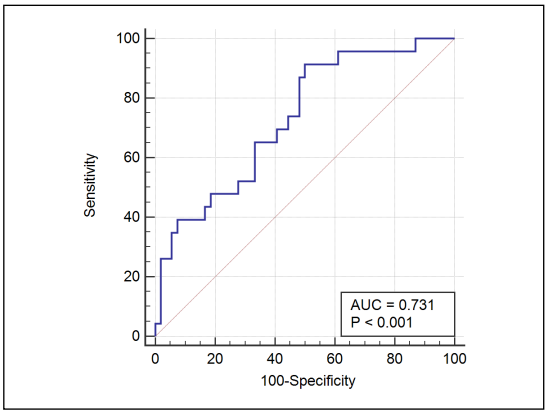

Figure 1. Receiver operator characteristic curve for the native thiol level in the prediction of mortality.

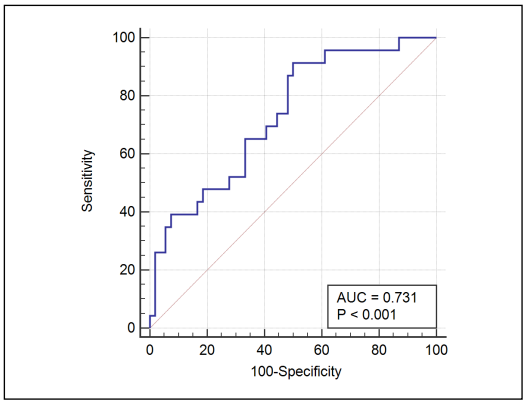

Figure 2. Receiver operator characteristic curve for the total thiol level in the prediction of mortality.

The median follow-up duration of the patient group was 35 months. According to the cut-off value (305 µmol/L and 326.3, respectively), the median overall survival (OS) was significantly shorter in patients with low native thiol (Figure 3) and total thiol levels (Figure 4) (p<0.001).

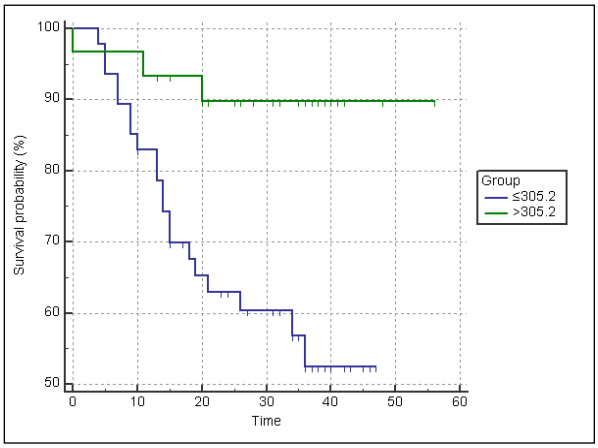

Figure 3. Kaplan-Meier survival analysis for native thiol with a cut-off point of 305.2 (Log-rank test, P=0.0001).

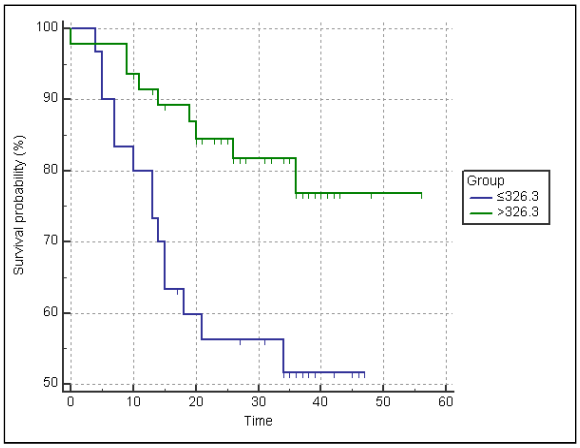

Figure 4. Kaplan-Meier survival analysis for total thiol with a cut-off point of 326.3 (Log-rank test, P=0.0110).

DISCUSSION AND CONCLUSION

This study demonstrated reduced native thiol and total thiol levels in patients compared to the control subjects. The percentage ratios of SS/SH, SS/SH+SS, and NT/SH+SS, which are indicators of oxidative stress, also reached a level of statistical significance in patients. The data of this study suggest that the status of SH-SS homeostasis may be altered in patients with early-stage lung cancer due to ROS-induced oxidation/reduction reactions. These results suggest that low plasma levels of native thiol and total thiol are associated with poor survival.

In a study, NT and TT levels were found to be low in patients with lung cancer. This study included patients with both early-stage and advanced-stage lung cancer. Despite this heterogeneous population, these low levels indicate that oxidant/antioxidant balance is impaired by lung cancer.[1]

While the native thiol and total thiol levels were lower in lung transplant patients compared to the control group, their disulfide levels were similar. Higher disulfide-to-native thiol (SS/SH) and disulfide-to-total thiol (SS/SH+SS) ratios have been reported in lung transplant recipients.[6] The lung is a major target organ for damage caused by exogenous oxidants. Oxidative mechanisms play a role in carcinogenesis initiation, promotion, and progression. The lungs need antioxidants to protect against possible oxidative injury due to direct exposure to cancer agents and very high quantities of oxygen.

The study by Erel showed higher disulfide levels in inflammatory diseases but significantly lower disulfide levels in renal cell carcinoma and colon carcinoma.[4]

The lowest disulfide levels have been observed in aggressively growing tumors; however, in slow-growing tumors like basal cell carcinoma, the decrease has been reported to be at a subnormal level.[7]

In our study, the native thiol and total thiol levels were lower in the patient group. Moreover, the disulfide levels were higher in the patient group. There was no difference between the deceased and surviving patients. This can be explained by the fact that all patients evaluated in this study may have had early-stage lung cancer. There are a limited number of studies investigating the effects of thiol-disulfide hemostasis on cancer patients. In our study, the native and total thiol levels were lower in those who died during the follow-up period (p<0.001).

A study on patients with diabetic ketoacidosis (DKA) found low levels of native, and total thiol. This indicates the presence of oxidative stress in these patients. Disulfide bonds are formed because of thiol metabolism. According to Otal et al.[8] the disulfide level, which does not increase with increased thiol use, indicates the possibility of exposure of advanced proteins to oxidation in DKA patients.

The total thiol levels of our patients were similarly low, but the disulfide levels increased compared with the control group. This result demonstrates the oxidation mechanism in patients with early-stage lung cancer, but no intensive oxidation mechanism as in DKA patients. Thiol-disulfide homeostasis is impaired in many diseases of unknown etiology.[9]

A review assessed thiol-disulfide homeostasis in various skin diseases and found a shift toward the disulfide or thiol side in patients; however, it remained unchanged in some patients. This may vary depending on the stage and severity of the disease, and the level of oxidative stress and antioxidant capacity.[10]

In this study, the cut-off value for the native thiol level to predict mortality was determined using ROC analysis. The sensitivity of the native thiol level and the specificity of the disulfide level were 91.3% and 50%, respectively. The sensitivity and specificity of the total thiol level were 65.2% and 70.4%, respectively. The decreased antioxidant levels in patients with early-stage lung cancer may be associated with poor prognosis.

There is no information on the level of antioxidants, particularly in lung cancer. Studies have reported an increase in antioxidant activity in lung cancer, whereas other studies have shown decreased antioxidant activity.[11]

High oxidative biomarker levels are associated with metastasis and tumor aggressiveness in lung cancer. Therefore, evaluating biomarkers for early-stage cancer is not applicable. However, our results revealed an association between reduced antioxidant activity and mortality in early-stage NSCLC.

In conclusion, these results may demonstrate the roles of oxidative stress in lung cancer and the biomarkers that may be used to predict prognosis.

Ethics Committee Approval: This study was approved by the Ethics Committee of Yıldırım Beyazıt University Faculty of Medicine (Date: 15.06.2020, decision no: 185). All participants provided informed consent. The study adhered to the principles of the Declaration of Helsinki and was conducted by the international declaration guideline.

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

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Can NLR, D-Dimer, and MPV Values Predict Mortality and Clinical Severity in Covid-19 Patients?

Kovid-19 Hastalarında NLR, D-Dimer ve MPV Değerleri Mortaliteyi ve Klinik Ciddiyeti Öngörebilir Mi?

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ABSTRACT

Objective: In this study, we aim to determine the relationship between neutrophil/lymphocyte ratio (NLR), D-dimer, and mean platelet volume (MPV) parameters with mortality and clinical severity in Covid-19 patients.

Materials and Methods: This retrospectively planned study included Covid-19 patients admitted to the emergency department between March 2020 and September 2020. Sociodemographic characteristics, laboratory parameters, and imaging results of the patients were obtained. The patients were grouped according to the development of mortality and clinical follow-up.

Results: Seven hundred patients were included in the study and the mean age of the patients was 49.1±18.2 years. Mortality developed in 5.4% (n=38) of the patients. NLR, D-dimer, and MPV levels of patients who developed mortality were higher than those who survived (p<0.001; p <0.001 and p=0.035, respectively). In ROC analysis, >6 NLR levels, >8.45 MPV levels, and >0.57 D-dimer levels were found to be predictive for mortality (p<0.001; p=0.019; p <0.001, respectively).

Conclusions: The high NLR, D-dimer, and MPV levels obtained at the time of admission in Covid-19 patients can be used as an indicator of mortality. Elevated NLR and D-dimer levels are useful in determining the severity of the disease and clinical follow-up.

Keywords: Covid-19, D-dimer, mean platelet volume, mortality, neutrophil/lymphocyte ratio

ÖZ

Amaç: Bu çalışmada amacımız Kovid-19 hastalarında nötrofil/lenfosit oranı (NLR), D-dimer ve ortalama trombosit hacmi (MPV) parametrelerinin mortalite ve klinik ciddiyet ile olan ilişkisini tespit etmektir.

Materyal ve Metot: Retrospektif olarak planlanan bu çalışmaya Mart 2020 ile Eylül 2020 tarihleri arasında acil servise başvuran Kovid-19 hastaları dahil edildi. Hastaların sosyodemografik özellikleri, laboratuvar parametreleri ve görüntüleme sonuçları hastanenin elektronik kayıt sisteminden elde edildi. Hastalar mortalite gelişimine ve klinik takip şekline göre gruplandırıldı.

Bulgular: Çalışmaya 700 hasta alınmış olup hastaların yaş ortalaması 49,1±18,2 yıldı. Hastaların %5,4'ünde (n=38) mortalite gelişti. Mortalite gelişen hastaların NLR, D-dimer ve MPV düzeyleri sağ kalanlardan daha yüksekti (p<0,001; p<0,001 ve p=0,035 sırasıyla). ROC analizinde >6 NLR düzeylerinin ((%71,05 sensitivite ve %92,15 spesifite; AUC: 0,900 (%95 GA 0,858-0,941, p<0,001)), >8,45 MPV düzeylerinin (%68,4 sensitivite ve %53,9 spesifite; AUC: 0,601 (%95 GA 0,564-0,638, p=0,019)) ve >0,57 D-dimer düzeylerinin (%81,6 sensitivite ve %73,9 spesifite; AUC:0,841 (%95 GA 0,812-0,867 p<0,001)) mortalite için belirleyici olduğu görüldü.

Sonuç: Kovid-19 hastalarında başvuru anında alınan NLR, D-dimer ve MPV düzeylerindeki yükseklik mortalite için bir gösterge olarak kullanılabilir. NLR ve D-dimer düzeylerindeki yükseklikler hastalığın ciddiyetini ve klinik takip şeklini belirlemede kullanışlıdır.

Anahtar Kelimeler: D-dimer, kovid-19, mortalite, nötrofil/lenfosit oranı, ortalama trombosit hacmi

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INTRODUCTION

At the end of 2019, a new coronavirus named 2019 novel coronavirus (Covid-19; SARS-nCoV-2) emerged in Wuhan, China, causing an unusual viral pneumonia epidemic. This novel coronavirus disease, also known as Covid-19, has spread rapidly worldwide as it is highly contagious.[1] The ongoing Covid-19 pandemic has posed an extraordinary threat to global public health.[2] Ultimately, on March 11, 2020, the World Health Organization (WHO) officially described the global Covid-19 outbreak as a pandemic.[3]

About 80% of infected individuals have mild to moderate symptoms. The remaining patients may be in a clinical condition severe enough to require hospitalization. Among the severely ill, the most serious complications are acute respiratory distress syndrome (ARDS)/diffuse alveolar damage.[4]

Neutrophil/Lymphocyte ratio (NLR) is a widely used marker to evaluate bacterial infections' severity, define the immune response to stress stimuli, and may have prognostic value in pneumonia and malignancies.[5] Recent studies have shown that changes in NLR may damage T lymphocytes caused by Covid-19, which may be responsible for disease worsening.[6] Platelet activation is linked to the pathophysiology of diseases prone to thrombosis and inflammation.

Studies suggest a relationship between the increase in mean platelet volume (MPV) and the risk of thrombosis. D-Dimer is a specific degradation product produced in the hydrolysis of fibrin.[7] It may reflect the effects of infection on coagulation in infectious diseases. Some studies have reported increased levels of D-Dimer in patients with pneumonia.[8] The level of D-Dimer in critically ill Covid-19 patients is significantly increased, with frequent coagulation disorders and microthrombotic formation in peripheral blood vessels.[9]

In this study, we aim to determine the relationship between NLR, D-dimer, and MPV parameters with mortality in Covid-19 patients.

MATERIALS AND METHODS

Ethical Approval: By the Declaration of Helsinki, it was gotten after the tertiary Training and Research Hospital ethics committee approval (Dated: 13.01.2021, decision no: 2021/1/19).

Study Population: Patients older than 18 with a positive PCR test for Covid-19 and a lung computed tomography who came to the emergency department of a tertiary hospital between March 2020 and September 2020 were included in the study. Patients younger than 18 years of age, with pregnancy status, with a diagnosis of hematological disease, with a diagnosis of oncological disease, with a diagnosis of rheumatological disease, who received immunosuppressive therapy, and whose adequate clinical information, laboratory and imaging data could not be reached were excluded from the study.

Data Collection: Sociodemographic characteristics such as age, gender, comorbidity, laboratory values such as neutrophils, lymphocytes, D-dimer and MPV, and thorax CT images and comments of the patients included in the study were obtained from the electronic registry system of the hospital. The clinical course of the patients was analyzed from the electronic record system. Regarding follow-up, the patients were divided into three groups: outpatients (discharged from the emergency room), patients admitted to the service, and patients admitted to the intensive care unit. As an outcome measure, patients who developed mortality during the hospital stay and patients who did not were divided into two groups. Sociodemographic characteristics and laboratory parameters were compared between all groups.

Statistical Analysis: Obtained data were analyzed using IBM SPSS Statistics 25 and MedCalc statistical software (version 20; MedCalc Software, Ostend, Belgium) package programs. Categorical variables are expressed as frequency and percentage. The mean±standard deviation for the numerical variables that fit the normal distribution, the median (minimum-maximum) for the variables that did not include the normal distribution; Student's t-test was used to compare numerical data with normal distribution in pairwise group comparisons, the Mann-Whitney U test was used to compare data that did not fit. The Chi-square or Fisher's exact test was used to compare categorical data. Kruskal-Wallis's analysis of variance was used for multiple group comparisons. Receiver operating characteristics (ROC) analysis was performed to determine the best NLR, D-dimer, and MPV levels in evaluating the mortality. Optimal cut-off values and sensitivity and specificity values were determined for these measurements. The area under the curve (AUC) and 95% confidence intervals (CI) are indicated. All statistical tests were two-tailed, and the statistical significance level was accepted as $p<0.05$ for all analyses.

RESULTS

Between the specified dates, the data of 1154 patients were examined for the study, and a total of 700 patients were eligible for the study after the exclusion criteria were applied. The mean age of the patients was 49.1 ± 18.2 years. The female/male ratio was 1.2/1. There was at least one other disease in 39% (n=273) of the patients. Mortality developed in 5.4% (n=38) of the patients. Pneumonia was observed in 58.6% (n=410) of the patients on thorax CT (Table 1).

Table 1. Sociodemographic characteristics, laboratory results, and imaging results of the patients.

Characteristic		Value
Age (years)	Mean ± Sd	49.1 ± 18.2
Gender n (%)	Female	388 (55.4)
	Male	312 (44.6)
Comorbidity n (%)	Comorbidity (-)	427 (61.0)
	Comorbidity (+)	273 (39.0)
	Hypertension	232 (33.1)
	Asthma	55 (7.9)
	Diabetes mellitus	54 (7.7)
	COPD	22 (3.1)
	Chronic kidney disease	7 (1.0)
	Heart failure	5 (0.7)
	Cerebrovascular disease	4 (0.6)
	Coronary artery disease	4 (0.6)
	Other*	6 (0.8)
Mortality (+) n (%)		38 (5.4)
NLR Med (min-max)		2 (0-40)
MPV (fl) Ort ± SS		8.4 ± 1.0
D-dimer (µg/ml) Med (min-max)		0.34 (0.02-35)
Thorax CT n (%)	Pneumonia (-)	290 (41.4)
	Pneumonia (+)	410 (58.6)

* Other; 2 patients with hypothyroidism, 1 patient with pulmonary arterial hypertension, 1 patient with chronic HBV, 1 patient with HCV, 1 patient with arrhythmia; CT: computed tomography; MPV: Mean Platelet Volume; NLR: Neutrophil Lymphocyte Ratio; COPD: Chronic Obstructive Pulmonary Disease.

The mean age of patients who developed mortality (75.6 ± 10.5) was significantly higher than that of survivors (47.5 ± 17.4) (p<0.001). It was observed that patients who developed mortality had a higher rate of another disease than those who did not (p<0.001). The median NLR value of patients who developed mortality was 8.5, while 2 in patients who did not (p<0.001). The median D-dimer level of patients who developed mortality was 1.16, while it was 0.32 in patients who did not (p<0.001). The median MPV level of patients who developed mortality was 8.6, while it was 8.4 in patients who did not (p=0.035). All three parameters were statistically higher in patients who developed mortality than in those who did not (Table 2).

Table 2. Comparison of demographic, clinical, and laboratory results of groups with and without mortality.

		Mortality (+) (n=38)	Mortality (-) (n=662)	p
Age (years)	Mean ± Sd	75.6 ± 10.5	47.5 ± 17.4	0.001†
Gender n (%)	Kadın	17 (44.7)	371 (56.0)	0.173††
	Erkek	21 (55.3)	291 (44.0)	
Comorbidity n (%)	Comorbidity (-)	6 (15.8)	421 (63.6)	0.001††
	Comorbidity (+)	32 (84.2)	241 (36.4)	
ICU (+) n (%)		35 (92.1)	23 (3.5)	0.001††
NLR Med (min-max)		8.5 (2-36)	2 (0-40)	0.001*
MPV (fl) Med (min-max)		8.6 (7.2-10.6)	8.4 (0.16-12.40)	0.035*
D-dimer (µg/ml) Med (min-max)		1.16 (0.2-35.0)	0.32 (0.02-9.7)	0.001*
Thorax CT n (%)	Pneumonia (-)	2 (5.3)	288 (43.5)	0.001††
	Pneumonia (+)	36 (94.7)	374 (56.5)	

†Independent samples t test; ††Chi-square test; *Mann Whitney U test; ICU: intensive care unit; CT: computed tomography; MPV: Mean Platelet Volume; NLR: Neutrophil Lymphocyte Ratio.

When outpatients (discharged from the emergency room) and inpatients were compared; The mean age of the patients hospitalized in the ICU was among the patients hospitalized in the ward; The mean age of patients hospitalized in the community was also higher than the mean age of patients discharged from the emergency department (p<0.001; for both). The comorbidities of the patients admitted to the ICU were more common than those admitted to the service and discharged from the emergency department (Table 3). NLR levels of the patients hospitalized in the ICU were higher than the patients hospitalized in the service (p<0.001); NLR levels of hospitalized patients were also higher than those discharged (p<0.001). It was determined that MPV levels were higher in those who were followed up in the ICU than those who were discharged (p=0.049) and were similar among the other groups. Patients admitted to the ICU had higher D-dimer levels than those released and disclosed to the ward (p<0.001; for each). The D-dimer levels of the patients who were

Table 3. Comparison of demographic, clinical, and laboratory results according to the follow-up type of patients.

		Discharged (n=342)	Service admission (n=300)	ICU admission (n=58)	p
Age[abc]	Mean ± Sd	40.5[a] ± 14.1	54.1[b] ± 17.2	73.5[c] ± 12.2	0.001[†]
Gender n (%)	Kadın	186 (54.4)	175 (58.3)	27 (46.6)	0.220[††]
	Erkek	156 (45.6)	125 (41.7)	31 (53.4)	
Comorbidity n (%)	Comorbidity (-)	278 (81.3)	140 (46.7)	9 (15.5)	0.001[††]
	Comorbidity (+)	64 (18.7)	160 (55.3)	49 (84.5)	
****NLR[abc]**		2 (0-26)[a]	2 (0-28)[b]	8 (2-40)[c]	0.001[*]
****MPV[abc] (fl)**		8.4 (0.16-12)[a]	8.3 (6.5-11.3)[b]	8.6 (6.8-12.4)[c]	0.045[*]
****D-dimer[abc] (µg/ml)**		0.28 (0.02-8.07)[a]	0.36 (0.04-9.29)[b]	0.93(0.2-35)[c]	0.001[*]
Thorax CT n (%)	Pneumonia (-)	225 (65.8)	62 (20.7)	3 (5.2)	0.001[††]
	Pneumonia (+)	117 (34.2)	238 (79.3)	55 (94.8)	

[†]One Way ANOVA test; [††]Chi-square test; *Kruskal Wallis test; **Median (minimum-maximum); CT: computed tomography; MPV: Mean Platelet Volume; NLR: Neutrophil Lymphocyte Ratio; ICU: intensive care unit; Age[a-b]:p<0.001; Age[a-c]: p<0.001; Age[b-c]: p<0.001; NLR[a-b]: p<0.001; NLR[a-c]: p<0.001; NLR[b-c]: p<0.001; MPV[a-b]: p=0.996; MPV[a-c]: p=0.049; MPV[b-c]: p=0.052; D-dimer[a-b]: p=0.003; D-dimer[a-c]: p<0.001; D-dimer[b-c]: p<0.001.

followed up in the community were also higher than those who were discharged (p=0.003) (Table 3).

In ROC analysis, it was observed that NLR (p<0.001), D-dimer (p<0.001), and MPV levels (p=0.035), respectively, were the only determinants of mortality. Accordingly, NLR levels >6 were an indicator for mortality with a sensitivity of 71.05% and a specificity of 92.15% (AUC: 0.900 (95% CI 0.858-0.941, p<0.001)); MPV levels >8.45 were an indicator for mortality with 68.4% sensitivity and 53.9% specificity (AUC: 0.601 (95% CI 0.564-0.638, p=0.019)), and >0.57 D-dimer levels 81%, It was found to be an indicator for mortality (AUC:0.841 (95% CI 0.812-0.867 p<0.001)) with a

Table 4. Diagnostic performance of NLR, MPV and D-dimer on mortality.

	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p
NLR	0.900	>6.0	71.05	92.15	20.1	98.7	0.001
MPV (fl)	0.601	>8.45	68.4	53.9	7.8	96.7	0.019
D-dimer (µg/ml)	0.841	>0.57	81.6	73.9	15.2	98.6	0.001

AUC: Area Under the Curve; PPD: positive predictive value; NPD: negative predictive value; MPV: Mean Platelet Volume; NLR: Neutrophil Lymphocyte Ratio.

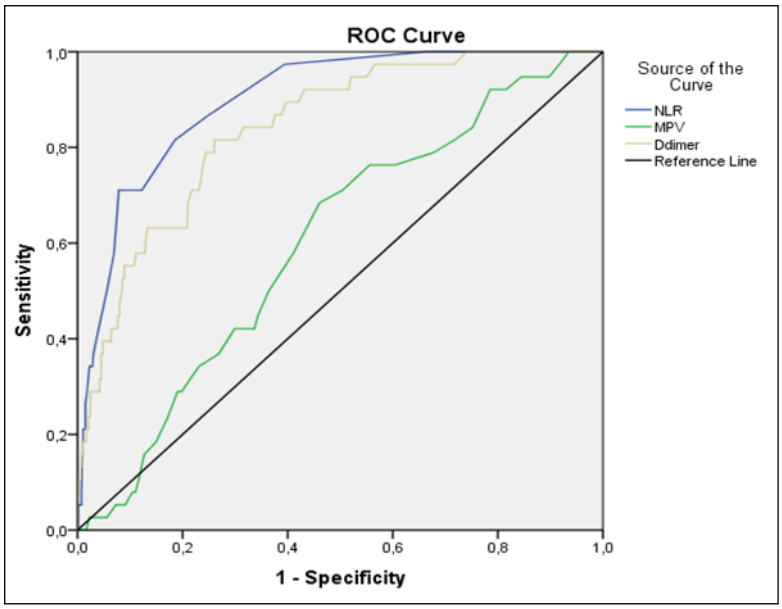

Figure 1. ROC curve for NLR, MPV, and D-dimer - mortality association.

sensitivity of 6 and a specificity of 73.9% (Table 4). The corresponding ROC curve is shown in Figure 1.

DISCUSSION AND CONCLUSION

Due to the rapid spread of the Covid-19 pandemic, there has been a need to define markers that can be used in diagnosing the disease, in the follow-up of its clinical course, and in determining the prognosis. Because tags that can show disease progression and severity can enable the identification of high-risk individuals and the optimal use of resources. However, the sensitivity of frequently used markers in clinical practice, such as platelet, hemoglobin, lactate dehydrogenase, prothrombin time, and transaminases, the diagnosis and severity of Covid-19 needs to be revised.[10-13] Therefore, the need to define easily accessible and widely used laboratory parameters arose to predict the disease's severity and outcome. Therefore, our study aimed to determine the NLR, D-dimer, and MPV values in the prognosis and severity of Covid-19.

The mortality rate of the patients included in our study was 5.4%. Although this rate is consistent with the literature data, it can be seen as relatively low since patients in the good clinical condition who are scheduled to be discharged are also included in the study. Patients planned for discharge were almost half of the patients in the study (48.8%). Although the patients with and without mortality in our study were similar in terms of gender, it was seen that the mean age and the rate of having other diseases were higher in patients who developed mortality following the literature data (Table 1). The relationship between age, gender, and comorbidity variables with the way of follow-up of the patients (discharge, hospitalization, and admission to the intensive care unit) was similar to the relationship with mortality (Table 2). As in other diseases, sociodemographic characteristics are insufficient for managing the disease, and there is a need to benefit from rapidly accessible and frequently used laboratory parameters. In this context, we evaluated the NLR, D-dimer, and MPV parameters we looked at among the groups we formed among the patients in our study.

In our study, NLR levels were higher in patients who developed mortality than in those who did not. We even found that an NLR level of >6 indicates mortality, with a sensitivity of 71.05% and a specificity of 92.15%. In a retrospective study of 681 clinically severe Covid-19 patients by Chen et al., it was reported that the mortality rate was 15.3%, and NLR levels >6.66 could be a strong indicator of mortality if myocardial damage is also present.[14] In our study, D-dimer levels were an important prognostic and clinical severity indicator in patients with Covid-19. However, many factors or diseases affect D-dimer levels, and the clinician should be kept in mind for the follow-up of patients with Covid-19.

In a prospective study of 61 patients by Liu et al., it was shown that NLR levels >3.13 were an independent factor in predicting critical illness, with a sensitivity of 87.5% and a specificity of 71.7%.[6] In our retrospective study, which we conducted with a more significant number of patients compared to this study, we found that high NLR levels were a determining parameter in the follow-up of patients (Table 3). In a prospective cohort study by Zeng et al. with 352 hospitalized and followed-up patients, NLR levels were reported to indicate clinical worsening.[15] In our study, blood parameters taken at the time of application were evaluated following the emergency service practice, but results similar to those of Zeng et al.'s study on NLR were obtained. In a compilation of 32 studies by Alkhatip et al., more than 7500 patients were evaluated. It was stated that the NLR level was higher in those with high disease severity among Covid-19 patients.[16] In light of all these data, NLR levels are a useful parameter in determining critically ill patients, regardless of study design and the number of patients.

The uncertainty of the initial phase of the Covid-19 disease and the information supported by the studies have directed clinicians to study D-dimer examination from patients even in the early stages of the pandemic. As a matter of fact, in the study of Santotoribio et al., it was reported that D-dimer levels are a valuable parameter even at the diagnosis stage in Covid-19 patients.[17] In our study, D-dimer levels were evaluated in terms of the prognosis and clinical severity of the disease, and we found that D-dimer levels were higher in patients with mortality than those who did not (Table 2). It can be thought that age and comorbidity also contributed to the higher D -dimer in the mortality group. This situation can be considered as a limitation of the study. Besides we found that D-dimer levels had 81.6% sensitivity and 73.9% specificity for values >0.57. In addition, we found that D-dimer elevation was determinant in the clinical follow-up of the disease (discharge, service admission, and admission to the intensive care unit) (Table 3). In a study by Emin et al., it was reported that D-dimer levels increased as the disease severity increased.[18] In a study of 248 patients by Yao et al., it was reported that high D-dimer levels were an indicator of mortality.[19] Although our study supports all these literature data, it should be kept in mind that there may be variability in various hemostasis parameters, including D-dimer, prothrombin time, and thrombocytopenia, due to the presence of both hyperinflammation and coagulopathy in Covid-19 disease.[20,21] In our study, D-dimer levels were found to be an important prognostic and clinical severity indicator in patients with Covid-19. However, we think that many factors or diseases affect D-dimer

levels, and the clinician should be kept in mind for the follow-up of patients with Covid-19.

Among the indicators of the inflammatory response, MPV is a strong candidate, and many studies have evaluated the relationship between inflammation and MPV.[22] Therefore, in our study, we evaluated the clinical significance of MPV in patients with Covid-19. Our study found that MPV levels were higher in the mortality group than in the non-developing group (p=0.035). However, MPV levels are not a valuable parameter for the clinical follow-up of the disease (Table 3). A study by Aydınyılmaz et al. reported that MPV levels of >10.45 were an indicator of mortality.[23] In our study, MPV >8.45 fl had a sensitivity of 68.4% and a specificity of 53.9% for mortality.

In conclusion, the high NLR, D-dimer, and MPV levels obtained at the time of admission in Covid-19 patients can be used as an indicator of mortality. Elevated NLR and D-dimer levels are useful in determining the severity of the disease and clinical follow-up.

Ethics Committee Approval: Our study was approved by the University of Health Science Samsun Education and Research Hospital Ethics Committee (Date: 13.01.2021, decision no: 2021/1/19). The study was carried out following the international declaration, guidelines, and the study was conducted following the international declaration, guidelines.

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Fibrosis-4 Index as a Predictor for Disease Severity and Mortality in Patients with COVID-19

COVID-19 Hastalarında Hastalık Şiddeti ve Mortalite için Bir Öngörücü Olarak Fibrozis-4 Indeksi

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ABSTRACT

Objective: The Fibrosis-4 (FIB-4) index is a noninvasive marker of liver fibrosis in various patient populations. We examined whether there is a relationship between the severity and prognosis of COVID-19 disease and the FIB-4 index.

Materials and Methods: This study is cross-sectional and retrospective. The patients were divided into two groups: those hospitalized in the intensive care unit (ICU) and those hospitalized in the clinic (non- ICU).

Results: Of the 158 cases,86(54%) were male. Age, total bilirubin, AST levels and FIB-4 index were higher in ICU patients compared to non-ICUs ($p<0.001$,$p=0.002$, $p=0.003$, $p<0.001$ respectively). FIB-4 index non-survivors were also higher ($p=0.002$). When the effect of the FIB-4 index on the severity of COVID-19 disease and mortality was evaluated by ROC analysis, both ICU and non-survivors were found to be significant (respectively, FIB-4 score; AUC=0.705,95%CI:0.624-785,$p<0.001$; AUC=0.654,95%CI:0.566-742,$p=0.002$). When the FIB-4 index cut-off value for disease severity was taken as 2.19, 70.0% sensitivity and 60% specificity were found in predicting disease severity. Moreover, when the FIB-4 index cut-off value for mortality was taken as 2.19, 71.2% sensitivity and 53% specificity were found in predicting mortality.

Conclusions: The FIB-4 index is an independent predictor of severity and mortality in COVID-19 patients requiring ICU.

Keywords: COVID-19, FIB-4 index, mortality

ÖZ

Amaç: Fibrosis 4 (FIB-4) index çeşitli hasta popülasyonlarında karaciğer fibrozisini gösteren non invaziv bir belirteçdir. Çalışmamızda COVİD-19 hastalığının ciddiyeti ve mortalite ile FIB-4 skoru arasında ilişki olup olmadığını incelemeyi planladık.

Materyal ve Metot: Bu çalışma kesitsel retrospektiftir. COVID-19 tanısı doğrulanmış olup hastanede yatan 158 hastayı içermektedir. Hastalar yoğun bakım ünitesinde (ICU) yatanlar ve kliniğe yatırılanlar (non-ICU) olarak iki gruba ayrıldı.

Bulgular: Toplam 158 olgunun 86 (50%)'sı erkek idi. Yaş, total bilirubin, AST ve FIB-4 index yoğun bakım ünitesinde yatanlarda yoğun bakımda yatmayanlarla karşılaştırıldığında sırasıyla ($p<0,001$, $p=0,002$, $p=0,003$, $p<0,001$) idi. FIB-4 index hayatta kalmayanlarda daha yüsekti ($p=0,002$). FIB-4 skorunun COVİD-19 ciddiyetini ve mortaliteyi öngörmedeki etkisi ROC analizi ile değerlendirildiğinde hem yoğun bakımda yatan hemde hayatta kalmayanlarda anlamlı bulundu (sırasıyla AUC=0,705, 95%CI: 0.624-785, $p<0,001$; AUC=0,654, 95%CI:0,566-742, $p=0,002$). Hastalık şiddeti için FIB-4 indeks için cut-off değeri 2,19 olarak alındığında hastalık şiddetini öngörmede %70,0 duyarlılık ve %60 özgüllük bulunmuştur. Ayrıca, mortalite için FIB-4 indeksi için cut-off değeri 2,19 olarak alındığında, mortaliteyi öngörmede %71,2 duyarlılık ve %53 özgüllük bulunmuştur.

Sonuç: FIB-4 indeksi yoğun bakım gerektiren COVİD-19 hastalarının cidiyetini ve mortaliteyi belirlemede bağımsız bir öngördürücüdür.

Anahtar Kelimeler: COVİD-19, FİB-4 indeks, mortalite

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INTRODUCTION

Coronavirus 2019 disease caused by SARS-CoV-2 has a broad spectrum of clinical features (asymptomatic disease, pneumonia, acute respiratory distress syndrome (ARDS), and death).[1] Approximately 5% of coronavirus 2019 patients require intensive care follow-up.[2] The need for intensive care and mortality occur in advanced age, comorbidity (chronic obstructive lung disease, diabetes mellitus, hypertension, cardiovascular disease), and immunesuppressive conditions.[3]

COVID-19 affects various organs. It has been shown that approximately 14-78% of affected individuals have increased liver function tests.[4] It is thought that this transaminase elevation is caused by hepatocellular damage.[5] Elevated AST/ALT ratio from liver function tests, hyperbilirubinemia, and hypoalbuminemia are associated with significant clinical adverse events.[6]

Liver fibrosis mainly develops in patients with fatty liver disease associated with metabolic dysfunction (MAFLD),[7] chronic hepatitis, cirrhosis, liver failure, and hepatic carcinoma.[8,9] A simple and noninvasive FIB-4 index has been developed to predict clinical courses and predict hepatic fibrosis in patients with chronic HCV, chronic hepatitis B, and HIV/HCV coinfection.[10,11] However, there is very little research evaluating the relationship of the FIB-4 index with the severity of COVID-19 and prognosis.[12-14]

In this study, we wanted to show whether there is a relationship between liver FIB-4 and the severity of COVID-19 and mortality.

MATERIALS AND METHODS

Ethics Committee Approval: This study was planned according to the Declaration of Helsinki. Ethical approval was obtained by applying to the ethics committee. (Date: 30.06.2021, decision no: 331).

Subjects: A total of 158 patients diagnosed with COVID-19 by the real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) and hospitalized in the tertiary hospital were included in the study. Cases with pregnancy, hematological disease, cirrhosis, and viral hepatitis were excluded. The patients were divided into two groups. The first group was hospitalized in the clinic (non-ICU), and the second group was in the intensive care unit (ICU). ICU patients were severe and critical (ARDS, mechanical ventilation, sepsis). The diagnosis of the disease severity was made according to the WHO severity definition.[15] In addition, patients were divided into two as survivors and non-survivors. Demographic/clinical, laboratory features, and FIB-4 scores were compared between groups.

Analysis: All data were analyzed retrospectively. Complete blood count (CBC) samples and other laboratory tests were carried out using routine methods. The FIB-4 index was calculated according to the formula: FIB-4 = age (year) × AST (U/L)/ [√ALT (U/L) × platelet count (109/L)].10

Statistical Analysis: The conformity of the variables to the normal distribution was investigated using Kolmogorov-Smirnov. The continuous variables were shown as mean and standard deviation (SD) or medians of the 25th–75th percentile. Categorical associations were evaluated by using the $\chi 2$ test. If parametric tests were fulfilled, independent groups were examined by t-test; if not, the Mann-Whitney U test was used. Receiver operating curve (ROC) analysis was used to calculate for the FİB-4 index the required cut-off values to differentiate ICU and non- survivor patients with maximum sensitivity and specificity. Statistical significance was indicated by $p \leq 0.05$. Statistical analyses were done using the SPSS version 20.0 statistics software (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the total 158 cases, 54% were male. The mean age was 71.0±10.9 years in ICU patients and 57.7±17.4 years in non-ICU patients, and the difference was significant (p<0.001). There was no difference between the two groups regarding gender (p=0.055). Descriptive statistics showing the demographic and clinical findings of the patients are shown in table 1. As a comorbid disease, HT and CVD were more common in ICU patients than in non-ICU patients, and there was a significant difference (respectively, p=0.002, p<0.001). Neutrophil count, WBC, procalcitonin, CRP, sedimentation, ferritin, LDH, d-dimer, INR, FBG (fasting blood glucose), creatinine, AST and lactate levels were significantly higher in ICU patients than non- ICU patients (respectively, p< 0.001, p=0.003, p<0.001, p<0.001, p=0.006, p< 0.001, p< 0.001, p<0.001, p=0.012, p<0.001, p<0.001, p=0.003, p=0.014). Although platelet count was higher in ICU patients compared to non- ICU, there was no significant difference. Total cholesterol, LDL levels, and lymphocyte count were lower in ICU patients (respectively p=0.006, p=0.032, P<0.001). When the FIB-4 index was compared, the results were higher in ICU patients than in non-ICU patients (median (IQR); 2 (1.1-3.0) vs. 3.1(2.0-4.7), p<0.001) (Table 1).

Table 1. Comparison of COVID-19 patients' demographics and clinic characteristics between patients with intensive care unit and non-intensive care unit.

Variables	Non-ICU patients (n=79)	ICU patients (n= 79)	p
Age, year	57.7±17.4	71.0±10.9	0.001
Gender, F/M (%)	42/37 (58.3/43.0)	30/49 (41.7/57.0)	0.055
Diabetes mellitus, n (%)	25 (31.6)	33(41.7	0.130
Hypertension, n (%)	30 (37.9)	48 (60.0)	0.002
COPD, n (%)	5 (6.3)	7 (8.8)	0.411
CVD, n (%)	5 (6.3)	25 (31.6	0.001
Chronic renal failure, n (%)	5 (6.3)	10 (12.6)	0.121
Malignancy, n (%)	1 (1.2)	4 (5.0)	0.132
White blood cell count, 10^3/ mm^3	6.4± 2.3	8.2 ± 3.4	0.003
Neutrophil count, $10^3/mm^3$	3.66 (3.0-5.5)	7.9 (4.6-10.7)	0.001
Lymphocyte count, $10^3/mm^3$	1.3 (0.9-1.9)	0.7 (0.4-1.0)	0.001
Platelet count, $10^3/mm^3$	196± (78)	206 ± (82)	0.941
C-reactive protein, (CRP), mg/L	32 (14-89)	110 (68-173)	0.001
Procalcitonin, ng/mL	0.06 (0.04-2.0)	0.28 (0.12-1.1)	0.001
Sedimentatin, mm/h	43 (27-70)	66 (44-74)	0.006
Alanine transferase, (ALT), U/L	27 (18-42)	27 (16-39)	0.675
Aspartate transferase, (AST), U/L	33 (24-42)	42 (30-61)	0.003
Total bilirubin, mg/dL	0.56±0.24	0.74±0.32	0.002
D-dimer, ng/mL	561 (300-1080)	1230 (642-1937)	0.001
Ferritin, ugFEU/L	262 (122-604)	748 (428-1777)	0.001
INR	1.1 (1.0-1.2)	1.2 (1.1-1.3)	0.012
Lactate dehydrogenase, (LDH),U/L	290 (220-351)	453 (386-594)	0.001
Total cholesterol, mg/dL	157 ±35	137±44	0.006
Low-density lipoprotein, (LDL), mg/dL	100±26	89±32	0.032
Lactate,,mmol/L	1.6 (1.3-2.0)	2 (1.6-2.5)	0.014
Glucose, mg/dL	121±46	186±95	0.001
Creatinine, mg/dL	0.7 (0.6-0.9)	1.0 (0.7-1.8)	0.001
FIB-4 index	2 (1.1-3.0)	3.1 (2.0-4.7)	0.001

Descriptive results for continuous variables were expressed as mean and standard deviation or as median and interquartile range, depending on the normality of their distribution; ICU: intensive care unit; COPD: chronic obstructive pulmonary disease; CVD: cardiovascular disease, INR: international normalized ratio; Fib-4: Fibrosis 4 index.

52 (32.9%) patients were non-survival. The mean age of survivors was significantly lower (p<0.001). WBC count, neutrophil count, total bilirubin, ferritin, LDH, D-dimer, CRP, procalcitonin, sedimentation, lactate, and FIB-4 index were significantly higher among non-survivors. Although AST was elevated in non-survivors, it was not significant. A comparison of laboratory and clinical features of non-survivor and survivor patients is shown in Table 2. When the results were compared in terms of FIB-4 score, it was higher in non-survivors than in survivors (median (IQR); 2.1 (1.1-3.0) vs. 3.1(2.0-4.7), p<0.002). There was a positive correlation between FIB-4 index and age, CRP, lactate, ferritin, and total bilirubin (p=0.001, p=0.017, p<0.002, p=0.002 and p=0.001, respectively). There was a positive correlation between FIB-4 index and age, CRP, lactate, ferritin, and total bilirubin (p=0.001, p=0.017, p<0.002, p=0.002 and p=0.001, respectively) (Table 2).

When the effect of the FIB-4 index on the severity of COVID-19 disease and mortality was evaluated by ROC analysis, both ICU and non-survivors were found to be significant (respectively, FIB-4 score; AUC=0.705, 95%CI:0.624-785, p<0.001; AUC=0.654, 95%CI:0.566-742, p=0.002). When the cut-off value for the FIB-4 score was taken as 2.19, it had 70.0% sensitivity and 60% specificity, vs. 71.2% sensitivity and 53% specificity in predicting disease severity and mortality, respectively (Figure 1).

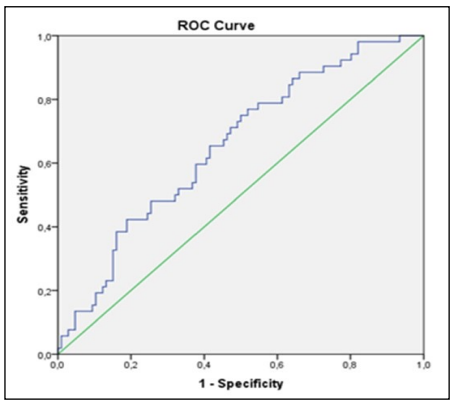

Figure 1. Receiver operating characteristic (ROC) curve for FIB-4 index in patients with survivor or non-survivor COVİD-19.

Table 2. Comparison of COVID-19 patients' demographics and clinic characteristics between survivors and non-survivors.

Variables	Survivor (n=79)	Non-survivors (n= 79)	p
Age, year	60.8± 17.1	71.7±9.9	0.001
Gender, F/M (%)	53 (50)/53(50)	19 (36)/33(64)	0.110
Systolic blood pressure, mm-Hg	123± 17	25±20	0.688
Diastolic blood pressure, mm-Hg	76±14	73±13	0.806
Diabetes mellitus, (%)	34 (32)	24 (46)	0.110
Hypertension, (%)	47 (44)	31 (59)	0.089
COPD, (%)	6 (5.6)	6 (11.5)	0.252
CVD, (%)	13 (12.2)	17 (32.6)	0.003
Chronic renal failure, (%)	7 (6.6)	8 (15.3)	0.105
Malignancy, (%)	1 (0.9)	4 (7.6)	0.031
Neutrophil count, $10^3/mm^3$	6.8±2.8	8.1 (3.2)	0.030
Lymphocyte count, $10^3/mm^3$	1.2 (0.8-1.6)	0.6 (0.4-1.0)	0.001
Platelet count, $10^3/mm^3$	199± (79)	205 ± (83)	0.674
C-reactive protein, (CRP), mg/L	52 (15-98)	113 (69-176)	0.001
Procalcitonin, ng/mL	0.08 (0.05-2.0)	0.32 (0.14-1.3)	0.001
Sedimentatin, mm/h	50 (30-70)	69 (39-78)	0.010
Alanine transferase, (ALT), U/L	27 (19-43)	27 (16-37)	0.510
Aspartate transferase, (AST), U/L	35 (25-46)	40 (25-57)	0.121
Total bilirubin, mg/dL	0.61±0.28	0.74±0.29	0.047
D-dimer, ng/mL	633 (330-1420)	1200 (589-2102)	0.009
Ferritin, ugFEU/L	351 (187-726)	758 (370-1487)	0.001
INR	1.1 (1.0-1.2)	1.2 (1.1-1.4)	0.008
Lactatedehydrogenase, (LDH),U/L	307 (242-397)	471 (385-650)	0.001
Total cholesterol, mg/dL	151 ±36	139±48	0.150
Low-density lipoprotein, (LDL), mg/dL	96±27	91±34	0.412
Lactate, mmol/L	1.6 (1.3-2.0)	2.1 (1.6-2.6)	0.004
Glucose, mg/dL	133±58	191±102	0.001
Creatinine, mg/dL	0.8 (0.6-1.0)	1.1 (0.7-1.9)	0.001
FIB-4 index	2.1 (1.3-3.4)	3.0 (2.1-4.6)	0.002

Descriptive results for continuous variables were expressed as mean and standard deviation or as median and interquartile range, depending on the normality of their distribution; ICU: intensive care unit; COPD: chronic obstructive pulmonary disease; CVD: cardiovascular disease, INR: international normalized ratio; Fib-4: Fibrosis 4 index.

DISCUSSION AND CONCLUSION

In this study, Neutrophil count, WBC, LDH, d-dimer, creatinine, AST, procalcitonin, CRP, ferritin levels, and advanced age of ICU patients were higher than the non-ICU patients. The effect of the FIB-4 score in predicting the severity and mortality of COVID-19 disease was statistically significant. The FIB-4 score was higher in ICU patients and non-survivors compared to non- ICU and survivors.

In previous studies, advanced age was shown to be associated with severe COVID-19.[16] Similarly, advanced age was present in ICU and non-survivor patients in our study. COVID-19 has a powerful and fatal course in individuals with comorbidity.[3] In studies, diabetes mellitus and coronary artery disease were shown to be the most common causes of comorbidity after hypertension, respectively.[17] In our research, similarly, hypertension, diabetes, and cardiovascular disease were common in ICU patients and non-survivors, respectively. In a previous study, the mortality rate in the ICU group was ≤49%, and in another study, 60%.[18,19] In this study, we found the mortality rate to be 34% in inpatients.

The most important process that plays a role in disease severity and prognosis in COVID-19 is the severe and uncontrolled inflammatory response caused by infection. Many factors that play a role in this inflammatory response are associated with severe illness and mortality. In a meta-analysis study, platelet count, AST, LDH, and ferritin were biomarkers for critical patients. On the other hand, in non-survivors, a relationship was found with platelet count, but no association was found between AST and LDH.[20] In other meta-analyses, significant decreases in lymphocyte count, platelets, and albumin were observed in patients with severe COVID-19 disease compared to non-serious. At the same time, elevated ALT, AST, total bilirubin, LDH, and d-dimer levels were found.[21,22] Similarly, in our study, procalcitonin, CRP, neutrophil count, WBC, creatinine, LDH d-dimer, ferritin, AST, and total bilirubin levels were significantly higher in ICU patients than in non-ICU patients. Although platelet count was higher in ICU patients than in non-ICU patients, there was no significant difference. Similarly, there was no difference in platelet count in non-survivor patients; although AST levels were high, there was no significant difference, and total bilirubin levels were significantly higher.

Liver damage is seen in severe COVID-19 patients.[1,22] The mechanisms behind COVID-19 liver injury are thought to be directly related to virus effects, the elevation of certain cytokine levels, hypoxemia, and shock.[23] Fibrosis may increase the risk of

developing an exacerbated inflammatory response in COVID-19 severe patients. Developing liver disease stimulates immune cells. Activated immune cells, cytokines, chemokines, and other inflammatory markers play a role in maintaining the chronic low-grade systemic inflammation state.[24] In an acute infection, IL-6 released from macrophages stimulates the synthesis of acute phase response proteins such as CRP, ferritin, complement, coagulation factors, etc., in hepatocytes.[25] We found correlations between CRP, ferritin, INR, and FIB-4 score. Similarly, another study found a correlation between CRP and FIB-4 score.[13] This suggests that the inflammatory response is exacerbated in patients with higher fibrosis markers. Studies have shown that elevated AST/ALT ratio, hyperbilirubinemia, and hypoalbuminemia among liver function tests are associated with significant adverse events in COVID -19 patients.[7] Ibanez-Samaniego et al. announced that the FIB-4 score is an independent predictor of disease prognosis in patients aged 35-65 years with COVID-19.[13] Li Y et al. showed that the FIB-4 score is associated with mortality in COVID-19 patients regardless of underlying conditions, including liver disease.[15] In another study, it was determined that the FIB-4 score was an independent predictor of mortality in COVID-19 cases, regardless of the severity of the disease.[13] In our study, AST and total bilirubin levels are high in ICU patients. In our study, the FIB-4 index was higher in ICU and non-survivors than in non-ICU and survivors ($p<0.001$; $p<0.002$). When the FIB-4 index cut-off value for disease severity and mortality was taken as 2.19, 70.0% sensitivity and 60% specificity were found in ICU patients in predicting disease severity; 71.2% sensitivity and 53% specificity in predicting mortality.

The FIB-4 score can be calculated quickly from blood sample data worldwide. Therefore, it can help predict the need for ICU and mortality in COVID-19 disease. At the same time, these bio-markers are vital for early diagnosing of patients at risk of critically ill and providing early intervention to improve prognosis.

In conclusion, the FIB-4 score is an essential and useful independent predictor of ICU need, disease severity, and mortality in COVID-19 patients. Although this study was conducted in a large cohort and a tertiary hospital, there were some limitations. These limitations are the single-center, retrospective design and the lack of anthropometric data due to the urgency of epidemics. FIB-4 index components are not liver-specific. FIB-4 index may be affected by disorders other than liver disease. Therefore, more prospective studies are needed.

Ethics Committee Approval: This study was planned according to the Declaration of Helsinki. The study was approved by Sakarya University Non -interventional Ethics Committee (Date: 30.06.2021, decision no: 331).

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

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